

RESEARCH ARTICLE

Master of all trades: thermal acclimation and adaptation of cardiac function in a broadly distributed marine invasive species, the European green crab, *Carcinus maenas*

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ABSTRACT

As global warming accelerates, there is increasing concern about how ecosystems may change as a result of species loss and replacement. Here, we examined the thermal physiology of the European green crab (*Carcinus maenas* Linnaeus 1758), a globally invasive species, along three parallel thermal gradients in its native and invasive ranges. At each site, we assessed cardiac physiology to determine heat and cold tolerance and acclimatory plasticity. We found that, overall, the species is highly tolerant of both heat and cold, and that it survives higher temperatures than co-occurring native marine crustaceans. Further, we found that both heat and cold tolerance are plastic in response to short-term acclimation (18–31 days at either 5 or 25°C). Comparing patterns within ranges, we found latitudinal gradients in thermal tolerance in the native European range and in the invasive range in eastern North America. This pattern is strongest in the native range, and likely evolved there. Because of a complicated invasion history, the latitudinal pattern in the eastern North American invasive range may be due either to rapid adaptation post-invasion or to adaptive differences between the ancestral populations that founded the invasion. Overall, the broad thermal tolerance ranges of green crabs, which may facilitate invasion of novel habitats, derive from high inherent eurythermality and acclimatory plasticity and potentially adaptive differentiation among populations. The highly flexible physiology that results from these capacities may represent the hallmark of a successful invasive species, and may provide a model for success in a changing world.

KEY WORDS: Acclimatory plasticity, Cardiac physiology, Local adaptation, Species invasion, Thermal tolerance

INTRODUCTION

Environmental temperature exerts a pervasive influence on the physiology of ectothermic animals, from the level of molecular processes to that of broad-scale biogeographic patterning (Hochachka and Somero, 2002). As global temperatures rise, there is increasing concern about the ability of ectothermic animals to survive in their warming environments, and considerable effort is being expended to identify the physiological traits that may be of greatest importance in adapting to rising temperatures and to learn how these traits differ among species (Somero, 2010).

One aspect of success in a changed thermal environment comprises a combination of broad intrinsic tolerance of acute changes in temperature and a high capacity for acclimatization

(phenotypic ‘plasticity’) during extended exposure to a new thermal regime (Simons, 2011; Healy and Schulte, 2012). These two abilities may largely govern how eurythermal an ectothermic species is, and thus help determine its chances for success in a warming world, particularly over short time scales.

Because maintenance of broad thermal tolerance may be metabolically costly and, indeed, unnecessary for animals inhabiting narrow thermal niches, ectothermic species range from extreme eurytherms to narrowly tolerant stenotherms (Pörtner and Farrell, 2008). Animals that have evolved in highly stable environments such as the Southern Ocean may have narrow thermal tolerance and a limited ability to acclimatize (Peck et al., 2010), whereas those that have evolved in habitats where temperatures show wide seasonal or diurnal variation are often eurythermal and manifest significant acclimatization potential (Fangue et al., 2006; Hopkin et al., 2006). Such differences in tolerance and acclimatization capacities are seen clearly in comparisons of closely related congeneric species that have evolved in different thermal environments (Stillman and Somero, 2000; Stenseng et al., 2005). For example, porcelain crabs (genus *Petrolisthes*) living very close to their upper thermal limits lacked a strong ability to acclimate beyond those limits. In contrast, cooler-water congeners were able to shift their thermal optima substantially beyond their environmental limit after acclimation to warmer temperatures (Stillman, 2003) (but see Calosi et al., 2008; Bozinovic et al., 2011).

Differences in thermal tolerance among conspecifics may also play important roles in governing the persistence of a species in a changing thermal environment. Recent studies have shown that reproductively isolated populations of a single species that have evolved under different thermal conditions differ in thermal tolerance (local adaptation) (Kuo and Sanford, 2009; Kelly et al., 2012). For example, populations of salmon may be thermally matched to the conditions they experience as they return to their natal waterways to spawn (Eliason et al., 2011). Acclimatization ability may also differ between thermally divergent populations. Laboratory thermal acclimation studies have revealed these potentials (Sorte et al., 2011), although studies comparing acclimatization or acclimation abilities across intraspecific populations are rare.

One of the most potentially powerful study systems for examining intraspecific differences in thermal sensitivities is invasion biology. Widespread invasive species often are successful across a range of habitat temperatures and thus offer a special opportunity for analysis of differences in acclimatization potential and genetic variation due to thermal selection (Chown et al., 2007; Sorte et al., 2010b; Lockwood and Somero, 2011). An ability to acclimatize to new conditions has long been suggested as an important factor in determining invasion success (Yeh and Price, 2004; Smith, 2009; Nyamukondiwa et al., 2010). Additionally, invasive species,

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especially those with short generation times and high levels of genetic variation, may have the ability to adapt rapidly to their new environments, as has been shown for *Drosophila subobscura* across multiple thermal clines in its non-native range (Balanyá et al., 2006).

An appropriate invasive species with which to explore variation in temperature tolerance and acclimatization ability across a thermal gradient is the European green crab, *Carcinus maenas* (Linnaeus 1758). Its native range extends from Iceland to northern Africa, encompassing an approximately 25°C range of average sea surface temperature. The species has successfully established non-native populations in Japan, Australia and Tasmania, South Africa, Argentina and both coasts of North America (Carlton and Cohen, 2003; Hidalgo et al., 2005). Previous work using mitochondrial DNA and a suite of microsatellites has established the global genetic structure of *C. maenas*, and has identified likely source populations for each independent introduction (Roman and Palumbi, 2004; Darling et al., 2008; Darling, 2011).

Here, we focused on populations of *C. maenas* in its native Europe and in North America, where the species has been introduced on at least three separate occasions. The first introduction, which is the oldest known globally, occurred in approximately 1817 near Long Island Sound, New York. This initial invasive population slowly spread north and south, to range between southern Nova Scotia and coastal Virginia (Carlton and Cohen, 2003). In the 1980s, the range of *C. maenas* rapidly advanced north through the Maritime Provinces of Canada, spreading to northern Nova Scotia, Prince Edward Island, and finally to Newfoundland in 2007 (Roman, 2006; Blakeslee et al., 2010). Genetic analysis revealed that this rapid northward expansion was concurrent with a second introduction of the species from northern Europe (Roman, 2006). Since that time, the two introduced populations have widely introgressed, with genetic signatures of the original invasion extending throughout the East Coast range, and signatures of the second introduction extending as far south as New York and still moving southward (Pringle et al., 2011). In contrast, the West Coast population was founded circa 1990 by individuals from the original East Coast introduction, and by 1998 had spread to its current distribution between Elkhorn Slough, CA, USA, and Vancouver Island, BC, Canada, with no evidence for multiple introductions (Behrens Yamada et al., 2005; Tepolt et al., 2009).

