

1 **Integrating metabolic performance, thermal tolerance,**
2 **and plasticity enables for more accurate predictions on**
3 **species vulnerability to acute and chronic effects of global**
4 **warming**

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1 Abstract

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

1 **Introduction**

2 Changes in latitudinal, altitudinal and bathymetric distribution caused by global warming
3 have been increasingly documented across terrestrial and aquatic taxa (e.g. Southward *et al.*,
4 1995; Menéndez & Gutiérrez, 1996; Parmesan & Yohe, 2003; Root *et al.*, 2003; Perry *et al.*,
5 2005). In general, understanding the mechanistic basis of sub-lethal and lethal thermal
6 tolerance (e.g. Pörtner, 2001, Helmuth *et al.*, 2005) allows the prediction of species responses
7 under future warming scenarios, and scaling up predictions to assemblages and ecosystems
8 (Bernardo *et al.*, 2007, Somero, 2010, 2011). To date, however, most studies investigating the
9 causal mechanisms underpinning species vulnerability to warming have focused on
10 physiological traits in isolation (e.g. Stillman & Somero, 2000; Pörtner & Knust, 2007; Calosi
11 *et al.*, 2008; Bartolini *et al.*, 2013).

12 The frequency and intensity of extreme acute thermal events are predicted to increase with
13 the global change (IPCC, 2012). Species short-term resilience to these acute thermal events
14 will depend on their upper thermal limits, as well as on their ability to adjust these limits,
15 when exposed to higher temperatures, through phenotypic plasticity (e.g. acclimatization).
16 Indeed, phenotypic plasticity may allow organisms to express broader thermal tolerance
17 windows (Ghalambor *et al.*, 2007; Charmantier *et al.*, 2008; Bozinovic *et al.*, 2011). In the
18 longer term, plasticity may define species scope for resilience to change *via* phenotypic
19 buffering (Waddington, 1942; Bradshaw, 1965), and, in part, species scope for adaptation *via*
20 genetic assimilation (Pigliucci *et al.*, 2006). Conventionally, plasticity of thermal limits has
21 been studied by characterising the magnitude of Reaction Norms (Schlichting & Pigliucci,
22 1998 and ref. therein). However, no comparative study has, so far, focused on both the
23 magnitude and shape of plasticity (*sensu* Schlichting & Pigliucci, 1998; Pigliucci, 2001; c.f.
24 Murren *et al.*, 2014).

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

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

1 shape) (Bozinovic *et al.*, 2011; Murren *et al.*, 2014) needs to be developed to more accurately
2 elucidate species vulnerability to global warming.

3 Here we integrate the investigation of metabolic performance, thermal tolerance, and their
4 plasticity to provide more accurate and balanced predictions on relative species vulnerability
5 to both chronic and acute effects of warming. In order to provide an empirical test for this
6 new paradigm, we applied our integrative, synthetic approach to an assemblage of six
7 caridean prawns occurring in the same geographic, hence macroclimatic, region but living in
8 different thermal habitats. Three species inhabit broader/more variable thermal environments
9 (including the invasive species *Palaemon macrodactylus*), and three species live in
10 narrower/more stable thermal conditions. Based on the current literature (e.g. Stillman, 2002,
11 2003; Deutsch *et al.*, 2008; Dillon *et al.*, 2010; see also Folguera *et al.*, 2009; Bozinovic *et al.*,
12 2013; Peck *et al.*, 2014; Rezende *et al.*, 2014), we hypothesise that, compared to species
13 occupying narrower/more stable thermal niches, species inhabiting broader/more variable
14 thermal environments possess: 1) higher upper thermal limits, 2) lower plasticity of these
15 limits, 3) higher metabolic rates, 4) higher metabolic plasticity, and 5) higher metabolic costs
16 at higher temperatures. As a consequence, species living in broader/more variable thermal
17 environments may be less vulnerable to extreme acute thermal events, but at greater risk from
18 chronic exposure to warming (Stillman, 20002, 2003; Deutsch *et al.*, 2008; Tewksbury *et al.*,
19 2008; see also Folguera *et al.*, 2009; Overgaard *et al.*, 2014), according to the idea that
20 evolutionary trade-offs may exist between these two forms of heat tolerance (Rezende *et al.*,
21 2014).

22 Caridean prawns are both ecologically and economically important. They represent a large
23 fraction of biomass of coastal shallow water assemblages of invertebrates (e.g. Bechmann *et*
24 *al.*, 2011), and exert a great ecological impact upon benthic trophic webs as either carnivores
25 or detritivores (Pihl & Rosenberg, 1984; Henderson, 1987; Oh *et al.*, 2001). Also, some

- 1 species are targets of commercial and artisanal fisheries (e.g. *Crangon crangon*, Attrill &
- 2 Thomas, 1996; Henderson *et al.*, 2006).

For Review Only

1 **Materials and Methods**

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

3 We investigated six caridean shallow water prawn species: *Palaemon elegans* (Rathke
4 1837), *Palaemon macrodactylus* (Rathbun 1902), *Palaemon serratus* (Pennant 1777) and
5 *Palaemonetes varians* (Leach 1814) (Palaemonidae), *Crangon crangon* (Linnaeus 1758)
6 (Crangonidae), and *Pandalus montagui* (Leach 1814) (Pandalidae). These species all occur in
7 the same geographic, hence macroclimatic, region but live in different thermal habitats.
8 *Palaemon elegans* and *P. varians* occupy broader/more variable thermal niches, inhabiting
9 intertidal rock pools and salt marshes respectively. *Palaemon serratus*, *C. crangon* and *P.*
10 *montagui* occupy narrower/more stable thermal niches, living in subtidal habitats. The
11 invasive species *P. macrodactylus* also lives in subtidal habitats, but withstands a broader
12 range of thermal conditions, which possibly explains its recent geographical expansion
13 (Spivak *et al.*, 2006; Lavesque *et al.*, 2010; Soors *et al.*, 2010).

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

19 Once in the laboratory specimens were transferred to tanks (approx. 4.6 L, max. 10 ind.
20 *per tank*) supplied with fully aerated sea water (salinity 33), and kept at their collection
21 temperature for 24 h in order to adjust to laboratory conditions. Subsequently, individuals
22 were haphazardly divided into four equal-size groups: 20 for *P. elegans*, seven for *P.*
23 *macrodactylus*, eight for *P. serratus*, 25 for *P. varians*, 28 for *C. crangon* and eight for *P.*
24 *montagui*. Each group was exposed to one of four incubation temperatures (10, 15, 20 and
25 25 °C) for 7 d. This exposure period is considered to be sufficiently long to acclimate

1 temperate species at 15-20 °C, but short enough to prevent the onset of longer-term negative
2 effects due to too much time spent in the laboratory (e.g. Terblanche *et al.*, 2006; Calosi *et al.*,
3 2008; Calosi *et al.*, 2010). Incubation temperatures were selected within the temperature
4 range experienced by the species across their geographical ranges, as well as potential future
5 warming scenarios (IPCC, 2014). Specimens were incubated stepwise in constant-temperature
6 (CT) rooms (12:12 h L/D regime), starting from their collection temperature. At each step
7 aquaria were ramped to the next temperature level, and kept at these conditions for 24 h
8 before being further ramped to the next level, with temperature increasing/decreasing with a
9 rate of approx. 0.015 ± 0.005 °C min⁻¹ (mean \pm SD). Once they had reached the desired
10 incubation temperature, aquaria were kept at these conditions for one week, with a maximum
11 water temperature fluctuation of approx. 0.6 °C.

