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RESEARCH ARTICLE

The male blue crab, *Callinectes sapidus*, uses both chromatic and achromatic cues during mate choice

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SUMMARY

In the blue crab, *Callinectes sapidus*, claw color varies by sex, sexual maturity and individual. Males rely in part on color cues to select appropriate mates, and these chromatic cues may be perceived through an opponent interaction between two photoreceptors with maximum wavelength sensitivities at 440 and 508 nm. The range of color discrimination of this dichromatic visual system may be limited, however, and it is unclear whether male blue crabs are capable of discriminating the natural variations in claw color that may be important in mate choice. By testing males' innate color preferences in binary choice tests between photographs of red-clawed females and six variations of orange-clawed females, we examined both the chromatic (opponent interaction) and achromatic (relative luminance) cues used in male mate choice. Males significantly preferred red-clawed females to orange-clawed females, except when the test colors were similar in both opponency and relative luminance. Our results are unusual in that they indicate that male mate choice in the blue crab is not guided solely by achromatic or chromatic mechanisms, suggesting that both color and intensity are used to evaluate female claw color.

Supplementary material available online at http://jeb.biologists.org/cgi/content/full/215/7/1184/DC1

Key words: blue crab, *Callinectes sapidus*, color vision, mate choice.

INTRODUCTION

Bright, conspicuous colors are often investigated within the context of sexual communication and sexual selection. Animals use color to communicate information such as sex (Butcher and Rohwer, 1989; Marquez and Verrell, 1991), reproductive readiness (McLennan and McPhail, 1990; Sköld et al., 2008), individual or species identity (Losos, 1985; Detto et al., 2006), social status (Rohwer, 1975; Watt, 1986) and individual quality (Endler, 1980; Hill and Montgomerie, 1994). However, colors can only be effective signals or cues if they can be perceived by the intended receiver (Rowland, 1979). It is necessary then, when investigating the role of an animal's coloration, to also consider the receiver's visual system, which varies widely across animal taxa (reviewed by Briscoe and Chittka, 2001; Osorio and Vorobyev, 2008; Kelber and Osorio, 2010).

The blue crab, *Callinectes sapidus*, is an excellent system for simultaneously investigating the perception of color cues and their role in sexual signaling. The blue crab is a colorful portunid species endemic to coastal Atlantic waters along North, Central and South America. Male and female blue crabs have sexually dimorphic claw coloration; adult males have white and blue claws, and adult females have orange or red claws (Fig. 1). The claw coloration of immature males and females ranges from pale blue or violet to orange (Fig. 1), and qualitative evidence suggests that claw coloration changes with sexual maturity in both males and females. These differences in claw color between sexes and between sexually immature and mature crabs suggest that claw color may act as a cue of sex and/or sexual maturity, while individual variation within each group invites speculation regarding individual quality or fitness.

Hypotheses regarding the signaling function of claw coloration must be evaluated within the parameters of the blue crab visual system. Blue crabs likely have a dichromatic visual system. Electroretinogram (ERG) data suggest the presence of photoreceptors with a peak sensitivity of 508 nm (green) over the entire eye and a second set of photoreceptors in the ventral portion of the eye with a peak sensitivity at about 440 nm (blue) (Martin and Mote, 1982). Thus, the detection of chromatic cues may occur *via* opponency between the green and blue photoreceptors in the ventral portion of the eye. Achromatic cues, those that are based on signal intensity alone, are likely detected *via* the 508 nm photoreceptor given that it is the predominant receptor in the eye (Martin and Mote, 1982).

Color vision was suggested in behavioral experiments where *C. sapidus* showed distinct responses to yellow, red and blue approaching objects (Bursey, 1984). However, these experiments did not control for the intensity of the colors used; thus, the responses may have been based on signal intensity rather than color. A more recent behavioral study demonstrated the blue crab's use of chromatic cues when males displayed a preference for images of red-clawed females over those of gray-clawed females that had the same luminance (as perceived by the blue crab eye) (Baldwin and Johnsen, 2009). Although males preferred red-clawed females to those with isoluminant gray claws, it was not determined how or whether male blue crabs can distinguish naturally occurring variations in the red and orange claw coloration of females. The blue crab's blue–green dichromatic visual system may have a restricted range of color discrimination because of its limited sensitivity to long-wavelength light. Thus, the nuances of orange and red colors that are evident to humans may not be perceived by the blue crab eye.

Here, we investigated natural variation in claw coloration in male and female blue crabs, documenting color differences between sexually immature and mature individuals of both sexes. We then estimated the appearance of claw color to the blue crab visual system by modeling perceived luminance (achromatic cues) and opponency (chromatic cues) using ERG data. Then, in binary choice tests using photographs of females with claws colored red and six variations of orange, we behaviorally tested the ability of male blue crabs to discriminate between various long-wavelength-dominated colors. These behavioral assays examine both chromatic and achromatic mechanisms of male mate choice and the likelihood of whether males are capable of discriminating between naturally occurring claw colors.

MATERIALS AND METHODS

Study species

Males and females of the blue crab, *C. sapidus* Rathbun 1896, were captured from Jarrett Bay near Smyrna, NC, USA (34°45′31″N, 76°30′44″W) in April 2011. Sex and sexual maturity were determined by visually examining claw color, abdomen shape and overall size. In males, sexual maturity is associated with size and individuals over 100 mm were assumed to be sexually mature (Milikin and Williams, 1984). In females, abdomen shape is a reliable indicator of sexual maturity. Immature females have triangular shaped abdomens while mature females have wider, rounder abdomens (Newcombe et al., 1949; Jivoff et al., 2007). Prepubertal female crabs are in a transitional stage of sexual maturity, meaning that they will become sexually receptive just prior to their next molt; these can also be identified by their abdominal shape and coloration.