To compare the thermal tolerances of these different populations, we focused on cardiac function, one of the most widely used physiological traits for indexing thermal sensitivities of ectotherms (Hochachka and Somero, 2002). Heart rate and metabolism are tightly coupled in ectotherms, and both increase as temperature rises due to Q_{10} effects (Frederich and Pörtner, 2000). However, near the

upper thermal tolerance limits of an ectotherm, cardiac rate typically shows a reduced ability to rise with increasing temperature and, at a critical temperature (CT_{max}), falls precipitously (e.g. Stillman, 2002). Such reductions in cardiac function at high temperatures lead to an inability to maintain aerobic scope, and thus represent a loss of key behavioral abilities such as the locomotory capacity needed in predator–prey interactions (Pörtner, 2002; Casselman et al., 2012). Cardiac limits can be shifted in response to short-term acclimation in some species (Stillman, 2003; Stenseng et al., 2005; Camacho et al., 2006), and have been shown to reflect long-term environmental adaptation to differences in habitat temperature due to latitude or altitude, e.g. to vertical position in intertidal habitats (Stillman, 2002; Stenseng et al., 2005; Eliason et al., 2011). Thus, cardiac function is well suited to comparative studies of thermal tolerance and acclimatory plasticity among and within species.

In this study, we compared high and low temperature tolerance and acclimatory plasticity between populations of *C. maenas* at seven sites across its native European and invasive North American biogeographic ranges. Given that the global range of *C. maenas* encompasses a wide range of environmental temperatures, the species may have potential for local adaptation despite high gene flow. By examining *C. maenas* in both its native and invasive ranges, we can ask whether there is any evidence for adaptation to temperature in the native range, and if so, how that adaptation has carried through to invasive populations. Further, we can compare sites within the invasive ranges to ask whether there is any evidence for rapid adaptation of thermal tolerance post-invasion. By examining tolerance and acclimatory plasticity in concert, we can explore the relationship between tolerance limits and plasticity at an intraspecific level and examine how these traits vary with the absolute temperatures and breadth of thermal ranges the different populations encounter. More generally, we provide insights into how adaptation to temperature may occur on a time scale relevant to global change.

RESULTS

Environmental temperature

Mean temperature at each collection site, measured using iButtons deployed during the experimental period, varied from 11.9°C at Newfoundland, the coldest site, to 24.2°C at New Jersey, the warmest site (Table 1). Long-term satellite data from offshore locations showed striking differences in thermal stability between regions, with the East Coast of North America (NA) sites (NL, ME, NJ; see Materials and methods) having much larger annual variability than the West Coast NA sites (BC, CA); the European sites (NO, PT) were intermediate in stability (Table 1; Fig. 1A). Annual mean temperatures varied from 6.5°C in Newfoundland to

Table 1. Site details for *C. maenas* collections

Site	Code	Size (mm)	% Red	Lat.	Long.	Date	Short-term temperature (°C)				Long-term temperature (°C)			
							Mean	s.d.	Max.	Min.	Mean	s.d.	Max.	Min.
Toquart Bay, BC, Canada	BC	75.5±6.9	45.5	49.0203	-125.3586	Aug. 2010	16.8	0.7	18.5	13.8	10.9	2.5	16.0	6.4
Seadrift Lagoon, CA, USA	CA	64.2±6.1	73.0	37.9079	-122.6726	Apr. 2011	16.8	1.2	20.2	14.5	12.7	1.2	17.1	10.0
North Harbour, NL, Canada	NL	61.1±4.9	53.2	47.8459	-54.0969	Sep. 2011	11.9	1.2	14.0	9.0	6.5	5.1	16.7	-0.6
Walpole, ME, USA	ME	59.7±5.3	35.4	43.9351	-69.5809	Aug. 2011	17.5	1.0	19.8	10.5	9.2	4.6	18.0	2.3
Tuckerton, NJ, USA	NJ	58.3±7.2	52.0	39.5095	-74.3248	Jul. 2011	24.2	2.1	32.4	19.5	12.5	7.3	25.1	0.6
Skitnepollen, Askøy, Norway	NO	63.2±5.9	22.6	60.5061	5.0226	Aug. 2012	16.1	0.9	17.8	14.0	9.8	3.4	17.9	4.0
Seixal, Portugal	PT	45.7±6.5	22.4	38.6455	-9.1036	Jul. 2012	15.0	0.7	17.1	13.8	17.8	2.0	22.0	14.2

Lat., latitude; Long., longitude; Size, mean carapace width of tested crabs ±s.d.; % Red, percentage of crabs in later intermolt (determined by a red integument color); Date, month and year of sampling.

Short-term temperatures are from iButtons deployed at collection sites during sampling. Long-term temperatures are from monthly AVHRR SST data from 1987 to 2011.