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

17

18 **Determination of metabolic rate**

19 Oxygen consumption rate ($\dot{M}O_2$) under resting conditions was used as a proxy for Standard
20 Metabolic Rate (SMR), as in Spicer & Eriksson (2003) and Small *et al.* (2010). $\dot{M}O_2$ was
21 measured in glass closed cell experimental chambers. Each chamber was supplied with fully
22 aerated sea water at the selected incubation temperature and sealed underwater to prevent air
23 bubbles trapping in the chamber. Furthermore, each chamber was equipped with a magnetic
24 flea, which was shielded on the bottom by a perforated Petri dish, and placed over a multi-
25 channel magnetic stirrer (MS-53M, Jeio Tech, Chalgrove, UK) to ensure moderate and

1 continuous water mixing, preventing the formation of a hypoxic layer around the prawn. Also,
2 to provide a substrate to reduce prawn activity levels, each chamber was supplied with one or
3 two marbles (42 mm x 42 mm x 16 mm), depending on specimen dimensions, resulting in a
4 volume of water of either 190.3 or 218.4 mL. Additionally, to monitor prawn behaviour in the
5 chambers, subsamples of six ind. for each species at each incubation temperature were filmed
6 with cameras. All the specimens were quiescent during the experimental trials, and no major
7 changes in behaviour were observed at elevated incubation temperatures (data are not
8 presented here); $\dot{M}O_2$ could, therefore, be effectively used as proxy for SMR. Each prawn was
9 introduced into an experimental chamber and allowed to recover from handling and to settle
10 into experimental conditions for 30 min (Small *et al.*, 2010; Magozzi and Calosi *pers. obs.*).
11 The chambers were then closed and maintained sealed for experimental trials of 1.5-2 h.
12 Preliminary tests defined this period to be sufficiently long to undertake $\dot{M}O_2$ measurements,
13 whilst preventing prawn exposure to hypoxic conditions: i.e. oxygen saturation was never
14 allowed to fall under 70 % (Vandonk & Dewilde, 1981; Small *et al.*, 2010; Oliphant *et al.*,
15 2011), with oxygen concentration remaining always $> 190 \mu\text{mol O}_2 \text{ L}^{-1}$. In order to avoid
16 sharp thermal variations, respiration chambers were kept in CT rooms inside temperature-
17 controlled open water baths at the selected incubation temperature during the experimental
18 trial. Oxygen concentration was measured both immediately before closing the chamber and
19 immediately after re-opening it at the end of the experimental trial by using an O_2 electrode
20 (1302, Strathkelvin Instruments, Glasgow, Scotland) connected to a calibrated oxygen meter
21 (929, Strathkelvin Instruments), and expressed as $\text{nmol O}_2 \text{ g wet weight}^{-1} \text{ min}^{-1} \text{ STP}$. Before
22 the experiments started, calibration was carried out at each incubation temperature by using 0
23 and 100 % O_2 saturation as calibration points.

24 After $\dot{M}O_2$ measurements were completed, wet weight (g) and body volume (mL) of each
25 prawn were measured and then used for correction of the $\dot{M}O_2$ values. Weight was

1 determined by using an electronic high precision scale (PF-203, Fischer Scientific UK Ltd,
2 Loughborough, UK), and specimen volume was determined by immersing each prawn in a
3 Pyrex graduated cylinder (100 mL, accuracy 1 mL, Fischer Scientific UK Ltd) supplied with
4 sea water, and measuring the water displaced by the introduction of the prawn. All individuals
5 were returned to their aquaria, at their incubation temperature, each inside a numbered
6 perforated screw top transparent container (either 82.0 or 149.0 mL, depending on specimen
7 dimensions), and left to recover for 24 h before upper thermal limits were determined.

8

9 **Determination of Upper Thermal Limits**

10 In order to measure Upper Thermal Limits (UTL) a number of observable responses were
11 identified during preliminary tests and used as end-points, as in Calosi *et al.* (2008) and
12 Massamba-N'Siala *et al.* (2012). A temporal sequence of responses to increasing temperature
13 was identified as follows: 1) Mouth Gaping (MG) (wide and continuous opening of the mouth
14 parts); 2) Tail Flipping (TF) (fast upside down flip of the tail bringing abdomen and
15 cephalothorax towards one another) (Arnott *et al.*, 1998); 3) Loss of Orientation (LO):
16 inability of a prawn to right itself after having turned onto a side or its dorsal surface
17 (Brattstrom, 1968; Hopkin *et al.*, 2006; Oliphant *et al.*, 2011); 4) Onset of Spasms (OS): first
18 uncontrolled, convulsive and spasmodic movements (Zweifel, 1957; Lutterschmidt &
19 Hutchison, 1997; Hopkin *et al.*, 2006); 5) Death (D) or total paralysis (prawn laying on the
20 bottom of the experimental well for more than 15 s with no movement/pleopod beating).

21 However, not all individuals showed this complete sequence of end-points: 94.5, 90.7 and
22 100 % of the experimental prawns showed LO, OS and D respectively, whilst only 79.3 and
23 64.8 % of them showed MG and TF respectively. Also, compared to MG and TF, LO, OS and
24 D showed lower variance. Given this variability, only LO, OS and D were assumed to
25 represent temperature-induced mechanisms underpinning the failure of fundamental

1 physiological functions (Lutterschmidt & Hutchison, 1997). However, in order to avoid
2 redundant utilization of these end-points in further analyses, correlation analyses among them
3 were performed to only include functionally independent traits. As there were significant
4 positive relationships between LO and OS, OS and D, LO and D (minimum $r_{263} = 0.840$, $p <$
5 0.0001), here we mainly focused on OS, which more closely fulfils the original definition of
6 critical thermal maximum (CT_{max}): ‘the thermal point at which locomotory activity becomes
7 disorganized and the animal loses its ability to escape from conditions that will promptly lead
8 to its death’ (Cowles & Bogert, 1944; see also Lutterschmidt & Hutchison, 1997).

9 Experiments to determine UTL were started at the temperature at which individuals of a
10 given group had been incubated during the exposure period, and carried out by employing a
11 ramping program, with temperature increasing at a rate of $0.75\text{ }^{\circ}\text{C min}^{-1}$ (realised $+0.668 \pm$
12 $0.016\text{ }^{\circ}\text{C min}^{-1}$; mean \pm SE) (Lutterschmidt & Hutchison, 1997; Rezende *et al.*, 2011; c.f.
13 Overgaard *et al.*, 2012) performed with a computer-controlled water bath (R5, Grant
14 Instruments Cambridge Ltd, Shepreth, UK). Each prawn was removed from its individual
15 aquarium using a small net and rapidly, but carefully, introduced into one well (diam. 51 mm,
16 depth 65 mm) of a generic six-well plate, whose bottom surface was painted white with Tipp-
17 Ex[®] to allow an easier and more accurate visualization of the end-points. A maximum of five
18 individuals were tested at any time and, in order to avoid observer biases (Terblanche *et al.*,
19 2007), measurements were all undertaken by one single observer (S.M.). To avoid prawn
20 escaping, wells were covered with a lid between additions of individuals; once the experiment
21 started, the lid was removed to allow full aeration. The actual temperature was measured
22 every 60 sec with a digital thermometer (HH802U, Omega[®] Engineering Inc., Stamford, USA)
23 placed in an empty well adjacent to the prawn wells to avoid disturbance.

24

25 **Definition and calculation of plasticity**

1 Here plasticity is intended as the ability of specimens to adjust their metabolic performance
2 (measured as $\dot{M}O_2$) and thermal tolerance (measured as UTL) following incubation to
3 increasing temperature. Plasticity was determined according to the concept of Reaction
4 Norms (Schlichting & Pigliucci, 1998 and ref. therein; Pigliucci, 2001) (Fig. S1), as well as
5 Q_{10} for metabolism. In order to determine both the magnitude and shape of the plastic
6 response (*sensu* Pigliucci, 2001; Murren *et al.*, 2014), plasticity was calculated both within
7 the whole temperature range examined (P_{tot}) and within smaller temperature intervals (10-15,
8 15-20 and 20-25 °C – P_{10-15} , P_{15-20} , P_{20-25} respectively) (Fig. S1). P_{tot} was calculated as the
9 difference between mean values of either $\dot{M}O_2$ or UTL measured at the two extreme
10 temperature treatments (10-25 °C – P_{10-25}). In order to include *P. montagui* in the computation,
11 P_{tot} was also calculated between 10 and 20 °C (P_{10-20}), as this species had no replicates at
12 25 °C due to 100 % mortality (Table S1; see also Results). P_{10-15} , P_{15-20} , P_{20-25} were calculated
13 as the difference between mean $\dot{M}O_2$ and mean UTL measured at two consecutive
14 temperature treatments. This complementary calculation allowed not only the quantification
15 of the magnitude of plasticity within smaller temperature intervals, but also the description of
16 the shape of Standard Metabolic Rate–Temperature (SMR–T) and Upper Thermal Limits–
17 Temperature (UTL–T) Reaction Norms, providing a more accurate understanding of plastic
18 responses and highlighting between-species differences (Schlichting & Pigliucci, 1998;
19 Pigliucci, 2001; Murren *et al.*, 2014). In addition, we calculated the temperature coefficient
20 for the change in $\dot{M}O_2$ with temperature (Q_{10}) both within the whole temperature range
21 (considering as extreme temperatures 10 and either 20 or 25 °C) and within smaller
22 temperature intervals. Whilst the calculation of Q_{10} values allows the distinction between
23 acclimation-induced changes in metabolism and just an expected physiological response to
24 temperature, it does not allow an appropriate interpretation of the increase in energy
25 expenditure associated with metabolic plastic responses to increasing temperature. Because

1 we wish to consider the energetic implications of the metabolic plastic response, as well as to
2 compare metabolic plasticity with plasticity of thermal limits, here we mainly focus on
3 Reaction Norms.

