Mar Biol (2013) 160:2723–2732
DOI 10.1007/s00227-013-2265-x

ORIGINAL PAPER

Thermal tolerance of larvae of *Pollicipes elegans*, a marine species with an antitropical distribution

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Received: 11 December 2012 / Accepted: 15 May 2013 / Published online: 29 May 2013

Abstract For the antitropical gooseneck barnacle *Pollicipes elegans,* population-specific physiological temperature tolerance of larvae may serve as a barrier to larval dispersal across the warmest regions of the tropical Pacific Ocean. Thermal tolerance ranges of larvae of three different populations of *P. elegans* sampled in 2011 and 2012 (Mexico [MX], El Salvador [ES], and Peru [PE]) were investigated by measuring three indicators of physiological performance: swimming activity, oxygen consumption, and lethality or LT_{50}. The thermal tolerance profiles, which include measurable optimum (maximum aerobic performance), pejus ("getting worse") and pessimum (worst aerobic performance) ranges, of larvae from the three populations were consistent with their characteristic environmental temperatures. In MX, larvae live close to the upper border of their optimum during warm months and so have a limited capacity to tolerate higher-than-normal temperatures. Larvae from the ES population likewise appear to live within their optimum temperature range, but these larvae lack a detectable pessimum range, suggesting they would be unable to cope with temperatures above their pejus range. Larvae from PE have a broad optimum but no pejus range. Different thermal tolerance ranges provide strong evidence for population-dependent physiological adaptations in *P. elegans*. For the southern (PE) and northern (MX) *P. elegans* populations, high tropical temperatures are likely to be a strong direct physiological barrier to larval survival and dispersal, which is in contrast to the more thermally tolerant ES population.

Communicated by J. P. Grassle.

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Introduction

For marine communities from temperate populations in the northern and southern hemisphere, the warm temperatures and strong currents in shallow tropical oceans function as one of Earth's oldest and strongest biogeographic barriers. The modern-day oceanographic structure of tropical oceans originated in the pre-Cretaceous, more than 145 million years ago, creating a long-standing barrier to marine dispersal and biotic interchange between temperate ecosystems on opposite sides of the equator (Kauffman and Johnson 1988; Lindberg 1991). Nevertheless, some species of barnacles (Newman and Foster 1987), fish (White 1986; Stepien and Rosenblatt 1996; Burridge and White 2000), mussels (Hilbish et al. 2000), gastropods (Koufopanou et al. 1999), shrimp (Anker and Ahyong 2007), and brachiopods (Shi and Grunt 2000) have an "antitropical" (Hubbs 1952) (sometimes "amphitropical" or "bipolar", see Burridge 2002) distribution in which they are found on both sides of the warmest tropical waters, but are absent from the equatorial tropical region.

Most discussions of antitropicality assert that temperature is a primary factor limiting antitropical species' distributions; dispersal across equatorial tropical regions may only be likely during times of cooling, such as during glacial periods of the Pleistocene, whereas both reduced connectivity and tropical extirpation will be most likely during episodes of interglacial warming (Hubbs 1952; Lindberg 1991; Newman and Foster 1987; Burridge and White 2000). For many benthic taxa with pelagic, feeding larvae, the physiological tolerances of larvae may be a

fundamentally important factor limiting species' distributions (Storch et al. 2009; Weiss et al. 2012). Planktonic larvae are the most likely vector for dispersal and colonization, but at the same time, larvae may also be the most physiologically sensitive life-history stage (Hamasaki 2003; Pörtner and Farrell 2008; Weiss et al. 2009; Carstensen et al. 2010; Walther et al. 2010). Despite the potential importance of larval thermal tolerances to species' distributions, particularly antitropical taxa, intra- and interspecific studies of thermal tolerance windows for larvae are rare (Storch et al. 2009; Walther et al. 2010; Zippay and Hofmann 2010; Weiss et al. 2012).

The physiological responses of larvae to temperature can be assessed using the concept of a "thermal tolerance window" (Frederich and Pörtner 2000; Pörtner 2001). This is an integrative measure of the temperature sensitivity of an organism and provides a bioenergetic framework for measuring physiological stress (Sokolova et al. 2012) that can help describe the potential for local persistence and adaptation, and influence dispersal across the species' range. Within the optimum range, aerobic performance of an organism is at its maximum and energy is available for development, growth, and storage. The first signal of temperature stress occurs at the pejus temperature (T_P), where rising temperatures create a mismatch between oxygen demand and an organisms' capacity to supply oxygen to internal tissues. This mismatch causes falling oxygen levels in the body fluids (Frederich and Pörtner 2000) that begin to limit organismal performance, measured by a reduction in activity (Storch et al. 2009).

