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Population-dependent acclimatization capacity of thermal tolerance in larvae of the rocky-shore barnacle *Pollicipes elegans*

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Abstract. As environmental temperatures increase and become more seasonally variable, the ability of individuals to plastically alter their physiological responses to temperature (=acclimatize) may affect the potential for species persistence. Among marine organisms, the larval stage is often the most physiologically sensitive; larvae are also often the main dispersal stage in the life history. However, studies that address the acclimatization of marine larvae are rare. We investigated whether larvae of the gooseneck barnacle *Pollicipes elegans* from two temperate populations, one from the Northern Hemisphere (Mexico) and one from the Southern Hemisphere (Peru), show patterns of seasonal acclimatization to temperature. We compared the effects of temperature on swimming activity, oxygen consumption, and mortality of larvae from the two populations in both warm and cold seasons. Larvae from Mexico had higher thermal tolerances when collected in the boreal summer compared to the boreal winter, while no similar indication of seasonal acclimatization was seen in larvae from Peru. The lack of acclimatization in larvae of *P. elegans* from Peru may be related to recent thermal history, low selection for acclimatization due to irregular patterns of seasonal temperature change during ENSO events, or to different phylogeographic histories of Northern- and Southern-hemisphere populations.

Additional key words: acclimatization, antitropical, barnacle, eastern tropical Pacific, nauplius

Marine organisms living in intertidal environments experience wide temporal fluctuations in both air and water temperature, and the geographic ranges of these species can also span thousands of kilometers and wide geographic temperature gradients (Stillman & Somero 2000; Helmuth et al. 2006). Members of these species can show considerable intrapopulation and geographic differences in the capacity for acclimatization of thermal tolerance (Stillman 2003; Stillman & Tagmount 2009; Sorte et al. 2011). These differences are receiving an increasing amount of attention because seasonal acclimatization can enhance survival and buffer the effects of temperature on key physiological processes, reducing the energetic costs of maintenance and freeing energy for non-maintenance functions such as activity, growth, and reproduction (Pörtner 2002; Pörtner et al. 2006). In a changing world, the acclimatization capacity of individuals may affect the resilience of a species or population to climate change (Somero 2010).

Phenotypic plasticity of thermal sensitivity will be key to dealing with both higher and more variable temperatures that are expected to occur with climate change (Easterling et al. 2000). The terms used to describe plasticity in thermal tolerance have traditionally been separated based on whether it is induced through exposure to changing temperatures in natural environments, termed temperature acclimatization, or through experimental manipulation of laboratory conditions, termed temperature acclimation (Hochachka & Somero 2002). Plastic responses are necessary for dealing with rates of temperature change that may outpace evolutionary responses (Kelly et al. 2012); capacity for acclima-

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tion/acclimatization can therefore provide insight into an organism's ability to deal with changing temperatures (Stillman 2003). Acclimatization responses are not always beneficial and may be non-adaptive in environments where temperatures change rapidly or seasonal changes are unpredictable. A broad range of thermal tolerance may be more optimal in these cases (Pörtner 2002; Jost et al. 2012).

In many intertidal marine invertebrate taxa, thermal tolerances of individuals are higher in the warm season and lower in the cool season, a phenomenon known as seasonal temperature acclimatization (Sommer et al. 1997; Pörtner 2002; Wittmann et al. 2008; Schröer et al. 2011). Such patterns have been found in many marine groups including polychaete worms (Sommer et al. 1997; Wittmann et al. 2008; Schröer et al. 2011), bivalves (Ansell et al. 1980; Chapple et al. 1998; Compton et al. 2007), corals (Berkelmans & Willis 1999) and crustaceans (Carvalho 1987; Cuculescu et al. 1998; Hopkin et al. 2006). While these patterns have been well established for adults, capacity for acclimatization has received less attention in earlier life history stages of marine organisms. The majority of studies on temperature acclimatization in larvae focus on crustaceans such as shrimp (*Crangon crangon* (Linnaeus 1758), Paschke et al. 2004; Urzúa & Anger 2013) and crabs (*Neohelice granulata* (Dana 1851) (as *Chasmagnathus granulatus*), Bas et al. 2007; *Halicarcinus planatus* (Fabricius 1775), Diez et al. 2012). Early developmental stages are generally considered to have narrower windows of stress tolerance than adults, and therefore present a bottleneck in a species' life cycle (Andronikov 1975; Hamasaki 2003; Pörtner & Farrell 2008; Weiss et al. 2009; Carstensen et al. 2010; Walther et al. 2010). Understanding patterns of seasonal acclimatization in larval stages can potentially provide important insight into the factors governing population dynamics, range limits, and the ability of marine species to persist in the face of changing global temperature regimes (Pechenik 1999; Storch et al. 2009; Weiss et al. 2012; Walther et al. 2013).

In the present study, we investigated seasonal temperature acclimatization of larvae of the gooseneck barnacle *Pollicipes elegans* (Lesson 1831) in the context of the thermal tolerance window (Frederich & Pörtner 2000; Pörtner 2001). *Pollicipes elegans* lives in the lower intertidal zone of rocky, wave-swept coastal regions of the Eastern Pacific Ocean in Mexico (MX) (between 26 and 19°N) and Peru (PE) (between 3 and 12°S) (Laguna 1990; Van Syoc 1994). While peripheral populations are large, in the central part of the range, in the intertropical convergence zone (ITCZ), this species is known only from an isolated population in El Salvador and a handful of observations of the species in southern Mexico and Costa Rica (Laguna 1985; Marchant 2014).

