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Original Article

Stomatopods detect and assess achromatic cues in contests

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Conspicuous, colorful displays are often used by animals to communicate within and between species. Previously, researchers have manipulated specific components of color signals (i.e., hue, total reflectance, and/or chroma) using paints, photographs, videos, or filters. However, these manipulations may not adequately mimic the spectrum of color signals outside the range of human perception. Thus, these methods are inappropriate for organisms with unconventional visual systems, such as stomatopods (mantis shrimp). Here, we describe a novel application of a femtosecond laser to increase total reflectance of the stomatopod meral spot, a distinct area on the raptorial appendage used in territorial contests. Ultrafast lasers provide a programmable way to precisely manipulate patch total reflectance of live stomatopods without causing collateral damage. We tested how experimentally increasing meral spot reflectance impacted receiver behavior during territorial contests. Contests in which receiver stomatopods faced an opponent with a lightened meral spot were shorter and receivers showed increased rates of agonistic behaviors. This result suggests that lighter meral spots indicate lower fighting ability; thus, receivers are more willing to engage in a contest. This research provides the first demonstration that stomatopods can detect and assess achromatic variation in contests. Furthermore, we demonstrate that ultrafast lasers provide a powerful tool to investigate achromatic signaling, particularly for organisms whose size, aquatic habitat, or visual system otherwise prevent realistic alterations to color signals (e.g., butterflies, jumping spiders, or decapod crustaceans). This study advances our knowledge about stomatopod visual communication and offers a valuable tool for future research.

Key words: **brightness, crustacean, cue, luminance, signal, visual ecology.**

INTRODUCTION

Animals use color signals during mate choice (Baeta et al. 2008; Baldwin and Johnsen 2012), agonistic encounters (Pryke et al. 2001; Whiting et al. 2006) and as warning signals (Prudic et al. 2007; Aronsson and Gamberale-Stille 2008). These signals convey information about the signaler to the receiver, such as fighting ability (Olsson 1994), aggression (Whiting et al. 2006), individual quality (Baeta et al. 2008), or social status (Tibbetts 2002). To investigate color signals, typically researchers alter colors of live animals (Tibbetts 2002; Gerlach et al. 2014) or create standard stimuli to vary the color signal (e.g., models, Yewers et al. 2016; photographs, Baldwin and Johnsen 2012; or videos, Künzler and Bakker 2001). For such methods to be biologically relevant, the manipulation must be understood within the context of the receiver's visual system (Bennett et al. 1994). For organisms with well understood or conventional visual systems, visual models can provide insight into how organisms might perceive these manipulations (Endler 1990; Vorobyev and Osorio 1998). However, for many species, we do not know how they process visual information. Consequently, visual models may not provide an accurate reflection of how a manipulation is perceived. Alternatively, if we can precisely manipulate color so that the spectral shape remains within natural variation, we would not need visual models to predict how a manipulation is perceived.

Stomatopods (mantis shrimp) are organisms that likely use color signals to communicate; however, due to a complex visual system, it is difficult to predict how they may perceive color manipulations. Stomatopods have up to 20 photoreceptor classes (Marshall and Arikawa 2014): 12 for color vision, 6 for polarization vision, and 2 for luminance vision. They also process visual information in a unique way that is not fully understood (Thoen et al. 2014). Studies investigating the role of chromatic and achromatic signals in stomatopod intraspecific communication suggest that such signals play

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an important role in mate choice (Chiou et al. 2011) and contests (Franklin et al. 2016). Notably, however, these color manipulations did not mimic natural variation. Thus, the altered behaviors observed may reflect either a response to the color signal or a recognition error (Liebert and Starks 2004).

Neogonodactylus oerstedii is a small stomatopod species (up to 65 mm length) found throughout the Caribbean. Located in shallow waters (<10 m depth), they are illuminated by broad band sunlight (including 300–700 nm). They reside in cavities in coral rubble or rock and these refuges are important for avoiding predators, processing food, mating, and brooding eggs (Caldwell 1987). Stomatopods are well known for competing aggressively over ownership of these refuges (Dingle and Caldwell 1969; Caldwell 1979; Caldwell and Dingle 1979; Caldwell 1987). During these contests, stomatopods can escalate to "strikes" where they strike one another at high speed with their second maxillipeds (up to 23 m s^{-1}; Patek et al. 2004). In many cases, before escalating to strikes, they perform a threat display known as the meral spread. In meral spread position, the second maxillipeds are pulled laterally, displaying the meral spots (Figure 1). In *N. oerstedii*, the meral spots are purple with a UV component, and the color is known to play a role in contests over refuges (Franklin et al. 2016).