As temperatures increase above T_P, organisms enter the pejus range in which they still function, but performance continues to decline and less energy is available to support nonmaintenance functions. Further warming leads to the critical temperature (T_C), which is defined by the onset of anaerobic metabolism and a progressive insufficiency of cellular energy supply to fuel essential maintenance costs, measurable by a decrease in oxygen consumption (Storch et al. 2009). In the pessimum range above T_C, short-term survival is possible but only at the expense of shutting down many ATP-demanding functions (Frederich and Pörtner 2000; Pörtner 2001; Pörtner et al. 2005; Sokolova et al. 2012). In the lethal range, beginning at the lethal temperature (T_L), the balance of ATP supply and demand is disrupted and extreme physiological stress responses are required for short-term survival (Sokolova et al. 2012), measurable by LT_{50} (Zippay and Hofmann 2010; Fowler et al. 2011).

The size and internal shape of the entire thermal tolerance window, including both the endpoints and scopes of the optimum, pejus, and pessimum ranges, are essential for predicting the ability of how a population will cope with different temperature regimes (Sokolova et al. 2012). For antitropical species, characterization of the thermal tolerance window of dispersive larvae may provide a mechanistic explanation for their present distributions, as well their past and future biogeographic responses to changing climate.

We investigated larval thermal tolerances and the potential for temperature to set a physiological limit on the antitropical distribution of the gooseneck barnacle *P. elegans* in the eastern Pacific Ocean. Laguna's (1990) biogeographic analysis of eastern Pacific barnacles reported that populations of *P. elegans* are abundant between 26°N and 19°N and between 3°S and 12°S, but absent in the eastern tropical convergence zone from 19°N to 3°S. Van Syoc (1994) found an isolated population in El Salvador (13°N), and there are museum records for one locality in Costa Rica (9°N; US National Museum of Natural History) (Fig. 1). Although other undiscovered, isolated populations may exist in the tropics, *P. elegans* are clearly very rare across more than 3,000 km of coastline within the tropical eastern convergence (compared to adjacent areas in the northern and southern hemisphere), and hence, this species is considered antitropical (Van Syoc 1994). Sea surface temperatures vary considerably across the species' range. Temperature on the west coast of South America is heavily influenced by the Humboldt Current, which transports cooler surface waters from the southern Pacific into lower latitudes but which also causes strong upwelling of cold water at low latitudes along the Peruvian coast. Populations in the Northern Hemisphere are similarly, but not as severely, influenced by the Californian Current, which flows from the north toward the equator. In contrast, temperatures on the El Salvadoran coast are influenced by the eastward flowing Equatorial Counter Current, which brings warm tropical water into the eastern Pacific ICZ all year long (Garrison 2009).

The goal of this study was to describe and compare the thermal tolerance windows of larvae of *P. elegans* from three different populations that experience different seasonal and annual temperature regimes (Mexico, El Salvador, and Peru). We wanted to determine whether larvae are adapted to local sea surface temperatures and whether their tolerances might explain the species' antitropical distribution.

Materials and methods

Collection localities

Adult *P. elegans* were collected from rocky intertidal shores at three different locations: Gaspareno, Mexico (MX, 23°10′58″N, 110°8′27″W), in October 2011; Mizata, El Salvador (ES, 13°30′43″N, 89°36′21″W), in March

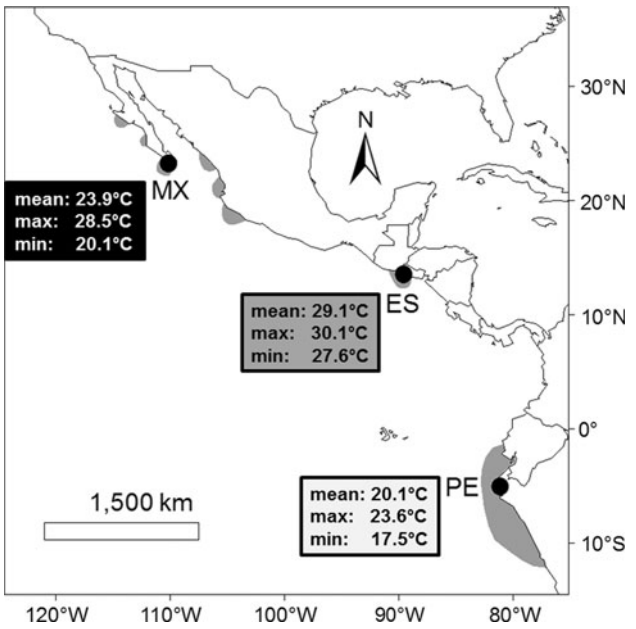

Fig. 1 Distribution of *P. elegans* (*gray shading*; after Van Syoc 1994) and sampling locations (*black shading*): MX, Gaspareno, Mexico (23°10′58″N, 110°8′27″W); ES, Mizata, El Salvador (13°30′43″N, 89°36′21″W); PE, El Arco, Peru (5°5′16″S, 81°10′15″W). Mean, maximum, and minimum annual sea surface temperature of each site. Mean calculated as average monthly mean temperatures from 2000 to 2012 (except El Niño years 2001/2002); maximum as warmest month each year; minimum calculated as average of coldest month each year for same period (disc.sci.gsfc.nasa.gov; Acker and Leptoukh 2007)

