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Latitudinal differences in thermoregulatory color change in *Uca pugilator*

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ABSTRACT

Animals can change color either rapidly in response to changes in their immediate environment or slowly as the seasons change. Such plasticity can permit local adaptation but it can also be constrained by physiological and behavioral mechanisms. Here, we explore how different temperature regimes along a latitudinal gradient spanning the natural range of a species of fiddler crab may affect the ability of crabs to change color rapidly in response to acute changes of temperature. *Uca pugilator* populations from New York (NY), North Carolina (NC) and Florida (FL) were exposed in air to 5 °C and 35 °C and carapace shade intensity (from dark to light color) and contrast was recorded. In general, individuals darkened when exposed to cold conditions, and lightened when exposed to warm conditions. Males from different populations differed in the magnitude of response to temperature, but had similar directional changes in shade (dark under cold conditions and light under warm conditions) suggesting differences among populations in the ability to change color. Females showed similar directional changes but there were no differences among populations. Differential responses of crabs to temperature suggest that crabs have local physiological adaptations and can acclimate quickly to withstand fluctuating or extreme temperatures.

1. Introduction

An organism's coloration is a conspicuous and widely studied trait. Color patterns have been associated with nutrient uptake, thermoregulation, camouflage, communication and species recognition (Bohlin et al., 2008; Cummings et al., 2008; Detto et al., 2008; Kingsolver, 1987). Variation in color among populations has been attributed to local adaptation affected by several mechanisms of natural selection (e.g. Rosenblum, 2005; Stuart-Fox et al., 2004). As species expand their range, they may encounter habitats with different environmental conditions. Natural selection on color variants may lead to regional differentiation in fixed color patterns, but many organisms can also respond to environmental change with plastic color response, such as the rearrangement of chromatophores (e.g. Stegen et al., 2004; Vestheim and Kaartvedt, 2006). Phenotypic plasticity is often responsible for the adaptability of individuals to novel environments (Via and Lande, 1985). Here, we show that an intertidal crab changes color rapidly and predictably in response to changes in temperature but that this ability differs along a latitudinal gradient, and we discuss potential mechanisms and constraints that affect local populations.

Many organisms change color in response to changing temperatures because different color intensities can affect thermal budgets (Angilletta, 2009). Yet, color patterns can vary with latitude (Lacey et al., 2010; Martin et al., 2010) and altitude (Karl et al., 2009; Parkash et al., 2009). The ability to change color and its potential variation across latitudes can be just as important as plastic responses to changes in local conditions. In a latitudinal and seasonal context we could expect greater plasticity where local thermal change is more variable. However, activation of chromatophores can be metabolically costly (e.g., Ibanez and Lindstrom, 1962; Petty and Jackson, 1979) and crabs are not the exception (Green, 1964). This cost creates a tradeoff between changing color to thermoregulate and camouflage in fiddler crabs (Kronstadt et al., in review) and there could be other tradeoffs as well. The net cost may differ between sexes due to different behaviors, morphologies or energy budgets involved in mating, foraging and other activities. The existence of such a cost suggests that natural selection will act to maximize color change in response to behavioral or physiological components. Because carapace color influences the absorption of solar radiation, and thus body temperature (Darnell et al., in review; Kronstadt et al., in review; Wilkens and Fingerman, 1965), one might expect daily and seasonal color changes in response to the thermal regime, and reflecting a rapid acclimation ability. Endogenous rhythms affect body color in fiddler crabs (Fingerman et al., 1958), yet fiddler crabs show strong responses to temperature regimes and these responses differ between sexes (Silbiger and Munguia, 2008), in part due to sexually dimorphic traits (Darnell and Munguia, 2011) and behavioral responses (Darnell et al., in review).

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Uca pugilator responds to changes in temperature by changing the color of its carapace (Silbiger and Munguia, 2008). Males and females have different changes in coloration throughout the diel cycle as well as in response to short-term (15 min) temperature changes. Decreases in temperature make both sexes dark and monochromatic. However, females are more sensitive to diel changes while males are more sensitive to increases in temperature. These color changes occur quite rapidly, within 5 min, suggesting a direct response of the chromatophores (Green, 1964) rather than an indirect, hormone-mediated response (Fingerman et al., 1958; Thurman, 1988).