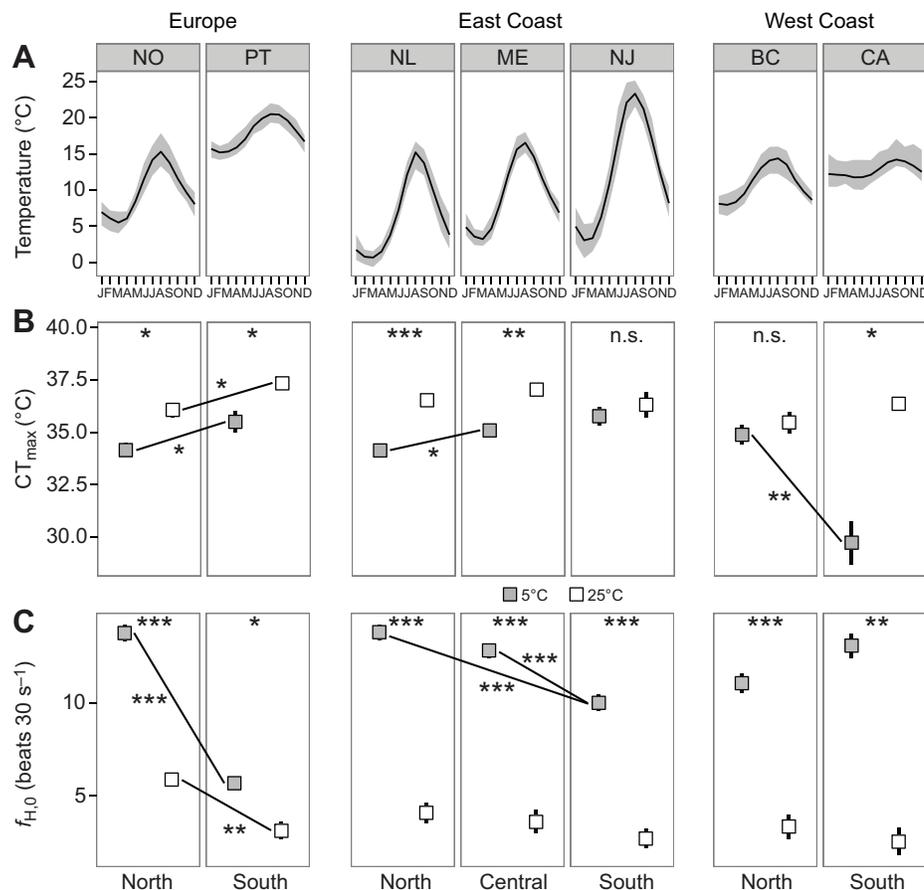


Fig. 1. Environmental temperature and thermal tolerance data for laboratory-acclimated *C. maenas* from seven sites across its native and invasive range. (A) Long-term sea surface temperature (SST) data near each site. Black line denotes mean; gray shadow indicates maximum and minimum values. Values are averaged monthly between 1987 and 2011. (B) Heat tolerance. (C) Cold tolerance. Within each site, asterisks indicate significant differences between animals acclimated to 5°C and those acclimated to 25°C. Within each region, lines indicate significant differences between animals from different sites after acclimation to a common temperature. CT_{max}, critical cardiac maximum temperature; f_{H,0}, heart rate at 0°C. **P*<0.05; ***P*<0.01; ****P*<0.001; n.s.=*P*≥0.05. Sites identified as in Fig. 5.

17.8°C in Portugal. Within all regions, mean temperatures were lower in the northern sites than in the southern sites.

All sites were sampled in summer with the exception of California, which was sampled in April. Preliminary data from animals collected at California in October 2010 were not significantly different for cold or heat tolerance than those collected in April 2011 (supplementary material Fig. S3). Because of this seasonal stability, both in temperature and in cardiac function, we feel that comparing the California data from April with the data collected in the summer months at other sites is valid.

High temperature tolerance

The mean high temperature at which heart function collapsed (CT_{max}) varied from 33.7 to 37.3°C across sites and acclimation

treatments, except in California where CT_{max} dropped to 29.7°C after 5°C acclimation (Table 2; Fig. 1B; Fig. 2A). One individual from British Columbia was an extreme outlier (CT_{max} 22.6°C), and was excluded from subsequent analysis. Within all sites except New Jersey and British Columbia, 25°C acclimation significantly increased CT_{max} relative to 5°C acclimation (Table 2; Fig. 1B). This acclimation-induced increase in CT_{max} was between 1.8 and 2.4°C for both European sites (both sites: *P*=0.01), Newfoundland (*P*=0.0006) and Maine (*P*=0.001), and was particularly extreme in California, which showed a 6.7°C difference (*P*=0.01) due largely to the low CT_{max} of cold-acclimated crabs. Because of this extreme decrease in tolerance in California, which may be due in part to an unnatural, closed environment causing a decrease in genetic diversity (C.K.T. and S. R. Palumbi, manuscript in preparation; see

Table 2. Thermal tolerance of *C. maenas* by site and acclimation treatment

Site	CT _{max} (°C)			<i>P</i>	f _{H,0} (beats 30 s ⁻¹)			<i>P</i>
	5°C	Field	25°C		5°C	Field	25°C	
NO	34.2±0.4 (8)	33.7±0.6 (12)	36.1±0.3 (10)	0.01	13.8±0.4 (10)	9.4±0.4 (12)	5.9±0.4 (10)	<0.001
PT	35.5±0.5 (12)	37.2±0.2 (11)	37.3±0.2 (11)	0.01	5.7±0.4 (12)	4.9±0.3 (10)	3.1±0.5 (11)	0.002
NL	34.1±0.3 (12)	34.4±0.5 (7)	36.5±0.2 (10)	0.001	13.9±0.4 (11)	12.5±0.5 (10)	4.2±0.6 (10)	<0.001
ME	35.1±0.3 (10)	35.6±0.4 (12)	37.0±0.0 (8)	0.001	12.9±0.4 (12)	5.5±0.9 (12)	3.6±0.6 (11)	<0.001
NJ	35.8±0.5 (12)	36.5±0.5 (6)	36.3±0.6 (7)	0.6	10.0±0.4 (11)	4.3±0.8 (6)	2.7±0.5 (8)	0.001
BC	34.9±0.5 (6)	35.0±0.7 (7)	35.5±0.5 (10)	0.4	11.1±0.5 (8)	6.8±2.1 (3)	3.3±0.7 (10)	0.001
CA	29.7±1.0 (6)	33.8±0.6 (9)	36.4±0.2 (5)	0.01	13.1±0.7 (5)	9.0±0.5 (6)	2.5±0.7 (5)	0.01

Data are given after laboratory acclimation to 5 or 25°C and after acclimatization in the field. Data are given as a group mean ± s.e.m.; numbers in parentheses indicate sample size for each group.

CT_{max}, critical thermal maximum; f_{H,0}, heart rate at 0°C.

P-values are comparison between 5°C- and 25°C-acclimated animals from each population after multiple test correction. Significant values (*P*<0.05) are indicated in bold.