Achromatic variation in meral spot color may convey valuable information to a receiver about opponent fighting ability, or Resource Holding Potential (RHP; Parker 1974). This is likely to be the case because the total reflectance (brightness) of meral spots increases immediately after molting, while the exoskeleton of the stomatopod is still soft (Reaka 1975). Quantitative measurements of meral spot color in *N. oerstedii* demonstrated that, after molting, the observed color change is due largely to an increase in total reflectance (Supplementary Figure S1; Supplementary Table S1). Molted individuals have soft exoskeletons and are thus unable to punch and defend their refuge from challenger stomatopods. This is vital information for an opponent attempting to win the refuge and gain safety from predators. If this is the case, meral spot total reflectance may act as an unwanted cue indicating RHP rather than a signal (i.e., it provides no benefit to the signaler; Searcy and Nowicki 2005). Despite the potential importance of achromatic cues in contests, whether total reflectance is a cue indicating RHP remains unknown.

To investigate whether total reflectance may be assessed during stomatopod agonistic encounters, we developed a technique using an ultrafast laser to increase the reflectance of the meral spot. Ultrafast lasers have a range of applications including micromachining transparent materials (Gattass and Mazur 2008), transfection of DNA in cells (Tirlapur and Konig 2002), control of chemical reactions (Assion et al. 1998), and nonthermal ablation of cells (Mondia et al. 2011). Here, we used an ultrafast laser to specifically target and break down pigment molecules contained within the stomatopod exoskeleton. By decreasing pigmentation, we were able to increase total reflectance to closely match the increase in total reflectance of recently molted stomatopods. These stomatopods were then used as subjects in behavioral experiments to investigate the role of meral spot total reflectance in stomatopod contests. Specifically, we investigated whether meral spot total reflectance could indicate RHP in stomatopods.

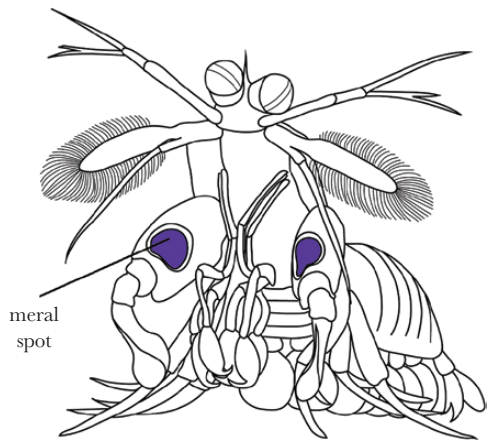

Figure 1
A stomatopod performing a threat display, the meral spread. The meral spots (purple) are located on the second maxillipeds.

METHODS

Husbandry

Neogonodactylus oerstedii were obtained commercially (KB MarineLife, Florida, USA) in May and September, 2014, and housed at Tufts University. Stomatopods were housed under full spectrum lighting (12:12 light:dark cycle) in 75 L aquaria, divided into 3 compartments. Stomatopods could not see one another, but water was cycled throughout the entire tank through a carbon filter. At least one male and one female were in each aquarium. All stomatopods received a 25 mL falcon tube with 7 mm of the tapered end sawn off and covered in duct tape to act as a refuge. Seawater (Instant Ocean, Blacksburg, USA) was maintained at 33–34 ppt salinity and 22–25 °C. One-third water changes occurred twice per week and stomatopods were fed frozen clams or squid 3 times per week. Stomatopods were fed 1 day before being used in experiments trials.

Ultrafast laser manipulation of meral spot total reflectance

Precise patterning and corresponding modification of the optical characteristics of the meral spots were obtained by using a previously described micromachining setup associated with the laser source (Applegate et al. 2015; Supplementary Figure S2). Pilot studies were conducted first on preserved stomatopods and then on anesthetized stomatopods to determine an appropriate protocol and the flexibility of the technique. Stomatopods were anesthetized by placing them in a container with 25 mL of seawater and cooling them in the freezer (−13 °C) for 20 min. They were then pinned in a small petri dish to expose the meral spot and covered with iced seawater. The meral spot was exposed to ultrafast (~150 fs) pulses of 810 nm light focused to a ~5 µm spot with a 0.2 NA 4× microscope objective at a pulse repetition frequency of 80 MHz. We determined that a laser power of 700 mW was appropriate; this increased meral spot total reflectance but did not burn the cuticle. Speed of laser movement could be varied to alter the degree of brightening (Supplementary Figure S3a). We opted to use a speed of 200 µm s^{-1} to create a visible (to our eyes) increase in reflectance and ensure stomatopods were not anesthetized for too long (at this speed the procedure was 10 min for 1 meral spot). This resulted in a radiant exposure of 67 kJ cm^{-2} at each exposed location and minimized risk of cuticle damage. The treatment was performed over 2 consecutive days with 1 meral spot treated on each day. Control stomatopods experienced the same treatment, except the laser was used on their carapace (dorsal surface), a location unlikely to be visible during territorial contests. Due to the relative weakness

of the exoskeleton at this location, the laser power was reduced to 525 mW to avoid causing injury. All stomatopods received 1 week recovery before behavioral trials.