Given the spatially variable microhabitat conditions (e.g., at the scale of meters within the intertidal) and their complex reproductive strategies driven by sexual selection (Christy, 1995; Christy and Salmon, 1984), fiddler crabs are a good taxon for studying latitudinal-scale variability in plastic responses to temperature change within a species. Vernberg and Tashian (1959) tested the thermal limits of six fiddler crab species occurring in temperate and tropical zones of North America. Results suggested that tropical species were less tolerant of cold conditions than were temperate species, but tropical species survived hot conditions longer relative to temperate species. Finally, the ability of northern species to acclimate to lower temperatures was highly variable whereas tropical species were more consistent; prompting the authors to suggest that northward expansion of tropical fiddler crabs was limited by their ability to acclimate to low temperatures (Vernberg and Tashian, 1959). Therefore, adjustments of color should provide an important adaptive mechanism to deal with thermal adaptations to different latitudes.

In a given locality, *U. pugilator* experiences wide thermal variation from hot sunny upper intertidal sands to shaded, wetter lower intertidal marsh habitats (Allen, 2007). *U. pugilator* is a broadly distributed intertidal crab occurring from Massachusetts to the Gulf of Mexico. These habitats show wide environmental variations, both in diel temperature regimes, temperature gradients from the surface to the bottoms of burrows, and percent cover by incoming tidal seawater. Here, we examine how three populations of *U. pugilator* differ in their ability to change color in response to temperature change over a broad latitudinal gradient. We were particularly interested in how temperature affects crabs that are actively foraging and searching for mates, both of which occur in air during low tide. We compared males and females in their ability to change color (i.e., carapace shade and contrast) in two different air temperature regimes, a cold treatment and a hot treatment. If crabs from three thermally disparate populations respond to temperature changes by adjusting their carapace patterns to a similar shade or texture state, this would suggest that there has been no local adaptation to this response mechanism. Alternatively, a difference among populations in color change could be a result of local adaptation because populations are subjected to different temperature ranges. The ability for crabs to recover to their pre-treatment shade state is a measure of the cost of color change (or the benefit of the pre-treatment color) and the degree of plasticity in color change. If populations return to their original states at the same rate, this would indicate that there are no differences in selective pressure to return to the original state (whether physiological or environmental). However, different environmental conditions could be present in these localities, and we discuss the potential sources behind variations in color change among populations.

2. Methods

2.1. Study sites

We collected *U. pugilator* from West Meadow Creek in Stony Brook, New York (40° 55′ N, 73° 08′W), Pine Knoll Shores, North Carolina (34° 42′ N, 76° 49′ W), and from Wakulla Beach, Florida (30° 06′ N, 84°15′ W), on the northern Gulf of Mexico. All crabs used in the experiment had not recently molted. The mean daily air temperatures in August are 29 °C, 27 °C, and 31 °C for the NY, NC, and FL sites, respectively. However the minimum and maximum yearly air temperatures range from, −3 °C to 30 °C, −1 °C to 30 °C, and −1 °C to 37 °C in NY, NC and FL, respectively. During the two weeks prior to collecting and working with crabs, the mean temperature and its coefficient of variation (CoV) obtained from nearby NOAA weather stations were 26.5 °C (CoV = 20%), 26.4 °C (CoV = 16%), and 22.5 °C (CoV = 21%) for FL, NC, and NY, respectively.

2.2. Experimental design

In all three locations crabs were subjected to the same experimental setup. In 2008 populations from New York and North Carolina were studied and compared to the same experiment conducted in 2006 in Florida. These studies were carried out in July, therefore during the breeding period for all three populations. Crabs were collected from the field and maintained in tanks with seawater and sand in the lab for 24 h at about 22 °C. Crabs were kept briefly in holding cups in air at room temperature and then were transferred to experimental cups, with crabs still in air, that were immersed in a water bath at one of three experimental temperatures (see Appendix 1 for details). The dorsal side of individual crabs was photographed at each of three stages: before, after 15 min exposure to hot (~35 °C), room temperature (~22 °C) or cold (~5 °C), and after a 15 min recovery period following exposure to test temperatures. Crab body temperature is not as extreme as water temperatures, in a pilot study hot treatment crabs were 12% cooler and cold treatment crabs were 15% warmer when using an internal thermocouple to measure body temperature (Munguia and Darnell, unpubl. data). Temperature-induced color change is complete after a 15 min exposure period (Kronstadt et al., in review). Crab carapaces were wiped dry to minimize specular reflection, and then photographed against a white background next to a ruler for scale. Photographs had a resolution of 2592 × 1944 pixels. We conducted the experiment with 30 individuals of each sex, for each of the 3 temperature treatments (total of 1440 photographs). We did not obtain recovery data for the Florida population, thus we only present the effects of treatment for this population. Fidlder crabs can change color due to circadian or circatidal rhythms (e.g., Fingerman et al., 1958; Palmer, 1995; Silbiger and Munguia, 2008) and therefore all experiments were conducted during daylight hours.