Measuring spectral reflectance of claw color

Blue crab claw color was measured in April 2011, during the first peak of the commercial soft crabbing season in North Carolina. During this time, juvenile and adult male and female blue crabs were available in running seawater enclosures at a commercial crab fishing facility in Smyrna. Crabs were separated into five groups based on sex and sexual maturity: immature males, mature males, immature females, prepubertal females and mature females. Spectral reflectance measurements of claws were taken in a darkened room. Prior to measurements, crabs were chilled on ice for 15–30 min to facilitate handling.

Spectral reflectance data were collected using methods outlined previously (Johnsen, 2005), using a fiber optic reflectance probe (R400-7 reflection probe, Ocean Optics Inc., Dunedin, FL, USA) coupled with a pulsed xenon light source (PX-2, Ocean Optics) and a multi-channel spectrometer (USB2000, Ocean Optics). The reflectance probe contained seven 400 μm diameter optical fibers in a six-around-one arrangement. The six outer fibers were coupled to the light source and illuminated the specimen. The central fiber collected the light reflected from the specimen and was coupled to the spectrometer. The end of the reflectance probe was held next to the claw surface at a 45 deg angle using a rigid optical mount, which illuminated and collected the back-reflection of the claw surface. This approach reduced the collection of specularly reflected light from the shiny claw surface and instead collected the diffuse reflectance, which is relatively independent of the angles of illumination and measurement (Palmer, 1995). The reflectance measurements were calibrated using Spectralon™, a diffuse reflectance standard that diffusely reflects nearly 100% of light from 200 to 800 nm (WS-1 Diffuse Reflection Standard, Ocean Optics). Spectral reflectance was taken at the center of the dactyls (moveable fingers) of each side of the claws, referred to as the interior and exterior claw faces relative to the crab. Measurements were taken from the right claws of 18–29 individuals of each group.

Calculation of relative luminance and opponency to the blue crab eye

Claw reflectance spectra, illumination spectra and the spectral sensitivity of the visual system were used to model the perceived chromatic and achromatic signals. The luminance (L) of reflected light perceived by the blue crab eye is given by:

$$L = C \sum_{\lambda=400}^{700} R(\lambda) I(\lambda) S(\lambda) \Delta\lambda \qquad (1)$$

where $R(\lambda)$ is the diffuse spectral reflectance of the object being viewed, $I(\lambda)$ is the downwelling spectral irradiance, $S(\lambda)$ is the spectral sensitivity of the crab eye, and C is a constant that includes factors such as eye size, etc., that are independent of wavelength and factor out when comparing different samples. The irradiance was chosen to be that of the test tank used in the behavioral experiments described later in this paper. The spectral sensitivity of *C. sapidus* was based on ERG data (Martin and Mote, 1982), and perceived luminance was found for both the blue photoreceptor (λ_{max}=440 nm) and the green photoreceptor (λ_{max}=508 nm). In many species, the medium wavelength photopigment functions as the achromatic channel, and in the blue crab the green photoreceptor is the predominant one found throughout the eye (Martin and Mote, 1982; Cronin and Forward, 1988). Therefore, the achromatic cues we report on here are based on the quantum catch of the green photoreceptor. In addition, for easier comparison we normalized all crab-perceived luminance by the perceived luminance of the red test color used in the behavioral experiments described below, referring to this ratio as 'relative luminance'.

To determine color as perceived by the blue crab eye, we assumed that color vision occurred *via* an opponency mechanism between the putative 440 nm and a 508 nm pigment in the blue crab eye (Martin and Mote, 1982; Baldwin and Johnsen, 2009). We used a simple model that assumes that the influence of the two pigments is balanced over the wavelength range of 400 to 700 nm, i.e.:

$$\text{Opponency} = \frac{L_{508} - L_{440}}{L_{508} + L_{440}}\ , \qquad (2)$$

where L_{λ} is the perceived luminance calculated in Eqn 1 for a pigment with a peak wavelength of λ. The relative gain of the two channels was adjusted to produce an opponency value of zero for an achromatic object.

While the blue crab may be sensitive to ultraviolet light, we report here only on the visible spectrum of light as a result of limitations of the known spectral sensitivity of the blue crab. In addition, preliminary analyses that extrapolated the visual sensitivity of the crab eye into the ultraviolet did not give significantly different results.

Behavioral experiments evaluating male color preference

Male blue crabs were collected from June to November 2009 and April to August 2010. Crabs were immediately placed into individual buckets with a shallow layer of water and transported to Duke University's central campus in Durham, NC, USA. There, crabs were kept in individual compartments within a 700 l recirculating artificial seawater system (salinity 29–31‰, temperature 25–26°C,

