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Color variation in signal crayfish *Pacifastacus leniusculus*

Roberto SACCHI***, Stefania C**ANCIAN**, Daniela G**HIA**, Gianluca F**EA**, and Alan C**OLADONATO**

Dipartimento di Scienze della Terra e dell'Ambiente, Università degli Studi di Pavia, Viale Taramelli 24, Pavia, I-27100, Italy

*Address correspondence to Roberto Sacchi. E-mail: roberto.sacchi@unipv.it

Abstract

External coloration in animals depends on the interaction of several different factors including the genetics and epigenetics processes that underlie the color expression, the mechanisms of color perception, and the general mechanisms controlling color evolution and function. Among all, camouflages from predators and conspicuousness are of particular interest because pose animal to choose between opposite adjustment in coloration. The external coloration of crustaceans is mainly due to the accumulation of carotenoids in the exoskeleton and the epidermal layer, and the trade-off between camouflage and communication had led to a variety of responses, involving signal partitioning, spectral sensibility, changing coloration, or signaling behavior. Here, we used digital images to explore intrapopulation variability of the external coloration of *Pacifastacus leniusculus* among body regions within an individual and between sexes. We found that 1) ventral coloration of claws are more saturated and brilliant than upper parts, 2) males express a more saturated and brightness coloration than females, especially on the lower portion of claws, 3) color intensity and brightness increases with size differently in different body regions, and 4) brightness is more variable in males than in females. All the above patterns support the hypothesis that color in this species could be the result of a compromise between camouflage from predators and conspicuousness for communication. The results of this study suggest that carotenoid might have something to do with intraspecific communication and perform more complex functions than that of a simple pigment.

Key words: crayfish, external coloration, HSV, individual variation, sexual dimorphism

The study of animal coloration is an interdisciplinary sector in great growth that tries to explain the different ways in which animals develop the colors, the genetics, and epigenetics processes that underlie the color expression, the mechanisms of color perception, and the general mechanisms controlling color evolution and function (Cuthill et al. 2017). A lot of effort has been directed to these mechanisms, with the understanding that animal coloration represents a compromise between pressures that may conflict one another, rather than the result of a single key factor (Endler 1981; Stuart-Fox and Moussalli 2009; Cuthill et al. 2017). Camouflage from predators and conspicuousness for communication requires opposite adjustment in coloration, as crypsis leads to reduce the contrast between color and background, whereas signals for communication promote bright and flashy colors, increasing the contrast with the background (Franklin et al. 2020). This trade-off between has several solutions both animals, including 1) signal partitioning, in which body regions visible to predators are used for camouflage and body regions hidden from potential predators are used for signaling

(Stuart–fox and Ord 2004), 2) colors that are not visible to predators but only to conspecifics, like ultra-violet or polarized light (Cummings et al. 2003; Siebeck et al. 2010; Abramjan et al. 2020), 3) spatial resolution of coloration in a way that color is conspicuous only at a short distance, whereas at distance colors blend matching the background (Barnett et al. 2018), and 4) changing colorations that become cryptic or conspicuous when necessary (Stuart-Fox et al. 2008), or 5) altering signaling behaviors when predators are present (Endler 1987).

Crustaceans show a huge variety of color patterns that are used both for camouflage to predators (Stevens et al. 2013, 2014; Nokelainen et al. 2017) and communication with conspecifics (Detto et al. 2008). As in other taxa, even in crustaceans, the trade-off between camouflage and communication had led to a variety of responses. For example, stomatopods employ signals that can be hidden from predators and use primary body coloration for camouflage (Gagnon et al. 2015; Franklin et al. 2020), male crabs of genus *Uca* possess a larger colored claw, which waves in species-specific undulations during courtship (Zeil and Zanker 1997), and several crabs display prominent color patterns on the carapace and claw that allow recognition and mating attraction (Cummings et al. 2008). Changing in camouflage requirements as well as in behavioral pattern causes ontogenetic color changes and this kind of response is quite common in crustaceans. For example, the isopods (*Idotea montereyensis*) change from red to green to match the background as they change the habitat from red to green algae (Lee 1966). Similarly, the shore crabs (*Carcinus maenus*) keep white spots on the carapace for camouflage in mussel beds, but lose them when grow and move into the algae (Todd et al. 2006). Although a lot of effort has been devoted to the analysis of the relationship between coloration and background (e.g., Thacker et al. 1993; Franklin et al. 2020), the variability in the color pattern within a population, including sexual dimorphic colorations and individual variation, has been often neglected in crustaceans. When sexually dimorphic colorations are found, they are usually interpreted in terms of adaptation for predator avoidance (e.g., *Idotea baltica*, Jormalainen et al. 1995; *Austinixa aidae*, Peiró et al. 2013). However, in some cases, sexually dimorphic colorations cannot be explained in terms of camouflage, such as the soft red patch in the crayfish male *Cherax quadricarinatus*, which possibly transmits information about the gender, size, and quality of its owner (Karplus et al. 2003). Intra-individual variability in coloration in crustaceans has been attributed to a range of factors including diet (Harpaz et al. 1988; Kaldre et al. 2015), background substrate color (Beingesser and Copp 1985; Thacker et al. 1993), light condition (Thacker et al. 1993), genetic variation (Dunham et al. 1979; Walker et al. 2000), and ontogeny (Beingesser and Copp 1985; Hartzell 2017). An additional factor increasing variability in color patterns is the ability of some crustaceans, and more in general arthropods, to individually recognize conspecifics through their morphological traits including color (Vannini and Gherardi 1981; Tibbetts 2004; Detto et al. 2006; Van der Velden et al. 2008; Detto et al. 2008). In order to fully understand the function of variation in the color pattern of crustaceans, it is necessary to first describe the naturally occurring diversity among individuals at a population level (Bauer 1981; Detto et al. 2008).