2.3. Estimation of color change

We use two approaches to evaluate color change in fiddler crab carapaces as a function of different temperatures. First, we quantified carapace shade across treatments and sexes to distinguish between shades (e.g., dark to light). Second, we used contrast (Haralick et al., 1973) as a quantitative measurement that provides information on body color pattern, independent of shade. Contrast is a relatively standard metric used in image analysis (e.g., Haralick et al., 1973; Hassanien and Ali, 2006; Puissant et al., 2005) and refers to the distribution of pixels that can produce either a speckled or monochrome pattern. The measures of shade and contrast are complementary, since previous observations have shown that carapaces can become speckled or monochrome in contrast while not changing in shade in response to temperature (Darnell et al., in review; Kronstadt et al., in review; Silbiger and Munguia, 2008).

The photographs obtained in the experiment were analyzed with ImageJ software (National Institutes of Health). The dorsal area of the carapace was transformed into an 8 bit grayscale image. From each individual's photograph a histogram of 256 pixel values (ranging from 0 = black, 255 = white) was extracted from a rectangle with corners at the carapace edges ensuring the largest area for pixel sampling. A gamma distribution was fitted to the pixel distribution,

$$f(x) = \left(1/m^{\gamma}\right)\left(x^{\gamma-1}e^{-x/m}\right)/(\Gamma(\gamma)) \quad (1)$$

where Γ is the gamma function, γ is the shape parameter, and m is the scale parameter, whose reciprocal is proportional to the maximum value of the frequencies (x), incorporated to ensure that it is a valid probability distribution and sums to one. Each photograph's pixel intensity distribution was fit by maximum likelihood estimates to a gamma distribution and thus we could extract estimates of γ for each individual. The gamma parameter was then used to describe a particular crab's shade and differences were assessed using parametric statistics. Low values of γ indicate dark shades and high values indicate light shades. Gamma parameters were natural-log transformed to normalize values and meet parametric assumptions.

Contrast was determined by quantifying the distribution of pixel intensities extracted from the individual crab's carapace photograph. An image was transformed into a matrix of pixel values from which different descriptive metrics can be obtained. Contrast, C, shows the number of local variations between high and low pixel values within the matrix,

$$C = \sum_{n=0}^{N_{q-1}} n^2 \left(\sum_{\substack{i=1 \\ |i-j|=n}}^{N_q} \sum_{j=1}^{N_q} p(i,j) \right) \quad (2)$$

where $p(i,j)$ is the (i,j)th entry in a normalized gray-tone value within a symmetrical matrix (with i rows and j columns), and N_q is the number of distinct gray levels in the image. As images show greater transitions in grayscale, C values increase, reflecting an increase in pixel heterogeneity. Contrast values are scaled to the area of the carapace (i.e. the total number of pixels in an image). These values can then be analyzed with parametric statistics to determine differences among sampled groups (see below).

These two metrics have the potential to measure distinct biologically relevant responses. First, γ values show how individuals respond to temperature (Silbiger and Munguia, 2008) by becoming lighter or darker in shade. C values will indicate how the texture of a carapace is affected through different pigment migratory patterns within chromatophores. The measure of contrast, C, can be indicative of the unique fingerprint of each individual. If C remains constant as crabs are subjected to changes in temperature, it suggests that animals may retain the same texture as a response to processes not associated to temperature, but which could be strongly related to other functions such as communication or camouflage (e.g., Cummings et al., 2008; Detto et al., 2006). While we found no consistent correlations between shade and contrast in our data, such interactions might present tradeoffs between responses to temperature and other factors.