Spectral measurements

The spectrum of the central, colored part of the meral spot was recorded using a JAZ spectrophotometer (Ocean Optics, Dunedin, USA) with a PX-2 pulsed xenon light source. The reflectance was recorded between 300 nm and 700 nm and measured relative to a WS-1 white standard. To mimic natural conditions, the light source was set at 45° to the meral spot surface and the collector was perpendicular. Light source and collecting probes were 600 µm UV-VIS fiber optic cables (Ocean Optics) with collimating lenses attached to the end. Both were fixed in position to ensure a fixed distance between probes and sample. Live stomatopods were immobilized by cooling them in a freezer (−13 °C) for 30 min in 25 mL of water, and then pinning them out in a petri dish with the meral spot facing up. The petri dish was filled with just enough seawater to cover the meral spot. All reflectance measurements were recorded in a dark box with only the light source illuminating the sample. We recorded 2 measurements of both the left and right meral spots from 11 control and 12 treated stomatopods.

The 4 spectra obtained from each stomatopod were averaged and a lowess smoother applied using RCLR (Montgomerie 2008). Variables obtained from spectral data included total reflectance (area under the curve from 300 to 700 nm), hue (wavelength values of the peaks in the UV) and spectral saturation (maximum reflectance ÷ minimum reflectance; Andersson 1999). These were compared between treated (n = 12) and control (n = 11) stomatopods. We did not compare these variables statistically because we do not know how stomatopods perceive color. Similarly, we did not conduct visual modeling (sensu Franklin et al. 2016) because it remains unknown how stomatopod process achromatic information.

Contest trials

Before the meral spot manipulation was performed, all stomatopods were allocated into groups of 3 or 4, based on body length (measured from the tip of the rostrum to the tip of the telson). The 2 stomatopods most similar in body length (<4% difference) were designated as "residents" and would experience the experimental manipulation (described above) either on their meral spots (treatment group) or on their carapace (control group). The remaining 1 or 2 stomatopods were designated at "intruders" and were the focal individuals of the behavioral trials (below).

A repeated measures experimental design was used to account for within individual variation in intruder behaviors. On consecutive days, intruders (focal individuals, n = 16) would face a control resident, then a treatment resident (or vice versa, order randomized). Any intruders that did not compete on both days were removed from the analysis (n = 2). Meral spot spectral reflectance was recorded after experiments (to minimize stomatopod handling) and we excluded any stomatopod groupings where the treatment resident's meral spots were not lighter than the control resident's meral spots (n = 4). This could occur if treatment residents had much darker meral spots than control residents prior to manipulation. This left 10 intruders in our analysis that competed in both treatment groups (a final sample size similar to other stomatopod behavioral experiments; Chiou et al. 2011; Franklin et al. 2016). Four intruders were larger than their resident opponents and 6 were smaller than their resident opponents. Each resident was used twice (5 treatment residents; 5 control residents). Trials were performed in the laboratory at Tufts University between 1000 and 1600 under full spectrum light. All trials were conducted in an opaque plastic tub (46 × 38 × 12 cm) with 8 cm of fresh Instant Ocean seawater heated to 23–24 °C and 3 cm of coarse sand covering the bottom. We included plastic grids of 12.5 mm grid size semiburied in the sand in all trials. These grids were used to calibrate the video.

At the beginning of the trial, the tub was divided in half with an opaque plastic divider. The resident was placed in one compartment in the refuge from its housing tank and the intruder placed in the other compartment with no refuge. After 10 min acclimation time, the barrier was removed and recording of behaviors commenced. All trials were recorded from above with a GoPro (Hero 3+ Black edition, USA) video camera (60 f.p.s., 1080p, medium f.o.v.) and scored blind. Behaviors recorded from the video footage included: latency until approached refuge, speed of approach, duration of fight, proportion of time intruder is out of resident refuge, number offensive behaviors (intruder and resident), number of "coils" (intruder), and the winner of the fight (see descriptions below). We recorded behaviors until the trial concluded or for the first 5 min of the interaction (whichever came first). If there was no interaction or no clear winner, the trial was ended after 30 min. At this time, stomatopods were returned to their housing tanks.