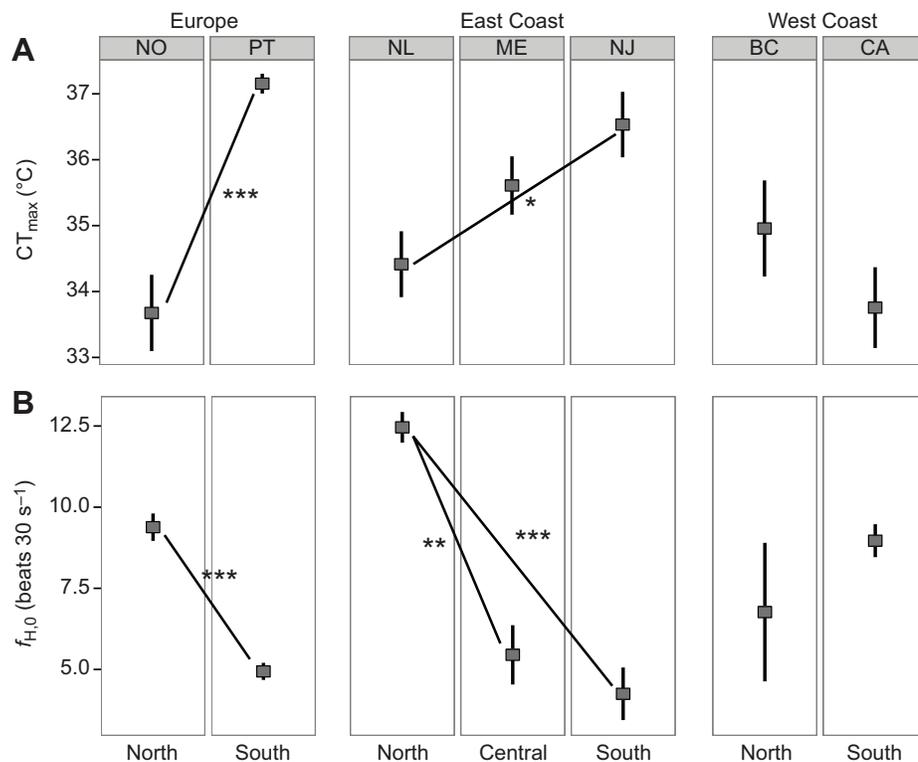


Fig. 2. Thermal tolerance data for field-acclimatized *C. maenas* from seven sites across its native and invasive range. (A) Heat tolerance. (B) Cold tolerance. Within each region, lines indicate significant differences ($P < 0.05$) between animals from different temperature sites. CT_{max}, critical cardiac maximum temperature; f_{H,0}, heart rate at 0°C. * $P < 0.05$; ** $P < 0.01$; * $P < 0.001$. Sites identified as in Fig. 5.**

below), we performed subsequent analyses both with and without California data. Heat tolerance of field-acclimatized animals ranged between 33.7°C (Norway) and 37.2°C (Portugal), and at all sites was not significantly outside the range of CT_{max} found for 5°C- and 25°C-acclimated animals (Table 2; Fig. 2).

After 5°C acclimation, southern sites had higher CT_{max} than did northern sites in Europe and East Coast NA (Fig. 1B; supplementary material Table S1). In Europe, Portugal had a CT_{max} that was 1.4°C higher than that in Norway ($P = 0.03$). In East Coast NA, CT_{max} in New Jersey was 1.6°C higher than in Newfoundland ($P = 0.05$); and in Maine it was 1.0°C higher than in Newfoundland ($P = 0.03$). Despite the higher magnitude of difference between New Jersey and Newfoundland, this was not significant after multiple test correction ($P = 0.05$), possibly due to the larger variance in the New Jersey data (s.e.m. ± 0.05) relative to the other two East Coast NA sites (s.e.m. ± 0.03). CT_{max} in Maine was not significantly different than that in New Jersey (0.7°C; $P > 0.05$). In West Coast NA, the pattern was reversed, due to the dramatically lower CT_{max} in California (29.7°C) relative to all other sites ($P = 0.008$).

After 25°C acclimation, CT_{max} increased significantly from north to south only in Europe (Fig. 1B; supplementary material Table S1), where it was 1.3°C higher in Portugal than in Norway ($P = 0.03$). There were no significant differences between any sites in North America after 25°C acclimation ($P > 0.05$). In field-acclimatized animals, CT_{max} increased from north to south by 3.5°C in Europe ($P < 0.001$) and by 2.1°C in East Coast NA (NL–NJ: $P = 0.04$; Fig. 2A). There were no significant differences in West Coast NA or between the central site (ME) and either the northern or southern East Coast NA sites in field-acclimatized animals ($P > 0.05$; supplementary material Table S1; Fig. 2A).

Cold temperature tolerance

Average heart rate at 0°C (f_{H,0}) varied from 2.5 to 13.9 beats 30 s⁻¹ across sites and acclimation treatments (Table 2; Fig. 1C). This measure was substantially and consistently plastic, with all sites

having significantly lower f_{H,0} after 25°C acclimation relative to 5°C acclimation (Table 2; Fig. 1C). This acclimation-induced difference ranged from 2.6 beats 30 s⁻¹ in Portugal ($P = 0.002$) to 10.6 beats 30 s⁻¹ in California ($P = 0.01$). Cold tolerance of field-acclimatized animals was within the range of f_{H,0} found for 5°C- and 25°C-acclimated animals at each site (Table 2).

In crabs acclimated to 5°C, f_{H,0} decreased from north to south by 8.1 beats 30 s⁻¹ in Europe ($P < 0.0001$) and by 3.9 beats 30 s⁻¹ between Newfoundland and New Jersey in East Coast NA ($P < 0.001$) (Fig. 1C; supplementary material Table S1). In East Coast NA, the central site in Maine had a f_{H,0} that was comparable to that of the northern site in Newfoundland (1.0 beats 30 s⁻¹; $P > 0.05$), and 2.8 beats 30 s⁻¹ higher than that of the southern site in New Jersey ($P = 0.001$). The difference in f_{H,0} between 5°C-acclimated crabs from the West Coast NA sites was not significant (2.0 beats 30 s⁻¹; $P > 0.05$).

After 25°C acclimation, there was a significant difference in cold tolerance between sites only in Europe, where Norway had a f_{H,0} 2.8 beats 30 s⁻¹ higher than that of Portugal ($P = 0.002$; Fig. 1C; supplementary material Table S1). In field-acclimatized animals, f_{H,0} decreased from north to south by 4.4 beats 30 s⁻¹ in Europe ($P < 0.001$) and by 7.0–8.2 beats 30 s⁻¹ between Newfoundland and the more southern sites of New Jersey and Maine in East Coast NA (NL–NJ: $P = 0.002$; NL–ME: $P < 0.001$; supplementary material Table S1; Fig. 2). There were no significant differences in West Coast NA or between Maine and New Jersey in East Coast NA for field-acclimatized animals ($P > 0.05$; supplementary material Table S1; Fig. 2).

Acclimatory plasticity

For heat tolerance, there was no significant relationship between acclimatory plasticity and maximum CT_{max} or mean sea surface temperature (SST), with or without the idiosyncratic site in California ($P > 0.05$; Fig. 3A,B). In contrast, acclimatory plasticity in cold tolerance significantly increased as maximum f_{H,0} increased

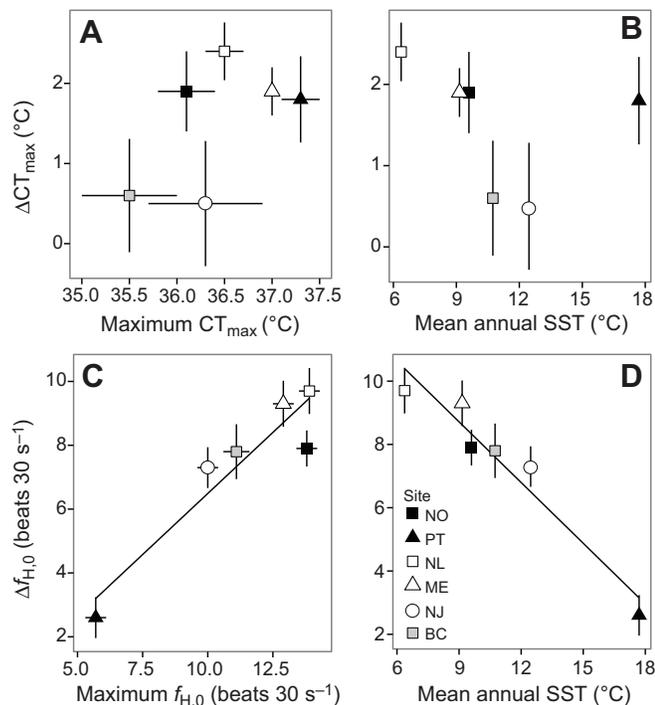


Fig. 3. Relationship between acclimatory plasticity and maximum tolerance or environmental temperature for all sites except CA.