Among crustaceans, freshwater crayfish form a group in which the knowledge on the meaning of color and color pattern is still largely incomplete, also because colors quickly disappear within a few days of being preserved in fixatives such as formalin or alcohol (reviewed in Schuster 2020). Variation in color patterns among crayfish results from contrasting color in a variety of structures in different body regions including antennae, rostral margins, leg joints, spines, claws, carapace, telson, and uropodes, just to name a few (but see Schuster 2020 for detail and pictures). Freshwater crayfish show a wide intra-population variability in coloration in terms of saturation, hues, and brightness (Schuster 2020), which has been the focus of numerous studies, especially resulting in a description of causative mechanisms of colorations, that is, pigments and their distribution in the skin, rather than an analysis of the environmental pressure maintaining color variation at population levels. For example, Beingesser and Copp (1985) reported that young *Procambarus clarkii* are green and match the background with physiological color changes, but shift to a morphologically fixed red coloration when adults. Hartzell (2017) found that intraspecific color variation in the *Cambarus bartonii* and *Faxonius obscurus* does not vary due to an ontogenetic shift in coloration, and hypothesized that some underlying genetic factors may be most likely responsible for the observed patterns in both crayfish species. Kaldre et al. (2015) supplied adult *Procambarus fallax* f. *virginalis* with different food and found a positive correlation between feed quality and the intensity of carapace coloration, but did not find any effect of feed quality on survival. Thacker et al. (1993) studied color variation in *Orconectes virilis* both in field and laboratory conditions and showed that adults can change color, brown morphs becoming blue, and vice versa.

The signal crayfish *Pacifastacus leniusculus* is native to northwest North America, but, due to introductions by humans, is now the most widely spread alien crayfish species in Europe (Kouba et al. 2014). Crayfish of this species are bluish-brown to reddish-brown, occasionally light- to dark-brown; underside of claws is red and the white-turquoise oval patch on the upper side of the claws is unique (Souty-Grosset et al. 2006). Signal crayfish have a predominantly nocturnal behavior (Styrishave et al. 2007; Johnson et al. 2014), but they remain relatively active during daylight hours, showing an adaptation to day-activity until to ∼34% of the total activity time (Lozan 2000). The species is able to a subtle deep visual detection at least against predators (Blake and Hart 1993). This article used digital image analysis to quantitatively describe color variability among individuals within a population and among different body regions within an individual as well, taking into account the effects of sexes and ontogeny (size).

Materials and Methods

We collected signal crayfish during autumn 2018 from 2 stretches in the Valla stream (Northern Italy) of ∼200 m long. Crayfish were searched by night when they are more active and outside the covers, collected by hand and placed into containers with stream water. All individuals that have just undergone a moult were excluded from the sample. After capture, each individual was sexed and was measured to record the cephalothorax length (CL, from the tip of the rostrum (RST) to the posterior median edge of the cephalothorax) using a digital caliper (accuracy 0.1 mm); then, crayfish have been taken into the laboratory of the University of Pavia and photographed for the color measure in ten different body regions using a Nikon D50 camera at a 1.2-million-pixel resolution, equipped with a Nikkor 60 mm AF-S Micro lens, and fixed on a stand at a distance of 18 cm. For each individual, we took high-resolution digital images of 10 different body parts (Figure 1), including thorax (TRX), RST, left and right upper claws (UCLWdx, UCLWsx), left and right lower claws (LCLWdx, LCLWdx), left and right upper oval patch (UOPdx, UOPsx), and left and right lower oval patch

(LOPdx, LOPsx). Each portion was photographed adjacent to a GretagMacBeth Mini ColorChecker chart (24 color references, 5.7 cm × 8.25 cm) in a 44 × 44 cm lightbox illuminated with 2 daylight 22 W circular neon tubes (Reporter 55100 Studio-kit). Overall, the sample included 152 individuals, 100 males, and 52 females. Males were on average 85.9 ± 17.3 mm long (range: 40.8–120.8 mm) with a cephalothorax of 43.8 ± 9.5 mm (range: 19.8–67.0 mm), whereas females were 80.8 ± 13.7 mm long (range: 61.0–114.1 mm) with a cephalothorax of 39.3 ± 6.5 mm (range: 29.3–55.0 mm).