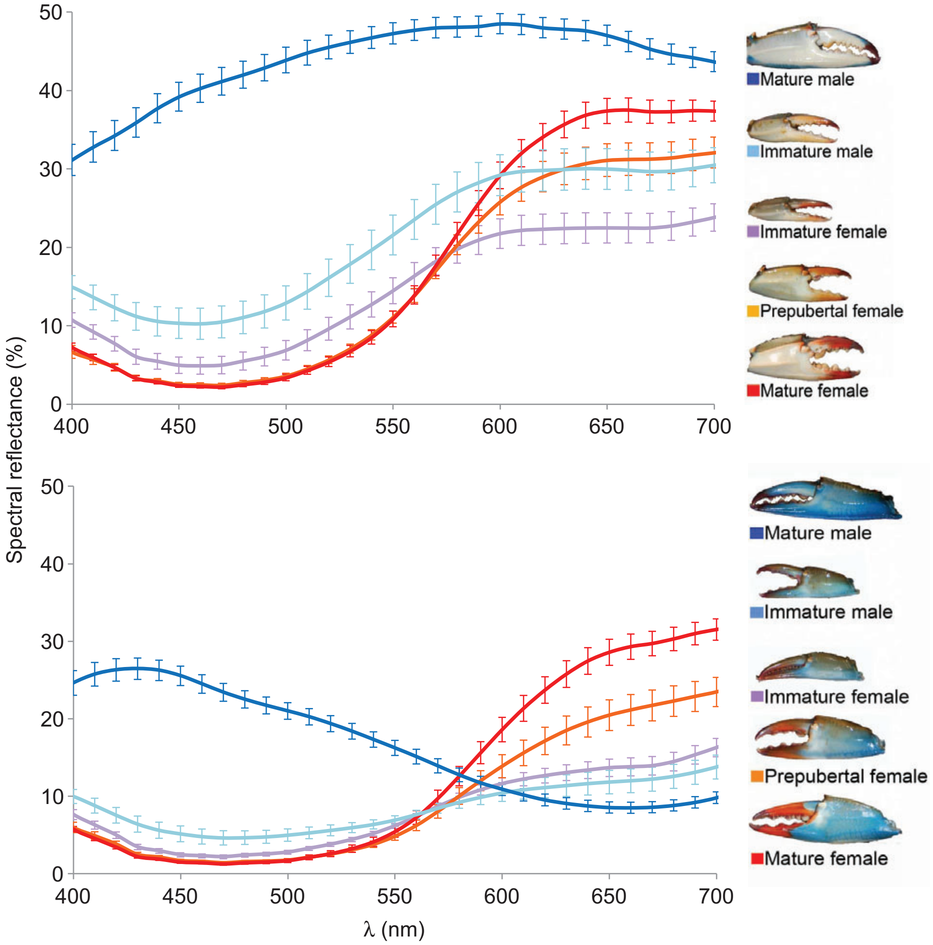

Fig. 1. Mean ± s.e.m. spectral reflectance against wavelength (λ), and photographs of both the exterior (top) and interior (bottom) claw surfaces of sexually mature males (*N*=28), immature males (*N*=30), immature females (*N*=27), prepubertal females (*N*=21) and sexually mature females (*N*=25).

natural light cycle). Compartment walls were opaque to minimize stress and agonistic behavior. Crabs were fed pieces of fish, shrimp or scallop every 2 days, and kept for at least 48 h before being used in experiments.

Binary choice experiments were conducted in three 100 l glass aquariums (32×91×46 cm) with gravel bottoms. During acclimation periods, water was filtered and at all times maintained at the same salinity and temperature as the holding tank. Experimental tanks were kept in a separate room and visually isolated on all sides by blue cloth. The tanks were observed *via* a video camera and lit using overhead fluorescent and incandescent lamps, resulting in a downwelling irradiance of ~8×10^{14} photons cm^{-2} s^{-1} (integrated from 400 to 700 nm).

Photographs of a sexually mature female in a receptive posture on a solid gray background were used in place of live females to limit confounding variables, such as chemical, tactile or motion cues. Photos were printed on Staples$^{\mathrm{TM}}$ brand recycled copy paper and mounted on white foam boards for stability. A photograph of a red-clawed female was tested against photographs with orange claws of varied relative luminance. The red color tested was selected from a photograph of a sexually mature female blue crab's claws using the Color Picker tool of Photoshop CS (Adobe Inc., San Jose, CA, USA). After selecting the red test color, we chose an orange color similar in relative luminance under the given illumination spectrum of the test arena. This color is described as orange 0.85, with the number corresponding to the relative luminance. The spectral reflectance of the red and orange colors was measured using a reflecting probe coupled with a light source and spectrometer. From orange 0.85, five other oranges were produced by increasing and decreasing the relative luminance of the color using the Brightness function in the Color Picker tool: one darker (orange 0.48) and four lighter variations (orange 2.6, 5.1, 7.7 and 12). These orange variations had similarly shaped reflectance curves, but varied in mean reflectance (Fig. 2). Thus, a total of six versions of the photograph were used: one image with red-colored claws (Fig. 3) and five images with orange-colored claws. The gray background used in the photographs had a relatively constant spectral reflectance (not shown), with a relative luminance of 3.8 and an opponency of 0.007.

All experimental trials were conducted between 07:00 h and 19:00 h local time from May to November 2009 and April to August 2010. Prior to the start of each experiment, one male was placed in an experimental tank and allowed to adjust to the surroundings for 3 h. Then, two photographs were presented to the crab – one at each end of the tank. Photograph positions were assigned randomly. Over the next hour, the male's behavior was recorded on video. Videos were later watched and scored blind to the experimental conditions. Most crabs made multiple stereotypic sexual displays that were unambiguously directed towards (and occurred within 5 cm of) one of the two images. During these 5–30 s displays, the crabs rose up on their walking legs, extended their claws, and waved their paddles while facing the photograph [see Baldwin and Johnsen (Baldwin and Johnsen, 2009) for further details]. The total number of sexual displays made towards each image over 1 h was counted. Choice was assigned to the image that received the greater number of displays. The results of each binary choice test, including first display

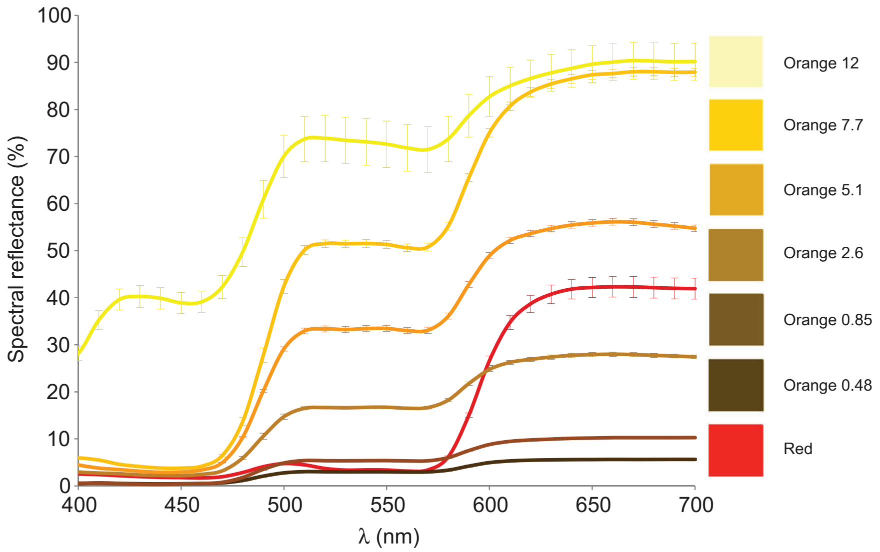

Fig. 2. Mean ± s.e.m. spectral reflectance of the red and orange colors used in behavioral experiments. N=20 for each color.

and number of displays, are given in supplementary material Table S1. We evaluated the statistical significance of choices for each variation of orange tested using two-tailed exact binomial tests. The statistical issues inherent in multiple testing were regulated using the Benjamini and Hochberg procedure, which controlled for false discovery rate (Benjamini and Hochberg, 1995).