Throughout the range of *P. elegans* sea surface temperatures (SSTs) differ markedly, and previous work has shown that the thermal tolerance windows of larvae of *P. elegans* differed between populations that experience contrasting thermal regimes (Walther et al. 2013). Walther et al. (2013) compared thermal tolerance windows for larvae collected from Mexico, Peru, and El Salvador during the warmest season at each locality (Walther et al. 2013). Here, we returned to the two seasonally variable locations (MX and PE) during their respective cold seasons, measured thermal tolerance windows of larvae, and compared these to data from the warm season (Walther et al. 2013) to look for evidence of seasonal temperature acclimatization. Climate change is expected not only to cause an increase in global temperatures, but also to lead to generally higher variance in environmental temperatures (Easterling et al. 2000). Capacity for phenotypic plasticity of thermal tolerance has been linked to species' ability to persist in the face of global climate change (Stillman 2003); thus, understanding the extent and distribution of acclimatization ability across life cycles is key to predicting the potential for populations to remain viable (Storey & Tanino 2012).

Methods

Collection localities

Adults of *Pollicipes elegans* were collected from rocky intertidal shores at two different locations during the locations' respective cold season of the year: Gaspareno, Mexico (MX: 23°10′58″N, 110°8′27″W) in February 2012, and El Arco, Peru (PE: 5°5′16″S, 81°10′15″W) in October 2012 (Fig. 1). At each location barnacles were collected from accessible rocks within 0.2 km from the listed coordinates for each location. Both locations experience temperate and seasonal climates, and warm-season thermal tolerances had previously been established for both populations (Walther et al. 2013). At the MX site, SSTs are generally warmer than those at the PE site, and seasonal variation is greater, with an average monthly minimum of 20.2°C and maximum of 28.5°C (averaged from the coldest and warmest months in the 10 years between 2002 and 2012) (disc.sci.gsfc.nasa.gov; Acker & Leptoukh 2007). At the PE site, water temperatures vary from

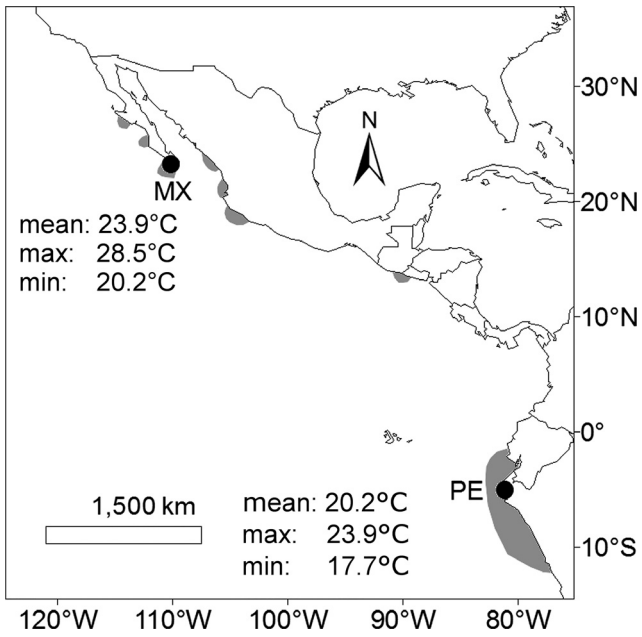

Fig. 1. Distribution of *Pollicipes elegans* (gray shading; after Van Syoc 1994) and sampling locations (black dots): MX, Gaspareno, Mexico (23°10′58"N, 110°8′27″W); PE, El Arco, Peru (5°5′16"S, 81°10′15″W). Mean, maximum, and minimum annual sea surface temperature at each location are shown. Means were calculated as average monthly mean temperatures from 2002 to 2012; maximums and minimums were calculated as the average of the warmest and coldest month for each year for the same period, respectively (disc.sci.gsfc.nasa.gov; Acker & Leptoukh 2007). Map redrawn with permission from Walther et al. (2013).

an average monthly SST of 17.7°C in the coldest winter months to 23.9°C in the warmest summer months (calculated as above for MX). Water temperatures at the time of cold-season collection were 20°C for MX and 15°C for PE (measured with a Temp 5 thermistor probe, OAKTON, Inc.). For comparison, the warm-season temperatures measured by Walther et al. (2013) at the same localities were 29°C (MX) and 23°C (PE). In other marine intertidal invertebrates, such as the mussel *Mytilus californianus* Conrad 1837, the most physiologically stressful temperatures are experienced during aerial exposures (Helmuth et al. 2002). Direct measurements of body temperatures of *P. elegans* do not exist, but aerial temperatures likely play a smaller role in determining the maternal body temperatures of *P. elegans* because they are exposed to air at only the lowest tides and live in areas of extreme wave activity (Kameya & Zeballos 1998), so we used SSTs as a proxy for the temperatures embryos are exposed to prior to larval release. Within 24 h after collection, adults were placed in plastic bags and transported in insulated coolers to Clemson University, South Carolina, USA.