Acclimatory plasticity is calculated as the difference in tolerance between 5°C- and 25°C-acclimated animals from a single site. (A) CT_{max} plasticity versus maximum CT_{max} ($P>0.05$); (B) CT_{max} plasticity versus mean annual SST ($P>0.05$); (C) $f_{H,0}$ plasticity versus maximum $f_{H,0}$ ($R^2=0.85$; $P=0.006$); and (D) $f_{H,0}$ plasticity versus mean annual SST ($R^2=0.92$; $P=0.002$).

($R^2=0.85$; $P=0.006$; Fig. 3C), and decreased as annual SST increased ($R^2=0.92$; $P=0.002$; Fig. 3D), excluding California. When California was included, the relationship between maximum $f_{H,0}$ and acclimatory plasticity in cold tolerance remained significant ($R^2=0.81$; $P=0.003$), but the relationship between maximum $f_{H,0}$ and mean SST did not ($R^2=0.49$; $P=0.05$).

DISCUSSION

Eurythermality

Temperature is likely of major importance in regulating the current and future distribution of *C. maenas* (Compton et al., 2010), and tolerances of both larval and adult stages may be critical in the species' biogeography. In North America, oceanic conditions during larval development have been correlated with recruitment strength (Behrens Yamada and Kosro, 2010). Laboratory trials suggest that green crab larvae have a narrower thermal tolerance than adults, and successfully developed only between 10 and 22.5°C (deRivera et al., 2007). While many abiotic factors doubtless impact green crab survival and recruitment at all life stages, our observations suggest that adults are highly eurythermal and this capacity may help govern the biogeographic range of the species.

Green crabs appear to show greater heat tolerance than other temperate species of crabs and lobsters with which they share their environment. We measured an average critical temperature of 34.5–36.5°C for green crabs, higher than the 30–35°C critical temperatures recorded for co-occurring species of crabs and lobsters studied with similar methods (Fig. 4; supplementary material Table S2). This fits with a growing body of research showing that invasive species are often more thermally tolerant than related non-

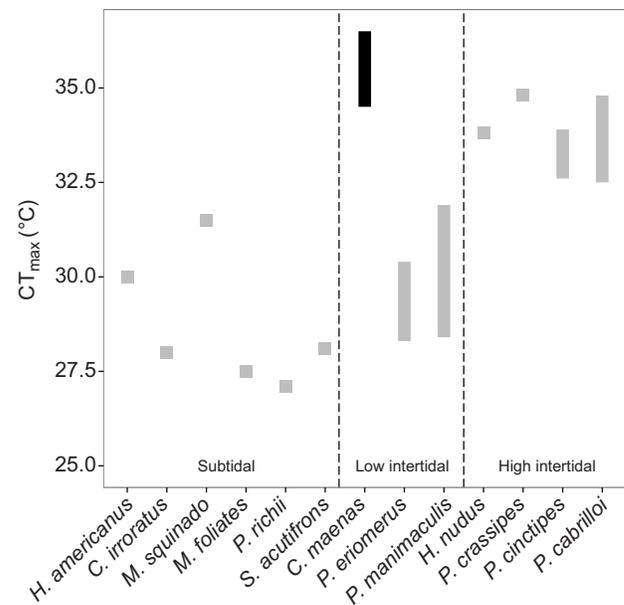


Fig. 4. Average CT_{max} values for *C. maenas* (black), and temperate marine crabs and lobsters with overlapping geographic distributions (gray). Data for comparison are published elsewhere (Frederich and Pörtner, 2000; Stillman, 2004; Camacho et al., 2006; Jost et al. 2012) (J. Thiemer and G.N.S., unpublished data). Additional information is given in supplementary material Table S2. Values are given as points where only one CT_{max} value was reported, or as bars if multiple CT_{max} values were reported for a single species after acclimation at multiple temperatures.

invasive species (Schneider, 2008; Sorte et al., 2010a; Lenz et al., 2011; Lockwood and Somero, 2011; Zerebecki and Sorte, 2011). A recent meta-analysis concluded that, in aquatic environments, invasive species performed significantly better at increased temperatures than did co-occurring native species (Sorte et al., 2013). Coupled with these results, our data suggest that green crabs may be more resilient to changing temperatures than their native counterparts.

One exception in our data is the heat tolerance for 5°C-acclimated California crabs, which showed a much lower mean CT_{max} (29.7°C) than recorded for *C. maenas* at any other site (34.1–34.8°C). This low value for California was consistent over two rounds of experimentation 6 months apart, so we are confident that it is not the result of experimental error (supplementary material Fig. S3). However, it contrasts with a value of 34.7°C found by a previous study for 6°C-acclimated animals collected 3 years previously from the same site (Kelley et al., 2011). While the methods of these two studies differ, this discrepancy suggests that the green crab population may not be stable over time at this specific site, an artificial lagoon with a recent history of heavy *C. maenas* removal, surrounded by suburban housing and potentially isolated from the surrounding environment (Ritter, 1970) (C.K.T., unpublished observation). In light of potential effects of the atypical environment experienced by the California population, species-wide comparisons were performed both with and without the California individuals.

In addition to high heat tolerance, green crabs also tolerate cold temperatures of 0°C or below. *Carcinus maenas* showed a linear decrease in heart rate with temperature between 15 and 0°C with no apparent breakpoint (supplementary material Fig. S2B), suggesting that the species' cold tolerance limit lies below 0°C. This is comparable to or lower than values recorded in all but one of six

species of porcelain crab, and that species (*P. cinctipes*) achieved this lower limit only after acclimation to 8°C (Stillman, 2004). Likewise, the spider crab *Maja squinado* had a lower critical temperature of -0.1°C (Frederich and Pörtner, 2000). In our experiments, all animals recovered after exposure to 0 to -1°C for 20–60 min even in the case of specimens acclimated to 25°C, suggesting that animals retain the ability to survive sub-zero temperatures regardless of acclimation temperature.