We used 126 male crabs during these binary choice tests. Because these males were wild-caught, it was not known how recently they had molted or mated. Both of these factors may have affected their sexual receptivity. If a male did not display mate preference during the 1 h trial, the trial was discarded and the male was tested again 5–7 days later. No males were used more than once in a 5 day period and males were not reused for the same test colors. After testing, the males were returned to the re-circulating seawater system.

RESULTS

Spectral reflectance of the claws of *C. sapidus*

Measurements of claw coloration revealed clear differences in the spectral reflectance of claws between male and female blue crabs and also between sexually immature and mature crabs of each sex (Fig. 1). In most individuals, the average reflectance of the exterior of the claw was greater than that of the interior of the claw. Spectral reflectance varied between claws of sexually mature males and females, with males reflecting more light at shorter (bluer) wavelengths and females reflecting more light at longer (redder) wavelengths. The spectral reflectance of both male and female blue crabs changed with sexual maturity (Fig. 1).

Relative luminance and opponency of natural claw colors

Based on our model of blue crab visual perception, the relative luminance and opponency values of claws differed between sexually immature males, mature males, immature females and mature females (Fig. 4). The perceived claw coloration appeared to be distinct between groups, although there was considerable individual variation. Claw coloration of prepubertal females overlapped with both immature and mature females, which is not surprising given that this is an intermediate molt stage that transitions females from sexually immature to mature.

Behavioral experiments

Male color preference did not appear to be mediated solely by chromatic or achromatic cues but rather appeared to have a specific range of preferred color and intensity. Males significantly preferred red-clawed females (with an opponency value of 0.33) to those with claws colored with orange 0.48 (opponency value 0.38; $P\leq0.004$), orange 5.1 (0.43; $P\leq0.013$), orange 7.7 (0.44; $P\leq0.001$) and orange 12 (0.16; $P\leq0.041$) (Figs 5 and 6). Males significantly preferred red-clawed females over females with orange claws with both lower opponency values (orange 12, gray 5.7 and gray 0.85) and higher opponency values (orange 0.48, orange 5.1 and orange 7.7; Figs 5

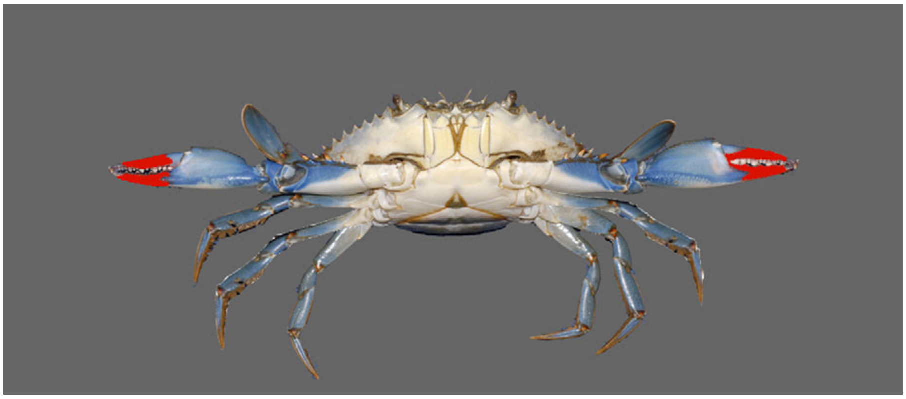

Fig. 3. One of the six photographs used in binary choice tests, pictured here with claws colored red. The crab is in a known sexually receptive posture.