2.4. Data analysis

We were interested in three questions. First, are there differences in shade and contrast among the three populations with no experimental exposure to temperature change? An ANOVA tested whether there were natural differences in γ and C before experimentation among the three populations. Second, are there population differences in the ability to change shade and texture when crabs are exposed to increased and decreased temperatures? We calculated the difference between before and after temperature exposure γ and C values standardized by the change in temperature in each experimental setup and performed a 3-way ANOVA testing for differences between sexes, treatments (cold or hot) and sites. Because of differences between sexes, including strong 2 and 3-way interactions, data were further split by sex and analyzed with a 2-way ANOVA for each sex. Differences between treatments would show crab sensitivity to particular changes in temperature. Third, do populations have similar recovery levels in color after 15 min? We used the difference in γ-values between 15 min after individuals had been removed from the temperature treatments and initial values for the New York and North Carolina populations; recovery data was not available for the Florida population. These data were analyzed with a 3-way ANOVA keeping site, treatment and sex as fixed factors. Analyses of shade and contrast were performed using MATLAB (Mathworks Inc., Natick, MA) and statistical analyses were performed with JMP (SAS Institute, Inc., Cary, NC).

3. Results

Crab carapace area differed among the three populations ($F_{5,449} = 303.75$, $P<0.001$), these differences were driven by site ($P<0.001$), sex ($P<0.001$) and the interaction between site and sex ($P=0.009$). The largest crabs were from the New York population, but males (mean area $\pm$ SE $= 2.53 \pm 0.03$ cm^2) and females (2.43 ± 0.03 cm^2) did not differ in size at any site. Florida (1.77 ± 0.05 cm^2) and North Carolina (1.49 ± 0.03 cm^2) males had intermediate sizes, followed by Florida females (1.46 ± 0.05 cm^2) and North Carolina females (1.26 ± 0.03 cm^2).

The γ-values (i.e. shade) of crabs at the time just before being exposed to experimental treatments were lowest in North Carolina crabs indicating that these crabs were the darkest ($F_{2,468} = 18.13$, $P<0.0001$) (Fig. 1). Populations also showed differences in contrast ($F_{2,468} = 108.28$, $P<0.0001$), where Florida was highest ($C = 53.69 \pm 3.2$), followed by New York ($C = 25.36 \pm 2.23$) and the lowest values were found for North Carolina crabs ($C = 6.85 \pm 2.33$).

Crabs from all localities showed similar changes in γ-values, becoming darker in the cold treatment and lighter in the hot treatment (3-way ANOVA, $F_{17,497} = 77.39$, $P<0.0001$; Table 1), but there were some interaction effects. Females became dark in cold and light in warm temperatures (2-way ANOVA, $F_{8,251} = 81.55$, $P<0.0001$) with small differences between populations (Fig. 2, top). But for males the three populations were displaced in their response to cold or hot treatments (2-way ANOVA, $F_{8,246} = 82.31$, $P<0.0001$), although the change of response from cold to warm temperature was similar (Table 1, Fig. 2, top).

Fiddler crab populations differed in changes in contrast values when individuals were exposed to different temperatures (3-way ANOVA, $F_{17,492} = 3.45$, $P<0.0001$; Table 2). Females showed strong treatment and site-by-treatment effects (2-way ANOVA, $F_{8,244} = 4.31$, $P<0.0001$; Table 2). Under cold temperatures, females from Florida became slightly more speckled and females from New York became slightly more monochromatic, with North Carolina maintaining their original contrast values (Fig. 2, bottom), however there were no statistical differences between the three populations (Table 2). Under hot temperatures, Florida females were more speckled with New York and North Carolina populations maintaining their contrast values with small differences between them (Fig. 2, bottom). Male change in contrast was not affected by treatments and a site by treatment interaction (2-way ANOVA, $F_{8,248} = 1.9$, $P = 0.06$; Table 2). In the cold treatment, males had small changes in contrast and all three populations were similar, with Florida males being slightly more speckled (Fig. 2, bottom). In the hot treatment, Florida and North Carolina males became slightly more monochromatic, while New York males were slightly more speckled, but the only differences were between New York and Florida males (Fig. 2, bottom). Florida crabs showed the largest range in plasticity of contrast between cold and hot treatments, whereas North Carolina and New York individuals showed smaller variation between treatments and sexes.

There were shade recovery differences between males and females. In the cold and hot treatments, females from both New York and North Carolina populations returned to original shade conditions after 15 min (Fig. 3, top), but males did not recover to the original shade. There were strong differences in recovery between treatments, sites and sexes, and an interaction between treatments and sites (3-way ANOVA, $F_{11,351} = 18.03$, $P<0.0001$; Table 3) suggesting that fiddler crab populations have specific temperature responses which are sex dependent. Crabs under normal temperatures did not return to the original gamma state as they were slowly responding to stress.