2012; and El Arco, Peru (PE, 5°5′16″S, 81°10′15″W), in January 2012 (Fig. 1). These three regions differ considerably in thermal regime: annual surface temperatures ranges are 20.1–28.5 °C for MX, 27.6–30.1 °C for ES, and 17.5–23.6 °C for PE (disc.sci.gsfc.nasa.gov; Acker and Leptoukh 2007). For this study, each population was sampled at a characteristically warm time of year; seawater temperatures at the collection localities when barnacles were sampled were 29 °C (MX), 29 °C (ES), and 23 °C (PE) (measured with a thermistor probe, OAKTON temp 5). The sampling times coincided with the lowest tides of the year, which is the only time the barnacles can be reliably collected from shore. We know of no studies on the seasonal reproductive cycles of *P. elegans*, but reproductive individuals were found on all sampling trips. Adults were placed in plastic bags and transported in an insulated cooler to Clemson University immediately after collection.

Larval culture

Prior to being opened, adults were rinsed with 5 % bleach solution, 95 % ethanol, and sea water (3 s wash with each solution) to limit contamination of larval cultures. Shells were cracked open and lamellae were removed with forceps and placed in single beakers containing 30 mL of artificial seawater (ASW; Instant Ocean, Aquarium Systems Inc., Mentor, Ohio, USA) made up to a salinity of 35 for several hours at 25 °C. To limit bacterial growth in larval cultures, the antibiotics penicillin (MP Biomedicals, LLC, cat. no. 194537) and streptomycin (MP Biomedicals, LLC, cat. no. 194541) were added to cultures to make a final concentration of 50 μg mL^{-1} penicillin and 25 μg mL^{-1} streptomycin (Strathmann 1987). Lamellae (number of lamellae depended on availability) from 4 to 12 adults with mature embryos (oldest embryonic stage right before hatching) were placed in 2-L beakers with 35 ASW treated with 50 μg mL^{-1} penicillin and 25 μg mL^{-1} streptomycin. To stimulate development and hatching of the first naupliar stage, beakers were illuminated by pointing the beam of a fiberoptic light at lamellae (Emlet and Sadro 2006). After larvae molted to stage II (~6 h after hatching), they were placed in 800 mL of gently bubbled ASW at concentrations of 1 larva mL^{-1}, maintained at 25 °C, and fed a combination of *Rhodomonas salina* and *Isochrysis galbana* (each at 10^4 cells mL^{-1}). Larvae were used for experiments within 1 day of reaching stage II (and in all cases before they molted to stage III).

LT_{50} (T_L)

Thermal tolerance experiments were conducted on stage II nauplii in an aluminum thermal gradient block with holes drilled to fit 20-mL glass scintillation vials. Recirculating chilling/heating water baths (Fisher Scientific, Isotemp 3006, 3016D) were connected to each end of the block (modified after Kuo and Sanford 2009) and the temperatures of the baths were set to create and maintain a temperature gradient from 25 to 45 °C. We tested larvae over 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. For each temperature, 5–20 larvae were placed in each of six replicate vials in 20 ml of ASW and brought to temperature at a rate of 1.8 °C min^{-1}. Larvae remained at the target temperature for 1 h and were allowed to recover at 25 °C for 3 h, after which mortality was assessed. Pilot studies showed that this protocol produced consistent results and was short enough that starvation was not a confounding factor. Pilot studies also indicated that oxygen drawdown was low in the vials and was not affected by temperature. For each set of vials, the number of alive/dead larvae at each temperature was counted; a logistic regression was then fitted to the binomial mortality data across the range of temperatures. LT_{50} was estimated from each logistic regression as the temperature at which 50 % of the larvae died using the reverse prediction function in JMP (version 10, SAS Institute Inc., Cary, North Carolina, USA). This experiment was repeated six times on separate batches of larvae from each

population. Data were normally distributed and variances were homogenous after log transformation, and a one-way ANOVA was run to test for differences in LT_{50} among populations ($N = 6$ per population; fixed factor). A Tukey's post hoc test was used to test for significant differences among populations. All statistical analyses were performed with JMP (version 10, SAS Institute Inc., Cary, North Carolina, USA).