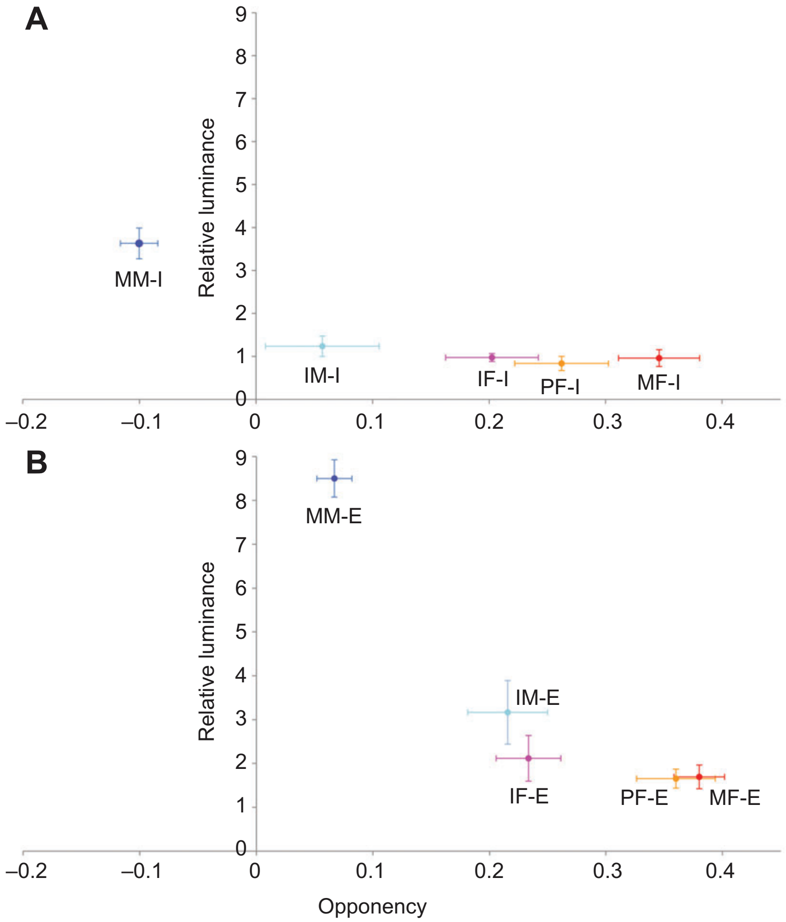

Fig. 4. Achromatic and chromatic cues of natural crab claws with 95% confidence intervals. Achromatic cues are shown as mean relative luminance, the perceived luminance of each color relative to red. Chromatic cues are reported as opponency values based on an opponent interaction between the blue (λ_{max}=440 nm) and green (λ_{max}=508 nm) channels. Data are reported for each side of the claw relative to the crab, referred to as the interior and exterior claw surface. (A) Interior claw surface. MM-I, mature male interior claws (*N*=28); IM-I, immature male interior claws (*N*=30); IF-I, immature female interior claws (*N*=27); PF-I, prepubertal female interior claws (*N*=21); and MF-I, mature female interior claws (*N*=25). (B) Exterior claw surface. MM-E, mature male exterior claws (*N*=28); IM-E, immature male exterior claws (*N*=30); IF-E, immature female exterior claws (*N*=27); PF-E, prepubertal female exterior claws (*N*=21); and MF-E, sexually mature female exterior claws (*N*=25).

and 6). However, males were either not able or not motivated to choose between red and orange claws similar in both opponency and relative luminance as perceived by the blue crab visual system. In the trials testing for preference between red and orange 0.85 and orange 2.6, males chose red slightly more often, but these preferences were not statistically significant.

DISCUSSION

In the present study, models of visual perception and behavioral tests of color vision were used to evaluate the ability of the blue crab to perceive differences in natural claw colors. Previous behavioral experiments have indicated that male blue crabs use chromatic cues when choosing a female mate by demonstrating male preference for red-clawed females over those with gray claws matched in relative luminance (Fig. 6B) (Baldwin and Johnsen, 2009). It remained unclear, however, whether males would be capable of discriminating between female claw colors found in nature (Fig. 1). The behavioral trials presented here were intended to probe the ability of the blue crab to choose between similar long-wavelength colors during mate choice. Unexpectedly, our results suggest that male blue crabs use a mixture of chromatic and achromatic cues to discriminate between long-wavelength colors. Additionally, males' preference for red-clawed females and their ability to discriminate red over variations of orange support the possibility that claw color may function as a sexual signal or cue.

We found that male blue crabs could distinguish between red and orange coloration, except when the test shades were similar in both relative luminance and opponency. The results suggest that both chromatic and achromatic cues function in the discrimination of colors dominated by long-wavelength light. Further, there may be a particular range of relative luminance (0.85–2.6) and opponency (0.3–0.45) values that stimulates male courtship behavior (Fig. 6). Additional studies using multiple shades of gray and other colors may help in understanding the limits of color vision in this species. Alternative models of relative luminance suggest that if the blue crab possessed a visual pigment with a λ_{max} between 540 and 600 nm, the choices we observed could be based solely on achromatic cues. However, no such photoreceptor has been detected through ERG or microspectrophotometry (MSP) (Martin and Mote, 1982; Cronin and Forward, 1988).

In invertebrate species, achromatic and chromatic cues are often used for different tasks. Achromatic vision may be more useful for tasks involving motion detection and shape or object recognition (Lythgoe, 1979; Kelber et al., 2003), while chromatic cues are often used when identifying and classifying objects, such as food, oviposition sites or potential mates (Vorobyev and Osorio, 1998; Sumner and Mollon, 2000). Most studies investigating innate visual behaviors of invertebrates show evidence of only chromatic cues or only achromatic cues being used during specific tasks, and the simultaneous use of both is at present unclear (Kelber and Osorio, 2010). However, experiments that involve color learning have shown evidence of the use of both. For example, studies on the honeybee, *Apis mellifera*, provide evidence that either chromatic or achromatic cues can be used for object identification, depending on the size of the object (Giurfa and Vorobyev, 1997; Giurfa and Vorobyev, 1998; Giurfa et al., 1997). The hawkmoth, *Macroglossum stellatarum*, can