Color measures

Images were analyzed in the Red-Green-Blue (RGB) color system following the method proposed by Bergman and Beehner (2008) and Sacchi et al. (2013). First, we used the Camera plug-in for Adobe Photoshop CS3 to create a new color profile that adjusted the color in the photographs (in the jpeg format) to the known color levels in each square of the ColorChecker chart. Then, for each image, we selected the "region of interest" using the "lazoo" tool, and pixels corresponding to point of reflected light were removed using the package "magick" in R (Ooms 2018). After that, we analyzed on average $27,554 \pm 14,082$ pixels for image, ranging from 10,478 to 46,079 of LOPdx and TRX, respectively. Finally, the RGB color values were rearranged in the Hue, Saturation, and Value (HSV) system. In this system the cubic geometry of the RGB color space is rearranged in a cylindrical-coordinate system in which the hue is the angle around the vertical axis and corresponds to the color lights, the saturation is the distance from the axis, and the brightness corresponds to the distance along the axis.

We were not able to achieve HSV values for all portions of the 152 individuals because some individuals lacked claws or had damaged RST. Therefore, the final sample included 1,420 images, of which 152 TRX, 149 RST, 141 UCLWdx, 140 UCLWsx, 142 LCLWdx, 136 LCLWdx, 141 UOPdx, 141 UOPsx, 142 LOPdx, and 136 LOPsx.

Statistical analysis

Color variation in sampled crayfish was assessed in a 2-step analysis: first, we generate the individuals' frequency distributions of hue, saturation, and brightness by using values computed on the whole samples of pixel analyzed for each individual; second, we used those distributions to compute single HSV values for each individual, and use it in statistical analyses through random intercept linear mixed models (LMMs). Frequency distributions of the HSV values were obtained by processing data of each image with a kernel density function (bandwidth = 0.02) and normalization to 1. In this way, we obtained a probability density distribution of the 3 color measures for each body region within an individual. These distributions were averaged to estimate the mean density distributions ($\pm 95\%$ CI) for the HSV values in each body region, separately for males and females. Because all distribution were bell-shaped (see Results section), we extracted the distribution modes of hue, saturation, and brightness as descriptors for the external coloration of crayfish, and we used them in the statistical analyses. LMMs to analyze intra- and inter-individual variation of crayfish' external coloration included the 3-way interaction sex × body region × size as the fixed effect. The size was expressed as the standardized CL of individuals and was used a proxy for individual age (Flint 1975). The individual entered the model as the random effect (random intercept) to account for unexplained variation at the individual level (σ^2_{ind}), after we controlled for the explanatory variables. The dependent variables were the H, S, and V modes of each individual, as estimated by
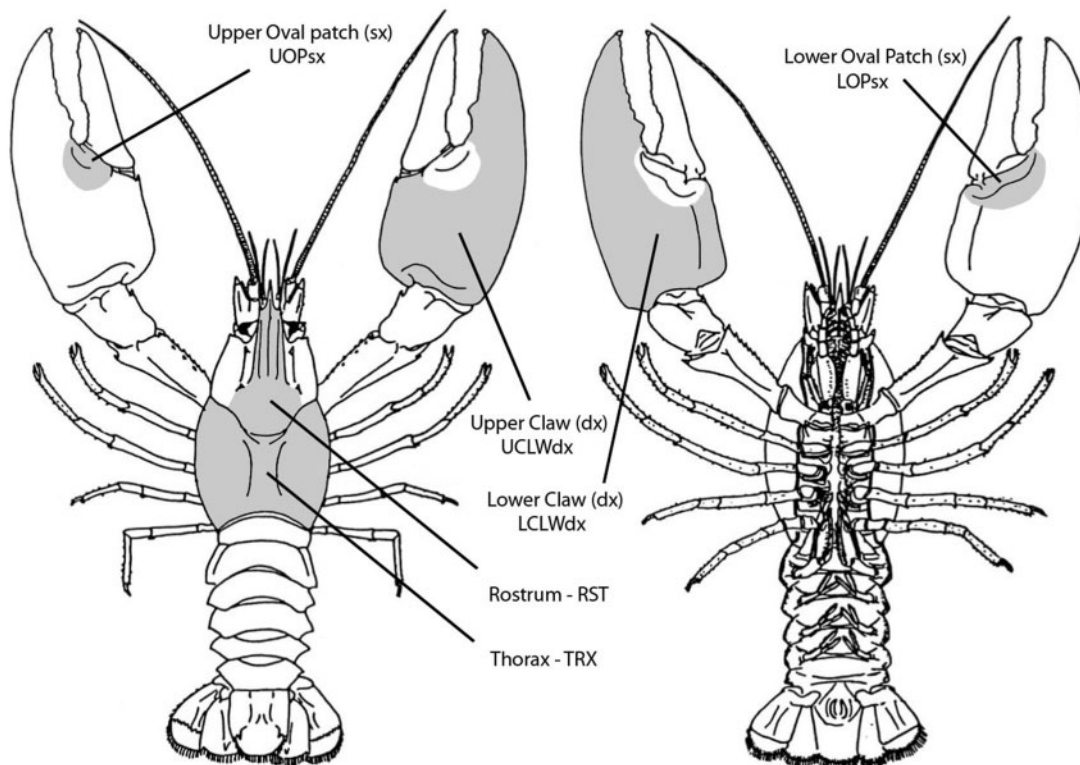