Activity (T_P)

To measure the thermal sensitivity of activity of stage II larvae, single larvae were acclimated for five min at 25 °C in a water-jacketed dish connected to a water bath to maintain the target temperature. Larvae ($N = 11$ or 12 population^{-1}) were monitored and video-recorded (JAI CV-S3300, Denmark) for 5 min, after which temperature was increased by 2 °C increments from 25 to 39 °C. Rates of temperature increase were 0.1 °C min^{-1} between 25 and 35 °C, and 0.5 °C min^{-1} between 35 and 39 °C. A 5-min video of each larva was recorded at each temperature. Because activity was not continuous, five one-second intervals during which each larva was swimming steadily were haphazardly chosen from each 5-min video. The fastest of these five measurements was then used as the maximum activity rate for each larva. Activity was estimated by counting antennule beats s^{-1} using iMovie (version 9.0.4). Lack of normality, heterogeneity of variance, and lack of sphericity prohibited the use of repeated-measures ANOVA. Therefore, maximum activity was analyzed using a mixed model for repeated measures using a Poisson distribution with larva nested within population; this model allowed for heterogeneous variance among populations (i.e., corrected for heterogeneity of variance) and heterogeneous repeated structures among populations (i.e., corrected for lack of sphericity) (Stroup 2012). Population and temperature were both treated as fixed factors in the model. The resulting model provided a good fit to the data (minimum AIC 230), and the residuals were normal. Fisher's pairwise *t* tests were used for post hoc comparisons. Analyses were performed with PROC GLIMMIX in SAS 9.3 (SAS Institute Inc., Cary, North Carolina, USA). Temperature break points for activity were estimated by calculating Arrhenius break temperatures (ABTs) (Stillman and Somero 1996). ABTs were calculated by plotting the natural logarithm of activity, using pooled activity data for each population, against temperature (1,000/K) and performing a segmented linear regression implemented in SegReg (Stinson et al. 1979). Pooled activity data were used instead of individual ABTs for each larva because for some larvae ($N = 4$ for MX, $N = 9$ for ES, $N = 5$ for PE), a single regression provided a better fit to the data than two separate regressions. If the 95 % confidence intervals of the break points estimated for the different populations did not overlap, the break points were considered significantly different.

Oxygen consumption (T_C)

Oxygen consumption rates of larvae were measured using the endpoint determination method (μBOD) of Marsh and Manahan (1999). Briefly, stage II larvae were suspended in filtered seawater (0.2-μm) in small respiration vials of known volume (~500–700 μL). Different numbers of individuals (12–338) were added to seven vials for each temperature treatment. Groups of seven vials were incubated at each of four temperatures, 25.1, 30.3, 35.4, and 41 °C, using a thermal block (see methods LT_{50}), after which 300-μL subsamples were taken from each vial with a temperature-equilibrated gas-tight syringe. Oxygen tension was measured in each sample with a temperature-calibrated polarographic oxygen sensor (Model 1302, Strathkelvin Instruments, UK). Larvae in each vial were counted, and for each temperature, oxygen consumption larva^{-1} was calculated as the slope of the regression line of oxygen consumed per hour against number of larvae in each vial. The error of each estimate was calculated as the standard error around the slope of the regression line.

Oxygen consumption data were normally distributed and variances were homogeneous, so a two-way ANOVA (fixed factors: population, temperature) was run to test for significant effects of population, temperature, and their interaction on oxygen consumption. Some of the regressions had a nonzero Y-intercept (see Marsh and Manahan 1999 for discussion of nonzero Y-intercepts using this method); in these cases, the data for individual vials were corrected by the intercept for each regression to allow for comparison of oxygen consumption rate among runs. Tukey's post hoc tests were used to test for significant differences among populations, temperatures, and their interaction. Statistical analyses were performed with JMP (version 10, SAS Institute Inc., Cary, North Carolina, USA).

Results

LT_{50} (T_L)

LT_{50}s of *P. elegans* differed significantly among populations (Table 1, one-way ANOVA, $P < 0.001$). The LT_{50} of larvae from PE was significantly lower than the LT_{50} of larvae from MX and ES (Tukey's post hoc test, $P < 0.001$ in both comparisons) (the LT_{50} was 37.8 ± 0.1 °C for PE, 39.1 ± 0.1 °C for MX, and 39.2 ± 0.1 °C for ES). No significant difference was detected between MX and ES (Tukey's post hoc test, $P = 0.85$).