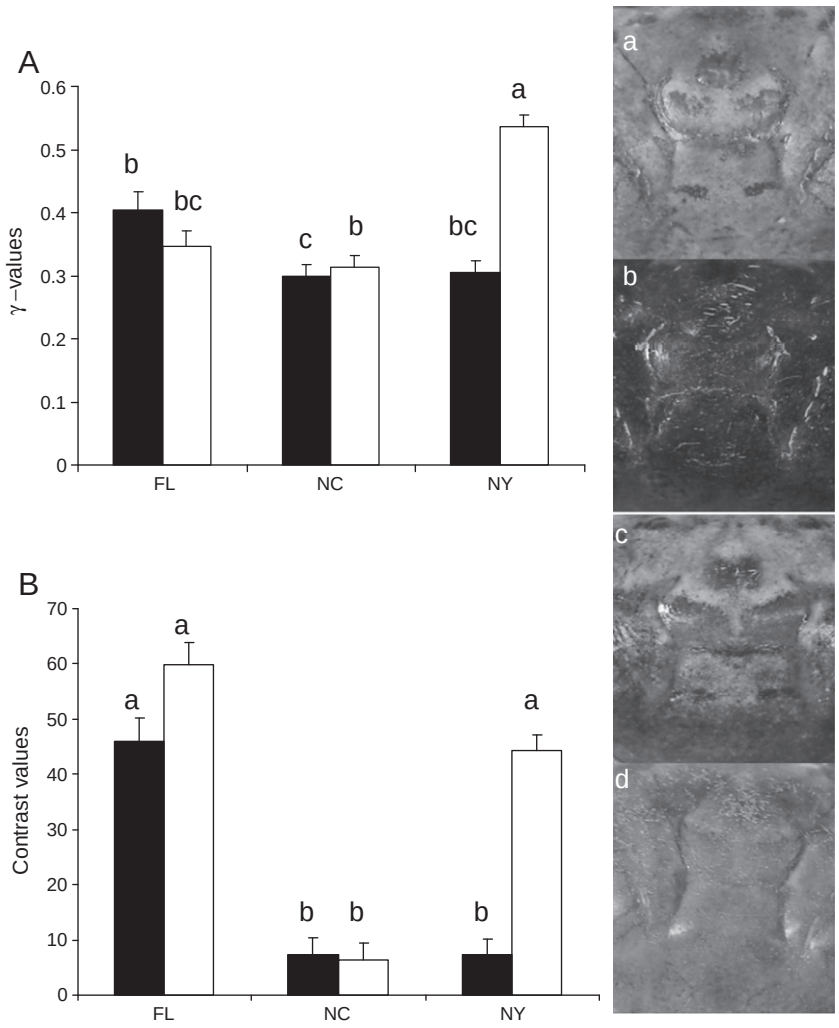

Fig. 1. Initial gamma, γ (i.e., shade) (A) and contrast, *C* (texture) (B) values before experimentation for the three populations. Black bars represent females, white bars represent males. Values are means ± SE. Different letters represent statistically different gamma and contrast values under a Tukey-HSD test. Pictures on the right are from male NY crab carapaces, the top two pictures are grayscale representations of high (light, a) and low (dark, b) gamma. The bottom two pictures show a high contrast (c) carapace and a low contrast (d) carapace.

Table 1
Source effects of 3-way ANOVAs on changes in gamma values between sexes, sites and treatments. Because of the strong 3-way interaction, 2-way ANOVAs were used for each sex testing for differences between sites and treatments. Changes in gamma were calculated as the difference between exposure and pre-treatment shades. SS = sum of squares. Significance at P <0.05 shown in bold.

