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 sub-lethal 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 seven 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 species *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.
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; Menéndez & Gutiérrez, 1996; Parmesan & Yohe, 2003; Root et al., 2003; Perry et al., 2005). In general, understanding the mechanistic basis of sub-lethal 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, 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 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 characterising 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).
Whilst 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).

Whilst 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, whilst 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. In order 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. Based on 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 hypothesise that, compared to species occupying narrower/more stable thermal niches, species inhabiting broader/more variable thermal environments possess: 1) higher upper thermal limits, 2) lower plasticity of these limits, 3) higher metabolic rates, 4) higher metabolic plasticity, and 5) 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, 20002, 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 in order 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 d. 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; Calosi et al., 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 ± 0.005 °C min\(^{-1}\) (mean ± SD). Once they had reached the desired
incubation temperature, aquaria were kept at these conditions for one 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 d in order 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 (Ṁ\(\text{O}_2\)) under resting conditions was used as a proxy for Standard
Metabolic Rate (SMR), as in Spicer & Eriksson (2003) and Small *et al.* (2010). \(\text{ṀO}_2\) 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 x 42 mm x 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); \( \dot{M}_{\text{O}_2} \) 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 \( \dot{M}_{\text{O}_2} \) measurements, whilst 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 \( \mu \text{mol O}_2 \text{ L}^{-1} \). In order 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 re-opening it at the end of the experimental trial by using an \( \text{O}_2 \) electrode (1302, Strathkelvin Instruments, Glasgow, Scotland) connected to a calibrated oxygen meter (929, Strathkelvin Instruments), and expressed as nmol \( \text{O}_2 \text{ g wet weight}^{-1} \text{ min}^{-1} \text{ STP}. \) Before the experiments started, calibration was carried out at each incubation temperature by using 0 and 100 % \( \text{O}_2 \) saturation as calibration points. After \( \dot{M}_{\text{O}_2} \) measurements were completed, wet weight (g) and body volume (mL) of each prawn were measured and then used for correction of the \( \dot{M}_{\text{O}_2} \) 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**

In order 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: 1) Mouth Gaping (MG) (wide and continuous opening of the mouth parts); 2) Tail Flipping (TF) (fast upside down flip of the tail bringing abdomen and cephalothorax towards one another) (Arnott *et al.*, 1998); 3) 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); 4) Onset of Spasms (OS): first uncontrolled, convulsive and spasmodic movements (Zweifel, 1957; Lutterschmidt & Hutchison, 1997; Hopkin *et al.*, 2006); 5) 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, whilst only 79.3 and 64.8 % of them showed MG and TF respectively. Also, compared to MG and TF, LO, OS and D showed lower variance. Given this variability, only LO, OS and D were assumed to represent temperature-induced mechanisms underpinning the failure of fundamental
physiological functions (Lutterschmidt & Hutchison, 1997). However, in order to avoid
redundant utilization of these end-points in further analyses, correlation analyses among them
were performed to only include functionally independent traits. As there were significant
positive relationships between LO and OS, OS and D, LO and D (minimum $r^2 = 0.840, p <
0.0001$), here we mainly focused on OS, which more closely fulfils the original definition of
critical thermal maximum ($C_{T_{max}}$): ‘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 °C min$^{-1}$ (realised $+0.668 \pm$
0.016 °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,
deep 65 mm) of a generic six-well plate, whose bottom surface was painted white with Tipp-
Ex® to allow an easier and more accurate visualization of the end-points. A maximum of five
individuals were tested at any time and, in order 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 sec with a digital thermometer (HH802U, Omega® 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) (Fig. S1), as well as $Q_{10}$ for metabolism. In order 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 °C – $P_{10-15}$, $P_{15-20}$, $P_{20-25}$ respectively) (Fig. 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 °C – $P_{10-25}$). In order to include *P. montagui* in the computation, $P_{\text{tot}}$ was also calculated between 10 and 20 °C ($P_{10-20}$), as this species had no replicates at 25 °C due to 100 % mortality (Table S1; see also Results). $P_{10-15}$, $P_{15-20}$, $P_{20-25}$ 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 accurate 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 °C) and within smaller temperature intervals. Whilst the calculation of $Q_{10}$ values allows 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 plasticity of thermal limits, here we mainly focus on Reaction Norms.