Larval rearing and physiological measurements

All larval culturing and physiological experiments were performed using the same methods as Walther et al. (2013) so that data would be directly comparable between animals acclimatized to warm temperatures (as in Walther et al. 2013) and those acclimatized to cooler temperatures. For clarity, the data collected for this manuscript are referred to as "MX (winter)" and "PE (winter)" throughout, and the data collected by Walther et al. (2013) are identified as "MX (summer)" and "PE (summer)."

Larval culture

To culture larvae, lamellae were removed from adults, placed in single beakers containing 30 mL of artificial seawater (ASW; Instant Ocean, Aquarium Systems Inc., Mentor, Ohio, USA), and maintained at 25°C for less than 1 week. When nauplii were visibly moving within lamellae, lamellae from 4 to 12 adults were combined, placed in 2 L of ASW, and stimulated to hatch with a fibreoptic light. All lamellae produced vigorously swimming larvae. After larvae hatched and molted to stage II (~6 h after hatching), 800 larvae were placed in 800 mL of ASW, gently bubbled with air, fed *Rhodomonas salina* and *Isochrysis galbana* (each at 10^4 cells mL^{-1}), and maintained at 25°C for no more than 4 d. Stage II larvae were used for all experiments. All ASW used in our experiments was made up to a salinity of 35 ppt and treated with the antibiotics penicillin (50 μg ml^{-1}; MP Biomedicals, LLC, cat no. 194537) and streptomycin (25 μg ml^{-1}; MP Biomedicals, LLC, cat no. 194541).

Thermal tolerance window

Temperature physiology of larvae of *P. elegans* was investigated using the thermal tolerance window concept (Frederich & Pörtner 2000; Pörtner 2001), which is an integrative approach that uses thermal sensitivity of multiple parameters such as activity, oxygen consumption rate, and survival to determine how temperature affects performance and the availability of energy for non-maintenance functions such as growth and development (Stillman & Somero 2000; Storch et al. 2009; Walther et al. 2010; Weiss et al. 2012; Walther et al. 2013). In this model, within the optimum temperature range high levels of available oxygen allow for maximum performance.

Outside of the optimum, activity decreases due to the metabolic demands of larval tissues outstripping oxygen supply. This decrease marks the pejus temperature, T_P (Frederich & Pörtner 2000; Pörtner 2002). Beyond T_P at the critical temperature, T_C, energetic demands continue to increase and aerobic scope is reduced. Finally, T_L (estimated from LT_{50}) marks the upper limit to short-term survival (Pörtner 2010; Sokolova et al. 2012).

Activity (pejus temperature, T_P)

We measured activity of larvae as antennule beats s^{-1} at 2°C intervals between 25 and 39°C (Storch et al. 2009, 2011; methods described in Walther et al. 2013). Single larvae (N=11 to 12 per population) were placed individually in a water-jacketed dish at 25°C and left undisturbed for 5 min prior to behavioral observations. Larvae were then video-recorded (JAI CVS-S3300, Denmark) for 5 min at each target temperature, and videos were imported into iMovie (version 9.0.4). Rate of temperature increase was 0.5°C min^{-1} or slower. Larval swimming is not continuous in barnacle larvae, so the maximum activity rate for a given temperature was taken as the fastest of five 1-s subsamples in which each larva was continuously swimming.

Performance limitation indicates the onset of the pejus range where increased energetic maintenance costs limit the oxygen and energy available for other functions such as activity (Pörtner 2010; Sokolova et al. 2012). To estimate the onset of performance limitation, or the pejus temperature (T_P), we calculated the Arrhenius break temperature (ABT) for activity; ABTs were considered significantly different if their 95% confidence intervals did not overlap (Walther et al. 2013). Within each population and season combination, activity data of individual larvae were pooled to determine ABTs because for some individual larvae, a single regression provided a better fit to the data than two separate regressions (2 of 12 for MX [winter], 4 of 11 for MX [summer], 5 of 12 for PE [winter], and 5 of 11 for PE [summer]; summer data from Walther et al. 2013). To compare the effects of temperature, population, season, and their interactions on activity we used a mixed model for repeated measures using a Poisson distribution with larva nested within population (fixed factors: temperature, population, season). Activity data had heterogeneous variance and lacked sphericity, so the model allowed for heterogeneous variance among populations and heterogeneous repeated structures among populations (Stroup 2012). Residuals were normal. Analyses were performed with PROC GLIMMIX in SAS 9.3 (SAS Institute Inc., Cary, North Carolina, USA).

Oxygen consumption (critical temperature, T_C)

In the pessimum range, oxygen demand exceeds oxygen supply and aerobic scope decreases (Pörtner 2001; Sokolova et al. 2012). To estimate the onset of the pessimum range, which occurs at the critical temperature (T_C) (Storch et al. 2009, 2011; Walther et al. 2013), we measured changes in oxygen consumption of larvae with increasing temperature. Oxygen consumption rates of larvae were measured using an endpoint determination method (μBOD) (Marsh & Manahan 1999) as described in Walther et al. (2013). In brief, different numbers of individuals (4–235) were added to 5–7 respiration vials (~500–700 μL) for each temperature treatment; this μBOD method of Marsh & Manahan (1999) determines oxygen consumption rate per larva from a regression between number of larvae $vial^{-1}$ and oxygen consumption $vial^{-1}$ h^{-1} across multiple vials. The error of each estimate was calculated as the standard error around the slope of the regression line. Vials were incubated at 25.1, 30.3, 35.4, and 41°C using a thermal block that created a gradient in experimental temperatures by using recirculating water baths to cool one end and heat the other end. Oxygen tension was measured after ~5–7.5 h with a temperature-calibrated polarographic oxygen sensor (Model 1302, Strathkelvin Instruments, UK). In the cases where regressions had a non-zero Y-intercept (see Marsh & Manahan 1999 for discussion of nonzero Y-intercepts using this method) we corrected the data for each individual vial by the intercept for each regression, which permitted us to compare oxygen consumption rates between runs (Walther et al. 2013).