Our cold tolerance results are consistent with the recent range expansion of *C. maenas* in Newfoundland, north of the tolerance range that would be predicted based on environmental temperatures in its native range (Compton et al., 2010). Buoy data from Placentia Bay, 7 km away from our collection site in Newfoundland, show daily average water temperatures of <5°C for 4–5 months annually, with winter temperatures falling to a -1.3°C minimum over the period of January 2011 to June 2013 (Come By Chance Point buoy, data available at www.smartbay.ca). Our experiments, coupled with the thriving green crab population in the cold waters of Newfoundland (Fisheries and Oceans Canada, 2011), strongly suggest that *C. maenas* may have considerable scope for expanding its range into waters that approach the freezing point of seawater.

Acclimatory plasticity

We found that *C. maenas* not only is highly eurythermal in its responses to acute temperature changes but also demonstrates acclimatory plasticity across its native and much of its invasive North American range. Individuals showed an average 1.5°C increase in heat tolerance between 5 and 25°C acclimation when California was excluded (with CA: 2.3°C average; Table 2; Fig. 1B; Fig. 3). This is slightly less than the maximum acclimation ability in cardiac thermal tolerance seen for the low intertidal porcelain crabs *P. eriomerus* and *P. manimaculis* (2.1 and 3.5°C, respectively), which had the highest acclimation ability of six congeneric species studied (Stillman, 2004). Lobsters were also quite plastic, with a 3°C increase in heat tolerance between 4 and 20°C acclimation (Camacho et al., 2006). While *C. maenas* was less plastic than these species, it maintains a considerably higher constitutive heat tolerance (Fig. 4).

Within *C. maenas*, there was no relationship between the magnitude of CT_{max} plasticity and either environmental temperature or maximum CT_{max} (Fig. 3A,B). Previous work with congeneric species has shown contradictory relationships between thermal tolerance, environmental temperature and acclimatory plasticity. In porcelain crabs, Stillman found that tropical species had the highest heat tolerance, but also the least ability to acclimate to increases in temperature (Stillman, 2002). On a larger scale, this pattern of increased vulnerability at lower latitudes was demonstrated in a meta-analysis of 38 terrestrial insect species (Deutsch et al., 2008). In contrast, in congeneric terrestrial beetles, Calosi and colleagues found that species with the highest heat tolerance had the greatest capacity to acclimate, shifting these limits even higher (Calosi et al., 2008). Several hypotheses have been proposed to resolve this discrepancy, which may reflect overarching evolutionary differences between terrestrial and marine environments, or differences between the specific taxa studied (e.g. in degree of phylogenetic relatedness or evolutionary trajectory) (Bozinovic et al., 2011). While this question has yet to be resolved, the contrast between studies strongly suggests that the relationship between adaptation temperature and acclimation capacity is not simple, and that we should be cautious in generalizing results.

Cold tolerance was significantly plastic at all sites, with heart rate at 0°C being 1.8–5.2 times higher after 5°C acclimation than after 25°C acclimation (Fig. 1C). A similar effect of acclimation on low-

temperature heart rate has been reported in lobsters (Camacho et al., 2006), mussels of the genus *Mytilus* (Braby and Somero, 2006) and snails of the genus *Chlorostoma* (formerly the genus *Tegula*) (Stenseng et al., 2005). Studies in fishes also have shown that low-temperature acclimation results in a broad suite of cardiac changes including enhanced ability to sustain function at low temperatures (Jayasundara and Somero, 2013), increased heart mass (Vornanen et al., 2005), and up-regulation of genes involved in protein synthesis and transcriptional regulation (Gracey et al., 2004). In the porcelain crab *P. cinctipes*, a transcriptomic response was detectable after 12 h of cold acclimation, and involved up-regulation of genes involved in glucose production and transcriptional regulation (Ronges et al., 2012). Thus, these studies suggest a broad importance among diverse taxa for acclimatization of cardiac function in the face of temperature change. Through this capacity for acclimatization, behavioral capacities may be conserved at low temperatures. Although we did not test this explicitly, we noted in this study that 5°C-acclimated animals regained normal movement after cold stress much more quickly than their 25°C-acclimated counterparts. In places like Newfoundland, where average winter water temperatures remain below 4°C for upwards of 4 months (Fig. 1A), the ability to increase heart rate and potential activity even marginally may be critical to survival.

In contrast to heat tolerance, we observed a significant negative relationship between the magnitude of cold tolerance plasticity and both environmental temperature and maximum $f_{H,0}$ (excluding CA; Fig. 3C,D). However, this may simply reflect the fact that we observed a much greater difference in $f_{H,0}$ among populations after 5°C acclimation (8.2 beats 30 s⁻¹) than after 25°C acclimation (3.4 beats 30 s⁻¹). Thus, the width of the acclimatory window is being driven largely by $f_{H,0}$ after 5°C acclimation, the same value used as maximum $f_{H,0}$. Several studies of congeneric insect species have found no relationship between cold tolerance and plasticity (Calosi et al., 2008; Strachan et al., 2011), although a meta-analysis of 15 arthropod species showed a correlation between increased plasticity and lower lethal temperature (Chown et al., 2008).

Adaptive divergence

Our study surveyed *C. maenas* across latitudinal gradients in two native range populations and five invasive populations founded between ~20 and ~200 years ago (Carlton and Cohen, 2003). We observed a strong, consistent relationship between heat tolerance and latitude in the European native range and a weaker pattern in the invasive range along the East Coast of North America. Because the experiment contrasts physiology after acclimation to common conditions, these differences suggest that *C. maenas* populations, especially in Europe, may be locally adapted. After acclimation to common temperatures, crabs from Portugal performed better in the heat than their Norwegian counterparts. This pattern also held true in the invasive range along the East Coast of North America, where the species has been established for only ~200 years. On the East Coast, however, this pattern may be an artifact of a complicated invasion history and not necessarily a newly evolved physiological cline (e.g. Balanyá et al., 2006). The southern East Coast population of *C. maenas* was founded in the early 1800s by a source from southern or central Europe (Darling et al., 2008). In contrast, the northern East Coast population was founded by a second, more recent introduction from northern Europe (Roman, 2006), perhaps introducing more cold-tolerant individuals into the East Coast and allowing the species to spread into colder northern waters. Subsequent research has shown extensive mixing between these introductions, with a rapidly moving introgression zone (Pringle et al., 2011).