Source	DF	SS	F ratio	P
Site	2	6.61	23.00	<**.0001**
Treatment	2	173.68	604.70	<**.0001**
Site∗Treatment	4	2.23	3.87	**0.004**
Sex	1	0.12	0.85	0.356
Site∗Sex	2	2.60	9.04	<**0.000**
Treatment∗Sex	2	0.77	2.67	**0.070**
Site∗Treatment∗Sex	4	3.79	6.60	<**.0001**

Source effects for 2-way ANOVAS

Sex	Source	DF	SS	F ratio	P
Males	Site	2	8.12	24.97	<**.0001**
	Treatment	2	96.17	295.80	<**.0001**
	Site∗Treatment	4	2.93	4.50	**0.002**
Females	Site	2	0.96	3.82	**0.023**
	Treatment	2	78.28	313.01	<**.0001**
	Site∗Treatment	4	3.07	6.13	<**0.000**

Recovery of contrast values (Fig. 3, bottom) showed strong differences between sites and sexes (3-way ANOVA, $F_{11,351} = 3.24$, $P < 0.001$; Table 3). North Carolina females did not recover their contrast in hot conditions relative to the other populations, while New York males did not recover their contrast values. Crabs under normal temperatures did return to original contrast states.

4. Discussion

The three *U. pugilator* populations showed strong differences in color change as a response to different temperatures. Males from different populations had similar directional and proportional changes in shade, but differed in the magnitude of response to temperature, suggesting differences among populations in the ability to change color, or in temperature sensitivity. Females had the same response to temperature regimes but did not show differences between populations. Local adaptation could be occurring through two different pathways: crab populations could differ because of males' ability to change color or populations may differ in male thermal tolerance, which may limit the amount of color change they exhibit when displaced from their thermal norm. Many poikilothermic species rely on behavioral mechanisms like color

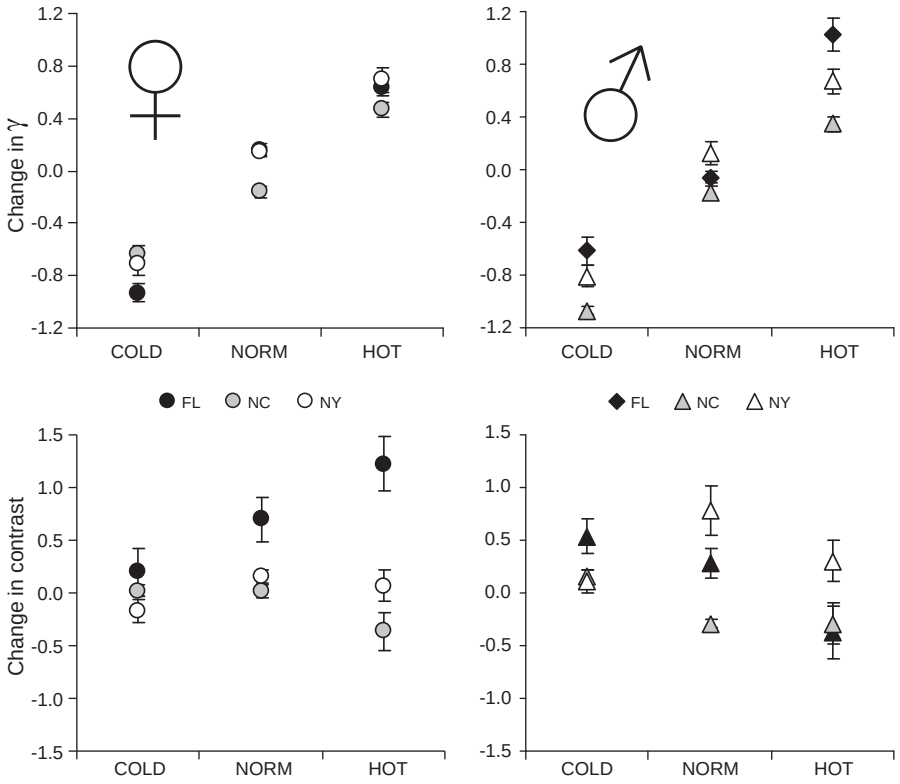

Fig. 2. Changes in carapace shade (γ-values) (top) and contrast (bottom) for females (left) and males (right). New York, North Carolina and Florida populations are represented by white, gray and dark symbols respectively. Norm is the treatment at normal temperatures. Values are means of change ± SE.

change to regulate temperature (Lagerspetz, 2006), but the ability to change color is also often associated with communication or camouflage. In fiddler crabs, it seems that changing color is overall a plastic response to environmental temperatures (Silbiger and Munguia, 2008) and crabs could have broad acclimation ability relative to crabs with more narrow distributions. Populations in this study, however, show patterns suggesting that local adaptation in crab physiology has evolved in males more strongly than females, perhaps because of male's sexually selected ornaments (Darnell and Munguia, 2011) or different activity

Table 2
Source effects of 3-way ANOVAs on changes in contrast values between sexes, sites and treatments. Because of the strong 3-way interaction, 2-way ANOVAs were used for each sex testing for differences between sites and treatments. Changes in contrast were calculated as the difference between exposure and pre-treatment shades. SS = sum of squares. Significance at $P < 0.05$ shown in bold.