Table 1 Effect of population on LT_{50} of larvae from three populations (MX, ES, and PE) of *P. elegans*; effects of population, temperature, and their interaction on activity and on oxygen consumption of larvae from three populations of *P. elegans* (significant *P* values in bold print)

	df	SS	MS	*F*	*P*
One-way ANOVA for LT_{50}					
Population	2	6.389627139	3.19481	35.8404	<**0.001**
Error	15	1.3371019			
Total	17	7.726729			
		Num *df*	Den *df*	*F*	*P*
Mixed-model repeated-measures for activity					
Population		2	31	0.50	0.6090
Temperature		8	248	232.46	<**0.0001**
Population × temperature		16	248	1.61	0.0655
	df	SS	MS	*F*	*P*
Two-way ANOVA for oxygen consumption					
Population	2	730.475	365.238	72.8377	<**0.0001**
Temperature	3	108.189	36.0632	7.1919	**0.0003**
Population × temperature	6	425.387	70.8978	14.1388	<**0.0001**
Error	62	310.8931	5.014		
Total	73	1,625.1430			

Activity (T_P)

Larvae from the warmest population (ES) had the highest ABT (34.5 °C), while ABTs were lower for larvae from MX (28.8 °C) and PE (30.2 °C) (Table 2). Confidence intervals overlapped for all contrasts except between ES and MX (Table 2). Temperature significantly affected activity in all populations, and activity was marginally different in the way it changed across temperatures among populations (mixed-model repeated-measures, temperature $P < 0.0001$, temperature × population $P = 0.0655$) (Table 1).

Oxygen consumption (T_C)

Temperature (two-way ANOVA, $P = 0.0003$) and population (two-way ANOVA, $P < 0.0001$) both had significant effects on oxygen consumption, and there was a significant interaction between population and temperature (two-way ANOVA, $P < 0.0001$) (Table 1; Fig. 2). Across all four temperatures, overall larval oxygen consumption was higher in PE than in ES or MX, and higher in ES than in MX (Tukey's post hoc, $P < 0.005$ for each of the three comparisons). For larvae from PE, the only significant decrease in oxygen consumption occurred between 30.3 °C

Table 2 Results of Arrhenius plot analysis of larvae from three populations (MX, ES, and PE) of *P. elegans*

	ABT (1,000/K)	CI (1,000/K)	ABT (°C)
MX	3.31	0.03	28.8
ES	3.25	0.02	34.5
PE	3.30	0.04	30.2

Arrhenius break temperatures (ABTs) (Tp) and 95 % confidence intervals (CI) reported both in 1,000/K and ABTs converted to °C

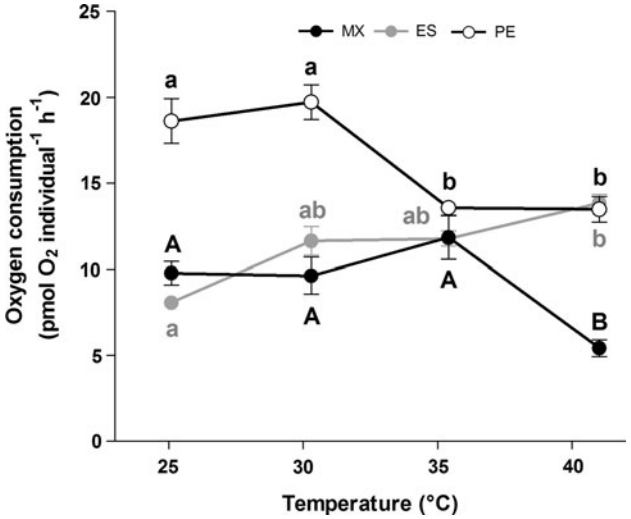

Fig. 2 Oxygen consumption (pmol O_2 individual^{-1} h^{-1} ± SE) of *P. elegans* larvae from each population (MX, *black*; ES, *gray*; PE, *white*) measured at 25.1, 30.3, 35.4, and 41 °C. Significant differences among temperatures in each population indicated with *letters* (MX: *black* upper cases; ES: *gray* lower cases; PE: *black* lower cases) (Tukey's post hoc, see "Results" section for details)

(19.7 ± 1 pmol O_2 h^{-1}) and 35.4 °C (13.6 ± 0.4 pmol O_2 h^{-1}) (Tukey's post hoc, $P < 0.0001$). No significant decrease in oxygen consumption with temperature was detected in larvae from ES (Fig. 2). In larvae from MX, oxygen consumption decreased significantly only between 35.4 °C (11.8 ± 1.3 pmol O_2 h^{-1}) and 41 °C (5.4 ± 0.5 pmol O_2 h^{-1}) (Tukey's post hoc, $P < 0.0001$).