There is a growing appreciation of the importance of intraspecific variation in thermal physiology to a species' ability to survive and thrive in novel conditions (Somero, 2010; Sanford and Kelly, 2011). Intraspecific variation may derive from adaptive genetic differences between populations (Pespeni et al., 2012), or from developmental plasticity, whereby the environmental conditions experienced early in an animal's development (or even in its mother's lifetime) can have a life-long, non-heritable impact on its physiology (West-Eberhard, 2003). In a study such as this, without multi-generational common-garden rearing, it is not possible to disentangle adaptation and developmental plasticity. However, integrating our results with other demographic, physiological and genetic data for this species suggests a role for both processes. In the East Coast of North America, the Maine and New Jersey populations are genetically similar to each other, while the Newfoundland population is genetically distinct due to an influx of novel genetic diversity from northern Europe (C.K.T. and S. R. Palumbi, manuscript in preparation) (Roman, 2006; Darling et al. 2008). CT_{max} differed between populations with different genetic backgrounds, and thus may reflect adaptive evolution. In contrast, our measure of cold tolerance appears to be more strongly related to environmental temperature than to genetic background, and thus may reflect long-term plasticity in response to environmental cues as well as underlying adaptive differences.

Adaptive variation among populations may enhance a species' survival, if poorly adapted populations can be replaced by recruits from populations already adapted to the new conditions (Sanford and Kelly, 2011). Conversely, in species with very limited dispersal, locally adapted populations may simply go extinct if they lack the capacity to evolve in response to new conditions (Atkins and Travis, 2010; Sanford and Kelly, 2011; Kelly et al., 2012). Several studies have found putatively adaptive differences in thermal physiology between populations of species with limited dispersal (Grosholz, 2001; Kuo and Sanford, 2009; Sorte et al., 2011) and even in certain widely dispersing species, e.g. the mussel *Mytilus californianus* (Rao, 1953; Logan et al., 2012). Intraspecific differences in both thermal tolerance and acclimatory plasticity have recently been demonstrated for the copepod *Tigriopus californicus* on the West Coast and three species of the snail *Littorina* on the East Coast of North America (Sorte et al., 2011; Kelly et al., 2012). However, these examples come from species studied in their native ranges, where they have had many generations to adapt to their environments. In our study, we see evidence of local adaptation in another widely dispersing marine species in its native range. Outside of its native range, the evidence for local adaptation is much less convincing, and variation between sites founded ~20–200 years ago

may derive largely from acclimatory plasticity (cold tolerance) and multiple founding events derived from differently adapted source populations (heat tolerance).

Conclusions and implications

The European green crab may owe its success in large part to its exceptionally flexible thermal physiology: the species can tolerate temperatures far in excess of those it experiences in the field, and it has the ability to shift its thermal limits further via acclimatory plasticity. Furthermore, *C. maenas* maintains this broad tolerance and plasticity even after a significant loss of neutral genetic diversity (Darling et al., 2008; Tepolt et al., 2009). Indeed, we observed that *C. maenas* is substantially more heat tolerant than co-occurring native crustaceans, and suggest that high and flexible tolerance may be hallmarks of success in a warming ocean.

In addition, *C. maenas* shows signs of having adapted over the long term to its local environment. Local adaptation in the native range in Europe may have contributed to the green crab's explosive expansion throughout the northern East Coast of North America. Our results support the hypothesis that secondary invasion from a northern source may have facilitated *C. maenas*' northward spread in the East Coast by introducing cold-adapted genotypes (Roman, 2006; Blakeslee et al., 2010). In a broader context, this example illustrates the potential importance of locally adapted populations in allowing a species to survive novel conditions. All populations are not necessarily physiologically equal, even in widely dispersing marine species, and population-level variation may be crucial to survival in a changing environment.

MATERIALS AND METHODS

Animal collection

Green crabs were collected from seven sites: Toquart Bay, BC, Canada, and Seadrift Lagoon, CA, USA, on the West Coast of North America; North Harbour, NL, Canada, Walpole, ME, USA, and Tuckerton, NJ, USA, on the East Coast of North America; and Skitnepollen, Askøy, Norway, and Seixal, Portugal, in Europe (Fig. 5; Table 1). Crabs were collected during the summer months (July–September) with the exception of California, which was sampled in April. Additional sampling of California, used to examine seasonal stability of tolerance at this site, took place in October.

At each site, animals were collected by trapping from shore in 0.5–4 m of water. Crabs selected for experiments were between 50 and 70 mm carapace width, except for in British Columbia and Portugal, where the majority of crabs selected were slightly larger (BC; 75.5±6.9 mm) or smaller (PT; 45.7±6.5 mm) (Table 1), respectively. At each site, both males and non-ovigerous females were selected. In *C. maenas*, integument color is an indication of an individual's point in the molt cycle, with green individuals being early in intermolt and red individuals being later in intermolt (McGaw

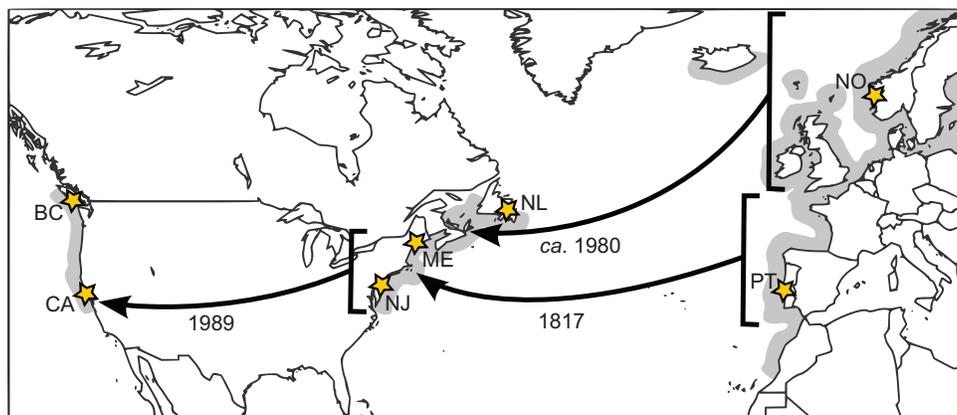


Fig. 5. Map showing collection locations and invasion history of *C. maenas*.

Locations are indicated by stars and adjacent codes. Current range is shown in gray. Approximate source populations are shown in brackets, with arrows leading to initial invasion sites. Dates indicate the first record of each invasion. Site codes as in Table 1.

et al., 1992). Crabs selected for these experiments spanned a range of integument colors. The proportion of later-intermolt individuals in a sample was dictated in part by the makeup of the local population, and varied from 22.4% in Portugal to 73.0% in California.

Environmental temperature

Environmental temperature data were collected at each site with two to four iButtons (DS1921G-F5#, Maxim Integrated, San Jose, CA, USA) deployed in fixed positions below the low-tide line, logging temperature every 15 min for 6–19 days preceding collection of field-acclimatized animals. Because *C. maenas* lives almost exclusively in shallow subtidal habitats, water temperature data provide a valid measure of body temperature (McGaw, 2003; Worden et al., 2006).

