

Claw coloration in the fiddler crab *Leptuca uruguayensis* has no correlation with male quality

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Abstract

Sexual selection is thought to play a major role in the evolution of color due to the correlation between a signaler's physiological state and the displayed color. As such, researchers often investigate how color correlates to the quality of the signaler. However, research on the relationship between color and individual quality is often taxonomically limited and researchers typically investigate how color phenotypes relate to one index of quality, such as a linear measure of body size. Here, we investigated the relationship among body size, claw size, claw muscle mass, lipid content, and the color of the claw in male fiddler crabs (*Leptuca uruguayensis*) which wield an exaggerated claw that varies in color from brown to red. We hypothesized that if the color was correlated to one or more indices of male quality, the color displayed on the claws of male *L. uruguayensis* could be under sexual selection. We found *L. uruguayensis* claw color varies substantially among the individuals we photographed. However, we did not find a correlation between claw color and indices of quality; neither brightness nor hue correlated to the indices of quality we measured. Our findings suggest that claw color in *L. uruguayensis* is unlikely to have evolved to signal quality, but may instead function as a species identity or as a non-indicator sexual signal.

Key words: animal coloration, animal communication, animal signals, Decapoda, non-indicator sexual signal, sexual selection.

Across animals, the function of color signals and the mechanisms that select for color signals vary (Otte 1974; Bradbury and Vehrencamp, 1998; Cuthill et al. 2017). For example, colors may serve as aposematic signals, such that signalers communicate their unprofitability toward potential predators (Guilford et al. 1990; Mappes et al. 2005; Prudic et al. 2007; Stevens and Ruxton 2012; Ruxton et al. 2018). Other animals use coloration for species or individual recognition where unique colors or patterns can be used to reliably discern between each other (Losos 1985; Couldridge and Alexander 2002; Detto et al. 2006; Siebeck et al. 2010; Caves et al. 2018; Dyson et al. 2020). Color has also been tied to sexual selection through female mate choice, male–male competition, or both (Andersson 1994; Houde 1997; Owens and Hartley 1998; Caro 2005; Ord and Martins 2006; Allen et al. 2011; Chen et al. 2012; Caro and Mallarino 2020). For example, female guppies prefer to mate with males with brightly colored fins

(Houde 1997), whereas in intraspecific contests, lizards in the genus *Anolis* advertise conspicuous colors with their extendable dewlap which serves as a territorial signal to other males (Henningsen and Irschick 2012). Thus, colors have functions that span both natural and sexual selection, with the latter usually being used for intraspecific signaling.

When color production is related to exogenous pigments such as carotenoids, color signals can serve as index signals that reliably communicate information about some characteristic of the signaler to other individuals of the same species, such as body size or condition (Hill 1996; Olson and Owens 1998; Biernaskie et al. 2014; Cuthill et al. 2017; Weaver et al. 2017). For instance, birds that consume and metabolically process carotenoid pigments can deposit these pigments into feathers and the resulting coloration is commonly used as a signal to convey individual fitness (Lozano 1994; Hill 1996, 1999; McGraw et al. 2002; Saks et al. 2003; Weaver et al. 2018). Presumably, only high-quality

birds can sequester enough carotenoids from their diet to invest them in producing vibrant colors. Thus, coloration can enable animals to signal their quality and fitness to receivers (Delhey and Peters 2008; Weaver et al. 2018; White 2020) as more colorful animals are often more likely to be selected by mates (Andersson 1994). However, investigations on the relationship between color signals and indices of quality are taxonomically limited, with a majority of these studies being conducted on vertebrates (Kodric-Brown 1989; Hill 1996; Grether et al. 1999; Hamilton et al. 2013), which leaves gaps in our understanding of the relationship between the evolution of animal coloration and sexual selection.

Furthermore, studies often investigate only how color relates to one putative index of quality related to sexual selection, such as body size, which may fail to fully assess quality signals by not accounting for other indices (Dobson et al. 2008; Stuckert et al. 2019; White 2020). For example, the red-blue polymorphism in coconut crabs (*Birgus latro*) is not correlated to age, sex, or size (Nokelainen et al. 2017; Caro et al. 2019), but this does not rule out the possibility for some other index of quality relates to coloration in this species. Other indices of size or condition such as lipid availability, or even the size of another phenotypic trait, may positively or negatively correlate with color traits. For example, coloration may correlate to an index of competitive ability, such that competitors with specific coloration are more likely to succeed in intraspecific contests compared to others (Govind and Pearce 1993; Huyghe et al. 2007, 2009; Lailvaux and Irtschick 2007; Hamilton et al. 2013). For instance, color is frequently tied to winning fights in species such as the frillneck lizard *Chlamydosaurus kingii* (Hamilton et al. 2013) and the jumping spider *Lyssomanes viridis* (Tedore and Johnsen 2012). In these scenarios, color may relate to the overall size of a structure used in combat or may be related to the performance capabilities of the structure, thus related to indices of quality that are not body size. To fully understand the function of coloration-based signals across animals, it is necessary to investigate diverse taxa, with a diverse range of visual systems, and link color phenotypes to multiple potential indices of quality. One of our goals is thus to expand on current knowledge by providing information on a new taxon with a different type of visual system.

Fiddler crabs (Decapoda: Ocypodidae) are an ideal group to study the relationship between color and sexual selection. Males wield an enlarged, sexually dimorphic claw that can be conspicuously colored (Crane 1975; Detto 2007; Callander et al. 2013; Dyson et al. 2020). Male fiddler crab claws are used in both male-male competition and female mate choice that usually occur in large aggregations in intertidal zones during the daytime (Crane 1975; Callander et al. 2013; Dyson et al. 2020). With nearly 130 fiddler crab species, male claw color varies across species, with a range of colors being displayed from relatively inconspicuous tan color, to bright yellow, orange, and red (Crane 1975). In some species, the color of the carapace is known to vary depending on the time of day or ontogeny and is thought to play a role in thermoregulation (Silbiger and Munguia 2008; Takeshita 2019), but the bodies of most fiddler crabs are cryptically colored compared with their often brightly colored claws (Crane 1975).

Studying the function of coloration in fiddler crabs has often involved a certain amount of speculation as we still have a relatively poor understanding of their color vision. Nevertheless, several studies provide evidence that fiddler crabs have the

basis for at least dichromatic color vision (Hyatt 1974; Horch et al. 2002; Jordão et al. 2007; Rajkumar et al. 2010; Jessop et al. 2020). This is supported by behavioral evidence of color discrimination within fiddler crabs (Hyatt 1975; Detto et al. 2006; Detto 2007; Detto and Backwell 2009; Dyson et al. 2020; Silva et al. 2022). For instance, studies have shown that fiddler crab coloration influences mating decisions; with females preferentially approaching yellow claws over gray claws in *Austruca mjoeberti* (Detto 2007), as well as in separate studies, preferring claws that reflected ultra-violet (UV) wavelengths compared with claws with UV wavelengths experimentally blocked (Detto and Backwell 2009; Silva et al. 2022). However, despite evidence that claw color can function for sexual selection, it remains to be tested whether claw coloration in fiddler crabs is correlated with male quality. One fiddler crab species that offers a good model system for investigating the relationship between claw coloration and male quality is *Leptuca uruguayensis*. The major claw of male *L. uruguayensis* varies in color (hue and saturation) and intensity (luminance) within a population, ranging from pale browns to vibrant reds (Figure 1). The lower outward-facing parts of the enlarged claw, which is the most visible during signaling, usually exhibit the most saturated and consistent coloration, which ranges from dark red to orange in *L. uruguayensis* (Crane 1975). As in other crustaceans, red coloration is maintained through the carotenoid astaxanthin which they sequester from algae, bacteria, and fungi within the sand particles they consume (Crane 1975; Tlustý and Hyland 2005; Helliwell 2010; Wade et al. 2017).