4

5 **Characterisation of phylogenetic relationships among species**

6 Sequences were obtained from GenBank (<http://www.ncbi.nlm.nih.gov/>) (see Table S2 for
7 accession numbers). The pool shrimp *Procaris ascensionis* (Chace & Manning, 1972) and the
8 banded cleaner shrimp *Stenopus hispidus* (Olivier, 1811) were used as outgroups.
9 Concatenated sequences were aligned using the ClustalW (Thompson *et al.*, 1994) algorithm
10 within MEGA 5.05 (Tamura *et al.*, 2011). The partition homogeneity test, otherwise known
11 as the incongruence length difference test (Farris *et al.*, 1994) was carried out in PAUP*
12 4.b.10 (Swofford, 2002) to assess if the data were significantly incongruent. The test was
13 implemented using maximum parsimony heuristic searches (100 replicates). All other settings
14 were left at their default values. The results of this test showed no significant incongruence
15 between genes ($p = 0.96$). Phylogenetic reconstruction was carried out using maximum
16 likelihood (ML) as implemented in MEGA with all settings left as their default options.
17 Support was measured with 1,000 bootstrap replicates. Only clades with significant support
18 values (defined here as ≥ 60 bootstrap) are shown.

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

25

1 **Statistical analyses**

2 The effects of species, incubation temperature, and their interaction on $\dot{M}O_2$ and UTL were
3 analysed by using a two-way ANCOVA test with 'Tank' as a random factor nested within
4 'Species' x 'Temperature', and 'Wet weight' as a covariate. Pairwise comparisons were based
5 on model-estimated marginal means with Least Significant Difference test correction ($\alpha =$
6 0.05). Data for $\dot{M}O_2$ and UTL were non-normally distributed even following various
7 transformations (minimum K-S₂₇₄ = 1.466, $p = 0.027$), and variances were not homogeneous
8 (minimum F_{2,252} = 2.383, $p = 0.001$). However, as our experimental design included 24
9 'Species' by 'Temperature' combinations with a minimum of seven replicates *per* treatment,
10 the ANCOVA design was assumed to be tolerant from the assumption of normality and
11 heteroscedasticity (Sokal & Rohlf, 1995; Underwood, 1997; see also Melatunan *et al.*, 2011).
12 The term 'Tank' did not have a significant effect on $\dot{M}O_2$ and UTL both among species and
13 temperature treatments (maximum F_{2,177} = 2.869, $p = 0.060$), therefore it was removed from
14 further analyses.

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

1 Results

2 Metabolic rate

3 Means \pm SE for oxygen consumption rate ($\dot{M}O_2$) are given in Fig. 1a and Table S3. The
4 minimum mean $\dot{M}O_2$ was observed in *P. serratus* incubated at 10 °C (46.1 ± 7.1 nmol O₂ g
5 wet weight⁻¹ min⁻¹), while the maximum was observed in *P. varians* incubated at 25 °C (799.9
6 ± 56.7 nmol O₂ g wet weight⁻¹ min⁻¹). In general, greater $\dot{M}O_2$ was observed at higher
7 incubation temperatures in all species. Nevertheless, $\dot{M}O_2$ response to increasing temperature
8 was significantly different in different species (Fig. 1a), as indicated by the presence of a
9 significant interaction between ‘Species’ and ‘Temperature’ ($F_{14,299} = 7.1$, $p < 0.0001$) (Table
10 1). At the lowest temperature tested (10 °C), *P. serratus* showed significantly lower mean
11 $\dot{M}O_2$ than all the other species, whilst *P. montagui* exhibited significantly higher mean $\dot{M}O_2$
12 (being comparable to *P. elegans* and *P. varians*). *Crangon crangon* and *P. macrodactylus*
13 showed intermediate mean $\dot{M}O_2$ between *P. elegans*, *P. serratus* and *P. varians*, with *P.*
14 *macrodactylus* being comparable to *P. elegans* and *P. varians* (Fig. 1a). At the highest
15 temperature tested (25 °C), *P. elegans* and *P. varians*, followed by *P. macrodactylus*, showed
16 significantly higher mean $\dot{M}O_2$ than *P. serratus* and *C. crangon*. Since no individuals of *P.*
17 *montagui* survived exposure to 25 °C, the highest temperature tested for this species was
18 20 °C. At this temperature, *P. montagui* showed significantly higher mean $\dot{M}O_2$ than the other
19 subtidal species (*C. crangon*, *P. serratus* and *P. macrodactylus*), being statistically
20 comparable to *P. elegans* and *P. varians* (Fig. 1a). Wet weight had a positive significant
21 effect on $\dot{M}O_2$ ($F_{1,299} = 166.3$, $p < 0.0001$) (Table 1).

22

23 Upper Thermal Limits

24 Means \pm SE for Upper Thermal Limits (UTL), measured as Onset of Spasms (OS), are
25 given in Fig. 1b and Table S3. Means \pm SE for UTL, measured as Loss of Orientation (LO)

1 and Death (D), are also given in Fig. S3a,b and Table S3. The minimum mean UTL (for all
2 end-points) was observed in *P. montagui* incubated at 10 °C (LO: 23.6 ± 0.2 °C; OS: $24.8 \pm$
3 0.3 °C; D: 27.1 ± 0.4 °C), while the maximum was observed in *P. macrodactylus* incubated at
4 25 °C (LO: 35.7 ± 0.4 °C; OS: 37.8 ± 0.5 °C; D: 39.9 ± 0.2 °C).

5 Whilst in general higher UTL were observed at higher incubation temperatures in all
6 species, UTL response to increasing temperature was significantly different in different
7 species (minimum $F_{14,275} = 2.2$, $p = 0.010$) (Fig. 1b and Fig. S3a,b) (Table 1). At 10 °C, *P.*
8 *macrodactylus*, *P. varians* and *C. crangon* exhibited significantly higher mean OS than *P.*
9 *elegans* and *P. serratus*, which in turn showed significantly higher mean OS than *P. montagui*
10 (Fig. 1b). Between-species differences identified for OS were maintained also for LO and D
11 with two exceptions: 1) *P. elegans* showed significantly higher mean LO than *P. serratus*,
12 being comparable to *P. macrodactylus* and *P. varians* (Fig. S3a); 2) *P. varians* showed
13 significantly higher mean D not only than *P. elegans* but also than *P. macrodactylus* and *C.*
14 *crangon* (Fig. S3b). At 25 °C, *P. macrodactylus* showed significantly higher mean UTL (for
15 all end-points) than all the other species, followed by *P. elegans*, *P. varians* and *C. crangon*,
16 and finally by *P. serratus* (Fig. 1b and Fig. S3a,b). UTL in *P. montagui* could not be tested at
17 25 °C, as no individuals survived exposure to this temperature. However, mean UTL in *P.*
18 *montagui* incubated at 20 °C were significantly lower than mean UTL in all the other species
19 incubated at both 20 and 25 °C (Fig. 1b and Fig. S3a,b). Wet weight did not have a significant
20 effect on UTL (maximum $F_{1,275} = 0.4$, $p < 0.528$ for LO), except on OS ($F_{1,263} = 15.7$, $p <$
21 0.0001) (Table 1).

22

23 **Plasticity of metabolic rate**

24 Data for plasticity of oxygen consumption rate ($\Delta\dot{M}O_2$) are given in Table S4. Within the
25 whole temperature range examined (10-25 °C), $\Delta\dot{M}O_2$ ranged from 125.3 nmol O₂ g wet

1 weight⁻¹ min⁻¹ in *P. serratus* to 719.8 nmol O₂ g wet weight⁻¹ min⁻¹ in *P. varians*. Whilst $\dot{M}O_2$
2 increased exponentially with increasing incubation temperature in all species (except in *P.*
3 *montagui* – quadratic) (minimum $R^2 = 0.672$, $F_{1,89} = 182.0$, $p < 0.0001$), there were between-
4 species differences in $\Delta\dot{M}O_2$, with *P. elegans* and *P. varians*, followed by *P. macrodactylus*,
5 showing higher $\Delta\dot{M}O_2$ than *P. serratus* and *C. crangon* (Fig. 1c and Fig. S4). Whilst showing
6 similar $\Delta\dot{M}O_2$ to *P. serratus* and *C. crangon* between 10 and 20 °C, *P. macrodactylus*
7 exhibited higher $\Delta\dot{M}O_2$ between 20 and 25 °C, ranking after *P. elegans* and *P. varians* (Table
8 S4). *Pandalus montagui* was the only species showing a quadratic increase in $\dot{M}O_2$ with
9 increasing temperature ($R^2 = 0.896$, $F_{2,17} = 73.5$, $p < 0.0001$) (Fig. 1c and Fig. S4), possibly
10 due to the lack of measurements at 25 °C. However, whilst between 10 and 15 °C this species
11 showed the lowest $\Delta\dot{M}O_2$, between 15 and 20 °C it showed high $\Delta\dot{M}O_2$, ranking after *P.*
12 *variens* (Table S4). Finally, Q_{10} values are reported in Table 2.