Source	DF	SS	F ratio	P
Site	2	0.10	1.73	0.178
Treatment	2	0.41	7.12	**0.001**
Site∗Treatment	4	0.68	6.01	**<.0001**
Sex	1	0.06	2.05	0.153
Site∗Sex	2	0.04	0.64	0.530
Treatment∗Sex	2	0.15	2.57	0.078
Site∗Treatment∗Sex	4	0.39	3.46	**0.008**

Source effects for 2-way ANOVAS

Sex	Source	DF	SS	F ratio	P
Males	Site	2	0.011	0.144	0.8663
	Treatment	2	0.515	6.808	**0.0013**
	Treatment∗Site	4	0.909	6.007	**0.0001**
Females	Site	2	0.12	3.21	**0.04**
	Treatment	2	0.03	0.85	0.43
	Treatment∗Site	4	0.14	1.84	0.12

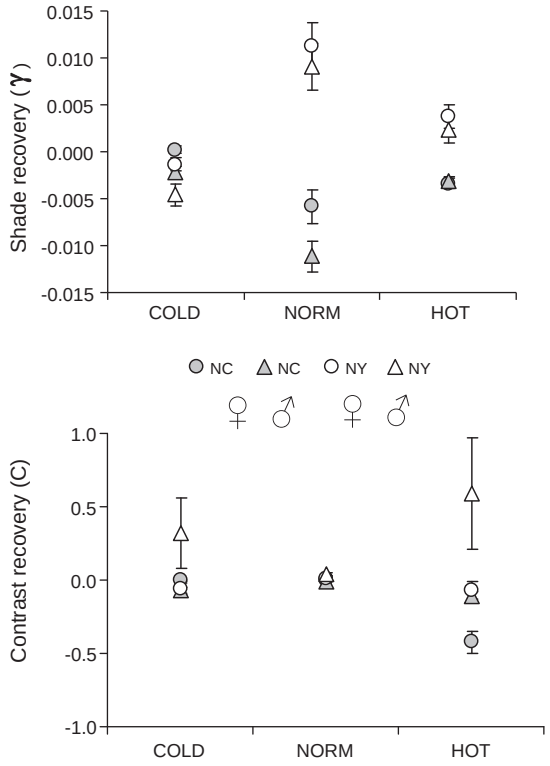

Fig. 3. Shade recovery (as changes in γ-values; top) and contrast recovery (as changes in C values; bottom) for the three populations (means ± SE). Gray symbols represent North Carolina populations, white symbols represent New York populations; circles are females and triangles are males. In New York, fiddler crabs do not return to pre-treatment shades as well as North Carolina.

Table 3
Results of a 3-way ANOVA on recovery levels of gamma values between sites, treatments and sexes. SS = sum of squares. Significance at P <0.05 shown in bold.

Source	DF	SS	F ratio	P
Recovery of gamma				
Treatment	2	0.0005	3.761	**0.024**
Site	1	0.0053	82.451	**<.0001**
Treatment∗Site	2	0.0064	49.679	**<.0001**
Sex	1	0.0005	7.853	**0.005**
Treatment∗Sex	2	0.0002	1.263	0.284
Site∗Sex	1	0.0000	0.027	0.869
Treatment∗Site∗Sex	2	0.0001	0.754	0.471
Recovery of contrast				
Treatment	2	0.179	0.159	0.853
Site	1	5.274	9.384	**0.002**
Treatment∗Site	2	4.069	3.619	**0.028**
Sex	1	4.226	7.518	**0.006**
Treatment∗Sex	2	3.625	3.224	**0.041**
Site∗Sex	1	1.788	3.181	0.075
Treatment∗Site∗Sex	2	0.671	0.597	0.551

patterns, since males are often exposed to strong sun while courting on high intertidal sand (Darnell et al., in review).