Thermal tolerance windows

Our estimations of the thermal tolerance windows of each population are shown in Fig. 3. We estimated the pejus temperature (T_P) as the Arrhenius break temperature of the swimming activity (Storch et al. 2009). The estimated T_P is an approximate and minimum value, because we measured activity in 2 °C temperature steps. The decrease in oxygen consumption marked the critical temperature (T_C) (also an approximate but minimum value). Because we had only 4 temperature points per population for measurements of oxygen consumption, it was not possible to calculate a breakpoint; instead, we set T_C as the temperature above which we saw a significant decrease in oxygen consumption (Storch et al. 2009). Because we used temperature increments of 2 and 5 °C, our estimates are limited in precision; this would, however, only reduce our power to find significant differences between populations in T_P and T_C. The temperature range between T_P and T_C was defined as the pejus range. We defined the LT_{50} value as T_L, and the temperature range between T_C and T_L temperature as the pessimum range. The thermal tolerance windows of larvae from MX consisted of pejus and pessimum ranges bracketed by ~30, ~35, and ~40 °C (Fig. 3a). In contrast, T_C and T_L overlapped for larvae from ES (Fig. 3b), meaning that there was no pessimum range in this population; ES larvae that survived beyond the LT_{50} showed no decrease in oxygen consumption. For the PE population, there was no pejus range due to coincident values of T_P and T_C (Fig. 3c).

Discussion

Comparing T_L, T_C, and T_P showed that all three populations had distinct thermal tolerance windows that varied in both the limits and scope of the pejus, pessimum, and lethal ranges. In MX larvae, the combined pejus and pessimum range were broad compared to PE and ES. In PE larvae, the decline in swimming performance (T_P) and oxygen consumption (T_C) occured at similar temperatures, meaning that the larvae had no detectable pejus range and there was no transition to the pessimum range. To our knowledge, the only previous example of a species with no detectable

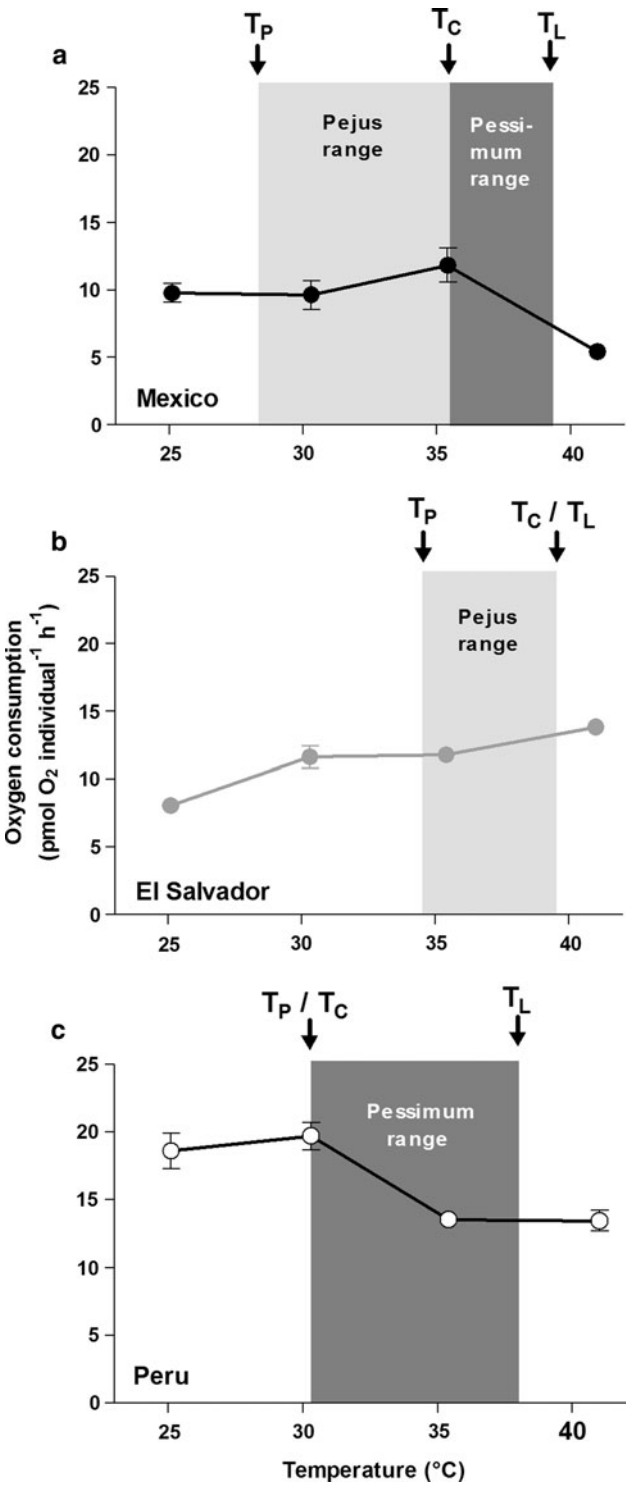

Fig. 3 Thermal tolerance ranges of *P. elegans* larvae from each population **a** MX, **b** ES, and **c** PE. Activity break points used to define T_P (T_P *arrow*) (MX: 28.8 °C; ES: 34.5 °C; PE: 30.2 °C), oxygen consumption decrease for T_C (T_C *arrow*) (MX: 35.4 °C; ES: 41 °C; PE: 30.3 °C), and LT_{50} value for T_L (T_L *arrow*) (MX: 39.1 ± 0.1 °C; ES: 39.2 ± 0.1 °C; PE: 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