Characterisation 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 1,000 bootstrap replicates. Only clades with significant support values (defined here as ≥ 60 bootstrap) are shown.

Our analysis highlights that, among palaemonid species, *P. elegans* and *P. serratus* are the most phylogenetically closely-related (Fig. 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’ x ‘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 a regression model – 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 ± SE for oxygen consumption rate (ṀO$_2$) are given in Fig. 1a and Table S3. The minimum mean ṀO$_2$ was observed in *P. serratus* incubated at 10 °C (46.1 ± 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 ± 56.7 nmol O$_2$ g wet weight$^{-1}$ min$^{-1}$). In general, greater ṀO$_2$ was observed at higher incubation temperatures in all species. Nevertheless, ṀO$_2$ 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 ṀO$_2$ than all the other species, whilst *P. montagui* exhibited significantly higher mean ṀO$_2$ (being comparable to *P. elegans* and *P. varians*). *Crangon crangon* and *P. macrodactylus* showed intermediate mean ṀO$_2$ 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 ṀO$_2$ 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 ṀO$_2$ 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 ṀO$_2$ (F$_{1,299}$ = 166.3, p < 0.0001) (Table 1).

Upper Thermal Limits

Means ± SE for Upper Thermal Limits (UTL), measured as Onset of Spasms (OS), are given in Fig. 1b and Table S3. Means ± SE for UTL, measured as Loss of Orientation (LO)
and Death (D), are also given in Fig. 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 ± 0.2 °C; OS: 24.8 ± 0.3 °C; D: 27.1 ± 0.4 °C), while the maximum was observed in *P. macrodactylus* incubated at 25 °C (LO: 35.7 ± 0.4 °C; OS: 37.8 ± 0.5 °C; D: 39.9 ± 0.2 °C).

Whilst 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 Fig. 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: 1) *P. elegans* showed significantly higher mean LO than *P. serratus*, being comparable to *P. macrodactylus* and *P. varians* (Fig. S3a); 2) *P. varians* showed significantly higher mean D not only than *P. elegans* but also than *P. macrodactylus* and *C. crangon* (Fig. 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 Fig. 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 Fig. 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 ($\Delta M\overline{O}_2$) are given in Table S4. Within the whole temperature range examined (10-25 °C), $\Delta M\overline{O}_2$ ranged from 125.3 nmol O$_2$ g wet
weight$^{-1}$ min$^{-1}$ in *P. serratus* to 719.8 nmol O$_2$ g wet weight$^{-1}$ min$^{-1}$ in *P. varians*. Whilst $ar{M}$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 ΔMO$_2$, with *P. elegans* and *P. varians*, followed by *P. macrodactylus*, showing higher ΔMO$_2$ than *P. serratus* and *C. crangon* (Fig. 1c and Fig. S4). Whilst showing similar ΔMO$_2$ to *P. serratus* and *C. crangon* between 10 and 20 °C, *P. macrodactylus* exhibited higher ΔMO$_2$ 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 MO$_2$ with increasing temperature ($R^2 = 0.896$, $F_{2,17} = 73.5$, $p < 0.0001$) (Fig. 1c and Fig. S4), possibly due to the lack of measurements at 25 °C. However, whilst between 10 and 15 °C this species showed the lowest ΔMO$_2$, between 15 and 20 °C it showed high ΔMO$_2$, 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 (ΔUTL) are given in Table S4. Between 10 and 25 °C, *P. serratus*, *C. crangon* and *P. varians* showed the lowest ΔUTL measured as LO (3.4 °C), OS (2.7 °C) and D (1.2 °C) respectively. By contrast, *P. macrodactylus* showed the highest ΔUTL measured as LO and OS (6.0 and 5.9 °C), and *P. serratus* exhibited the highest ΔUTL measured as D (6.8 °C). Between 10 and 20 °C, *P. montagui* showed lower ΔUTL 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, Fig. S3c and Fig. S5a,b,c), highlighting between-species differences in ΔUTL. 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
Whilst UTLs 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 ∆UTL than *P. varians*, as regression lines for LO, OS and D in this species had greater slopes (Fig. 1d and Fig. S3c).