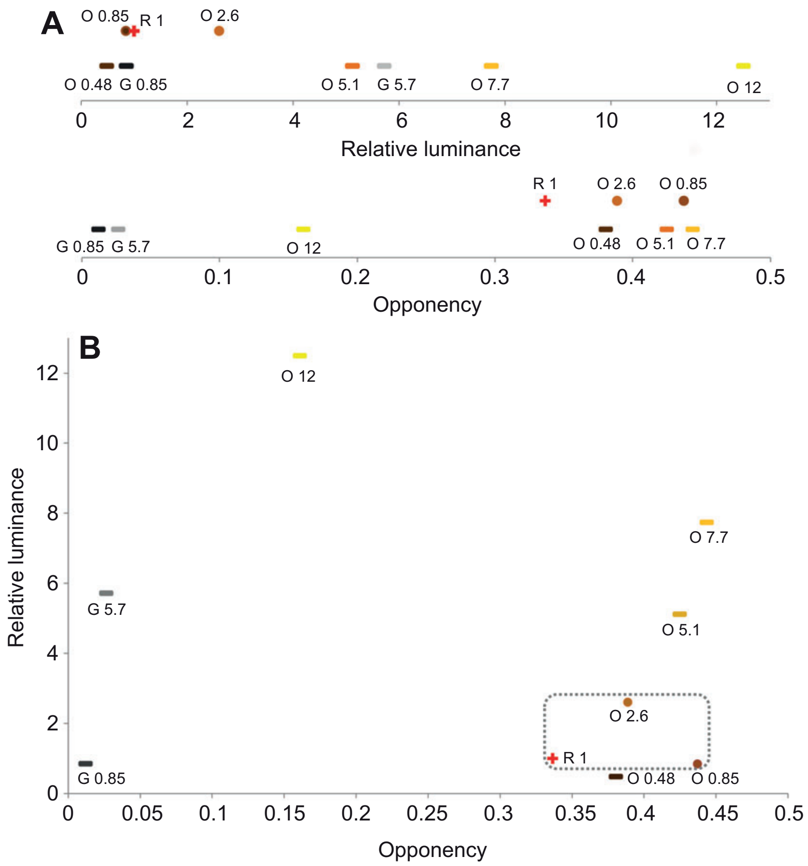

Fig. 5. (A) One-dimensional plots of relative luminance and opponency of the experimental colors used during binary choice trials. (B) Relative luminance and opponency plotted together to represent how the blue crab may perceive both achromatic and chromatic cues. In both, the red cross shows the value of the red test color. Orange colors that were chosen significantly less often than red are represented by minus signs. Circles represent orange colors that were not chosen significantly less often than red. Data in B are offset for clarity. R, red; G, gray; O, orange; numbers indicate the luminance relative to the red test color.

learn to associate rewards with either the chromatic or the achromatic aspect of a color, but appears to more readily learn the chromatic aspect (Kelber, 2005).

True color vision has been infrequently documented in crustaceans. Certainly, spectral sensitivity and the presence of different spectral classes of photoreceptors have been widely documented across crustacean species (Cronin and Forward, 1988; Frank and Widder, 1999; Rajkumar et al., 2010), but the behavioral evidence needed to confirm color vision (and discount confounding achromatic cues) is not common. Aside from tests in *C. sapidus*, color vision has been demonstrated in a fiddler crab, *Uca mjoebergi*, where females chose males with painted yellow claws over those with claws painted various shades of gray (Detto, 2007). Color vision has also been shown in a stomatopod, *Odontodactylus scyllarus* (Marshall et al., 1996). This mantis shrimp was capable of discriminating red, green and yellow from different grays. However, individuals of *O. scyllarus* were not able to discriminate blue from gray, possibly because of similarities between the blue and gray stimulation profiles (Marshall et al., 1996). The authors suggest that there may be a threshold value between photoreceptor catch ratios for color detection to occur.

Role of claw color

Some crustaceans, most notably crabs and stomatopods, have colorful displays paired with well-developed visual systems, and visual cues can play a role in their communication and social behavior (Schöne, 1968; Marshall et al., 2006). Fiddler crabs (*Uca* spp.) have been shown to use color cues in sex recognition, individual recognition and mate choice (Detto et al., 2006; Detto, 2007). In brachyuran crabs, the claws, in particular, are commonly used in sexual and agonistic communication (reviewed by Schöne, 1968; Christy, 1987). Claw color in the semaphore crab, *Heloecius cordiformis*, corresponds with sex and age (Detto et al., 2004), and may indicate sexual maturity. Given these examples, it is reasonable to assume that claw color may act as a signal or cue in the blue crab.

There are several possible roles for claw coloration in *Callinectes*. First, claw color may play a role in species recognition. There are as many as 16 species of *Callinectes*, 10 of which have overlapping habitats with *C. sapidus* in the Caribbean Sea (Robles et al., 2007). Claw color and pattern vary with species (Fig. 7) and may potentially be used to identify conspecific mates. It is possible that certain claw colors tested here were not recognized as belonging to potential mates and thus did not receive male preference.

Measurements of crab claw coloration and their estimated appearance to the blue crab eye indicate that claw color may also function in sex identification. Color differences between males' blue claws and females' red claws should be apparent to the blue crab's dichromatic visual system (Fig. 4). While we have not conducted tests between photographs of blue- and red-clawed crabs, in previous tests between photographs of red- and white-clawed crabs, males often addressed the white-clawed crab photographs with agonistic behavior (Baldwin and Johnsen, 2009). As the exterior face of male claws is largely white, this may indicate that male test subjects viewed our white-clawed crab photographs as male competitors. Also, previous behavioral tests of color vision in the blue crab showed that male and female blue crabs had significantly different reaction times to blue-, yellow- and red-colored approaching objects (Bursey, 1984).

Claw color may also act as a cue of sexual maturity. Spectral reflectance measurements show that claw color changes with sexual

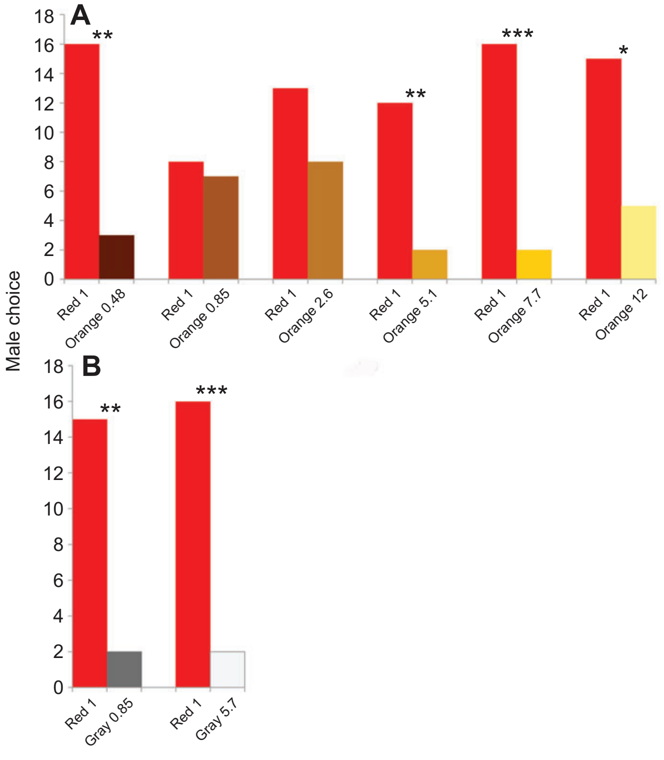

Fig. 6. (A) Results of the binary choice experiments testing male preference between red and orange female claws. (B) Previous results of binary choice tests showing male preference for females with red claws over those with white claws and dark gray claws that were isoluminant to the red claws (Baldwin and Johnsen, 2009). Asterisks denote significance (*$P \leq 0.05$, **$P \leq 0.01$ and ***$P \leq 0.001$).