13

14 **Plasticity for Upper Thermal Limits**

15 Data for plasticity of Upper Thermal Limits (ΔUTL) are given in Table S4. Between 10
16 and 25 °C, *P. serratus*, *C. crangon* and *P. varians* showed the lowest ΔUTL measured as LO
17 (3.4 °C), OS (2.7 °C) and D (1.2 °C) respectively. By contrast, *P. macrodactylus* showed the
18 highest ΔUTL measured as LO and OS (6.0 and 5.9 °C), and *P. serratus* exhibited the highest
19 ΔUTL measured as D (6.8 °C). Between 10 and 20 °C, *P. montagui* showed lower ΔUTL than
20 all the other species (except than *P. varians* for OS and D, and *C. crangon* for D). Different
21 species increased their UTL with increasing temperature by following different patterns (Fig.
22 1d, Fig. S3c and Fig. S5a,b,c), highlighting between-species differences in ΔUTL . In more
23 detail, UTL increased linearly in *P. macrodactylus* and *P. varians* (minimum $R^2 = 0.079$, $F_{1,53}$
24 = 4.5, $p = 0.038$), logarithmically in *P. elegans* (except for LO), *P. serratus*, and *C. crangon*
25 (minimum $R^2 = 0.410$, $F_{1,77} = 53.6$, $p < 0.0001$), and quadratically in *P. montagui* (except for

1 LO) (minimum $R^2 = 0.814$, $F_{2,15} = 32.9$, $p < 0.0001$) (Fig. 1d, Fig. S3c and Fig. S5a,b,c).
 2 Whilst UTLs of *P. elegans*, *P. serratus*, *C. crangon* and *P. montagui* showed an asymptotic
 3 trend within the examined temperature range, those of *P. macrodactylus* and *P. varians* did
 4 not. Nevertheless, *P. macrodactylus* showed greater Δ UTL than *P. varians*, as regression
 5 lines for LO, OS and D in this species had greater slopes (Fig. 1d and Fig. S3c).

6

7 **Relationships between $\dot{M}O_2$, UTL, and their plasticity**

8 Over the whole temperature range examined (10-25 °C), a significant positive logarithmic
 9 relationship between $\dot{M}O_2$ and UTL (for all end-points) was found in all species (minimum R^2
 10 = 0.093, $F_{1,53} = 5.4$, $p = 0.023$) (Fig. 2 and Fig. S6a,b). *Pandalus montagui* represented the
 11 only exception showing a marginally significant positive logarithmic relationship between
 12 $\Delta\dot{M}O_2$ and UTL measured as LO ($R^2 = 0.219$, $F_{1,13} = 3.7$, $p = 0.078$), and no significant
 13 relationships between $\Delta\dot{M}O_2$ and UTL measured as OS and D (maximum $R^2 = 0.211$, $F_{2,17} =$
 14 2.3, $p = 0.133$).

15 In addition, a significant negative relationship between UTL and Δ UTL was found when
 16 UTL was measured as D (Fig. 3), indicating that species showing the highest UTL also
 17 showed the lowest Δ UTL. Particularly, as UTL increased, Δ UTL decreased linearly between
 18 10 and 15 °C ($R^2 = 0.826$, $F_{1,3} = 19.0$, $p = 0.012$) (Fig. 3a), and quadratically between 15 and
 19 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
 20 significant relationship between UTL and Δ UTL was found at 20-25 °C, while a marginally
 21 significant negative linear relationship was found at 10-25 °C (Fig. 3c). *Palaemon*
 22 *macrodactylus* showed both high UTL and high Δ UTL (Fig. 3). A significant logarithmic
 23 relationship between $\dot{M}O_2$ and $\Delta\dot{M}O_2$ was also found, but only between 20 and 25 °C ($R^2 =$
 24 0.890, $F_{1,3} = 24.3$, $p = 0.016$), and is therefore not represented here.

1 **Discussion**

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

19

20 **Metabolic performance**

21 As already demonstrated for caridean prawns (e.g. Vandonk & Dewilde, 1981; Dalla Via,
22 1985; Salvato *et al.*, 2001; Oliphant *et al.*, 2011), exponential Standard Metabolic Rate–
23 Temperature (SMR–T) Reaction Norms are observed in all the species examined (except in *P.*
24 *montagui* – quadratic). Indeed, metabolic rate increases exponentially with temperature due to
25 increased kinetic energy of biochemical reactions (Gillooly *et al.*, 2001). However, between-

1 species differences in metabolic response are also observed, possibly due to differences in
2 mitochondrial density and aerobic capacity (e.g. Pörtner, 2001; see also Morley *et al.*, 2009)
3 emerging from the adaptation to different thermal habitats (Clarke, 2004; Clarke & Fraser,
4 2004; see also Watson *et al.*, 2013).

5 Overall, species inhabiting broader/more variable thermal environments (particularly *P.*
6 *elegans* and *P. varians*, but also, to an extent, *P. macrodactylus*) exhibit steeper SMR–T
7 Reaction Norms than those occupying narrower/more stable thermal conditions (*P. serratus*,
8 *C. crangon* and *P. montagui*). On average, these species also show higher mean Q_{10} values
9 (3.78 both between 10 and 20 °C, and between 10 and 25 °C) relative to subtidal species (2.67
10 and 2.56 between 10 and 20 °C, and between 10 and 25 °C respectively) (see Table 2). In the
11 assemblage investigated, the evolution of species metabolic response seems, therefore, to
12 have been driven by species evolutionary ecology (e.g. type of thermal habitat), rather than by
13 their phylogenetic history. If such response had been driven by species phylogeny, in fact, the
14 response of *P. serratus* would have resembled more closely that of other palaemonid species,
15 rather than that of *C. crangon* and *P. montagui* (see Fig. S1).

16 Whilst high metabolic plasticity may allow the maintenance of aerobic scope during fast
17 and frequent temperature fluctuations (Via *et al.*, 1995; Bozinovic *et al.*, 2011), it also implies
18 high energetic costs (Hulbert & Else, 2000; Rastrick & Whiteley, 2011; see also Watson *et al.*,
19 2013). Based on Q_{10} results (Table 2), species living in broader/more variable environments
20 (particularly *P. elegans* and *P. varians*) seem to better compensate for temperature increases
21 by increasing their metabolism to a greater extent, compared to species living in
22 narrower/more stable thermal conditions. However, when evaluating SMR–T Reaction Norms,
23 *P. elegans* and *P. varians* do show higher metabolic plasticity, but also incur considerably
24 higher energetic costs. In general, species living in broader/more variable thermal habitats
25 may be at great risk from the negative effects of chronic exposure to warming due to the

1 higher metabolic costs they incur, compared to species inhabiting narrower/more stable
2 thermal environments.

3 Based on SMR–T Reaction Norms, the invasive species *P. macrodactylus* maintains
4 relatively low metabolic rates, and associated energetic costs between 10 and 20 °C, but
5 expresses remarkably high metabolic plasticity between 20 and 25 °C. Between 10 and 25 °C,
6 this species also shows a lower mean Q_{10} than *P. elegans* and *P. varians* (Table 2), but a
7 higher Q_{10} in comparison to the other subtidal species (*C. crangon* and *P. serratus*) (Table 2).
8 *Palaemon macrodactylus* seems, therefore, to have evolved a remarkably high metabolic
9 control, which possibly explains its recent geographical expansion (Bates *et al.*, 2013), and
10 may make it especially resilient to future warming scenarios. By contrast, compared to the
11 other subtidal species, *P. montagui* exhibits higher metabolic rates, showing reduced
12 metabolic plasticity at low temperatures (10–15 °C) and elevated metabolic plasticity at
13 relatively high temperatures (15–20 °C). This, together with the fact that *P. montagui* exhibits
14 the lowest mean Q_{10} between 10 and 20 °C, suggests that this species may not be able to
15 beneficially adjust its metabolic performance to temperatures within the tested range (10–
16 20 °C), therefore being especially vulnerable to global warming.