Figure 1. Localization Color and codes of the ten body regions we used to characterize hue, saturation, and brightness in males and females of signal crayfish.

the peak of the density distributions. So 3 different models were run, 1 for each color measure. The initial model was simplified using backward elimination of the not-significant terms (likelihood-ratio χ^2 test, Zuur et al. 2009), and we checked residuals of the initial model for normality and homoscedasticity (Zuur et al. 2009). Finally, pseudo-R^2 accounting for the variance explained by the model (fixed and random efects combined) and for the fixed efects alone were computed according to Nakagawa and Schielzeth (2013). In order to test if males showed a larger among-individual variance than females, we used the F-test for homogeneity of variances on the HSV values predicted by the LMM models obtained at the end of the simplification procedure. Analyses were performed in R version 3.2.4 (R Core Team 2014) using the packages lme4 (Bates et al. 2015), LmerTest (Kuznetsova et al. 2017), and merTools (Jared and Frederick 2019) to generate models and perform statistical tests, whereas the package MuMIn (Barton 2019) was used to compute the pseudo-R^2. Otherwise stated, data reported are means ± standard errors.

Results

The density distribution curves of the HSV values computed over the 10 body regions in males and females (Figures 2–4) revealed a substantial variation in external coloration of crayfish as much between sexes as among portions within the same individual. The mean density distributions of hue values had a bell-shaped appearance in all analyzed portions in both sexes (Figure 2), ranging from red-orange ($H = 0.05$) to lemon-yellow ($H = 0.20$), with a peak near to orange-yellow (H: 0.12–0.15). Males appeared to have a redder shade than females, and the same was much more evident for lower portions with respect to upper portions in both sexes. Mean density distributions of saturation (S values, Figure 3) differed much more between sexes and body regions than hue values did. Color was more saturated in males than females, and the same occurred in lower portions with respect to upper portions of the same individual. Finally, the mean density distribution of brightness (V values, Figure 4) confirmed differences previously observed: males had a more brilliant aspect, as well as lower with respect to upper portions within an individual.

Analyses with LMMs supported statistically all the observed pattern, as for each one of the 3 color measures we found a significant effect of the interactions body part × sex and body part × size (Table 1, and Supplementary Table S1 for means and standard errors). Models were able to explain a large proportion of the total variance of color variation: fixed effects captured 36.9, 61.0, and 77.2% of the variation in H, S, and V values, respectively. These values raised to 81.9, 70.5, and 83.9%, respectively, when we included the proportion of variance associated with the random effect.

The body part × sex interaction suggested that 1) the color of the exoskeleton differed among body regions within an individual, but with different patterns in males and females and 2) the color of the exoskeleton differed between males and females but only limitedly to somebody regions (all statistics for pairwise comparisons of HSV of body regions within and between sexes are reported in Supplementary Tables S2–S4). In both males and females, the color of lower claws and oval patches was significantly redder, more saturated, and more brilliant than in all other body regions (Supplementary Figure S8). Still in both sexes, the RST was significantly more orange (higher hue), more saturated but less brilliant than TRX, upper claws, and oval patches (Supplementary Figure S8). The color of both upper claws and oval patches of males was
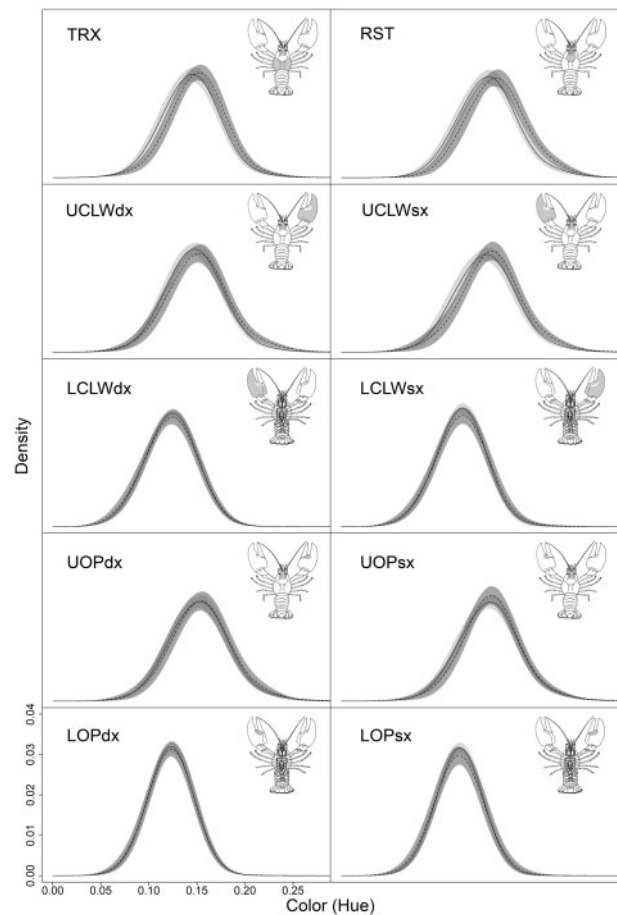

Figure 2. Distribution frequency of hue for different portions of the body and claws of male (solid line and light gray area) and female (dashed line and dark gray area) signal crayfish. Lines are means and area represents 95% CI.

redder and more saturated than the color of TRX, whereas the brightness was similar (Supplementary Figure S8). In females, the color was more saturated in upper claws and oval patches than in TRX, but no difference occurred for hue and brightness (Supplementary Figure S8). Finally, upper oval patches were redder and showed a more saturated coloration than upper claws in both sexes (Supplementary Figure S8).