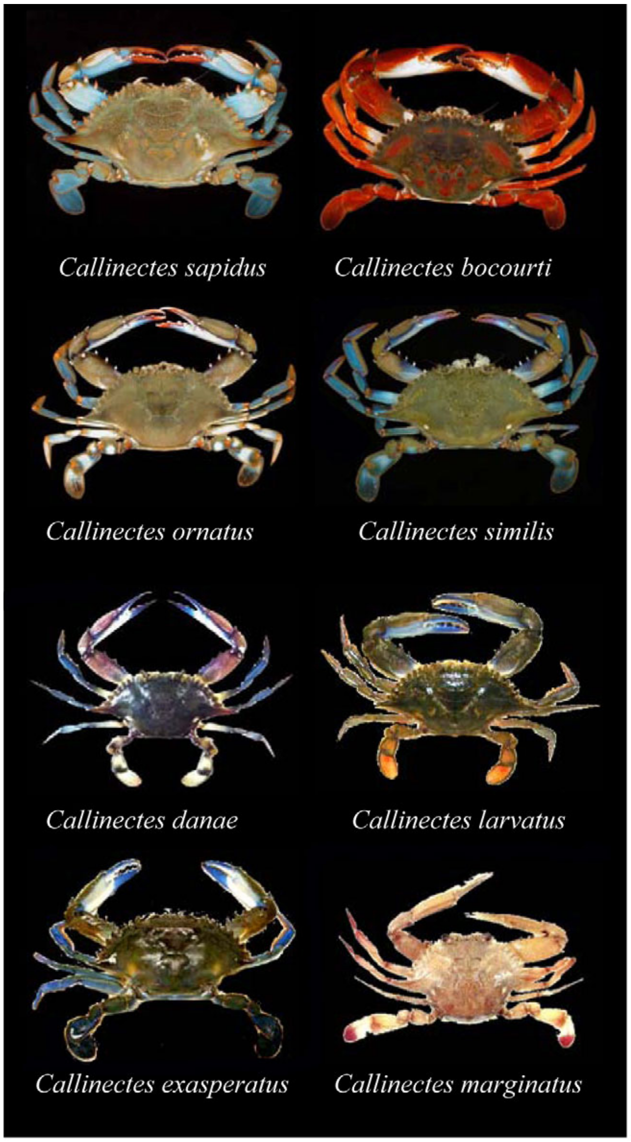

Fig. 7. Coloration of species in the genus *Callinectes*. Of the 16 species of *Callinectes*, up to 10 species may overlap in coastal regions of the Caribbean Sea. Eight of the 10 Caribbean species are pictured here (*C. maracaiboensis* and *C. affinis* are not shown). Photographs courtesy of the Southeastern Regional Taxonomic Center (SERTC).

maturity in male and female crabs (Figs 1 and 4). Also, claw colors of reproductively ready female crabs (prepubertal and sexually mature females) fall within the boundaries of observed male preference of both relative luminance and opponency (Fig. 5B). Together, these data provide evidence that males may use claw color to identify sexually mature female blue crabs. Examples of color cues indicating sexual maturity are found in many species and are most often described in males (Kodric-Brown, 1985; Frischknecht, 1993; Bakker and Mundwiler, 1994), but are being increasingly documented in females (McLennan, 1995; McLennan, 2000; Amundsen and Forsgren, 2001). Additionally, our color measurements show similarities between immature males and immature females (Figs 1 and 4). In species where juveniles and adults have different coloration, juvenile coloration may be a non-threatening cue to adult conspecifics, indicating a lack of competition for territory, food or mates (Neal, 1993; Mahon, 1994).

Finally, individual variation in claw color also invites speculation regarding claw color and individual quality. In the blue crab, both the blue and red colors are due to carotenoid-based pigments located in the hypodermis (Smith and Chang, 2007). As carotenoids cannot be synthesized *de novo* in animals, they must be ingested and may reflect an individual's foraging ability (Bagnara and Hadley, 1973; Brush and Power, 1976). In environments where carotenoids are limited, carotenoid-based pigmentation may serve as an indicator of individual quality (Endler, 1980; Hill and Montgomerie, 1994). In a number of species, carotenoid-based coloration may reflect an individual's parasite load, immunological health and overall condition (Borgia and Collis, 1989; McGraw and Hill, 2004; Clotfelter, 2007). Connections between coloration and mate attractiveness have been documented in several species of fish (Frischknecht, 1993; Bakker and Mundwiler, 1994; Evans and Norris, 1996) and birds (Hill and Montgomerie, 1994; Saks et al., 2003). Such results imply that both male and female blue crab claw coloration could be indicative of individual quality. While a relationship between claw color and individual quality has not yet been established for either male or female blue crabs, the possibility that claw color could advertise individual quality has intriguing applications for future studies of mate choice and intraspecific competition.

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Supplementary Table S1. The results of binary choice experiments testing male preferences for photographs of females with red or orange claw coloration are presented. The number of displays made to each color, the side of first display, and the preferred side are presented. Totals are shown with results of two-tailed exact binomial tests.