17

18 **Upper Thermal Limits**

19 As already demonstrated for other crustaceans (e.g. Bradley, 1978; Layne *et al.*, 1985;
20 Lagerspetz & Bowler, 1993; Cuculescu *et al.*, 1998; Stillman, 2003; Hopkin *et al.*, 2006),
21 Upper Thermal Limits increase with increasing temperature in all the species investigated.
22 Nevertheless, between-species differences in UTL response to increasing temperature are also
23 observed (e.g. Stillman, 2003; Hopkin *et al.*, 2006; Faulkner *et al.*, 2013; see also Araújo *et*
24 *al.*, 2013). In addition, UTL are significantly positively $\dot{M}O_2$ -dependent through a
25 logarithmic relationship (except in *P. montagui* – no relationship), supporting the idea that

1 aerobic scope is maintained by increasing $\dot{M}O_2$ until a *pejus* temperature is reached, then
2 thermal tolerance becomes time-dependent (Pörtner, 2001; Verberk & Bilton, 2011). The
3 *pejus* temperature is assumed to correspond to the point at which the relationship between
4 $\dot{M}O_2$ and UTL reaches the asymptote. In this instance, species showing higher metabolic
5 plasticity (*P. elegans* and *P. varians*) possess: 1) a lower $\dot{M}O_2$ -control for UTL, and 2) lower
6 *pejus* temperatures (i.e. the relationship between $\dot{M}O_2$, and UTL in these species appears to
7 tend to the asymptote at a lower temperature). Again, compared to species inhabiting
8 narrower/more stable thermal habitats, species occupying broader/more variable thermal
9 niches may, therefore, be at greater risk from the sub-lethal effects of global warming, since
10 their scope for critical processes such as locomotion, growth and reproduction is likely to
11 become compromised at a lower temperature.

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

17 A significant negative relationship between UTL and plasticity of thermal limits was also
18 found, with species showing the highest UTL (*C. crangon*, *P. varians* and *P. elegans*) also
19 showing the lowest plasticity of thermal limits. This suggests that the evolutionary trade-off
20 found in porcelain crabs (Stillman, 2002, 2003; see also Bozinovic *et al.*, 2011; Araújo *et al.*,
21 2013) may also apply to the prawn assemblage examined here. In this instance, given that
22 prawns with the highest UTL and plasticity of thermal limits are neither ecologically similar
23 (i.e. type of thermal habitat) nor the most phylogenetically closely-related, the evolutionary
24 basis of such trade-off cannot be inferred, highlighting the need of exploring a larger
25 phylogeny. However, it is relevant for conservation and commercial purposes that *P. elegans*,

1 *P. varians* and *C. crangon* are likely to be less vulnerable to extreme acute temperatures when
2 compared to *P. serratus* and *P. montagui*. Once again, the invasive species *P. macrodactylus*
3 stands out showing both elevated UTL and plasticity for thermal limits. Also, as this species
4 occurs in subtidal habitats, it is likely to possess greater thermal safety margins (*sensu*
5 Stillman, 2000; Deutsch *et al.*, 2008; see also Diederich & Pechenik, 2013; Overgaard *et al.*,
6 2014), and may, therefore, be the least vulnerable species to extreme acute thermal events. By
7 contrast, the low UTL and low plasticity of thermal limits observed in *P. montagui*, together
8 with their low $\dot{M}O_2$ -control on UTL, further indicate that this species is likely to be the most
9 vulnerable to warming. An alternative, not exclusive, view is that *P. montagui* shows very
10 limited plasticity of both metabolism and UTL because it is being exposed to temperatures
11 already above its *pejus* temperature.

13 **Towards a more integrative prediction of species and assemblage vulnerability to global** 14 **warming**

15 Integrating the investigation of metabolic performance, thermal tolerance, and their
16 plasticity helps to more accurately elucidate species and assemblage vulnerability to global
17 warming (Bozinovic *et al.*, 2011). Indeed, while thermal tolerance and metabolic performance
18 represent useful measures of acute and chronic resilience to warming respectively (Bozinovic
19 *et al.*, 2011), plasticity reflects the extent to which taxa are able to adjust their physiological
20 abilities to the global change (Ghalambor *et al.*, 2007; Charmantier *et al.*, 2008; see also
21 Murren *et al.*, 2014). In the long-term, sub-lethal temperatures associated with global
22 warming are likely to compromise organismal performance in critical processes like
23 locomotion, growth and reproduction (Pörtner & Knust 2007; Somero, 2011), which will
24 ultimately reduce species ability to maintain healthy populations at a specific location,
25 possibly leading to local extinctions and/or shifts along environmental gradients (Buckley,

1 2008; Kearney & Porter, 2009; Cheung *et al.*, 2010). Furthermore, these sub-lethal
2 temperatures are species-specific (c.f. Araújo *et al.*, 2013), leading to changes in assemblage
3 structure and dynamics including new ecological processes such as niche competition and
4 species invasions (Milazzo *et al.*, 2012). In the short-term, taxa may be also threatened by the
5 lethal effects of global warming, especially due to increasing intensity and frequency of
6 extreme acute thermal events (IPCC, 2012).

7 In the assemblage investigated, compared to species inhabiting narrower/more stable
8 thermal environments, species occupying broader/more variable thermal niches are likely to
9 be less vulnerable to extreme acute thermal events (c.f. Diederich & Pechenik, 2013), but may
10 be at greater risk from the negative effects of chronic exposure to warming (e.g. Folguera *et*
11 *al.*, 2009). Within this study, *P. montagui* and *P. macrodactylus* highlight the types of
12 responses seen at the ends of this acute/chronic response trade-off spectrum. The former
13 shows both extremely low thermal limits and metabolic control, whilst the latter possesses
14 high thermal limits, elevated plasticity for these limits, and a high metabolic control. On this
15 basis, under future global change scenarios, we predict that in the English Channel area *P.*
16 *montagui* may suffer a reduction in presence and abundance, whilst *P. macrodactylus* may
17 experience a further expansion (e.g. Bates *et al.*, 2013). In general, on-going environmental
18 changes may cause shifts in the presence and abundance of the prawn species along the
19 European Atlantic coasts, leading to considerable changes in assemblage structure and
20 dynamics, and ecosystem functioning, as already predicted based on results from laboratory
21 mesocosms for other marine assemblages (e.g. Hale *et al.*, 2010; Christen *et al.*, 2013).
22 However, it must be noted that our conclusions are solely based on responses of adult
23 individuals for the species investigated. Future work should include various developmental
24 stages, accounting for differences in stage-specific vulnerability, especially since early life
25 stages often represent physiological bottlenecks (Pörtner & Farrell, 2008; Storch *et al.*, 2010;

1 Bartolini *et al.*, 2013). In any case, the greater understanding of the mechanistic basis of acute
2 and chronic thermal tolerance, and their evolutionary trade-offs, achieved in our study can be
3 used to implement conservation policies aimed at protecting ecologically and economically
4 valuable resources (Bernardo *et al.*, 2007; see also Helmuth *et al.*, 2005), such as caridean
5 prawns.

For Review Only

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1 **References**

- 2 Angilletta MJ, Wilson RS, Navas CA, James RS (2003) Tradeoffs and the evolution of
3 thermal reaction norms. *Trends in Ecology & Evolution*, **18**, 234-240.
- 4 Araújo MB, Ferri-Yáñez F, Bozinovic F, Marquet PA, Valladares F, Chown SL (2013) Heat
5 freezes niche evolution. *Ecology Letters*, **16**, 1206-1219.
- 6 Arnott SA, Neil DM, Ansell AD (1998) Tail-flip mechanism and size-dependent kinematics
7 of escape swimming in the brown shrimp *Crangon crangon*. *Journal of Experimental*
8 *Biology*, **201**, 1771-1784.
- 9 Attrill MJ, Thomas RM (1996) Long-term distribution patterns of mobile estuarine
10 invertebrates (Ctenophora, Cnidaria, Crustacea: Decapoda) in relation to hydrological
11 parameters. *Marine Ecology-Progress Series*, **143**, 25-36.
- 12 Bartolini F, Barausse A, Poertner H-O, Giomi F (2013) Climate change reduces offspring
13 fitness in littoral spawners: a study integrating organismic response and long-term time-
14 series. *Global Change Biology*, **19**, 373-386.
- 15 Bates AE, McKelvie CM, Sorte CJB, Morley A, Jones NAR, Mondon JA, Bird TJ, Quinn G
16 (2013) Geographical range, heat tolerance and invasion success in aquatic species.
17 *Proceedings of the Royal Society B*, **280**, 20131958.
- 18 Bechmann RK, Taban IC, Westerlund S *et al.* (2011) Effects of ocean acidification on early
19 life stages of shrimp (*Pandalus borealis*) and mussel (*Mytilus edulis*). *Journal of*
20 *Toxicology and Environmental Health*, **74**, 424-438.
- 21 Bernardo J, Ossola RJ, Spotila J, Crandall KA (2007) Interspecies physiological variation as a
22 tool for cross-species assessments of global warming-induced endangerment: validation
23 of an intrinsic determinant of macroecological and phylogeographic structure. *Biology*
24 *Letters*, **3**, 695-698.