pejus range is the green crab *Carcinus maenas* (Jost et al. 2012). *C. maenas* live in highly variable intertidal habitats, and Jost et al. (2012) suggested that the absence of a pejus range in this species might reflect selection for a broad optimum range in a variable environment. Green crabs also had a higher heart rate than other crabs, potentially due to metabolic demands placed on them by their highly variable environments (Jost et al. 2012). While we did not measure lower thermal limits and so cannot estimate the breadth of the optimum range in *P. elegans*, PE larvae not only lacked a detectable pejus range but also had a higher metabolic demand than the MX and ES larvae, and PE larvae maintained swimming activity at much higher temperatures (up to 31.6 °C) than their normal field temperatures (17.5–23.6 °C). While the Peruvian upwelling region experiences little seasonal temperature fluctuation (a maximum 5 °C range over 2 years, Urban 1994), El Niño events have a strong effect on temperatures in this region, regularly increasing temperatures to 26.5 °C (Acker and Leptoukh 2007) and occasionally even higher (up to 28 °C, Kameya and Zeballos 1988). With their wide optimum range, PE larvae may have increased capability to tolerate high temperatures during El Niño events without experiencing limitation in energy supply. In contrast to PE larvae, however, other southern Pacific invertebrate larvae are much more sensitive to high El Niño-like temperatures, illustrated by higher mortality in bivalve larvae (Carstensen et al. 2010) and reduced oxygen consumption in crab larvae (Storch et al. 2009; Weiss et al. 2009, 2012).

Comparing the absolute oxygen consumption rates of populations showed that *P. elegans* from PE used oxygen at a higher rate than larvae from MX and ES in the physiological range of all three populations. This pattern suggests cold compensation in PE larvae; cold-compensated organisms often show higher mass-specific oxygen consumption rates than organisms from warmer regions (Hochachka and Somero 1984; Tschischka et al. 2000; Sommer and Pörtner 2004). Similar patterns have been seen in other taxa such as larvae of kelp crabs (Storch et al. 2009). In *P.elegans,* larval size differed among populations, but these size differences did not entirely drive the differences in metabolic rate; larvae from PE and MX had similar carapace lengths (0.33 ± 0.005 mm and 0.33 ± 0.003 mm, respectively), though larvae from ES were significantly smaller (0.32 ± 0.003 mm) (one-way ANOVA, $P = 0.0485$). In the PE population, aerobic larval metabolism reached its upper limit at a lower temperature than in the MX or ES populations, suggesting larvae from PE had limited capacities to adjust to further temperature increments.

Unlike larvae from the PE population, the ES population had a broad pejus range but the pessimum range was not detected because the T_L/LT_{50} (39.3 °C) was close to T_C, the temperature where oxygen consumption declined (41 °C). Lack of a pessimum range means that above T_C larvae lacked physiological mechanisms to permit short-term survival; thus, compared to MX and PE, the ES population may have a reduced ability to cope with short-term exposure to high temperatures. However, while the ES population routinely experiences the highest temperatures of the three, the annual habitat temperature fluctuates over only ~3 °C (from 27.6 to 30.1 °C), and thus, these larvae may only rarely encounter temperatures above their optimum range. Larvae of ES also had a broader thermal tolerance range and a higher thermal tolerance overall (as shown by higher T_P, T_C and T_L) than either the MX or PE population; their critical temperature appeared to be far beyond their environmental temperatures, which is similar to patterns seen in some tropical reef fish (Mora and Ospina 2001). This is in contrast to the general paradigm that tropical species have thermal limits close to their actual maximum habitat temperature and thus are vulnerable to increasing environmental temperatures (Somero 2005; Sunday et al. 2011). Nevertheless, the lack of a larval pessimum range in the ES population indicates a reduced tolerance of temperatures above their T_C, suggesting these populations are not adapted to extreme increases in water temperature.