Males and females did not differ for hue values in all body portions but RST, which had lower values (i.e., was redder) in males (Supplementary Figure S8). Saturation of color in upper and lower claws was significantly higher in males than in females, and the same was true for lower oval patches (Supplementary Figure S8). Finally, the color was more brilliant in males than in females in lower claws and lower oval patches (Supplementary Figure S8).

The body part × size interaction revealed ontogenetic effects on the expression of color in the exoskeleton of *P. leniusculus*, but with different patterns in the different body regions. So, differences among body regions in the external coloration within individual varied according to the body size (Figure 5, all statistics for pairwise comparisons of slopes among body regions within an individual are reported in Supplementary Tables S5–S7).

The hue value did not have a consistent pattern of variation among body regions, as it slightly decreased, remained unchanged, or even slightly raised with increasing individual size (Figure 5). However, none of the slopes significantly differed from zero
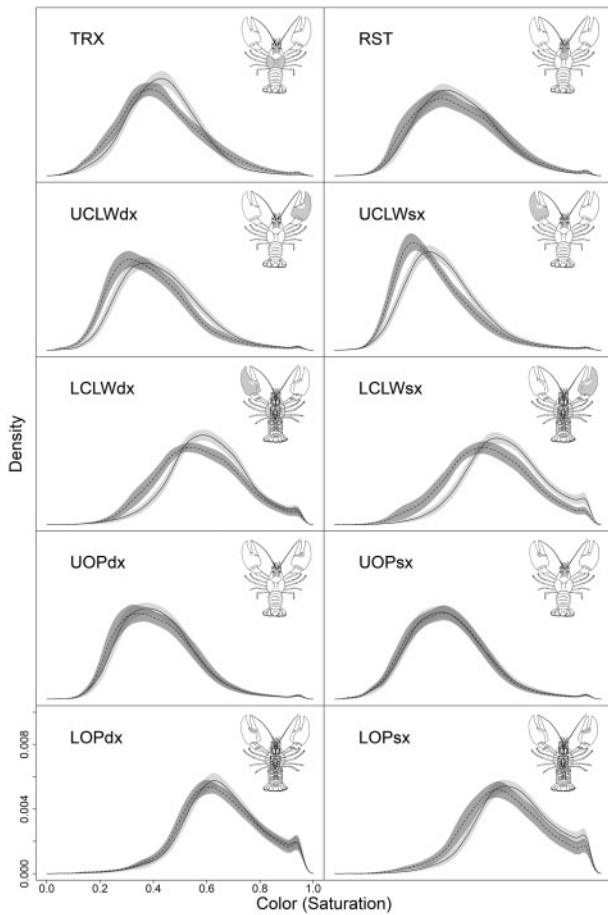
Figure 3. Distribution frequency of hue for different portions of the body and claws of male (solid line and light gray area) and female (dashed line and dark gray area) signal crayfish. Lines are means and area represents 95% CI.

Figure 4. Distribution frequency of brightness for different portions of the body and claws of male (solid line and light gray area) and female (dashed line and dark gray area) signal crayfish. Lines are means and area represents 95% CI.

(Supplementary Table S5). Despite this, pairwise differences in some cases became significantly more pronounced in larger individuals as the results of the opposite tendencies observed TRX and RST with respect to claws and, especially, oval patches (Figure 5). Notably, the difference between TRX and RST respect to upper oval patches significantly increased by 4–7 times in larger individuals, whereas the opposite occurred with respect to lower oval patches, which give the difference to significantly decrease by 1.5–2 times (Figure 5). TRX and RST besides shared the same pattern of variation.

Saturation significantly increased with growing crayfish size in all body regions, but upper oval patches. The effect was statistically significant for TRX, left upper claw, lower claws, and left lower oval patch, whereas in the remnant parts the relationship was marginally significant ($0.063 \leq P \leq 0.087$, Supplementary Table S5). As a consequence, coloration in upper oval patched was significantly less saturated with respect to all other body regions in larger individuals. Furthermore, smaller individuals had a TRX significantly less saturated then RST, and this difference disappeared in larger individuals (Figure 5 and Supplementary Table S5).