Our main goal was to test if claw color was correlated with indices of individual quality. Given the link between vibrant colors and mate attraction, we hypothesized that if color was correlated to indices of male quality, the color displayed on the claws of male *L. uruguayensis* could be under sexual selection. In this study, we used digital photography to quantify the color of male *L. uruguayensis* claws and then correlate the measurements of claw brightness and hue with multiple suspected indices of quality, including body size, claw size, claw muscle mass, and lipid content in the body (hereafter referred to as body condition). Given the extensive literature tying color to sexual selection, we expected that the color displayed would be positively associated with any indices of male quality we measured. If color is used during male-male competition, we expected muscle mass to be positively associated with claw color. Alternatively, claw color may function outside of sexual selection, such as predator deterrence or species recognition. If this is the case, then we expect color to be uncorrelated with any indices of male quality.

Materials and Methods

Animal collection and sampling site

We collected 39 adult male *Leptuca uruguayensis* at Guaraú (24°36'90" S, 47°01'92" W) in São Paulo State, Brazil, on the 23 November 2018. The sampling site was on the transition zone between a mangrove and a littoral zone, where the substrate was a mixture of sand and mud. We sampled where mangrove trees become sparse, avoiding sand dunes and associated flora were abundant as these areas had few fiddler crabs.

After collection, we stimulated crabs to autotomize their major claw and immediately photographed the claw (see details below) to avoid color change. We euthanized crabs



Figure 1. Variation in color of adult male *Leptuca uruguayensis* claws. Scale bars = 1 cm.

soon after they autotomized their major claw, putting them in plastic vials with 100% ethanol to preserve their lipid and muscle contents. The crab's body and the claws were stored separately to avoid mixing lipids and muscles. We ensured to only collect individuals with original, non-regenerated claws by visually observing features known from original claws, such as tubercles on the pollex and dactyl as well as by observing the manus shape (McLain and Pratt 2011; Graham et al. 2021).

Digital photography and image analysis

To quantify claw color, we used a similar approach to previous studies that used digital photography to quantify animal body color and pattern (e.g., Marshall et al. 2016; Smithers et al. 2017, 2018; Green et al. 2019). We used a Canon Eos Rebel T6i camera with a 100 mm Macro lens, positioned directly above the claw using a tripod for stabilization. Two Yongnuo 565ex II flashes were positioned inside a white Teflon diffuser tent to ensure homogeneous lighting and minimize shadows. All claw photos were taken against the same white background (a white medium-density fiberboard; MDF) on the same day in the field. At the start of the session, we first photographed a set of gray reflectance standards (Datacolor SCK200 SpyderCHECKR 24, Datacolor AG Europe) that were calibrated beforehand (see below). When generating the calibrated images during image analysis (see below) we selected the brightest (86.25% reflectance) and darkest (2.54% reflectance) gray squares as the reference standards. Each claw was photographed in the same position and lighting conditions as the reflectance standards. A scale bar was included in each photo and all images were taken in RAW format with manual camera settings (F9.0, ISO 100, Shutter speed 1/100s) and white balance. All photos were taken in the visible spectrum.

To calibrate the gray reflectance standards, we used an Ocean Optics USB 4000 spectrophotometer and a halogen light source. With the spectrophotometer, we measured

reflectance between 400 and 700 nm at 1 nm intervals with reference to a white standard (white side of a Kodak R-27 Gray card) and black standard (light source disabled). We oriented the probe at a 90° angle to the surface we intended to measure. Dark current and white standard reference measurements were taken immediately before measuring each square of the color checker to minimize any error associated with the drift of the light source and sensor.

We conducted image analysis by using the Multispectral Image Calibration and Analysis (MICA) Toolbox (Troschianko and Stevens 2015; van den Berg et al. 2020). Briefly, each image underwent linearization and standardization to control for non-linear image responses to light levels and illuminating conditions (Stevens et al. 2007; Troschianko and Stevens 2015). Following this, the manus of each claw was selected as a “region of interest.” The output from the MICA Toolbox was percentage reflectance for each of the camera's three-color channels, red, green, and blue, which we then used for the subsequent analysis described below.

Calculation of hue and brightness

The next step was to quantify the achromatic and chromatic aspects of claw color in a way that, to the best of our ability and knowledge, takes into consideration the visual system of the fiddler crabs. It should be noted that we do not attempt to directly model fiddler crab vision, but rather our approach aims to analyze the color data in the most biologically meaningful way given that so much about fiddler crab color vision and processing (including any possible opponency channels) is still unknown. As mentioned in the introduction, available evidence suggests that fiddler crabs, like many other crustaceans, most likely have a dichromatic visual system (Hyatt 1975; Horch et al. 2002; Johnson et al. 2002; Cronin and Porter 2008; Rajkumar et al. 2010; Jessop et al. 2020). When dealing with trichromatic visual systems (such as that of humans), and often also higher systems (such as the tetrachromatic system of birds), it is common within the literature

for the chromatic component of light to be defined according to two basic dimensions (hue and saturation), whereas the achromatic component is often defined as brightness (Kelber et al. 2003; Kelber and Osorio 2010). Brightness is the subjective measure of light intensity on a scale of black to white (Kelber et al. 2003; Kelber and Osorio 2010). Hue is used as a measure of color type and is the attribute denoted by terms such as red, yellow, green, and blue (Kelber et al. 2003; Kelber and Osorio 2010). In the context of studies that model animal visual systems, hue is often calculated as a ratio of the activity in one or more color channels over the activity in one or more other color channels (e.g., Spottiswoode and Stevens 2011; Smithers et al. 2018; Green et al. 2019; Duarte et al. 2021). This approach is broadly based on how opponent color channels in animal vision are thought to work. Finally, saturation is a measure of spectral purity (the amount of a given color type compared with white light) and is defined as the distance from the achromatic gray point (Kelber et al. 2003; Kelber and Osorio 2010).

Although this approach to describing and reporting color is common within the color literature, it is not directly transferable or applicable to a dichromatic visual system. This is because, unlike trichromatic (or higher) color space, dichromatic color space only has 2 dimensions meaning that hue and saturation are not independent. Hence, changes in wavelength can be readily confounded by changes in spectral purity and vice-versa (Wachtler et al. 2004; Roth et al. 2007; Kelber and Osorio 2010). Therefore, in this study, we calculated only 2 (brightness and hue) of these 3 dimensions. Horch et al. (2002) reported 2 visual pigments in the fiddler crab *Leptuca thayeri*. One with a peak absorption in the blue part of the spectrum (λ_{\max} 430 nm) and another with a peak absorption in the green part of the spectrum (λ_{\max} between 500 nm and 540 nm) (Horch et al. 2002). Therefore, for our first measure of brightness and hue, we calculated Brightness 1 as:

$$\text{Brightness 1} = \text{blue} + \text{green}$$

and Hue 1 as:

$$\text{Hue 1} = \frac{\text{blue}}{\text{green}}$$

where blue and green are the reflectance values from the camera's blue and green color channels respectively.