Shade and contrast metrics responded differently to temperature treatments. While shade is a proxy for overall dispersion of pigments, contrast reflects how uniformly pigments are dispersed or concentrated. In *U. pugilator*, different pigments respond differently to different stimuli, which may reflect the poor correlation between γ and contrast responses. Black and white pigments concentrate during the night and disperse during the day, suggesting a circadian rhythm and high sensitivity to light (Brown and Sandeen, 1948; Brown et al., 1953; Zeil and Hemmi, 2006). With increasing temperatures, black pigments concentrate while white pigments disperse, making carapaces lighter. Dark pigments also disperse in response to UV light in *U. pugilator* (Coohill and Milton, 1975; Coohill et al., 1970) and other crustaceans (e.g., Fuhrmann et al., 2011; Miner et al., 2000), suggesting an adaptive response to solar radiation. Therefore, changes in contrast may reflect adaptive responses from the different pigments involved or heterogeneous distribution of pigments throughout the carapace, even when carapace shade is becoming darker or lighter as a response to temperature (Kronstadt et al., in review; Silbiger and Munguia, 2008). This point seems evident in the recovery data where normal crabs get darker or lighter in captivity much slower than the temperature treatments. The different response times to normal temperatures between the gamma and contrast parameters is further evidence that coloration has different components, suggesting different functions.

Why do fiddler crab populations differ in their ability to change shades? Janzen (1967) would suggest that populations in temperate latitudes where temperature is variable should have higher thermal acclimation ability relative to populations in tropical latitudes where temperature is more constant (see also Vernberg and Tashian, 1959). *U. pugilator* populations differ in color change; yet counter to Janzen's hypothesis, populations at lower latitudes show greater shade (γ) changes than populations at higher latitudes. Of the three populations, Florida male crabs show the greatest color changes in response to hot temperature, especially in the ability to lighten (Fig. 2), and take a long time to return to the original state (pers. obs.).

Mechanisms regulating temperature could be morphological, physiological or behavioral. The male's major claw has been shown to affect heat balance in *Uca panacea* (Darnell and Munguia, 2011), and such morphological difference could explain a differential selective pressure between males and females in the role of color change; it is interesting to note that major claws are conspicuously light colored in many species (Crane, 1975). Alternatively, there could be physiological adaptations associated with each locality, perhaps tied to tidal fluctuations given that North Carolina has a greater fluctuation in tidal ranges than either Florida or New York (NOAA, http://co-ops.nos.noaa.gov/). Studies have shown a correlation between hormonal control of color change and tidal regimes (Palmer, 1995; Thurman, 1988); fiddler crabs living above the tidal mark do not show a tidal rhythm in pigment dispersion, while crabs burrowing in the intertidal zone do (Fingerman et al., 1958). If different populations experience different tidal regimes, then circatidal rhythms could influence color changing ability at a local scale. The high intertidal zone is a particularly strong source of thermal and water stress to males on the surface who wave and attract females, and a common thermoregulatory behavior involves individuals retreating to burrows to allow cooling and reduction of water loss (Allen, 2007). Herreid and Mooney (1984) found that *U. pugilator* would change to a light shade when fatigued, and these changes were driven through factors present in the hemolymph. In our study, crabs were not allowed to burrow, yet there could be burrowing differences among populations, perhaps also influenced by predation intensity and tidal regimes. In the Florida Gulf Coast population, tides are diurnal and irregular, while North Carolina and New York populations experience regular semi-diurnal tides. Under unpredictable tidal regimes during times of winds blowing offshore, crabs could be spending less time above ground. Color change could be a behavioral mechanism in response to changing temperature (Darnell et al., in review; Silbiger and Munguia, 2008), but individuals may prefer to maintain a particular color pattern for conspecific recognition or camouflage (Cummings et al., 2008; Detto et al., 2008).

5. Conclusions

Uca pugilator populations respond generically to cold and warm temperatures but differ by locality in their ability to change color. Florida males respond more to hot stress whereas North Carolina populations respond more to cold temperatures, suggesting differential adaptation to local temperature regimes or a highly plastic ability to acclimate to local conditions given the broad distribution of the species. North Carolina populations recover to the original shade better than New York populations. Such recovery capacity could occur if fiddler crab coloration is associated with mechanisms such as mate recognition or camouflage, and only under high thermal regimes and in higher latitude do crabs use color change as a mechanism to thermoregulate. Color change differences between males and females could be a result of morphological (Darnell and Munguia, 2011) or behavioral traits (Detto et al., 2006).

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