TRX, left lower claw and left oval patch were significantly more brilliant in larger than smaller individuals (Supplementary Table S6), whereas all other body regions did not vary with increasing size (Figure 5). As a consequence, the differences between TRX and RST, upper claws and upper oval patches were significantly amplified in larger crayfish (Supplementary Table S6).
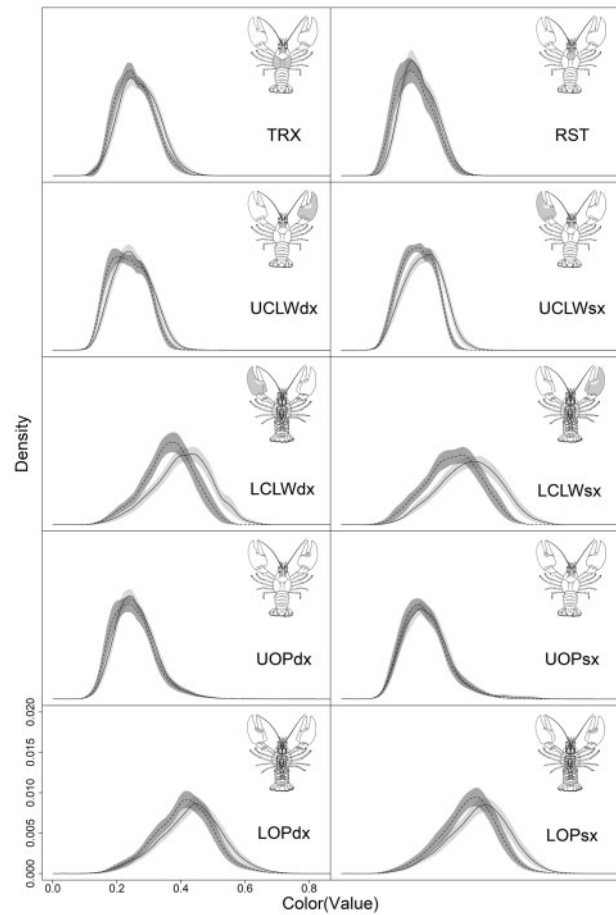
Table 1. Minimum significant LMMs for the effect of sex, body region, and size on the color of the exoskeleton of *Paciastacus leniusculus* measured in the HSV system

| Model	F	df	P-value
Hue			
Sex	0.175	1,246	0.68
Body region	300.215	9,1241	<0.001
Size	0.038	1,152	0.84
Sex × Body region	3.927	9,1242	<0.001
Size × Body region	3.680	9,1242	<0.001
Saturation			
Sex	7.468	1,163	0.007
Body region	276.321	9,1246	<0.001
Size	14.703	1,152	<0.001
Sex × Body region	2.633	9,1247	0.005
Size × Body region	2.342	9,1250	0.013
Value			
Sex	16.743	1,169	<0.001
Body region	637.485	1,1247	<0.001
Size	9.568	1,154	0.002
Sex × Body region	4.482	9,1248	<0.001
Size × Body region	2.685	9,1251	<0.001
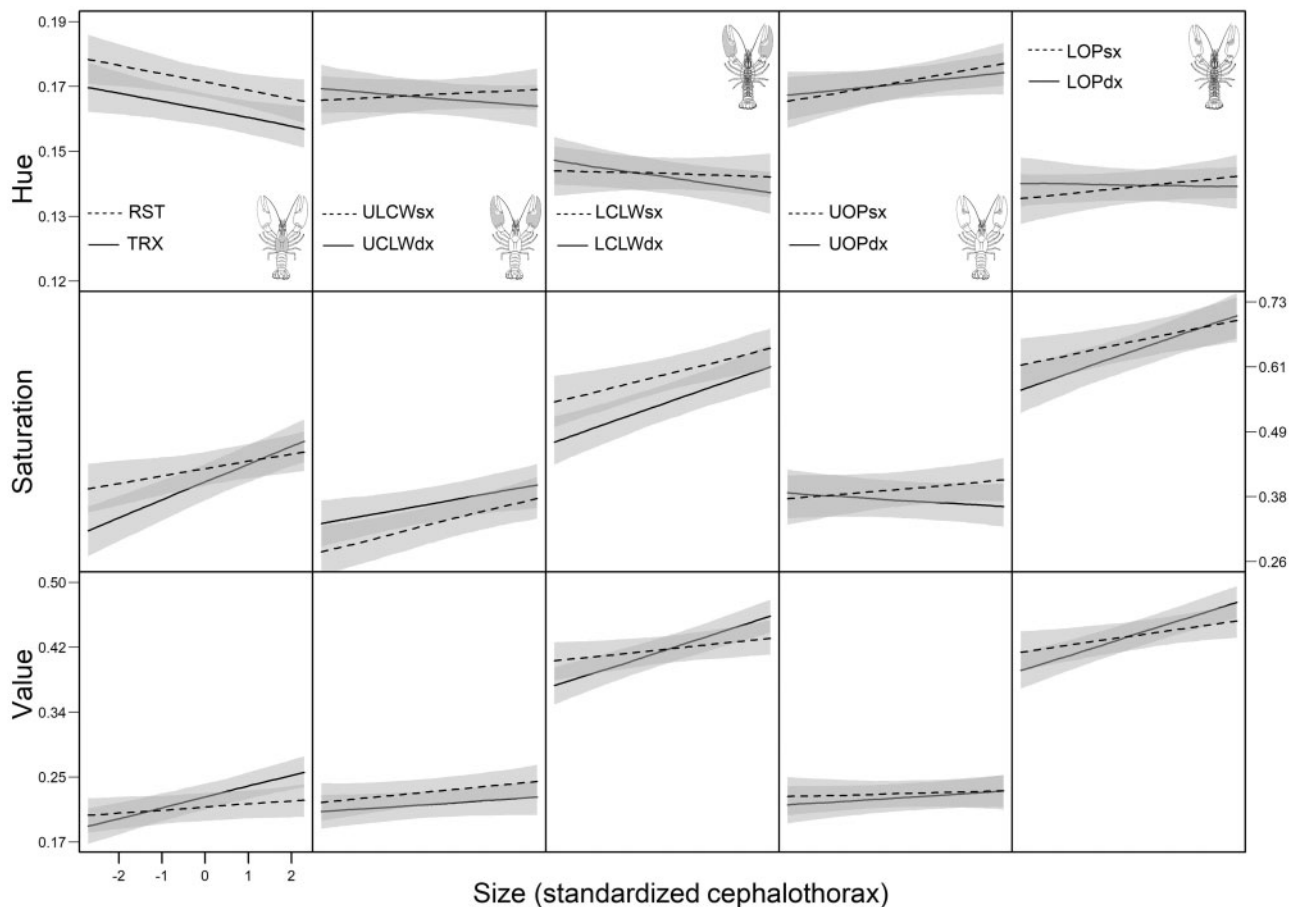
Figure 5. Relationships between body size and the 3 components of external coloration (hue, saturation, and brightness) in different portions of the body and claws of signal crayfish. Lines are means and area represents 95% CI.