We used a three-way ANOVA (fixed factors: temperature, season, population) to test for differences in oxygen consumption among temperatures, seasons, populations, and their interactions (summer data from Walther et al. 2013). Oxygen consumption data were normally distributed after log transformation, and variances were homogeneous. We used Tukey's post hoc tests to determine whether there were significant differences among temperatures and the interaction factors. All analyses were performed with JMP (version 10, SAS Institute Inc.).

LT_{50} (lethal temperature, T_L)

Beyond the lethal temperature (T_L), short-term exposure to higher temperatures results in mortality.

To determine the lethal temperature (T_L) we measured LT_{50} as described in Walther et al. (2013). Briefly, 5–13 larvae were placed in 20 mL glass scintillation vials that sat in an aluminum thermal gradient block set to maintain 12 temperatures: 25.1, 26.9, 28.6, 30.3, 32.0, 33.7, 35.4, 37.3, 39.2, 41.0, 42.9, 44.9°C. We assessed mortality after larvae were held at the target temperature for 1 h followed by a 3 h recovery period at 25°C. Ramping rates were 1.8°C min^{-1}. Using both our winter data and summer data from Walther et al. (2013), a two-way ANOVA was run to test for differences in LT_{50} among populations, seasons, and their interaction (fixed factors: population, season). LT_{50} data were normally distributed and variances were homogeneous. Tukey's post-hoc test was used to test for significant differences among the interaction between population and season. All statistical analyses were performed with JMP (version 10, SAS Institute Inc.).

Results

Swimming activity (pejus temperature, T_P)

Both winter populations had ABTs above which limb beat activity was compromised (MX [winter], 31.1°C; PE [winter], 30.2°C). These winter ABTs did not differ significantly between populations, nor were there significant differences between seasons or populations when winter ABTs were compared with summer ABTs from the same two populations (MX [summer], 28.8°C; PE [summer], 30.2°C; from Walther et al. 2013) (Table 1). Across both populations and seasons, temperature had a significant effect on activity ($p<0.0001$) with declining activity at the highest temperatures in all groups. None of the other terms significantly affected activity (Table 2).

Table 1. Results of Arrhenius plot analysis of stage II nauplii of *Pollicipes elegans* from the four population and season combinations (MX [winter], PE [winter], MX [summer], PE [summer]). Arrhenius break temperatures (ABTs) (T_P) and 95% confidence intervals (CI) both in 1000/K and ABTs converted to degrees Celsius (summer data from Walther et al. 2013).

	ABT (1000/K)	CI (1000/K)	ABT (°C)
MX (winter)	3.29	0.03	31.1
PE (winter)	3.30	0.02	30.2
MX (summer)	3.31	0.03	28.8
PE (summer)	3.30	0.04	30.2

Oxygen consumption (critical temperature, T_C)

Temperature, population, season, and their interactions all had significant effects on the oxygen consumption of larvae of *Pollicipes elegans* (Fig. 2, Table 2; three-way ANOVA, $p\leq0.00127$ in all comparisons), with the exception of the interaction between temperature and season (Table 2, three-way ANOVA, $p=0.8151$). MX (summer) larvae had significantly lower oxygen consumption than MX (winter), PE (summer), or PE (winter) larvae (Tukey HSD, $p<0.001$ in all three comparisons; population x season), while oxygen consumption among the other population and season combinations was not significantly different ($p\geq0.4765$ in all three comparisons). For T_C, as in the analyses performed on summer data by Walther et al. 2013, larvae from MX (summer) showed a significant drop in oxygen consumption between 35.4°C and 41°C (6.4 pmol O_2 $individual^{-1}$ h^{-1}, Tukey HSD, $p=0.0155$). In larvae from PE (summer), oxygen consumption significantly decreased between 30.3°C and 35.4°C (5.4 pmol O_2 $individual^{-1}$ h^{-1}, Tukey HSD, $p=0.0007$). In the winter larvae from MX the significant drop in oxygen consumption occurred between 30.3°C and 35.4°C (6.2 pmol O_2 $individual^{-1}$ h^{-1}, Tukey HSD, $p=0.0275$). No significant drops in oxygen consumption were detected in winter larvae from PE (Tukey HSD, $p\geq0.5981$ in all three comparisons).

LT_{50} (lethal temperature, T_L)

Population ($p=0.0002$), season ($p=0.0015$), and population x season ($p=0.0086$; Table 2) each had a significant effect on LT_{50} of *P. elegans* larvae (Fig. 3). Larvae from PE had a significantly lower LT_{50} than larvae from Mexico ($p=0.0002$), and summer larvae had a significantly higher LT_{50} than larvae from the winter ($p=0.0015$). LT_{50} of larvae from MX (summer) (39.1±0.1°C) was significantly higher than the LT_{50} of larvae from MX (winter) (38.0±0.2°C), PE (summer) (37.8±0.1°C), or PE (winter) (37.8±0.2°C) ($p\leq0.0008$ in all three comparisons), while no significant differences were detected among the other treatments ($p\geq0.6756$ in all three comparisons; summer data from Walther et al. 2013).