There is evidence to suggest that the spectral sensitivity of the R1-7 photoreceptors in some fiddler crabs can extend into the orange-red region of the spectrum (Jordão et al. 2007). Thus, crabs could have at least some sensitivities to variation in claw redness. This is somewhat analogous to how a red-green color-blind human may sometimes perceive certain red colors as dark greens. To account for the possibility of extended sensitivity into the red part of the spectrum, we calculated a second measure of brightness as follows:

$$\text{Brightness 2} = \text{blue} + \text{green} + \text{red}$$

and an additional measure of hue was calculated as:

$$\text{Hue 2} = \frac{\text{blue}}{(\text{green} + \text{red})}$$

where blue, green, and red are the reflectance values from the camera's blue, green, and red color channels, respectively.

It is important to note that there is evidence that some fiddler crab species are sensitive to ultraviolet (UV) wavelengths (Detto and Backwell 2009; Rajkumar et al. 2010; Jessop et al.

2020; Silva et al. 2022). However, we were unable to photograph the UV part of the spectrum because the camera had not undergone the necessary quartz conversion to enable UV sensitivity (Trosianko and Stevens 2015). Thus, although UV is not accounted for in any of the above calculations of brightness or hue, its absence should not negate its potential, but unknown, importance for *L. uruguayensis*.

Morphology, claw muscle mass, and lipid measurements

To investigate the potential correlation between color (brightness and hue) and indices of quality, we collected measurements of body size, claw size, claw muscle mass, and body condition (i.e., stored lipids), for each crab. For measurements of size, we measured both carapace width and claw length using a caliper with 0.01 mm precision. Carapace width was measured across the widest point of the carapace, whereas claw length was measured from the base of the propodus to the tip of the pollex.

We also collected data on claw muscle mass, which may serve as a proxy for an individual pinching force and fighting ability (Palaoro et al. 2020; Graham et al. 2021). To estimate the muscle mass of each claw, we first removed the preserved claw from the alcohol, dried it in an oven at 55 °C for 20 h, and then measured its mass on a precision scale (0.1 mg) to obtain the dry mass value. Next, we immersed the claws in 10% potassium hydroxide (KOH) for 20 h to digest the muscular tissue. We then dried the claw again in an oven at 55 °C for 20 h and reweighed them. The difference between the dry mass and the final mass of each claw was taken as a proxy for claw muscle mass.

Lastly, we measured the body condition of each crab based on measurements of the total amount of lipids stored within an individual (Koop et al. 2011). Across animals, lipid content is used as an index of body condition and individual quality due to its correlation to food quality (Cranford 1999; Blanckenhorn et al. 2003; Svensson et al. 2004; McKinney et al. 2014; Aoki et al. 2021). Thus, by estimating the amount of lipids, we can infer if color is associated with male quality. Measuring lipid content consisted of 2 steps: (i) the extraction of the lipids from the tissue, and (ii) the measurement of total lipids in the sample. For extracting lipids, we used the protocol from Folch et al. (1957) and Parrish (2002) and adjusted it for small tissue samples. To do this, we dissected the individuals and weighed the internal contents of the carapaces (e.g., stomach, gonads, gills, and hepatopancreas). After a series of dilutions, homogenizations, and drying, the lipids were diluted in 250 µL of chloroform and stored at -20 °C until we determined the total lipids. Given the number of details in this protocol, we added a detailed explanation to the [Supplementary Material](#) where each step of the extraction is thoroughly explained.

For the second step, measuring total lipids, we used an adapted protocol from three papers that have previously measured lipid content (see Frings and Dunn 1970; Frings et al. 1972; Knight et al. 1972). The protocol consisted of suspending the lipids with sulfuric acid and adding a phosphovanillin reagent to color the lipids. We then put the suspension in a spectrophotometer and measured the absorbance of each sample at 540 nm calibrated with a solution prepared only with reagents (i.e., without a lipid sample). The absorbance values of the lipid samples obtained from the crabs were used in the linear equations calculated for the standard curve,

and thus we estimated the expected amount of lipids for each absorbance value of the samples. Because the estimated lipid mass of the samples was calculated for each 20 μL of lipid extract sample, we calculated the mass of lipids for the total 250 μL of the sample (which contained all the lipids extracted from the individuals). Knowing the total mass of the entrails of each collected individual, we obtained the amount of lipids (in mg) that each crab had per gram of entrails (mg/g), and this value was used to infer the quality of the collected individuals (i.e., the more stored lipids, the higher the quality of the individual). A detailed explanation of all the steps performed here, including dilutions with concentrations and use of standard curves can be found in the [Supplementary Material](#).

Statistical analyses

All statistical analysis and plotting were performed in R (R Core Team: 2019). We first plotted the percentage reflectance for each of the camera's color channels (red, green, and blue) in a ternary plot to identify which color channel(s) exhibited the most variation between individuals.

Our goal was to test if color correlated to indices of quality; this entailed testing the correlation of both brightness and hue to our indices of quality. Each measure of brightness (1 and 2, see above) and hue (1 and 2, see above) was a dependent (response) variable in separate analyses, and each index of

quality was an independent variable in separate models. Thus, we built four models for each dependent variable: (i) brightness 1 on carapace width, (ii) brightness 1 on claw length, (iii) brightness 1 on claw muscle mass, and (iv) brightness 1 on lipid percent. The same models were built for brightness 2, hue 1, and hue 2. However, the package “*performance*” showed that the residuals of these models were heteroscedastic, which could lead to spurious correlations (Lüdecke et al. 2021). Further, the package also showed that some independent variables were highly correlated with one another ($r > 0.8$). Given that the index signals have clear predictions for all these measures—and these can be traced back to the theory, we did not discard any of the independent variables. Instead, we opted to perform a Spearman's rank test to test the correlation between our measures of color (brightness and hue) and each index of quality individually. We maintained the structure of the models but used a non-parametric test to test the strength of the association between our variables using the function “*cor.test()*” implemented in R software (R Core Team: 2019).

We also regressed red reflectance (dependent variable) against each index of quality in separate models. Each index of quality was the independent variable in our model. This approach entailed building four regression models: (i) red reflectance on carapace width, (ii) red reflectance on claw length, (iii) red reflectance on claw muscle mass, and (iv) red