Random effects were highly significant in each of the 3 models (H: LR $\chi^2 = 1{,}264$, $df = 1$, $P < 0.001$; S: LR $\chi^2 = 177.21$, $df = 1$, $P < 0.001$, V: LR $\chi^2 = 248.9$, $df = 1$, $P < 0.001$), suggesting that a large portion of the variance unexplained by sex, portion, and size (i.e., the fixed effects) depended on some unconsidered features of the individuals, especially for the hue. These proportions were 70.7, 23.4, and 28.8% for H, S, and V, respectively.

Finally, the color appeared to be more variable in males rather than females, notably for S and V measures (Supplementary Figure S9). This ratio ranged from 0.88 to 1.05 for the hue and did not significantly deviate from 1 in anybody region. The ratio for the saturation was >1 in all body regions, varying from 1.18 to 1.46, even none of the ten values still deviated significantly from 1 (but deviation was marginally significant for the TRX: $F_{99} = 1.462$, $P = 0.13$). In the case of brightness, the ratios varied from 1.85 to 2.25 and all were significantly >1 ($F > 1.852$, $P < 0.021$).

Discussion

Our data show that Signal crayfish possess an extremely variable pattern of coloration within the same population, and such variability relies on sex and size. Color variability even occurs within body regions of the same individual, notably between upper and lower portions of the body, and also among dorsal ones. Lower claws and oval patches had a redder hue, with a much more saturated and brilliant coloration that both upper claws and oval patches. We also

found significant differences between TRX and RST, especially in color saturation and brightness. Furthermore, we found ontogenetic patterns of color expression specific to different body regions, resulting in shaper color difference among body regions with increasing body size. The ontogenetic pattern was noteworthy in upper claws and oval patches, which showed a much larger increase in saturation and brightness than other body regions.

Patterns of intra- and inter-individual phenotypic differences might yield insights into the processed, namely selective pressures, which have governed its evolution. In particular, color patterns can make an individual conspicuous or cryptic, depending on the prevalent function of coloration, where duller colorations can be easily explained through natural selection for predator avoidance, whereas brilliant and full of pigment coloration may be evolved as signals for intraspecific communication. Intra-population variation of the exoskeleton in freshwater crayfish has been constantly interpreted as an adaptation to the background of environmental light to improve mimicry and reduce predation risk (Kent 1901; Thacker et al. 1993; Nokelainen et al. 2017). Our data for TRX and upper claws were consistent with this general idea, but the pattern of variation for both saturation and brightness of the redder portion, especially in males, were not. The specific reason is that red pigmentations in crustaceans are primarily due to the accumulation of carotenoids (notably astaxanthin, but also lutein and zeaxanthin, Latscha 1989; Pan et al. 2001) into the exoskeleton and in the epidermal layer between the exoskeleton and abdominal muscle (Katayama et al.

1972; Tanaka et al.1976; Simpson et al. 1981; Sagi et al. 1995; Boonyaratpalin et al. 2001). Crustaceans, including crayfish, are incapable to synthesize carotenoids but need to acquire them through diet (Katayama et al. 1971). Carotenoids are among the most effective antioxidants, and their primary function in the organism is scavenging free radicals (Svensson and Wong 2011). Because immune cells are highly sensitive to oxidative stress, carotenoids can enhance immunity (Svensson and Wong 2011), establishing the basis for a trade-off for carotenoids between coloration and immunity (Lozano 2001). Crayfish possess a complex visual system including well-developed compound eyes contains multiple lenses (Kubec et al. 2019), which makes them able to receive and analyze complex visual signals, and examples of communication though visual signals are not lacking (Bruski and Dunham 1987; Detto et al. 2006; Galeotti et al. 2006, 2007; Rubolini et al. 2007; Van der Velden et al. 2008; Kubec et al. 2019). The fact that larger males in signal crayfish were redder than small ones, and in general males were redder than females makes plausible the hypothesis that conspicuous red colorations might be involved in intraspecific communication rather than in crypsis. This hypothesis is further supported by the fact that the most saturated and brilliant coloration located in the lower claws, which can be rapidly hidden in the presence of a predator. Such a kind of response is also consistent with the signal partitioning as a solution for the trade-off between camouflage from predators and conspicuousness for communication. Thus, the dorsal body pattern is likely to provide signal crayfish with camouflage from predators (namely fish), whereas and the portion of claws hidden from potential predators can be used for signaling to conspecifics.