- 1 Bozinovic F, Calosi P, Spicer JI (2011) Physiological correlates of geographic range in
2 animals. *The Annual Review of Ecology, Evolution, and Systematics*, **42**, 155-179.
- 3 Bozinovic F, Catalan TP, Estay SA, Sabat P (2013) Acclimation to daily thermal variability
4 drives the metabolic performance curve. *Evolutionary Ecology Research*, **15**, 579-587.
- 5 Bradley PB (1978) Increase in range of temperature tolerance by acclimation in the copepod
6 *Eurytemora affinis*. *Biological Bulletin*, **154**.
- 7 Bradshaw AD (1965) Evolutionary significance of phenotypic plasticity in plants. *Advances*
8 *in Genetics*, **13**, 115-155.
- 9 Brattstrom BH (1968) Thermal acclimation in anural amphibians as a function of latitude and
10 altitude. *Comparative Biochemistry and Physiology*, **24**, 93-111.
- 11 Buckley LB (2008) Linking Traits to Energetics and Population Dynamics to Predict Lizard
12 Ranges in Changing Environments. *American Naturalist*, **171**, E1-E19.
- 13 Calosi P, Bilton DT, Spicer JI (2008) Thermal tolerance, acclimatory capacity and
14 vulnerability to global climate change. *Biology Letters*, **4**, 99-102.
- 15 Calosi P, Bilton DT, Spicer JI, Votier SC, Atfield A (2010) What determines a species'
16 geographical range? Thermal biology and latitudinal range size relationships in European
17 diving beetles (Coleoptera: Dytiscidae). *Journal of Animal Ecology*, **79**, 194-204.
- 18 Charmantier A, Mcleery RH, Cole LR, Perrins C, Kruuk LEB, Sheldon BC (2008) Adaptive
19 phenotypic plasticity in response to climate change in a wild bird population. *Science*,
20 **320**, 800-803.
- 21 Cheung WWL, Lam VWY, Sarmiento JL, Kearney K, Watson R, Zeller D, Pauly D (2010)
22 Large-scale redistribution of maximum fisheries catch potential in the global ocean under
23 climate change. *Global Change Biology*, **16**, 24-35.
- 24 Christen N, Calosi P, McNeill CL, Widdicombe S (2012) Structural and functional
25 vulnerability to elevated $p\text{CO}_2$ in marine benthic communities. *Marine Biology*.

- 1 Clarke A (2004) Is there a Universal Temperature Dependence of metabolism? *Functional*
2 *Ecology*, **18**, 252-256.
- 3 Clarke A, Fraser KPP (2004) Why does metabolism scale with temperature? *Functional*
4 *Ecology*, **18**, 243-251.
- 5 Compton TJ, Rijkenberg MJA, Drent J, Piersma T (2007) Thermal tolerance ranges and
6 climate variability: A comparison between bivalves from differing climates. *Journal of*
7 *Experimental Marine Biology and Ecology*, **352**, 200-211.
- 8 Cowles RB, Bogert CM (1944) A preliminary study of the thermal requirements of desert
9 reptiles. *Bulletin of the American Museum of Natural History*, **83**, 261-296.
- 10 Cuculescu M, Hyde D, Bowler K (1998) Thermal tolerance of two species of marine crab,
11 *Cancer pagurus* and *Carcinus maenas*. *Journal of Thermal Biology*, **23**, 107-110.
- 12 Dalla Via J (1985) Oxygen consumption and temperature change in the shimp *Palaemon*
13 *elegans*. *Marine Ecology-Progress Series*, **26**, 199-202.
- 14 Deutsch CA, Tewksbury JJ, Huey RB, Sheldon KS, Ghalambor CK, Haak DC, Martin PR
15 (2008) Impacts of climate warming on terrestrial ectotherms across latitude. *Proceedings*
16 *of the National Academy of Sciences of the United States of America*, **105**, 6668-6672.
- 17 Diederich CM, Pechenik JA (2013) Thermal tolerance of *Crepidula fornicata* (Gastropoda)
18 life history stages from intertidal and subtidal subpopulations. *Marine Ecology Progress*
19 *Series*, **486**, 173-187.
- 20 Dillon ME, Wang G, Huey RB (2010) Global metabolic impacts of recent climate warming.
21 *Nature*, **467**, 704-U788.
- 22 Farris JS, Källersjö K, A.G., Bult C (1994) Testing significance of incongruence. *Cladistics*,
23 **10**, 315-319.

- 1 Faulkner KT, Clusella-Trullas S, Peck LS, Chown SL (2013) Lack of coherence in the
2 warming responses of marine crustaceans. *Functional Ecology*, DOI: 10.1111/1365-
3 2435.12219.
- 4 Folguera G, Bastías DA, Bozinovic F (2009) Impact of experimental thermal amplitude on
5 ectotherm performance: Adaptation to climate change variability? *Comparative*
6 *Biochemistry and Physiology a-Molecular & Integrative Physiology*, **154**, 389-393.
- 7 Ghalambor CK, McKay JK, Carroll SP, Reznick DN (2007) Adaptive versus non-adaptive
8 phenotypic plasticity and the potential for contemporary adaptation in new environments.
9 *Functional Ecology*, **21**, 394-407.
- 10 Gillooly JF, Brown JH, West GB, Savage VM, Charnov EL (2001) Effects of size and
11 temperature on metabolic rate. *Science*, **293**, 2248-2251.
- 12 Hale R, Calosi P, McNeill L, Mieszkowska N, Widdicombe S (2011). Predicted levels of
13 future ocean acidification and temperature rise could alter community structure and
14 biodiversity in marine benthic communities. *Oikos*, **120**, 661-674.
- 15 Helmuth B, Kingsolver JG, Carrington E (2005) Biophysics, physiological ecology, and
16 climate change: Does mechanism matter? *Annual Review of Physiology*, **67**, 177-201.
- 17 Henderson PA (1987) On the population biology of the common shrimp *Crangon crangon* (L)
18 (Crustacea, Caridea) in the Severn Estuary and Bristol Channel. *Journal of the Marine*
19 *Biological Association of the United Kingdom*, **67**, 825-847.
- 20 Henderson PA, Seaby RM, Somes JR (2006) A 25-year study of climatic and density-
21 dependent population regulation of common shrimp *Crangon crangon* (Crustacea:
22 Caridea) in the Bristol Channel. *Journal of the Marine Biological Association of the*
23 *United Kingdom*, **86**, 287-298.
- 24 Hopkin RS, Qari S, Bowler K, Hyde D, Cuculescu M (2006) Seasonal thermal tolerance in
25 marine Crustacea. *Journal of Experimental Marine Biology and Ecology*, **331**, 74-81.

- 1 Hulbert AJ, Else PL (2000) Mechanisms underlying the cost of living in animals. *Annual*
2 *Review of Physiology*, **62**, 207-235.
- 3 IPCC (2012) Special Report on Managing the Risks of Extreme Events and Disasters to
4 Advance Climate Change Adaptation (eds Field CB, Barros V, Stocker TF, Dahe Q,
5 Dokken DJ, Ebi KL, Mastrandrea MD, Mach KJ, Plattner G-K, Allen SK, Tignor M,
6 Midgley PM) pp Page, New York, NY, USA.
- 7 IPCC (2014) The Working Group II (WGII) contribution to the Fifth Assessment Report on
8 impacts, adaptation and vulnerability will be considered in Yokohama, Japan, on 25-29
9 March 2014.
- 10 Kearney M, Porter W (2009) Mechanistic niche modelling: combining physiological and
11 spatial data to predict species' ranges. *Ecology Letters*, **12**, 334-350.
- 12 Lagerspetz KYH, Bowler K (1993) Variation in heat tolerance in individual *Asellus aquaticus*
13 during thermal acclimation. *Journal of Thermal Biology*, **18**, 137-143.
- 14 Layne JRJ, Manis ML, Claussen DL (1985) Seasonal variation in the time course of thermal
15 acclimation in the crayfish *Orconectes rusticus*. *Freshwater Invertebrates Biology*, **4**, 98-
16 104.
- 17 Lavesque N, Bachelet G, Beguer M *et al.* (2010) Recent expansion of the oriental shrimp
18 *Palaemon macrodactylus* (Crustacea: Decapoda) on the western coasts of France.
19 *Aquatic Invasions*, **5**, S103-S108.
- 20 Lutterschmidt WI, Hutchison VH (1997) The critical thermal maximum: data to support the
21 onset of spasms as the definitive end point. *Canadian Journal of Zoology-Revue*
22 *Canadienne De Zoologie*, **75**, 1553-1560.
- 23 Massamba-N'siala G, Calosi P, Bilton DT, Prevedelli D, Simonini R (2012) Life-history and
24 thermal tolerance traits display different thermal plasticities and relationships with