Long-term temperature data were also collated for each site, using SST analyses created by the NOAA/NWS National Centers for Environmental Prediction, based on AVHRR satellite data and buoy observations (Reynolds et al., 2007). These data comprise monthly offshore SST averages for the 1×1 deg gridboxes closest to study sites. Mean, s.d., maximum and minimum temperatures were calculated for each month and overall for the 25 years between 1987 and 2011. Though these data are collected largely offshore of our study sites, they provide long-term comparative information about temperature means and variances.

Acclimation

At each site, crabs were acclimated to 25°C for 18–27 days ($N=24-32$) or to 5°C for 25–31 days ($N=28-32$) prior to experimentation. These acclimation temperatures were chosen to span a significant proportion of the temperatures experienced by the species in its global range (Compton et al., 2010).

The same portable acclimation tanks were used at all sites, and constructed from 49 l coolers (Igloo Products Corp., Katy, TX, USA) fitted with 50 W titanium aquarium heaters with built-in temperature controllers (ViaAqua, Commodity Axis Inc., Camarillo, CA, USA; 25°C tanks), or three thermoelectric aquarium chillers (CoolWorks IceProbe, Nova Tec Products, San Rafael, CA, USA) run by an outside temperature controller (Newport Corporation, Irvine, CA, USA; 5°C tanks). Two iButtons (wrapped in Parafilm) were placed in each tank for the duration of the acclimation period to verify temperature. All tanks were aerated, filtered (Marina i25 Internal Filters, Hagen Inc., Montreal, Quebec, Canada), and kept on a 12 h:12 h light:dark cycle with light from 08:00 h to 20:00 h local time. Tank filters were replaced once per week, and 60% of tank water was exchanged every 3 days except for British Columbia, where 20% of tank water was exchanged every day. Artificial seawater was made up from distilled water and InstantOcean aquarium salt (Spectrum Brands, Madison, WI, USA) to 32 ppt salinity. Crabs were fed daily to satiety with Crab & Lobster Bites commercial crab food (HBH Pet Products, Springville, UT, USA).

Thermal tolerance testing

Heat tolerance was indexed by the temperature at which heart function was impaired during a heating protocol. Cold tolerance was indexed by heart rate after a cooling protocol. After acclimation, crabs were subjected to a high or low temperature ramp. Field-acclimatized crabs were also collected and tested within 1 week of collection. All ramps started with a 1 h hold at 15°C to minimize post-handling stress, during which the heart rate settled to a steady rate within approximately 5 min. For high temperature tolerance, after the initial hold at 15°C, tank temperature was increased by 5°C h⁻¹ until all crabs' hearts had stopped beating (35–43°C), which was lethal for the animal. For low temperature tolerance, temperature was decreased by 5°C h⁻¹ to 0 or -1°C, ending with a 20–60 min hold. (Some ramp rates were slightly slower when approaching 0°C.) Low temperature tolerance could not be tested to lethality: in preliminary testing, crabs survived exposure at -1.8°C for 5 h, and tank temperature could not be decreased below -1.8°C without extensive ice formation.

Temperature ramps were carried out in a 16 l Igloo cooler fitted with four chillers and one aquarium heater controlled by a temperature controller, and with an aerator to oxygenate and circulate the water. Each tank contained 12 l of artificial seawater (for laboratory-acclimated crabs) or local seawater (for field-acclimatized crabs). Throughout each ramp, tank temperature was recorded every minute via the temperature controller's thermocouple. Two

iButtons wrapped in Parafilm were included in each ramp to verify the temperature record.

Measuring heart rate

Cardiac activity was monitored continuously and non-invasively via photoplethysmography (Depledge, 1984). Infrared transducers were attached to each crab's carapace at a position directly over the heart, using quick-drying epoxy adhesive. Crabs were suspended by the transducer wires into the ramp tank and immersed 2–4 cm below the surface of the water. One to four crabs were tested simultaneously. Heart rate (as a voltage signal) was recorded in LabView SignalExpress 2009 (National Instruments Corporation, Austin, TX, USA). To ensure a clear signal, the voltage signal was initially recorded at 1000 Hz, filtered to remove high frequency noise due to environmental electrical interference (>10 Hz), and resampled at 50 Hz before being exported to a text file (supplementary material Fig. S1). For each heart rate record, heartbeats were counted for the first 30 s interval of each minute (beats 30 s⁻¹). Counting was carried out with a fast Fourier transform implemented in Python, supplemented by hand-counting for intervals with irregular or very low heart rates. Analyses started with the last 15°C point before the temperature rose or fell.

For high temperature tolerance, segmented linear regression implemented in JoinPoint v3.5.2 (Kim et al., 2000) was used to determine cardiac critical temperature (CT_{max}), defined as the point at which a record of beats 30 s⁻¹ versus temperature made an abrupt, irreversible drop (supplementary material Fig. S2A). We used average heart rate at 0°C ($f_{H,0}$), measured by beats 30 s⁻¹, as our cold tolerance index (supplementary material Fig. S2B).

Statistical analysis

To examine the impacts of acclimation, within-site differences were compared between 5°C- and 25°C-acclimated crabs using Mann–Whitney *U*-tests and a Benjamini–Hochberg multiple test correction (Benjamini and Hochberg, 1995). To examine differences in physiology due to adaptation or prolonged acclimatization prior to collection, sites within the same region were compared after the same acclimation treatment using Mann–Whitney *U*-tests (two sites: Europe, West Coast NA), or Kruskal–Wallis tests with *post hoc* pairwise Mann–Whitney *U*-tests and a Benjamini–Hochberg multiple test correction in the event of significance (more than two sites: East Coast NA).

We used the difference in thermal tolerance between 5°C- and 25°C-acclimated animals from the same site to represent that population's short-term acclimatory plasticity. To examine the relationship between plasticity and thermal limits, acclimatory plasticity at each site (excluding California; see Results) was plotted against maximum tolerance (tolerance after 25°C acclimation for CT_{max} or after 5°C acclimation for $f_{H,0}$) and mean annual SST. The relationship between these values was assessed using linear regression.

All statistical analysis was carried out in R (R Development Core Team, 2012), and plotted using the R package ggplot2 (Wickham, 2009).

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Competing interests

The authors declare no competing financial interests.

Author contributions

C.K.T. conceived and was involved in designing this study, carried out the experiments, analyzed the data, and prepared this manuscript. G.N.S. was involved in designing this study, analyzing the data, and co-authoring this manuscript.

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Supplementary material

Supplementary material available online at

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