Discussion

Larvae of the barnacle *Pollicipes elegans* from Mexico (MX) showed clear evidence for seasonal acclimatization, while larvae from Peru (PE) did

Table 2. Results of analyses testing for significant effects of temperature, season and population on swimming activity and oxygen consumption, and results from a two-way ANOVA testing for significant effects of season and population on LT_{50} of stage II nauplii from two populations (MX and PE) of *Pollicipes elegans* during the winter and summer. Significant *P* values are in bold ($p<0.05$).

	Num *df*	Den *df*	F	p
Mixed-model for swimming activity				
Temperature	8	336	443.76	**< 0.0001**
Population	1	42	0.00	0.9463
Season	1	336	0.10	0.7510
Temperature×Population	8	336	0.17	0.9946
Temperature×Season	8	336	0.65	0.7354
Population×Season	1	336	2.91	0.0891
Temp.×Pop.×Season	8	336	0.84	0.5672
	df	SS	MS	F	p
Three-way ANOVA for oxygen consumption					
Temperature	3	490.44111	136.4803	20.1841	**< 0.0001**
Population	1	257.40876	257.40876	31.7809	**< 0.0001**
Season	1	168.68176	168.68176	20.8263	**< 0.0001**
Temperature×Population	3	93.17681	31.0589	3.8347	**0.0127**
Temperature×Season	3	7.63334	2.5444	0.3141	0.8151
Population×Season	1	345.28115	345.28115	42.6301	**< 0.0001**
Temp.×Pop.×Season	3	147.90647	49.3022	6.0871	**0.0009**
Error	81	656.0572	8.0995		
Total	96	2143.8233			
	df	SS	MS	F	p
Two-way ANOVA for LT_{50}					
Population	1	3.2646545	3.2646545	20.3410	**0.0002**
Season	1	2.1578705	2.1578705	13.4450	**0.0015**
Population×Season	1	1.3605463	1.3605463	8.4771	**0.0086**
Error	20	3.2099216	0.16050		
Total	23	9.9929928			

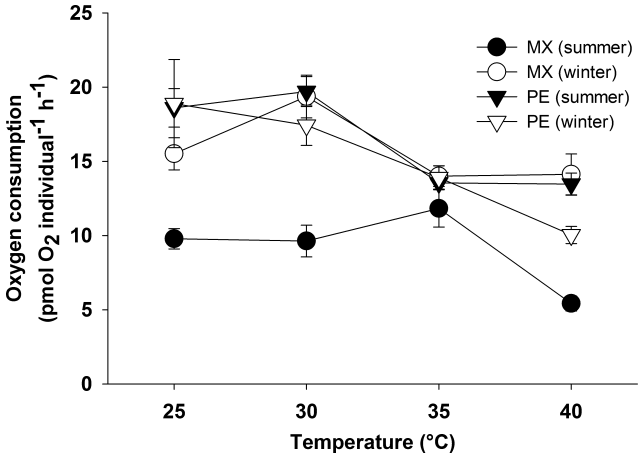

Fig. 2. Oxygen consumption (pmol O_2 individual^{-1} h^{-1}±SE) of *Pollicipes elegans* larvae from each population and season combination (MX (summer) black circle; MX (winter) white circle; PE (summer) black triangle; PE (winter) white triangle) measured at 25.1, 30.3, 35.4 and 41°. Summer data are from Walther et al. (2013).

not. Acclimatization was not seen across the entire upper range of the thermal tolerance window, however; T_P (the temperature at which activity began to slow) was similar in both seasons (Walther et al. 2013; this study, Table 1) suggesting that each population experienced a mismatch between oxygen supply and demand at close to the same temperature regardless of season. In contrast, in MX (winter) the onset of T_C (the temperature at which oxygen consumption drops and larvae enter the pessimum range) was significantly lower than in summer larvae from the same population (30.3°C vs. 35.4°C, respectively; data for summer from Walther et al. 2013). This shows a narrowing of the thermal tolerance window in winter conditions when compared to summer conditions, a phenomenon seen in other intertidal organisms that helps conserve energy when animals are not living close to their upper thermal limits (Wittmann et al. 2008; Sokolova et al. 2012). The onset of T_C in PE (winter) was not detectable

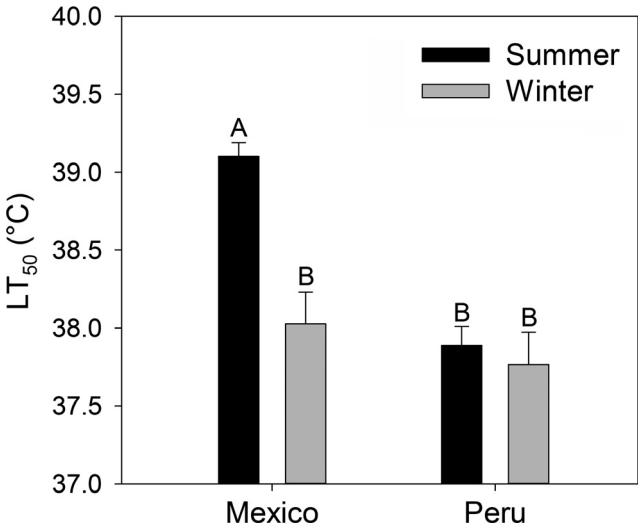

Fig. 3. Upper lethal limits (LT_{50}) of *Pollicipes elegans* larvae from each population and season combination. Bars represent the mean LT_{50} (±SE) for each population in summer (black) and winter (light gray). Shared letters indicate LT_{50} values that did not differ (Tukey HSD, $p>0.05$).