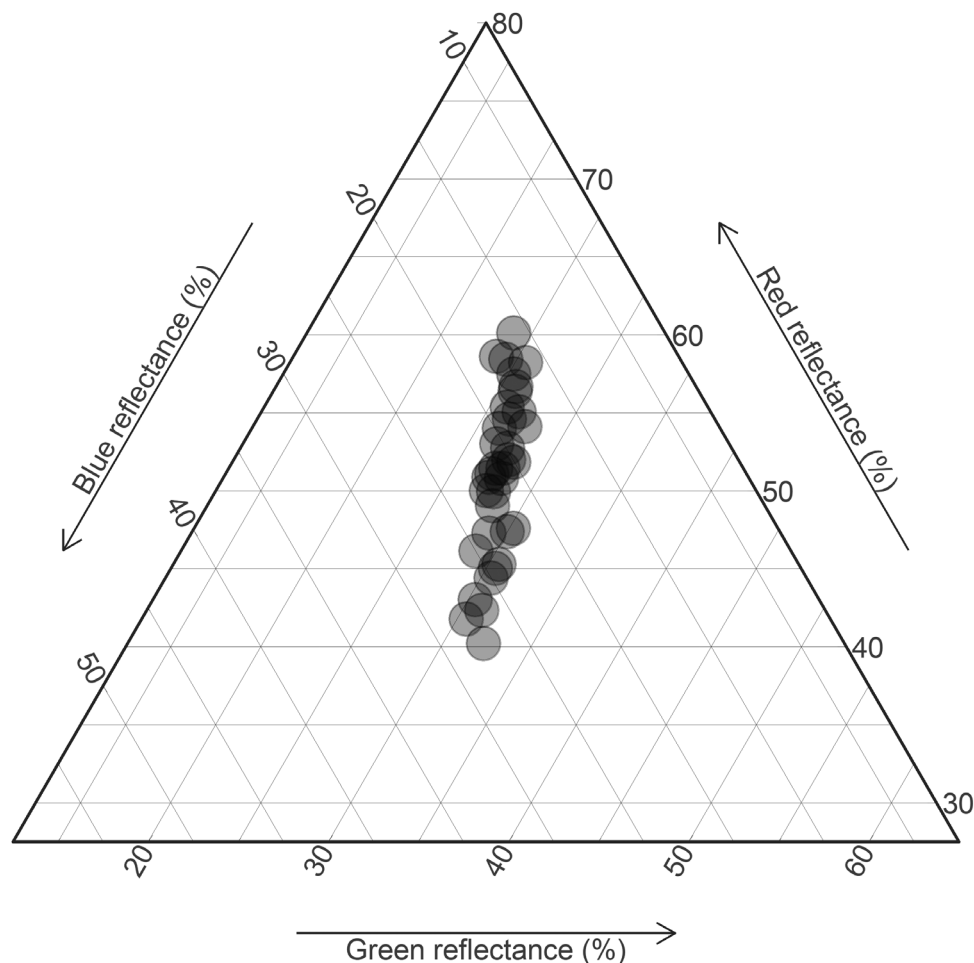


Figure 2. Ternary plot depicting the percent reflectance of red, green, and blue wavelengths, based on the corresponding RGB color channels from the camera, measured from 39 male *Leptuca uruguayensis* claws.

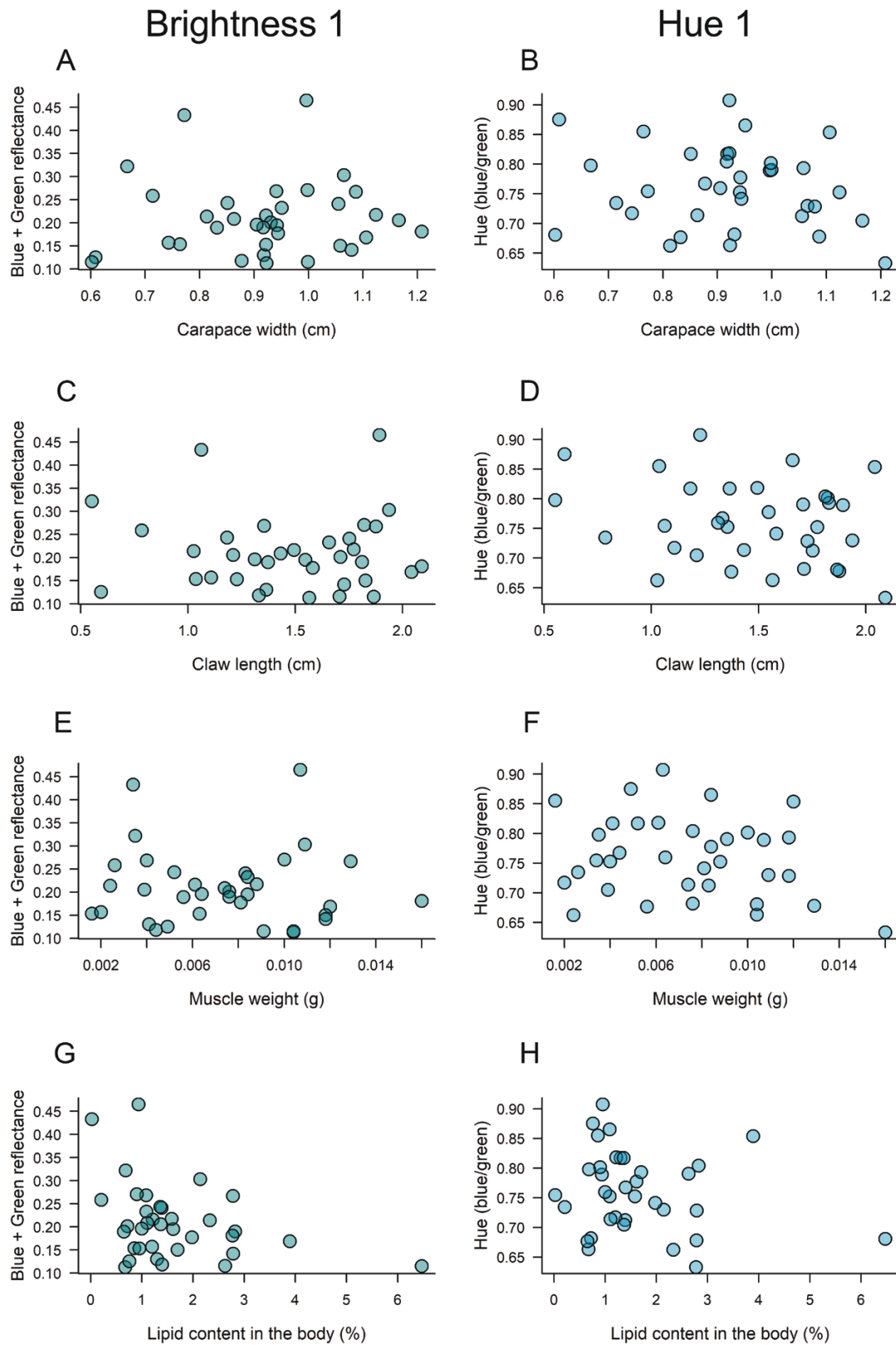


Figure 3. The relationship between brightness 1 (left column) and hue 1 (right column) with indices of quality. Brightness 1 is the sum of the green and blue reflectances, whereas hue 2 represents a ratio of the blue reflectance by the green reflectance. Indices of quality are represented in rows, hence carapace width (A, B), claw length (C, D), claw muscle mass (E, F), and lipid content in the body (G, H). There was no significant correlation between these variables. All figures represent data from 39 *Leptuca uruguayensis* males.