### Relationships between \( \dot{M}O_2, \) 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 Fig. 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, F\(_{1,13}\) = 3.7, p = 0.078), and no significant relationships between ∆\( \dot{M}O_2 \) 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 ∆UTL was found when UTL was measured as D (Fig. 3), indicating that species showing the highest UTL also showed the lowest ∆UTL. Particularly, as UTL increased, ∆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 relationship between UTL and ∆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 ∆UTL (Fig. 3). A significant logarithmic relationship between \( \dot{M}O_2 \) and ∆\( \dot{M}O_2 \) was also found, but only between 20 and 25 °C (R\(^2\) = 0.890, F\(_{1,3}\) = 24.3, p = 0.016), and is therefore 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; Dillon et al., 2010; Tewksbury et al., 2008). 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 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 Fig. S1).

Whilst high metabolic plasticity may allow the maintenance of aerobic scope during fast
and frequent temperature fluctuations (Via et al., 1995; Bozinovic et al., 2011), it also implies
high energetic costs (Hulbert & Else, 2000; Rastick & 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 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.*, 2013; see also Araújo *et al.*, 2013). In addition, UTL are significantly positively MO₂-dependent through a logarithmic relationship (except in *P. montagui* – no relationship), supporting the idea that
aerobic scope is maintained by increasing $\dot{\text{MO}_2}$ 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

$\dot{\text{MO}_2}$ and UTL reaches the asymptote. In this instance, species showing higher metabolic

plasticity (*P. elegans* and *P. varians*) possess: 1) a lower $\dot{\text{MO}_2}$-control for UTL, and 2) lower

*pejus* temperatures (i.e. the relationship between $\dot{\text{MO}_2}$, 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 sub-lethal 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 $\dot{\text{MO}_2}$-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 occur 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, 2000; 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 MO2-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 long-term, sub-lethal 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,
Furthermore, these sub-lethal 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., 2012). 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, 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, whilst 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, whilst *P. macrodactylus* may experience a further expansion (e.g. Bates et al., 2013). In general, on-going 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., 2010; 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., 2010;
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 caridean prawns.
Acknowledgments

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Supporting Information legends

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 ± SE for oxygen consumption rate (ṀO₂) and Upper Thermal Limits (UTL).

Table S4 Mean plasticity of oxygen consumption rate (ΔṀO₂) and Upper Thermal Limits (ΔUTL).

Figure S1 Representation of plasticity.

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

Figure S3 Mean ± 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 ṀO₂ and temperature.

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

Figure S6 The relationship between ṀO₂ and UTL measured as LO and D.
Table 1 Results for two-way ANCOVAs testing the effect of ‘Species’, ‘Temperature’, and their interaction on the oxygen consumption rate (ṀO₂) 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 d 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.

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Table 2 Temperature sensitivity of $\dot{\text{MO}_2}$, expressed as the temperature coefficient for the change in $\dot{\text{MO}_2}$ 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 °C 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. *nr = not recorded

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Figure legends

Figure 1 The effect of temperature on (a) mean oxygen consumption rate (ṀO₂) (here used as a proxy for Standard Metabolic Rate, SMR), (b) Upper Thermal Limits (UTL), measured as Onset of Spasms (OS), and (c,d) MO₂ 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 ± SE for (a) MO₂ or (b) UTL after 7 d 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 MO₂ or UTL in response to increasing incubation temperature according to the best-fit regression model. UTL are measured as OS. Raw data, regression equation and relevant statistics for MO₂ and UTL are provided in Figure S4 and Figure S5b respectively.

Figure 2 The relationship between MO₂ and UTL, measured as OS, for the prawn species investigated in this study. Circles represent individual prawn MO₂ and UTL measured after 7 d 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**: $p > 0.05$.

Figure 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.