In contrast to both PE and ES, the MX population had broad and well-defined pejus and pessimum ranges; this population experiences cooler temperatures than the ES population but with greater annual fluctuations (between 20.1 and 28.5 °C). Both the ES and MX populations were collected when habitat temperatures were the same (29 °C) and so were both likely to have been similarly acclimatized. However, in comparison with ES larvae, which appeared to live well within their optimum temperature, larvae from the MX population had a narrower upper thermal tolerance and appeared to be close to the upper limits of the optimum range during the hottest season. A small increase in temperature during the warmest season would bring the MX population into the pejus range, where energy would have to be diverted from growth and development to maintain homeostasis.

Van Syoc (1994) combined museum collection records and SST data to show that the geographic distribution of adult *P. elegans* is related to temperature, with the species nearly absent from the warmest portions of tropical coastline immediately north of the equator. With respect to larval temperature physiology, some aspects of our data were consistent with the hypothesis that temperature limits the geographic distribution of *P. elegans*, whereas other aspects, such as the extremely high larval temperature tolerance of the ES population, were not. Temperatures both north and south of the ES populations appear to be well within the population's larval thermal tolerances. That

might explain the occasional and rare reports of *P. elegans* from sites in between the ES population and the north end of the PE population (e.g., Costa Rica; US National Museum of Natural History record 61749). Temperature regimes of those regions appear well within the physiological tolerances of larvae from the ES population, suggesting that either larval mortality unrelated to the effects of temperature on stage II larvae (e.g., predation, insufficient food supply, or more restrictive thermal tolerances at later stages), limitations on physical dispersal of larvae, or some aspect of the juvenile or adult habitat prevents the ES population from expanding north or south. Larval transport may be important because larvae may be less likely to disperse from ES out of the ICZ against the equatorial-bound California and Humboldt Currents (but see Dawson et al. 2010). The wind jets that create the Tehuantepec and Papagayo upwelling systems fall to the west and east of El Salvador, possibly isolating this region of the coast by carrying larvae offshore (Pennington et al. 2006; O'Dea et al. 2012).

However, our data do indicate that tropical temperatures likely act as a barrier to dispersal for populations from MX toward the south and, to a lesser extent, from PE to the north. Larvae from the MX population had narrow upper thermal tolerance limits, placing limitations on how larvae might cope with high temperatures to the south. In particular, during the El Niño events that occur about every 5 years, temperatures up to 3 °C above normal in southern Mexico (Carriquiry et al. 2001) may have a strong negative effect because the larvae would be in the pejus range. ES larvae, in contrast, would still be within their optimum. Studies on corals which live close to their thermal limits and cannot tolerate higher temperatures (Coles et al. 1976) show extreme mortality during El Niño events in southern Mexico (Carriquiry et al. 2001).

In conclusion, stage II larvae from the three collection sites have strikingly different thermal tolerance windows, particularly in the breadth of their pejus and pessimum ranges. Our results suggest that the larval thermal tolerances of populations of *P. elegans* are locally adapted and that larval physiology is dependent on source population. Additionally, our data suggest that warm tropical temperatures are not likely to be a barrier to dispersal for larvae from ES, which are the most thermally tolerant of the three populations. However, high seawater temperatures in the tropics could be a physiological barrier to dispersal for larvae from MX and PE, because larvae from MX that went southward would go through development within their pejus range, meaning that energy for growth and development would be limited; northward-moving larvae from PE would face constant exposure to their pessimum range, where only short-term survival is possible. We did not examine thermal tolerances of later stages, which may be different (e.g., Walther et al. 2010; Storch et al. 2011); however, the thermal tolerances of the earliest stages would provide a bottleneck to dispersal even if the later stages were more tolerant. Other factors driving the modern-day distribution of this species could include ocean currents, juvenile and adult environment, and other aspects of the larval habitat (e.g., food availability, predation, competition). Phylogeographic studies could shed light on whether ocean currents impact dispersal and connectivity among populations, and whether the ES population is either an ancestral (relictual) source population or a population of more recent origin from either the north or the south. The vicariance hypothesis for the origin of antitropicality suggests that temperate populations will show evidence of having been divided into northern and southern components by the origin of the tropics due to a long history of cooling and warming cycles (Lindberg 1991). From the thermal tolerance perspective, it seems possible that ES is a source "tropical" population from which PE and MX populations were established during cool periods. Our data suggest that once larvae from populations inhabiting cooler regions have become adapted to local temperature regimes, connectivity across the tropics would be further impaired by larval physiological tolerances, enhancing the isolation of antitropical populations.

Acknowledgements This work was supported by Clemson University's Department of Biological Sciences and by the National Science Foundation (OCE-0961996 to PBM and ALM). We thank Carmen Yamashiro from the Marine Invertebrates Research unit of IMARPE in Peru and Enrique Barraza from the Natural Resources and Environmental Department in El Salvador for providing collection permits and logistical support. We also thank L. Plough and C. Genovese for help with collection and technical support.

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