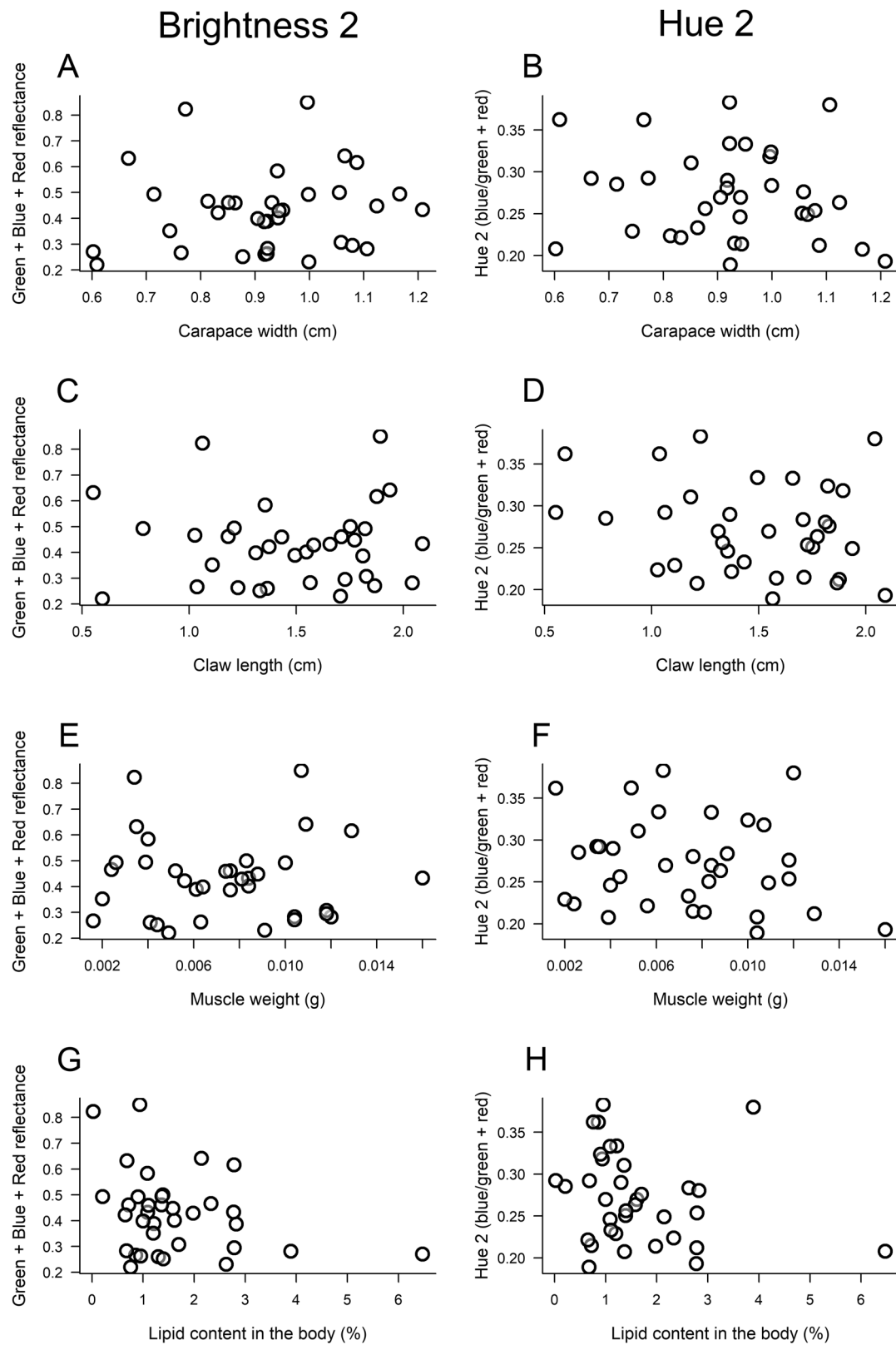


Figure 4. The relationship between brightness 2 (left column) and hue 2 (right column) with indices of quality. Brightness 2 is the sum of the reflectances of all color channels (green, red, and blue), whereas hue 2 represents a ratio of the blue reflectance by the green and red reflectances summed. Indices of quality are represented in rows, hence carapace width (A, B), claw length (C, D), claw muscle mass (E, F), and lipid content in the body (G, H). There was no significant correlation between these variables. All figures represent data from 39 *Leptuca uruguayensis* males.

Table 1. Results from the Spearman rank's tests of correlation between brightness, hue, and indices of quality. From each model, we report rho (the correlation coefficient of the Spearman correlation test) and the associated *P*-value. The residuals of all models were heteroscedastic, so we used a non-parametric test to avoid biased results. Results are reported from the analysis of 39 male *Leptuca uruguayensis* claws. Formulas presented in the table are in the following format: Dependent variable ~ independent variable; the tilde denotes that the dependent variable is in function of the independent variables.

Model (formula: $y \sim x$)	Rho (ρ)	<i>P</i> -value
Brightness		
(a) Brightness 1 ~ carapace width	0.104	0.552
(b) Brightness 1 ~ claw length	-0.004	0.980
(c) Brightness 1 ~ muscle mass	-0.100	0.570
(d) Brightness 1 ~ lipid content in body	-0.272	0.114
(e) Brightness 2 ~ carapace width	0.182	0.296
(f) Brightness 2 ~ claw length	0.065	0.711
(g) Brightness 2 ~ muscle mass	-0.023	0.897
(h) Brightness 2 ~ lipid content in body	-0.204	0.239
Hue		
(i) Hue 1 ~ carapace width	-0.135	0.441
(j) Hue 1 ~ claw length	-0.186	0.282
(k) Hue 1 ~ muscle mass	-0.152	0.382
(m) Hue 1 ~ lipid content in body	-0.145	0.402
(n) Hue 2 ~ carapace width	-0.185	0.287
(o) Hue 2 ~ claw length	-0.217	0.210
(p) Hue 2 ~ muscle mass	-0.176	0.313
(q) Hue 2 ~ lipid content in body	-0.266	0.112

reflectance on lipid percent. Given that reflectance is given in percentage, we opted to use a generalized linear model with a beta distribution (a beta regression) implemented in the package “betareg” (Cribari-Neto and Zeileis 2010). The beta distribution is bounded by values between 0 and 1, and thus, can deal with the distributions of the reflectance. To test how blue and green reflectance correlates to the indices of quality, we used the same approach as for the red reflectance, but we changed the dependent variable (from red reflectance to blue reflectance and then to green reflectance).

Results

We found high intraspecific variation in red and blue wavelength reflectance of the major claw of male *Leptuca uruguayensis*, whereas there was little intraspecific variation within the green channel (Fig. 2). The highest percentage reflectance was in the red channel (mean = 20.646; median = 22.002; SD = 7.84), with lower reflectance in the blue (mean = 8.497; median = 8.493; SD = 3.491), and green channels (mean = 11.217; median = 11.528; SD = 4.471).

Overall, we found no correlation in any of our variables. Brightness 1 and hue 1 were not correlated with any indices of quality (Figure 3). Similarly, we also found no correlation between either brightness 2 or hue 2 and the indices of quality (Figure 4). The correlation values and associated *P*-values can be found in Table 1.

Similarly, we found no correlations for most of the raw reflectance values for red (Figure 5) and green reflectance (Figure 6). However, there was a weak negative association between blue reflectance and lipid content (Figure 7), where each increase in percentage of lipid in the body decreased blue reflectance by 0.03 (or 3%; Figure 7). Estimates, standard errors, test statistics and *P*-values can be found in Table 2.

Discussion

We found that the enlarged claw of male *Leptuca uruguayensis* varied widely in their reflectance measurements, specifically within the blue and red regions of the visible spectrum. Despite this variation, we found little evidence to support the hypothesis that claw color (brightness and hue) correlates with the indices of male quality we measured. Interestingly, even the raw values of red reflectance, which is the most salient color perceivable by the human visual system, did not correlate with body size, claw size, claw muscle mass, or body condition (lipid content in body) (Figure 5 and Table 2). By contrast, when looking at the values for blue reflectance, there was a weak negative relationship between lipid content in the body and the percentage of blue wavelengths reflected (Figure 7 and Table 2). Nevertheless, this weak relationship was not apparent in our main results for hue 1 and hue 2, nor for brightness 1 and brightness 2, suggesting it is unlikely to be important for selecting mates or winning fights in these crabs. This highlights the importance of accounting for an animal's visual system as much as possible when studying the role of color signals.