- 1 temperature in the marine polychaete *Ophryotrocha labronica* La Greca and Bacci
2 (Dorvilleidae). *Journal of Experimental Marine Biology and Ecology*, **438**, 109-117.
- 3 Melatunan S, Calosi P, Rundle SD, Moody AJ, Widdicombe S (2001) Exposure to elevated
4 temperatures and pCO₂ reduces respiration rate and energy status in the periwinkle
5 *Littorina littorea*. *Physiological and Biochemical Zoology*, **84**, 583-594.
- 6 Menendez R, Gutierrez, D (1996) Altitudinal effects on habitat selection of dung beetles
7 (Scarabaeoidea: Aphodiidae) in the northern Iberian peninsula. *Ecography*, **19**, 313-317.
- 8 Milazzo M, Mirto S, Domenici P, Gristina M (2012) Climate change exacerbates interspecific
9 interactions in sympatric coastal fishes. *Journal of Animal Ecology*.
- 10 Morley SA, Lurman GJ, Skepper JN, Poertner H-O, Peck LS (2009) Thermal plasticity of
11 mitochondria: A latitudinal comparison between Southern Ocean molluscs. *Comparative*
12 *Biochemistry and Physiology a-Molecular & Integrative Physiology*, **152**, 423-430.
- 13 Murren CJ, Maclean HJ, Diamond SE *et al.* (2014) Evolutionary change in continuous
14 Reaction Norms. *The American Naturalist*, **183**.
- 15 Oh CW, Hartnoll RG, Nash RDM (2001) Feeding ecology of the common shrimp *Crangon*
16 *crangon* in Port Erin Bay, Isle of Man, Irish Sea. *Marine Ecology-Progress Series*, **214**,
17 211-223.
- 18 Oliphant A, Thatje S, Brown A, Morini M, Ravaux J, Shillito B (2011) Pressure tolerance of
19 the shallow-water caridean shrimp *Palaemonetes varians* across its thermal tolerance
20 window. *Journal of Experimental Biology*, **214**, 1109-1117.
- 21 Overgaard J, Kristensen TN, Sorensen JG (2012) Validity of Thermal Ramping Assays Used
22 to Assess Thermal Tolerance in Arthropods. *Plos One*, **7**.
- 23 Overgaard J, Kearney MR, Hoffmann AA (2014) Sensitivity to thermal extremes in
24 Australian *Drosophila* implies similar impacts of climate change on the distribution of
25 widespread and tropical species. *Global Change Biology*.

- 1 Parmesan C, Yohe G (2003) A globally coherent fingerprint of climate change impacts across
2 natural systems. *Nature*, **421**, 37-42.
- 3 Peck LS, Clark MS, Morley SA, Massey A, Rossetti H (2009) Animal temperature limits and
4 ecological relevance: effects of size, activity and rates of change. *Functional Ecology*, **23**,
5 248-256.
- 6 Peck LS, Morley SA, Richard J, Clark MS (2014) Acclimation and thermal tolerance in
7 Antarctic marine ectotherms. *Journal of Experimental Biology*, **217**, 16-22.
- 8 Perry AL, Low PJ, Ellis JR, Reynolds JD (2005) Climate change and distribution shifts in
9 marine fishes. *Science*, **308**, 1912-1915.
- 10 Pigliucci M (ed) (2001) *Phenotypic Plasticity Beyond Nature and Nurture*, Baltimore,
11 Maryland, The Johns Hopkins University Press.
- 12 Pigliucci M, Murren CJ, Schlichting CD (2006) Phenotypic plasticity and evolution by
13 genetic assimilation. *Journal of Experimental Biology*, **209**, 2362-2367.
- 14 Pihl L, Rosenberg R (1984) Food selection and consumption of the shrimp *Crangon crangon*
15 in some shallow marine areas in Western Sweden. *Marine Ecology-Progress Series*, **15**,
16 159-168.
- 17 Pörtner HO (2001) Climate change and temperature-dependent biogeography: oxygen
18 limitation of thermal tolerance in animals. *Naturwissenschaften*, **88**, 137-146.
- 19 Pörtner HO, Knust R (2007) Climate change affects marine fishes through the oxygen
20 limitation of thermal tolerance. *Science*, **315**, 95-97.
- 21 Pörtner HO, Farrell AP (2008) Physiology and Climate Change. *Science*, **322**, 690-692.
- 22 Rastrick SPS, Whiteley NM (2011) Congeneric Amphipods Show Differing Abilities to
23 Maintain Metabolic Rates with Latitude. *Physiological and Biochemical Zoology*, **84**,
24 154-165.

- 1 Rezende EL, Tejedo M, Santos M (2011) Estimating the adaptive potential of critical thermal
2 limits: methodological problems and evolutionary implications. *Functional Ecology*, **25**,
3 111-121.
- 4 Rezende EL, Castañeda LE, Santos M (2014) Tolerance landscapes in thermal ecology.
5 *Functional Ecology*.
- 6 Root TL, Price JT, Hall KR, Schneider SH, Rosenzweig C, Pounds JA (2003) Fingerprints of
7 global warming on wild animals and plants. *Nature*, **421**, 57-60.
- 8 Salvato B, Cuomo V, Di Muro P, Beltramini M (2001) Effects of environmental parameters
9 on the oxygen consumption of four marine invertebrates: a comparative factorial study.
10 *Marine Biology*, **138**, 659-668.
- 11 Schlichting CD, Pigliucci M (eds) (1998) *Phenotypic evolution: a reaction norm perspective*
12 Sunderland, Massachussets, Sinauer.
- 13 Small D, Calosi P, White D, Spicer JJ, Widdicombe S (2010) Impact of medium-term
14 exposure to CO₂ enriched seawater on the physiological functions of the velvet
15 swimming crab *Necora puber*. *Aquatic Biology*, **10**, 11-21.
- 16 Sokal RR, Rohlf FJ (eds) (1995) *Biometry: The Principles and Practice of Statistics in*
17 *Biological Research*, New York.
- 18 Sokolova IM, Pörtner HO (2003) Metabolic plasticity and critical temperatures for aerobic
19 scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: Littorinidae)
20 from different latitudes. *Journal of Experimental Biology*, **206**, 195-207.
- 21 Somero GN (2010) The physiology of climate change: how potentials for acclimatization and
22 genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology*,
23 **213**, 912-920.

- 1 Somero GN (2011) Comparative physiology: a "crystal ball" for predicting consequences of
2 global change. *American Journal of Physiology-Regulatory Integrative and Comparative*
3 *Physiology*, **301**, R1-R14.
- 4 Soors J, Faasse M, Stevens M, Verbessem I, De Regge N, Van Den Bergh E (2010) New
5 crustacean invaders in the Schelde estuary (Belgium). *Belgian Journal of Zoology*, **140**,
6 3-10.
- 7 Southward AJ, Hawkins SJ, Burrows MT (1995) 70 years observations of changes in
8 distribution and abundance of zooplankton and intertidal organisms in the Western
9 English-Channel in relation to rising sea temperature. *Journal of Thermal Biology*, **20**,
10 127-155.
- 11 Spicer JI, Eriksson SP (2003) Does the development of respiratory regulation always
12 accompany the transition from pelagic larvae to benthic fossorial postlarvae in the
13 Norway lobster *Nephrops norvegicus* (L.)? *Journal of Experimental Marine Biology and*
14 *Ecology*, **295**, 219-243.
- 15 Spivak ED, Boschi EE, Martorelli SR (2006) Presence of *Palaemon macrodactylus* Rathbun
16 1902 (Crustacea: Decapoda: Caridea: Palaemonidae) in Mar del Plata harbor, Argentina:
17 first record from southwestern Atlantic waters. *Biological Invasions*, **8**, 673-676.
- 18 Stillman JH (2002) Causes and consequences of thermal tolerance limits in rocky intertidal
19 porcelain crabs, genus *Petrolisthes*. *Integrative and Comparative Biology*, **42**, 790-796.
- 20 Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change. *Science*,
21 **301**, 65-65.
- 22 Stillman JH, Somero GN (2000) A comparative analysis of the upper thermal tolerance limits
23 of eastern Pacific porcelain crabs, genus *Petrolisthes*: Influences of latitude, vertical
24 zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology*, **73**, 200-
25 208.