An alternative explanation for the occurrence in signal crayfish of color variation among body regions within individual is that the orange-red color might be used as a way to communicate with conspecifics while avoiding detection from predators. In the species' native range of North America, the main predators of signal crayfish include teleost fish like smallmouth bass *Micropterus dolomieu* (Pflug and Pauley 1984), brown trout *Salmo trutta* and mountain whitefish *Prosopium williamson* (Lewis 1999), northern pikeminnow *Ptychocheilus oregonensis* (Jeppson and Platts 1959; Lewis 1999), and European perch *Perca fluviatilis* (Appelberg and Odelstrom 1988). In general, the crayfish are consumed during the major activity months (Stein 1977; Hepworth and Duffield 1987). The young crayfish, 0- and 1 years old, are the most frequently found in the stomachs of predatory fish (Stein 1977). In general, mammals and avian predators feed on crayfish too, for example, racoon *Procyon lotor*, otter *Lutra lutra*, and mink *Mustela vison* (Hogger 1988). Whereas among the avian predators, there are heron *Ardea cinerea* and kingfisher *Alcedo atthis*. All age classes of crawfish are preyed upon by mammals and birds but the diet is made up mostly by larger and heavier crayfish (Correia 2001). Mammals with nocturnal activity have reduced ability to detect colors, above all long-wavelength (notably, orange-red), due to the marked prevalence of rods on cones in the retina, and short-wavelength-sensitive (S-) cones on long-wavelength-sensitive (L-) cones (Peichl 2005). In contrast, the spectral sensitivity of crayfish peaked toward red, between 550 and 600 nm (reviewed in Schuster 2020), and did not correlate with the different habitat preferences among the species (Crandall and Cronin 1997). Therefore, by using orange-red colorations signal, crayfish may produce signals conspicuous to conspecifics using a property of light that is invisible to predators, allowing them to avoid being vulnerable to predators. The hypothesis is not unrealistic because such a kind of adaptation has been already described in crustaceans (Cummings et al. 2003; Siebeck et al. 2010;

Gagnon et al. 2015; Abramjan et al. 2020). For example, the mantis shrimp *Gonodactylaceus falcatus* produces strongly circularly polarized body patterns, which are discriminated by conspecifics to avoid occupied burrows when seeking refuge but are not seen by potential predators (Gagnon et al. 2015).

A further result of this study was that the exoskeleton color differed between sexes: males having a redder, more saturated, and brilliant coloration, whereas females a duller and drabber coloration. This difference varied among body regions, and notably, was especially noticeable in lower claws and lower oval patches. Sexual dichromatism is normally thought to have evolved in response to selection pressures that differ between the sexes: males evolving bright coloration due to sexual selection for increased ornamentation (reviewed by Badayev and Hill 2003), whereas females missing colorful pattern due to natural selection for cryptic coloration (Wallace 1868; Soler and Moreno 2012). In birds, sexual dimorphism in carotenoid-based colorations has been shown to depend on intense sexual selection by female mate choice for honest signals (Badayev and Hill 2000). Substantial variability of sexual dichromatism depends on the balance between the exaggerated trait in males and the environmental conditions affecting trait expression (Badayev and Hill 2003). Accordingly, we found sexual dichromatism vary between the upper and lower portion of claws, and was at the best in the lower portion, where the cost due to predation risk can be minimized. Support for a possible role of sexual selection in maintaining sexual dichromatism comes from the finding that inter-individual variation of color brightness was significantly larger in males with respect to females. Notably, just lower claws and lower oval patches were the most variable among males than females. However, color dichromatism may arise in some other way than sexual selection, including thermoregulation, biases in the visual system, species identification, and interspecific communication, which with a correlative approach such that we used in this study cannot be disentangled. In conclusion, carotenoids shaping the coloration of the exoskeleton of freshwater crayfish are well suited for testing ideas on color signals and their potential role in conveying information in these species.

In conclusion, crayfish color patterns have not been widely studied to better understand their meaning and adaptive function in crayfish, and only in recent years, the patterns of variation in the external coloration at both inter- and intra-specific level and have begun to be analyzed not only for camouflage, but also in the light of possible role in intraspecific communication and, more in general, crayfish behavior. The next steps will necessarily pass through experimental investigations to try to test hypotheses of communicative function of crayfish coloration and assess how color affects male-male competition or mate choice by females, or if juvenile coloration confers some sort of protection from predators because juveniles are subject to higher predator risk than adults.

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Supplementary Material

References

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