because we did not find a significant drop in oxygen consumption between any of our experimental temperatures, so comparison of T_C between seasons was not possible for PE. Overall rates of oxygen consumption were similar between the two seasons in PE but were lower in MX (summer) than in MX (winter); this pattern is consistent with seasonal acclimatization in MX and a lack thereof in PE. In fact, overall oxygen consumption rates were higher in MX (summer) compared to all other population and season combinations, a pattern that suggests cold-compensation in MX (winter) and PE (Hochachka & Somero 2002). The uppermost indicator of the thermal tolerance window, T_L (the lethal temperature), was also lower in the winter than in the summer for larvae from MX, but did not differ between seasons in PE. Thus, larvae from the Mexican population showed seasonal acclimatization of thermal tolerance while the Peruvian population did not (Fig. 4).

Differences between populations in absolute thermal tolerance and widths of the thermal tolerance windows are common (Stillman & Somero 2000; Clark et al. 2013), but why would individuals from populations within a single species differ in their ability to acclimatize when both populations experience considerable temperature variation? Stillman (2003) suggested that among species, taxa with greater absolute thermal tolerance have less capacity for acclimatory responses to temperature; such evolutionary tradeoffs in thermal adaptation may be key to setting distributional limits (Pörtner et al. 2006). Little is known about whether similar tradeoffs occur within populations of a single species (Angilletta 2009; Seebacher et al. 2012). In contrast to Stillman's (2003) hypothesis, we found that larvae from the population with the greatest absolute thermal tolerance (MX, which during its warm season had the highest LT_{50} of any group) also had the greatest capacity for thermal acclimatization. Larvae from PE, which had a lower absolute thermal tolerance, showed no measurable difference in T_P, overall oxygen consumption, or T_L between seasons, suggesting that they do not acclimatize to seasonal temperature variation in their natural habitat. Similarly, Calosi et al. (2008) found that diving beetles in the genus *Deronectes* with the highest absolute thermal tolerance also had the greatest capacity for plasticity in thermal tolerance. While evolutionary trade-offs between absolute thermal tolerance and plasticity may exist for some groups, this is evidently not a universal pattern; indeed, a recent meta-analysis by Gunderson & Stillman (2015) found little support for a relationship between maximum thermal tolerance and magnitude of thermal tolerance plasticity.

These differences in thermal tolerance and acclimatization capacity between larvae from PE and MX might cause these two populations to differ in their susceptibility to climate change. In both the summer and winter seasons in our study, Peruvian larvae were far from their upper limits of thermal tolerance (23.9°C, averaged from warmest months in the 10 years between 2002 and 2012), but our study was not conducted during an El Niño year when SSTs are typically warmer. Historically, El Niño events have resulted in SST anomalies of 2–6°C. In more recent years SSTs during extreme El Niño events have been as high as 10°C above long-term averages in PE (Tarazona & Arntz 2001), which would place PE larvae outside of their optimum and within their pessimism range (T_C=30.3°C). These more extreme El Niño events are expected to increase in frequency with climate change (Cai et al. 2014). Reproductive failure is likely during extreme El Niño years in PE, but warming temperatures may also result in an increased frequency of temporary poleward range expansions similar to a past range shift of *P. elegans* that coincided with an extreme El Niño event (Tarazona et al. 1988).

In contrast with larvae from PE, which are likely to experience temperatures outside their optimum only during El Niño years, larvae from MX routinely experience SSTs outside of their optimum during the summer (29°C, measured with a Temp 5