Red vs. Orange 0.48

	Number of Displays				
Preferred Color	**Red**	**Orange**	**Total**	**First Display**	**Preferred Side**
Red	3	1	4	Red	Right
Red	4	2	6	Red	Left
Red	3	0	3	Orange 0.48	Left
Orange 0.48	1	8	9	Red	Left
Orange 0.48	0	8	8	Orange 0.48	Right
Red	14	3	17	Red	Right
Red	17	5	22	Red	Left
Red	4	0	4	Red	Right
Red	1	0	1	Red	Right
Orange 0.48	1	8	9	Orange 0.48	Right
Red	4	0	4	Red	Right
Red	5	0	5	Red	Right
Red	3	0	3	Red	Right
Red	3	0	3	Red	Left
Red	3	1	4	Red	Right
Red	3	0	3	Red	Left
Red	11	9	20	Red	Left
Red	14	5	19	Orange 0.48	Right
Red	7	0	7	Red	Right
	Red 16: Orange 3			**Red 15: Orange 4**	**Left 7: Right 12**
	p≤0.004			**p≤0.019**	**n.s.**

Red vs. Orange 0.85

	Number of Displays				
Preferred Color	**Red**	**Orange**	**Total**	**First Display**	**Preferred Side**
Red	4	1	5	red	Right
Orange 0.85	0	7	7	Orange 0.85	Right
Orange 0.85	1	3	4	Orange 0.85	Left
Red	2	1	3	Red	Right
Red	8	3	11	Red	Right
Orange 0.85	1	5	6	Orange 0.85	Left
Red	10	1	11	Red	Right
Orange 0.85	6	3	9	Orange 0.85	Right
Red	3	1	4	Red	Left
Red	1	0	1	Red	Left
Orange 0.85	1	4	5	Orange 0.85	Left
Orange 0.85	0	4	4	Orange 0.85	Right
Orange 0.85	1	5	6	Orange 0.85	Left
Orange 0.85	2	7	9	Orange 0.85	Left
Red	3	1	4	Red	Right
	Red 8: Orange 7			**Red 8: Orange 7**	**Left 7: Right 8**
	n.s.			**n.s.**	**n.s.**

Red vs. Orange 2.6

	Number of Displays				
Preferred Color	**Red**	**Orange**	**Total**	**First Display**	**Preferred Side**
Orange 2.6	0	2	2	Orange 2.6	Right
Red	3	0	3	Red	Right
Orange 2.6	0	2	2	Orange 2.6	Right
Orange 2.6	0	4	4	Orange 2.6	Left
Red	6	3	9	Red	Right
Orange 2.6	2	4	6	Orange 2.6	Left
Orange 2.6	1	10	11	Orange 2.6	Left
Red	1	0	1	Red	Right
Red	11	0	11	Red	Left
Red	14	1	15	Red	Left
Red	10	0	10	Red	Right
Orange 2.6	3	10	13	Orange 2.6	Right
Red	14	0	14	Red	Right
Red	5	2	7	Red	Left
Red	15	9	24	Orange 2.6	Left
Red	6	4	10	Red	Left
Orange 2.6	0	10	10	Orange 2.6	Left
Orange 2.6	0	9	9	Orange 2.6	Right
Red	4	0	4	Red	Left
Red	16	0	16	Red	Right
Red	16	2	18	Red	Left
		Red 13: Orange 8		**Red 12: Orange 9**	**Right 10: Left 11**
		n.s.		**n.s.**	**n.s.**

Red vs. Orange 5.1

	Number of Displays				
Preferred Color	**Red**	**Orange**	**Total**	**First Display**	**Preferred Side**
Red	8	0	8	Red	Left
Orange 5.1	3	6	9	Red	Left
Red	3	2	5	Red	Left
Red	19	11	30	Red	Right
Red	11	2	13	Red	Left
Orange 5.1	0	5	5	Orange 5.1	Right
Red	5	4	9	Red	Right
Red	16	1	17	Red	Left
Red	12	3	15	Red	Left
Red	3	0	3	Red	Right
Red	10	3	13	Red	Right
Red	4	1	5	Orange 5.1	Right
Red	16	1	17	Red	Right
Red	11	0	11	Red	Left
		Red 12: Orange 2		**Red 12: Orange 2**	**Left 7: Right7**
		$p \leq 0.001$		**$p \leq 0.001$**	**n.s.**

Red vs. Orange 7.7

	Number of Displays				
Preferred Color	**Red**	**Orange**	**Total**	**First Display**	**Preffered Side**
Red	8	0	8	Red	Right
Red	6	1	7	Red	Left
Red	5	1	6	Red	Left
Red	10	3	13	Red	Left
Red	2	0	2	Red	Left
Red	2	0	2	Red	Left
Orange 7.7	3	9	12	Orange 7.7	Right
Red	2	0	2	Red	Right
Orange 7.7	1	7	8	Orange 7.7	Left
Red	4	0	4	Red	Left
Red	17	2	19	Red	Left
Red	3	0	3	Red	Left
Red	5	3	8	Red	Right
Red	5	0	5	Red	Right
Red	3	0	3	Red	Left
Red	3	1	4	Red	Right
Red	5	1	6	Red	Left
Red	5	0	5	Red	Right
		Red 15: Orange 2		**Red 15: Orange 2**	**Left 11: Right 6**
		$p \leq 0.002$		**$p \leq 0.002$**	**n.s.**

Red vs. Orange 12

	Number of Displays				
Preferred Color	**Red**	**Orange**	**Total**	**First Display**	**Preferred Side**
Red	9	5	14	red	Left
Red	4	0	4	red	Left
Red	3	0	3	red	Right
Orange 12	0	6	6	Orange 12	Left
Red	4	1	5	red	Right
Orange 12	0	5	5	Orange 12	Right
Red	3	0	3	red	Right
Red	21	0	21	Orange 12	Right
Red	13	0	13	red	Right
Orange 12	0	11	11	Orange 12	Right
Orange 12	0	6	6	Orange 12	Left
Orange 12	2	17	19	Red	Left
Red	9	0	9	Red	Right
Red	4	2	6	Red	Right
Red	11	0	11	Red	Left
Red	2	0	2	Red	Right
Red	11	1	12	Red	Left
Red	15	3	18	Red	Left
Red	8	4	12	Red	Right
Red	3	1	4	Red	Right
		Red 15: Orange 5		**Red 15: Orange 5**	**Left 8: Right 12**
		p≤0.041		**p≤0.041**	**n.s.**