Overall, our results imply that the color (at least within the visible spectrum) of the enlarged claw of *L. uruguayensis* is unlikely to be under sexually selected pressure as a signal to communicate the indices of individual quality measured in this study. However, it is important to note that in this study we were unable to measure UV reflectance so we cannot rule out the possibility that UV coloration could correlate with male quality, even if visible reflectance does not. This is important to note because there is evidence that some fiddler crab species are sensitive to ultraviolet (UV) wavelengths (Detto and Backwell 2009; Rajkumar et al. 2010; Jessop et al. 2020; Silva et al. 2022). Nevertheless, our finding still raises questions regarding the role that claw color plays in

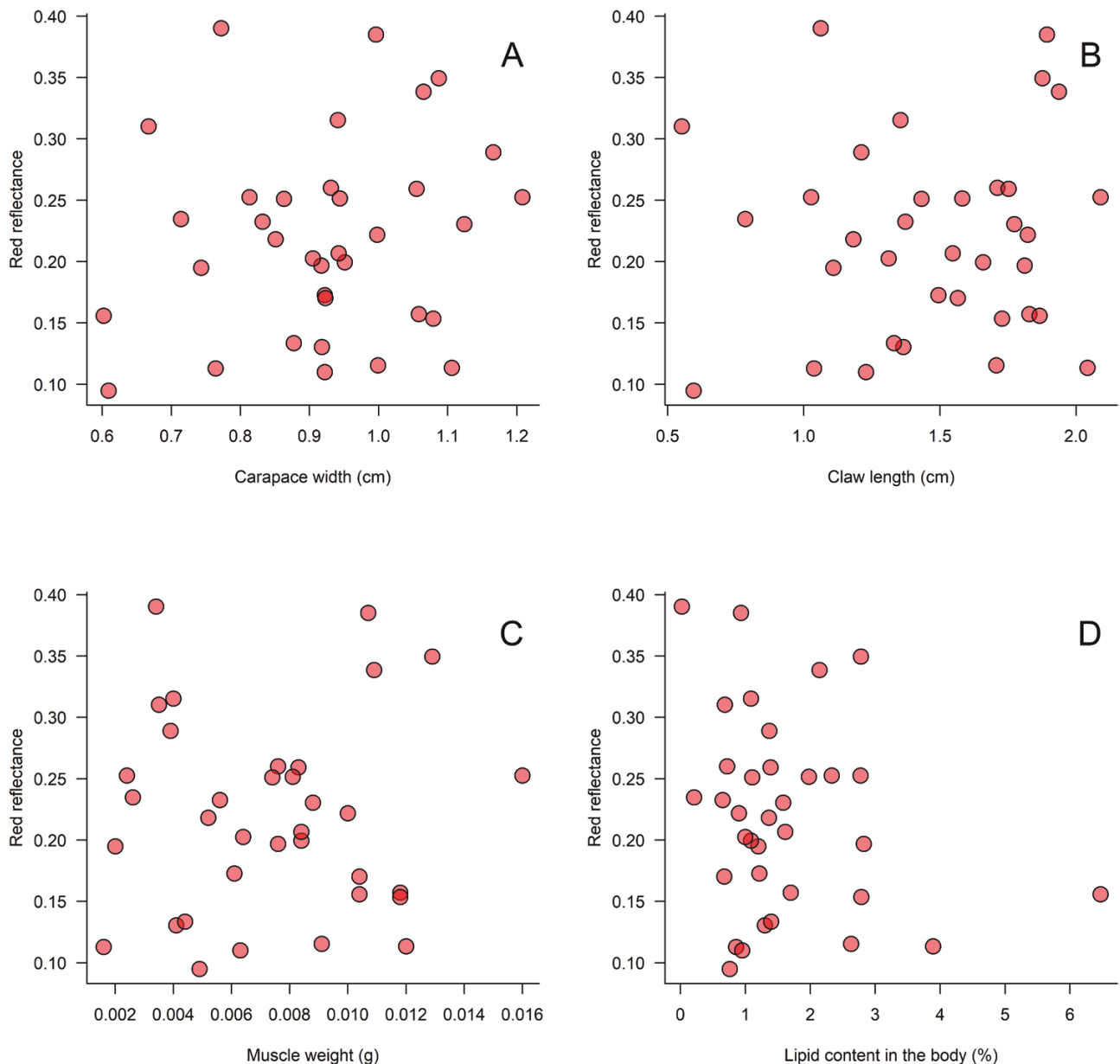


Figure 5. The relationship between reflectance within the red channel and (A) carapace width, (B) claw length, (C) claw muscle weight, and (D) lipid content in the body. There was no significant relationship between these variables. All figures represent data from 39 *Leptuca uruguayensis* males. Because none of the correlations were significant, we did not draw tendency lines.

mate choice and male–male interactions. Below, we discuss potential explanations for the function of the coloration of *L. uruguayensis* claws and evidence of color vision in fiddler crabs more broadly.

Despite evidence that suggests the color of male fiddler crabs and their enlarged claw is under sexual selection (Detto et al. 2006, 2008; Detto and Backwell 2009; Silva et al. 2022), there is little evidence to date that color is related to male quality or condition. Much of the work on the evolution of sexually selected colors assumes a relationship between color and some component of the signaler that correlates to individual quality or fitness (von Schantz et al. 1999; Biernaskie et al. 2014; Higham 2014). Thus, there is often an assumed cost to producing, maintaining, or wielding a conspicuous color signal that relates to the assumption that color is an index signal (Andersson 1994; Hill 1996, 1999; Weaver et al.

2017). In arthropods specifically, the costs of color signals are seldom studied, which leads to unfulfilled assumptions about the evolution of these traits. Further, even if a color signal is condition-dependent such that reliable information can be signaled based on the coloration, behavioral evidence must be provided before there can be a well-supported link between color's role in sexual selection. For example, Taylor et al. (2014) found that although the jumping spider *Habronattus pyrrithrix* shows red coloration that is condition-dependent, this color does not function as a signal in mating interactions (Taylor et al. 2014).

An additional explanation of why claw color in fiddler crabs might not be an index signal comes from the development and growth of fiddler crabs. Detto (2008) shows how the most dramatic changes in color occur during molting in fiddler crabs. Individuals can have small changes in brightness