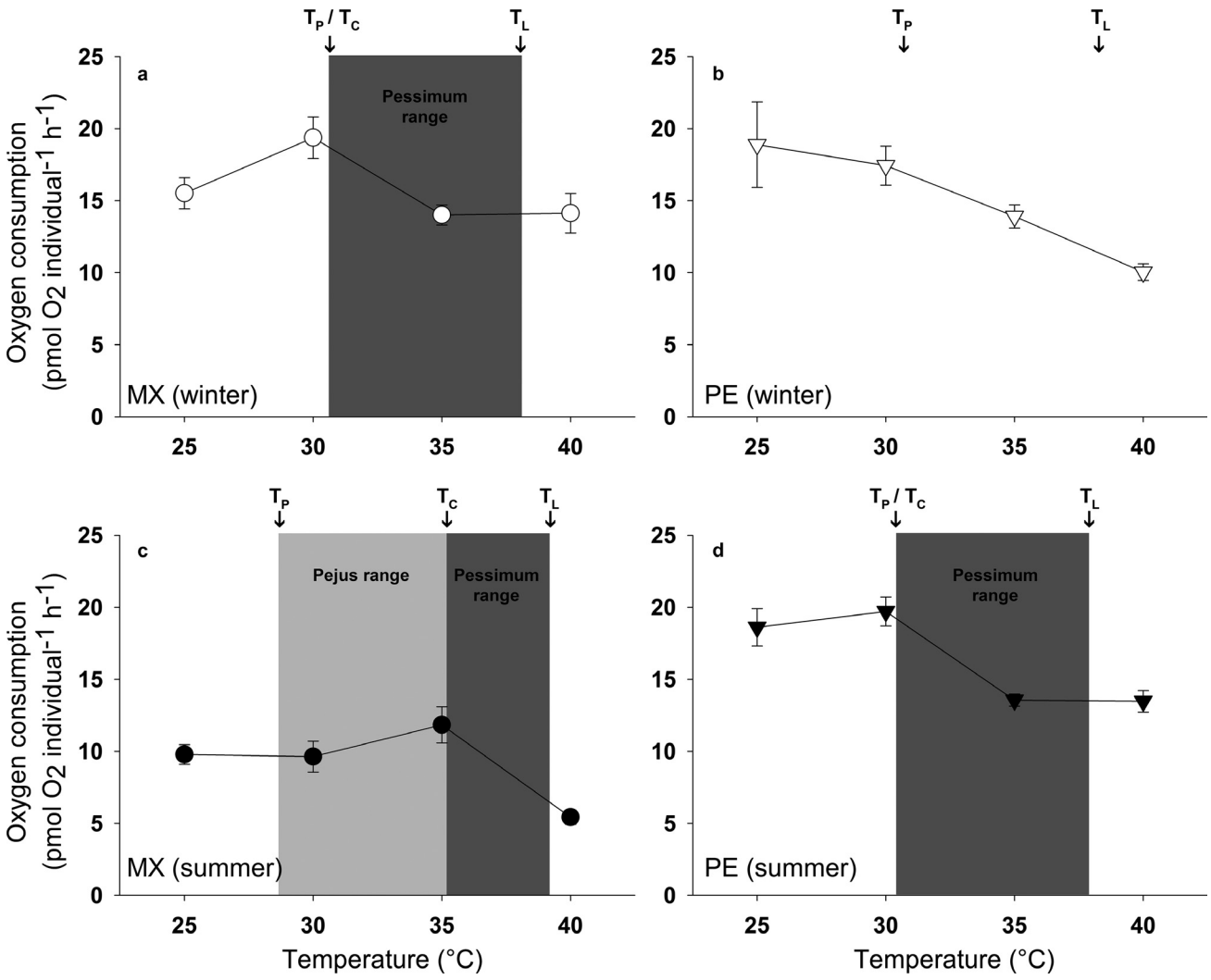

Fig. 4. Thermal tolerance windows of *Pollicipes elegans* larvae from each population and season combination **a**. MX (winter). **b**. PE (winter). **c**. MX (summer). **d**. PE (summer). Activity break points used to define T_P (T_P arrow) (MX [winter] 31.1°C; PE [winter] 30.2°C; MX [summer] 28.8°C; PE [summer] 30.2°C), oxygen consumption decrease for T_C (T_C arrow) (MX [winter] 30.3°C; MX [summer] 35.4°C; PE [summer] 30.3°C) and LT_{50} value for T_L (T_L arrow) (MX [winter] 38.0±0.2°C; PE [winter] 37.9±0.2°C; MX [summer] 39.1±0.1°C; PE [summer] 37.8±0.1°C). Pejus range (light gray shading) was between T_P and T_C. Pessimum range (dark gray shading) was between T_C and T_L. No significant drop in oxygen consumption was detected for PE (winter), so pejus and pessimum ranges were undefined. Summer figures reprinted with permission from Walther et al. (2013).

thermistor probe, OAKTON, Inc.; monthly maximum of 28.5°C between 2002 and 2012; T_P=28.8°C). A few degree increase in summer SSTs for the MX population may therefore lead to increased exposure to pejus temperatures, which represent the long-term temperature limits (Pörtner 2002), and reduce or inhibit reproductive success during the summer. If winter SSTs increase a few degrees larvae will remain well within their optimum. Patterns of *P. elegans* recruitment are unknown in Mexico; in the more extensively studied *P. pollicipes,* reproductive effort is lowest during the winter (Franco 2014). If this pattern is the same for *P. elegans* in Mexico, warming temperatures may threaten the persistence of populations in this region.

Population-level differences in the ability to acclimatize may be attributable to a number of factors. One possibility is that for any given time period, temperatures prior to collection may be higher or lower than seasonal averages, such that animals will not show their "normal" pattern of seasonal acclimatization. If the brooded larvae of *P. elegans* in Peru experienced temperatures different from seasonal averages in the weeks prior to our sampling,

the signal of seasonal acclimatization may not have been present. The exact duration of embryonic development of *P. elegans* is unknown, but in three congeneric species, embryonic development lasts approximately 3 weeks (Lewis 1975; Molares et al. 1994; Franco 2014). During January of 2012 (austral summer) the Peruvian coastline experienced weak La Niña conditions (http://iri.columbia.edu/our-expertise/climate/enso/). When we compared the daily mean SSTs in Peru during the 3 weeks before sampling we found that winter and summer averages were only 3.6°C apart (PE [winter] mean 16.8°C; PE [summer] mean 20.4°C; Fig. 5). Larvae from MX experienced larger and more consistent differences in SSTs before our winter and summer sampling at that location (MX [winter] mean 20.5°C; MX [summer] mean 29.0°C; Fig. 5) (Reynolds et al. 2007), which might have led to a higher degree of temperature acclimatization in that population. However, acclimation of other marine crustacean larvae to temperature changes similar in magnitude to the temperature difference between the seasons in PE have caused measurable physiological responses (Storch et al. 2009; Weiss et al. 2009). Therefore, the lack of evidence for seasonal acclimatization in any of the parameters we measured in the PE population may indicate that the capacity for temperature acclimatization is diminished in that group.