- 1 Storch D, Fernandez M, Navarette SA, Pörtner HO (2011) Investigations on the Chilean kelp
2 crab *Taliepus dentatus*. *Marine Ecology Progress Series*, **429**, 157-167.
- 3 Swofford DL (2002). PAUP* Version 4b10. Sinauer Associates Inc. Sunderland,
4 Massachusetts.
- 5 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S (2011) MEGA5: Molecular
6 Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and
7 Maximum Parsimony methods. *Molecular Biology and Evolution*, **28**, 2731–2739.
- 8 Terblanche JS, Klok CJ, Krafur ES, Chown SL (2006) Phenotypic plasticity and geographic
9 variation in thermal tolerance and water loss of the tsetse *Glossina pallidipes* (Diptera:
10 Glossinidae): implications for distribution modelling. *The American Journal of Tropical
11 Medicine and Hygiene*, **74**, 786-794.
- 12 Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL (2007) Critical thermal
13 limits depend on methodological context. *Proceedings of the Royal Society B-Biological
14 Sciences*, **274**, 2935-2942.
- 15 Tewksbury JJ, Huey RB, Deutsch CA (2008) Ecology - Putting the heat on tropical animals.
16 *Science*, **320**, 1296-1297.
- 17 Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: improving the sensitivity of
18 progressive multiple sequence alignment through sequence weighting, position specific
19 gap penalties and weight matrix choice. *Nucleic Acids Research*, **22**, 4673-4680.
- 20 Truebano M, Burns G, Thorne MaS, Hillyard G, Peck LS, Skibinski DOF, Clark MS (2010)
21 Transcriptional response to heat stress in the Antarctic bivalve *Laternula elliptica*.
22 *Journal of Experimental Marine Biology and Ecology*, **391**, 65-72.
- 23 Underwood AJ (ed) (1997) *Experiments in Ecology: Their Logical Design and Interpretation
24 Using Analysis of Variance.*, Cambridge, Cambridge University Press.

- 1 Vandonk E, Dewilde P (1981) Oxygen consumption and motile activity of the brown shrimp
2 *Crangon crangon* related to temperature and body size. *Netherlands Journal of Sea*
3 *Research*, **15**, 54-64.
- 4 Verberk W, Bilton DT (2011) Can Oxygen Set Thermal Limits in an Insect and Drive
5 Gigantism? *Plos One*, **6**.
- 6 Via S, Gomulkiewicz R, Dejong G, Scheiner SM, Schlichting CD, Vantienderen PH (1995)
7 Adaptive phenotypic plasticity – Consensus and controversy. *Trends in Ecology &*
8 *Evolution*, **10**, 212-217.
- 9 Waddington CH (1942) Canalization of development and the inheritance of acquired
10 characters. *Nature*, **150**, 563-565.
- 11 Watson S, Morley SA, Bates AE *et al.* (2013) Low global sensitivity of metabolic rate to
12 temperature in calcified marine invertebrates. *Oecologia*, **174**, 45-54.
- 13 Zweifel RG (1957) Studies on the critical thermal maxima of salamanders. *Ecology*, **38**.

1 **Supporting Information legends**

2

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

5

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

7

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

9

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

12

13 Figure S1 Representation of plasticity.

14

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

16

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

19

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

21

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

23

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

1 Table 1 Results for two-way ANCOVAs testing the effect of ‘Species’, ‘Temperature’, and
 2 their interaction on the oxygen consumption rate ($\dot{M}O_2$) and Upper Thermal Limits (UTL),
 3 measured as Loss of Orientation (LO), Onset of Spasms (OS) and Death (D), for the prawn
 4 species investigated in this study after 7 d exposure to one of four incubation temperatures (10,
 5 15, 20 and 25 °C) using ‘Wet weight’ as a covariate. Degrees of freedom (df), mean of square
 6 (MS), *F*-ratio (*F*) and probability level (*p*) are reported.

Trait	Source	df	MS	F	p
$\dot{M}O_2$	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

1 Table 2 Temperature sensitivity of $\dot{M}O_2$, expressed as the temperature coefficient for the
 2 change in $\dot{M}O_2$ with temperature (Q_{10}), in the prawn species investigated in this study. The
 3 temperature ranges used for the determination of Q_{10} are 10-20 °C and 10-25 °C. Mean Q_{10}
 4 for species living in broader/more variable thermal habitats and for species occupying
 5 narrower/more stable thermal niches are also reported. *nr = not recorded

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>	3.52	3.91	3.78	3.78
<i>Palaemon macrodactylus</i>	2.41	2.79		
<i>Palaemonetes varians</i>	5.42	4.64		
<i>Palaemon serratus</i>	3.20	2.40	2.67	2.56
<i>Crangon crangon</i>	2.80	2.72		
<i>Pandalus montagui</i>	2.01	nr*		

6

1 **Figure legends**

2

3 Figure 1 The effect of temperature on (a) mean oxygen consumption rate ($\dot{M}O_2$) (here used as
4 a proxy for Standard Metabolic Rate, SMR), (b) Upper Thermal Limits (UTL), measured as
5 Onset of Spasms (OS), and (c,d) $\dot{M}O_2$ and UTL plasticity in the prawn species investigated in
6 this study: *Palaemon elegans*, *Palaemon macrodactylus*, *Palaemon serratus*, *Palaemonetes*
7 *varians*, *Crangon crangon* and *Pandalus montagui*. Histograms represent mean \pm SE for (a)
8 $\dot{M}O_2$ or (b) UTL after 7 d exposure to one of four incubation temperatures: 10 (yellow), 15
9 (light orange), 20 (dark orange) and 25 °C (red). Significantly different mean values ($p < 0.05$)
10 at different incubation temperatures for the same species are indicated by different letters
11 placed above the histograms, while significantly different mean values ($p < 0.05$) at the same
12 incubation temperature among different species are indicated by different numbers placed
13 inside the histograms. Finally, overall significant differences in mean values ($p < 0.05$) among
14 different species are indicated by different numbers preceded by a star placed at the top of the
15 graph above each species. Pairwise comparisons were conducted using the Estimated
16 Marginal Means test with Least Significant Difference test correction. Lines in (c,d) represent
17 Standard Metabolic Rate–Temperature (SMR–T) and (d) Upper Thermal Limits–Temperature
18 Reaction Norms (UTL–T) respectively: i.e. the patterns through which species increased their
19 $\dot{M}O_2$ or UTL in response to increasing incubation temperature according to the best-fit
20 regression model. UTL are measured as OS. Raw data, regression equation and relevant
21 statistics for $\dot{M}O_2$ and UTL are provided in Figure S4 and Figure S5b respectively.

22

23 Figure 2 The relationship between $\dot{M}O_2$ and UTL, measured as OS, for the prawn species
24 investigated in this study. Circles represent individual prawn $\dot{M}O_2$ and UTL measured after 7
25 d exposure to one of four incubation temperatures: 10 (yellow), 15 (light orange), 20 (dark

1 orange) and 25 °C (red). Full lines represent the best-fit significant regression models
 2 respectively; regression equation and relevant statistics are as follows: *P. elegans*: $y =$
 3 $2.082\ln(x) + 21.708$, $R^2 = 0.609$, $F_{1,61} = 94.9$, $p < 0.0001$; *P. macrodactylus*: $y = 3.290\ln(x) +$
 4 18.692 , $R^2 = 0.887$, $F_{1,21} = 165.2$, $p < 0.0001$; *P. serratus*: $y = 3.071\ln(x) + 17.766$, $R^2 = 0.599$,
 5 $F_{1,23} = 34.3$, $p < 0.0001$; *P. varians*: $y = 1.60\ln(x) + 27.491$, $R^2 = 0.656$, $F_{1,53} = 101.3$, $p <$
 6 0.0001 ; *C. crangon*: $y = 1.460\ln(x) + 26.957$, $R^2 = 0.244$, $F_{1,77} = 24.9$, $p < 0.0001$; *P.*
 7 *montagui*: $p > 0.05$.

8

9 Figure 3 The relationship between UTL, measured as D, and plasticity for Upper Thermal
 10 Limits (Δ UTL) at four temperature intervals: (a) 10-15 °C ($y = -0.276x + 11.106$, $R^2 = 0.826$,
 11 $F_{1,3} = 19.0$, $p = 0.012$), (b) 15-20 °C ($y = -0.366x^2 + 25.504x - 406.748$, $R^2 = 0.966$, $F_{2,3} =$
 12 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)
 13 10-20 °C ($y = -0.176x^2 + 10.739x - 157.930$, $R^2 = 0.975$, $F_{2,3} = 19.0$, $p = 0.004$) °C. Δ UTL is
 14 calculated as the difference between mean values of D either between consecutive or extreme
 15 incubation temperatures (considering either 25 or 20 °C as upper extreme temperature). Data
 16 points represent individual species UTL (measured at the incubation temperature indicated in
 17 brackets) and Δ UTL; different symbols indicate different species: *P. elegans* (circle), *P.*
 18 *macrodactylus* (triangle), *P. serratus* (square), *P. varians* (diamond), *C. crangon* (cross) and
 19 *P. montagui* (plus). Full and dotted lines represent the best-fit significant ($p < 0.05$) and
 20 marginally significant ($0.05 < p < 0.08$) regression models respectively.