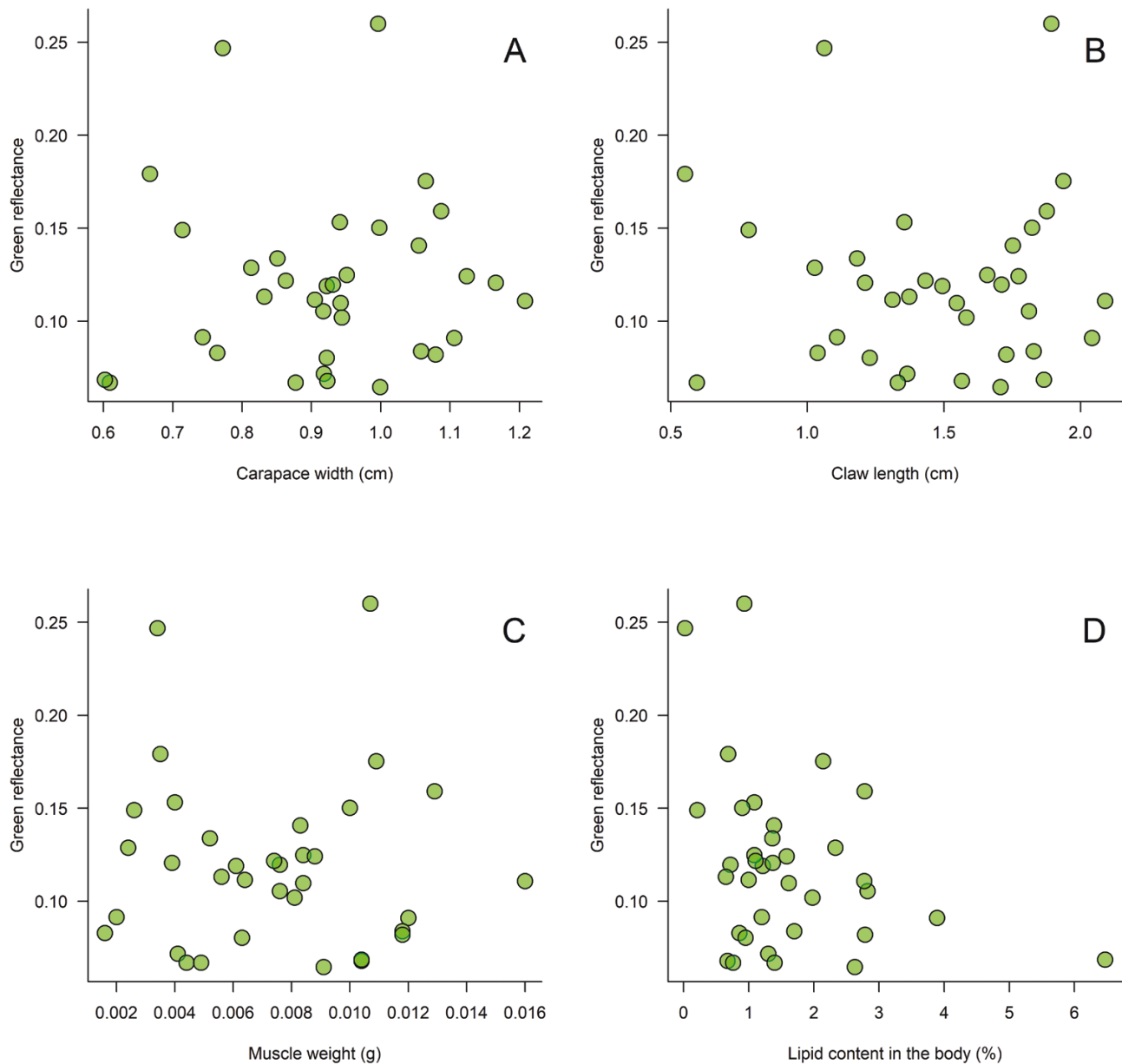


Figure 6. The relationship between reflectance within the green channel and (A) carapace width, (B) claw length, (C) claw muscle weight, and (D) lipid content in the body. There was no significant relationship between these variables. All figures represent data from 39 *Leptuca uruguayensis* males. Because none of the correlations were significant, we did not draw tendency lines.

when they are in intermolt period, but the current literature does not mention if these changes can happen in the claw itself or if they are specific to the carapace. Birds go through a similar process, where color change happens mostly when the feather is growing. After feathers are fully developed, color changes are mostly stopped (Willoughby et al. 2002; Delhey et al. 2017; Valdez and Benitez-Vieyra 2023). Thus, birds and fiddler crabs, color apparently can be seen as a snapshot of quality during the last molt. The critical issue is the timing of the molt. Some birds molt before reproduction (Valdez and Benitez-Vieyra 2023), which creates less problems for interpretations. Fiddler crabs, however, do not necessarily molt before breeding. As fiddler crabs age, molting becomes less frequent, and thus, color becomes a more distant proxy of their current quality. However, younger (i.e., smaller) individuals should provide a more accurate snapshot of their current

quality because of their frequent molting. If the color change is tied to the molting period, then any color signal being conveyed might provide unreliable information about the sender, decreasing the likelihood of claw color being an index signal.

However, claw color in *L. uruguayensis* could be sexually selected if it is either related to a Fisherian process of evolution or if it increases the detectability of the male. In a Fisherian runaway process, any arbitrary trait can evolve if it is related to mating preference. If there is any genetic correlation between color and mating preference for color, claw color may be selected regardless of any production and/or maintenance costs associated with the trait (Prum 2010). Several theoretical models have already shown that traits associated with female preference will be selected under a Fisherian process of evolution (Pomiankowski and Iwasa 1998; Tazzyman et al. 2014; Millan et al. 2020). Another non-mutually exclusive

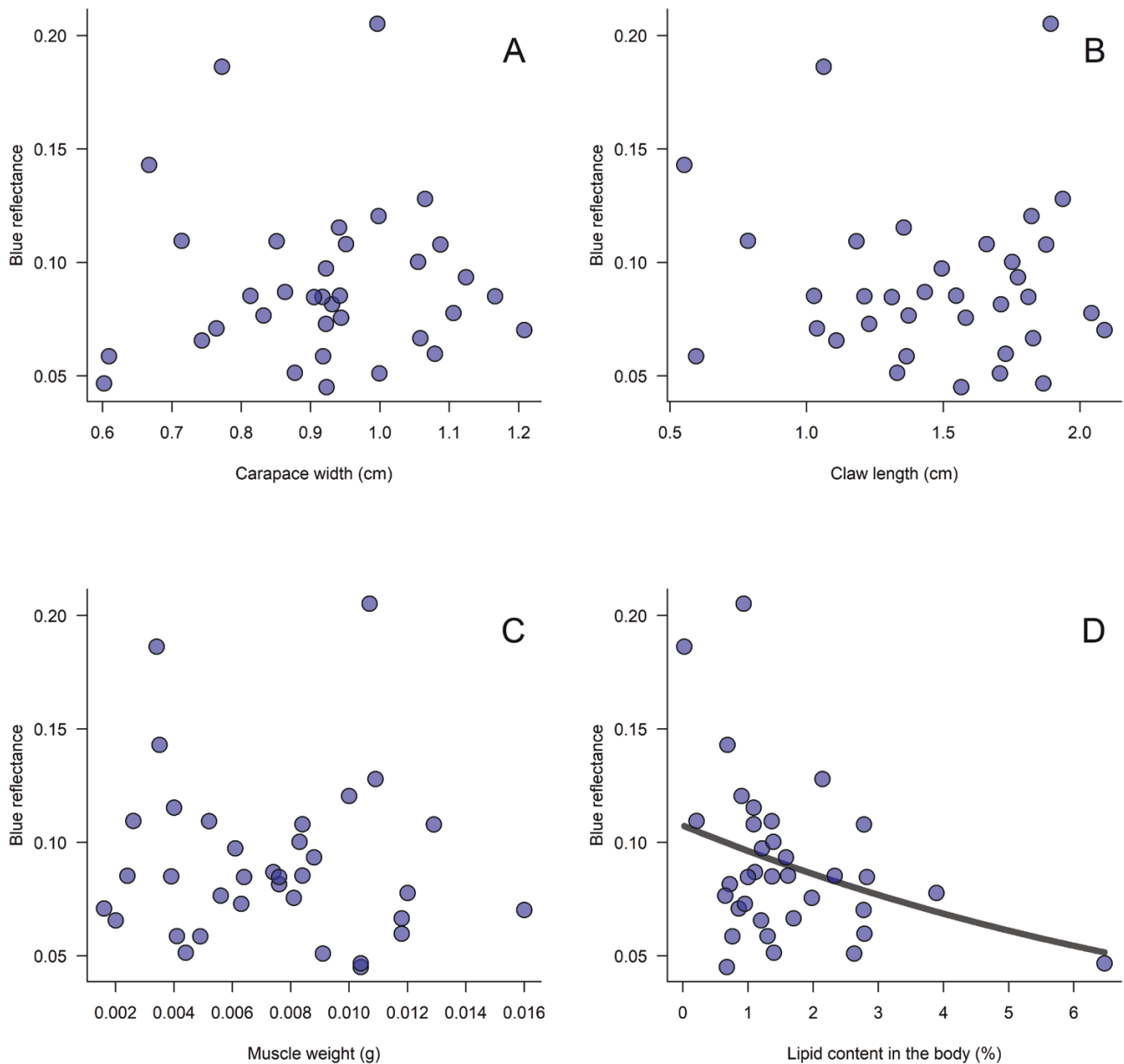


Figure 7. The relationship between reflectance within the blue channel and (A) carapace width, (B) claw length, (C) claw muscle weight, and (D) lipid content in the body. The solid line represents the significant correlation between lipid content and blue reflectance using a beta regression (hence the non-linearity of the curve). There was no significant relationship between these variables aside from a significant negative relationship in (D). All figures represent data from 39 *Leptuca uruguayensis* males.

hypothesis is that claw coloration might have evolved via sensory drive by increasing detectability to potential mates or competitors (Endler 1992). Both these processes predict signals that are under sexual selection but that do not necessarily correlate with individual fitness. Testing for either of these processes is hard, but when we combine: (i) previous evidence suggesting that claw coloration is used in mate choice in other species of fiddler crabs and that UV can be used during mate choice (Detto et al. 2006; Detto 2007; Detto and Backwell 2009; Silva et al. 2022), and (ii) our evidence that color is not correlated to index signals, it suggests that claw coloration in fiddler crabs might be selected through one of these processes.