If capacity is indeed limited, one possible explanation is that the regular occurrence of ENSO events in the region of the PE population, which are associated with anomalously warm temperatures during El Niño years and cold temperatures during La Niña years (Holton et al. 1989), may make seasonal changes in temperature inconsistent from year to year. When large seasonal changes occur irregularly, the ability to acclimatize may not be favored by natural selection because the physiological costs of acclimatization may outweigh the benefits when seasonal changes in temperature cannot be accurately predicted (Pörtner 2002; Tomanek 2008). In such highly variable and unpredictable environments a broad thermal tolerance window may be favored over a high degree of plasticity in thermal tolerance, despite the high physiological costs of maintaining a broad window (Jost et al. 2012). We did not investigate the lower end of the thermal tolerance window so we cannot say whether larvae from PE had a broader window overall, but the lack of a pejus range in larvae from PE did widen the optimum range of the upper portion of the thermal tolerance window.

Alternatively, or in combination, differences in acclimatization capacity may be linked to divergent population histories (Donelson & Munday 2012). As one example, Seebacher et al. (2012) found that historical patterns of colonization likely affected thermal tolerance and acclimation capacity of the invasive mosquito fish *Gambusia holbrooki* Girard 1859 in Australia. For *P. elegans*, Marchant (2014) suggested that the PE populations diverged relatively recently from a central, tropical, and most likely warm-adapted population while the MX population has been isolated in a seasonal environment for considerably longer. The ancestral population was tropical, and therefore it likely experienced comparatively stable, warm temperatures throughout the year (Walther et al. 2013). In tropical environments where seasonal variation in temperature is low, selection is

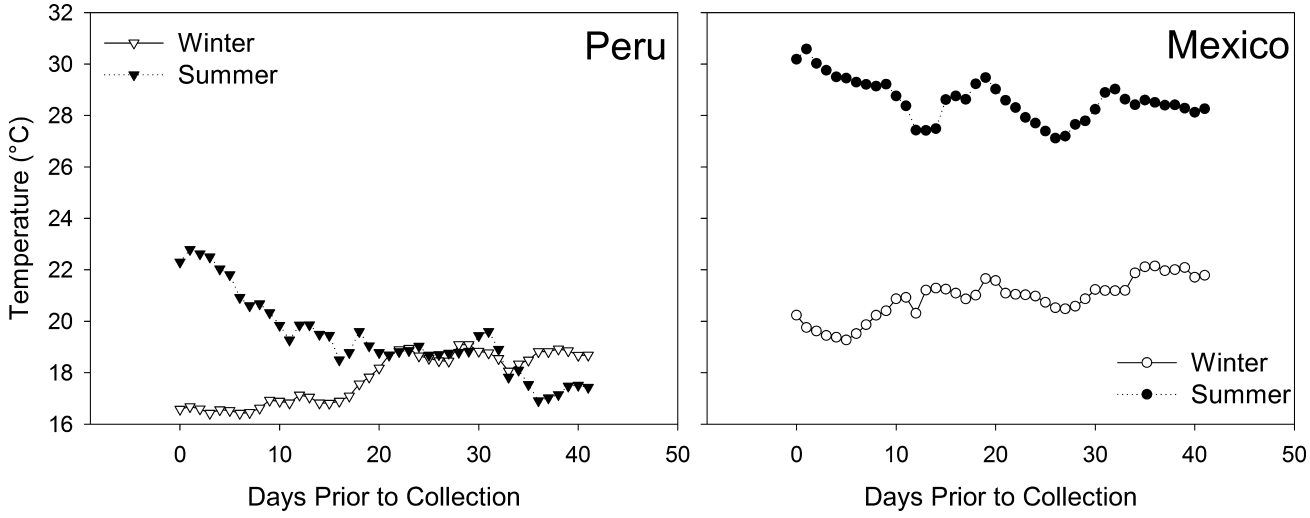

Fig. 5. Daily sea surface temperatures (Reynolds et al. 2007) in the 6 weeks prior to collection of larvae from Peru (triangles) and Mexico (circles) during the winter (black symbols) and summer (white symbols) at each location.

not likely to favor the energetically expensive process of changing thermal tolerance windows through acclimatization (Pörtner 2002; Angilletta 2009); thus, ancestral populations likely had reduced ability to acclimatize to temperature. A history of more recent gene flow between PE and a non-seasonal, tropical population may have contributed to reducing seasonal acclimatization in PE.

Definitively separating these possibilities would require sampling in different years and locations, common-garden experiments, or multigenerational laboratory studies, and is beyond the scope of the current work. However, our data are consistent with previous studies (Donelson & Munday 2012; Seebacher et al. 2012) that suggest not all populations are likely to respond physiologically in the same way to climate change. Historical changes in ocean temperature are thought to be responsible for the currently disjunct distribution of *P. elegans* (Van Syoc 1994), and increasing temperatures may result in even more widely distributed populations that vary in the ability to acclimatize, and therefore the capacity to persist, in warmer and more variable environments.

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