The lack of a strong correlation between claw color and male quality could also suggest that the coloration of *L. uruguayensis* is a non-costly species-identification signal (Dale

2006). Using color to identify other members of the same species would certainly be beneficial given that *L. uruguayensis* is sympatric with up to nine other species of fiddler crab within the northern part of its range (Crane 1975; Rosenberg 2020). For example, *L. uruguayensis* is known to share the same habitat as *Minuca rapax*, *Leptuca thayeri*, *Uca maracoani*, *Minuca burgersi*, *Leptuca cumulanta*, and *Leptuca leptodactyla* (Crane 1975; Checon and Costa 2018; Arakaki et al. 2020). Many of these species have claws with different coloration from *L. uruguayensis*. For instance, the claws of some of these species differ in color, from yellow and tan in *L. thayeri* and *M. rapax*, to orange and orange-red in *U. maracoani* and *M. burgersi*. At the location where we collected *L. uruguayensis*, we also saw *M. rapax* and *L. leptodactyla* in the same micro-habitat, both of which have a yellowish-tan

Table 2. Results from the tests of a relationship between red, green, and blue reflectance and body/claw measurements. From each model, the estimate (β), standard error (SE), z -value, and P -value are reported. All models assumed a beta distribution. Results are reported from the analysis of 39 male *Leptuca uruguayensis* claws. Formulas presented in the table are in the following format: Dependent variable ~ independent variable, the tilde denotes that the dependent variable is in function of the independent variables. Significant effects are bolded.

Model (formula: $y \sim x$)	Red			
	Estimate (β)	SE	z -value	P -value
(a) Percent red reflectance ~ carapace width	0.543	0.505	1.076	0.282
(b) Percent red reflectance ~ claw length	0.098	0.194	0.507	0.612
(c) Percent red reflectance ~ muscle weight	6.177	21.524	0.287	0.774
(d) Percent red reflectance ~ lipid content in body	-0.079	0.065	-1.200	0.23
	Blue			
(e) Percent blue reflectance ~ carapace width	0.161	0.435	0.369	0.712
(f) Percent blue reflectance ~ claw length	-0.064	0.164	-0.390	0.697
(g) Percent blue reflectance ~ muscle weight	-9.324	18.44	-0.506	0.613
(h) Percent blue reflectance ~ lipid content in body	-0.123	0.057	-2.153	0.031
	Green			
(i) Percent green reflectance ~ carapace width	0.262	0.484	0.584	0.559
(j) Percent green reflectance ~ claw length	-0.009	0.171	-0.058	0.954
(k) Percent green reflectance ~ muscle weight	-3.893	19.038	-0.205	0.838
(l) Percent green reflectance ~ lipid content in body	-0.013	0.006	-2.003	0.053

major claw. Therefore, at our sampling site, rather than serve as a signal of quality, the different claw coloration and waving displays of *L. uruguayensis* and sympatric fiddler crabs may function as a species identification signal, used to distinguish between the males of these species.

A possible species-identity function of claw coloration is supported by other studies on fiddler crabs. For example, the enlarged male claw of the fiddler crab *Austruca mjoebergi* vary in the degree of yellowness (Dyson et al. 2020), comparable to the variation in redness that we observed in *L. uruguayensis*. Dyson et al. (2020) found that female *A. mjoebergi* discern between robotic crabs with claws artificially colored with colors that fall outside the natural range exhibited by this species (Dyson et al. 2020). However, despite distinguishing between colors within and outside of the typical range of claw coloration, females did not discriminate between the hue, chroma, or intensity of claws that fell within the natural range of male claws. The authors concluded that despite exhibiting variation in claw coloration, these colors were unlikely to signal information to receivers about the quality of the signaler but were instead likely to serve a function in species recognition. Other research has demonstrated that *Tubuca capricornis* can discern between co-occurring fiddler crab species based on both color patterns on their carapace and the coloration of the male's enlarged claw (Detto et al. 2006). Color has also been found to be used in mate/species recognition in other Brachyura (Baldwin and Johnsen 2009, 2012; Chan et al. 2021). Future studies should combine data on claw color with behavioral experiments within *L. uruguayensis* to explicitly test the hypothesis that claw color serves as a signal in species recognition. Alternatively, claw coloration and variation in *L. uruguayensis* and other fiddler crab species could be a neutral and conserved trait (Caro et al. 2019; Caro 2021)—but again, behavioral studies are required before adaptive or non-adaptive hypotheses of claw color can be supported.

Despite evidence from the literature that fiddler crabs can discriminate color, we still have a relatively poor

understanding of their color vision compared with other taxonomic groups. This is partly due to the fact that characterizing the visual pigments within the photoreceptors of fiddler crabs has proved challenging over the years (Forward et al. 1988; Jordão et al. 2007). For example, Jordão et al. (2007) reported finding a single visual pigment within the R1-7 photoreceptors of four species of fiddler crabs. However, they were unable to measure the visual pigment within the much smaller R8 photoreceptor that is thought to house the shorter wavelength (UV/blue) sensitive pigment that, together with the pigment in the R1-7 photoreceptors, would provide the bases for color vision (Jordão et al. 2007). Nevertheless, although a definitive consensus is still lacking, physiological evidence generally supports the suggestion that fiddler crabs likely have a dichromatic color vision system (Hyatt 1975; Horch et al. 2002; Rajkumar et al. 2010; Jessop et al. 2020), which we attempted to take into account, but not directly model, in this study. As previously discussed, a limitation of our study is that we were not able to measure and account for the UV part of the spectrum which is known to be important for other species of fiddler crabs (Detto and Backwell 2009; Silva et al. 2022). Another important aspect of fiddler crab vision we did not consider in this study is their sensitivity to polarization (How et al. 2012, 2015; Smithers et al. 2019). This is a particularly interesting area for future research given that there is evidence that fiddler crabs process polarization as an independent channel of information (Smithers et al. 2019).

In conclusion, we reveal substantial variation in the coloration of male *L. uruguayensis* claws. Although evidence from other studies on crabs suggests that claw coloration can serve as a signal of male quality (Detto et al. 2004; Baldwin and Johnsen 2009), we found little support for this hypothesis in *L. uruguayensis*. Instead, we suggest that fiddler crab claw coloration, at least in the visible spectrum, likely serves either as a species-identification signal or as a signal selected through a Fisherian runaway process or sensory drive.

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Conflict of interest

None declared.

Data availability

All data and code can be found on GitHub (https://github.com/alexandrepaoro/fiddler_color). If you want the photographs of the claws, please email the corresponding author—they are too heavy to host on GitHub.

Supplementary Material

Supplementary material can be found at <https://academic.oup.com/cz>.

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