In general, we classified behaviors following Dingle and Caldwell (1969). Approach began when the intruder moved directly toward the resident's refuge (Dingle & Caldwell 1969). We determined distance from the refuge at the start and end of the approach to calculate speed of approach (mm s^{-1}). There were minimal vertical movements by intruders during approach. The contest started at the end of the intruder's approach. Fight duration was timed from the end of the approach until 1 stomatopod fled the contest. The stomatopod that remained in the refuge was classified as the winner. Offensive behaviors included "strike", a blow delivered by one or both of the enlarged second maxillipeds; "lunge", a short, rapid forward movement toward opponent; and, "meral spread", an outward spreading of raptorial second maxillipeds (Dingle and Caldwell 1969). The meral spread displays the meral spots located on the inside of the second maxillipeds. A coil is where the stomatopod curls up so that its head is above the telson (Dingle and Caldwell 1969). Meral spreads tend to occur near the beginning of the contest, after intruder approach. The meral spread may be followed by a lunge, or, in many size-matched contests, stomatopods escalate to strikes. Coils occur throughout the contest, either immediately after an offensive behavior, in response to an opponent offensive behavior or sometimes independently.

Statistical analysis

All analyses were conducted using R v. 3.1.1 (R Core Team 2014). The effect of treatment on intruder behaviors was assessed using generalized linear models (GLMs; R: glm) or generalized linear mixed models (GLMMs; R: glmer; Bates et al. 2015). All models included *treatment* (control or brightened) as a fixed factor. Trial order (first or second) and stomatopod ID (intruder ID for intruder behaviors or resident ID for resident behaviors) were initially included as random effects to account for repeated measures (except where indicated below). In almost all cases, the random effects explained very little variation in the data (variation of random effects terms were less than 1) and did not improve the fit of the model according to Akaike Information Criterion (AIC; comparison of full vs. reduced models). Thus, order was removed from all models and stomatopod ID was removed from all except for *latency to approach*. Continuous variables (*latency until approach*, *speed of*

approach, *duration of fight* and *time outside refuge*) were analyzed using log normal GLMs. Some fights did not conclude; thus, we only assessed differences in fight duration of the fights that concluded (control: $n = 5$; treatment: $n = 5$). Due to the lower sample size, we did not include any random effects for fight duration. For the variable *time outside refuge*, duration recording behaviors was included as a covariate to account for variation in trial duration. All count variables were analyzed with a Poisson error distribution and assessed for over-dispersion. We included a trial level error term in models exhibiting over-dispersion (*total intruder offensive behaviors, intruder coil rate, total intruder coils, resident offensive behavior rate* and *total resident offensive behaviors)*. There was a clear pattern in the residuals for *total intruder coils*, so we instead accounted for over-dispersion with a negative binomial error distribution for this variable. This fit the data better according to diagnostic plots. Models for *intruder offensive behavior rate*, *resident offensive behavior rate* and *intruder coil rate* included an offset of *time intruder outside refuge*, to account for different durations of recording these behaviors (these behaviors could only be recorded when an intruder was outside the refuge). An outlier was observed for *offensive behavior rate*, so we ran the analysis with and without the outlier. To analyze *total number of intruder offensive behaviors*, *total number of resident offensive behaviors* and *total number of intruder coils*, we did not include an offset. For fights that concluded, we investigated whether treatment influenced the intruder winning with a GLM with a binomial error distribution (no random effects were included). For all models, significance of the treatment term was assessed using marginal hypothesis tests (R: Anova; Fox and Weisberg 2011; Table 1).

RESULTS

Ultrafast laser manipulation of meral spot total reflectance

We used a femtosecond laser to increase reflectance of the meral spots of live *N. oerstedii* by exposing the meral spots to irradiation with focused ultrashort pulses of light (Figure 2a). We were able to precisely control the area that was treated by moving the stomatopod relative to the focal point via a computer controlled 3-axis

Table 1
The effects of increasing meral spot total reflectance on stomatopod behavior

Response variable	MLE (95% CI)		Probability distribution	χ^2 or F	df	P value
	Control	Treatment				
Proportion of contests won by intruder	0.60 (0.20–0.92) $n = 5$	0.40 (0.08–0.80) $n = 5$	Binomial	0.40	1	0.53
Duration of fight (seconds)	6 min 59 s (1 min 23 s–35 min 33 s) $n = 5$	0 min 23 s (4 s–2 min 55 s) $n = 5$	Log-normal	6.18	1	**0.013**
Time intruder out of refuge (seconds)	46 (20–102) $n = 10$	30 (14–67) $n = 10$	Log-normal	0.54	1	0.46
Latency until approach* (seconds)	136 (51–367) $n = 10$	129 (48–347) $n = 10$	Log-normal	0.01	1	0.92
Speed of Approach (mm s^{-1})	44 (27–72) $n = 10$	50 (31–83) $n = 10$	Log-normal	0.15	1	0.69
Intruder coils (# min^{-1})**	2.0 (1.2–3.1) $n = 10$	5.2 (3.3–8.6) $n = 10$	Poisson	8.66	1	**0.003**
Total intruder coils (#)	4.1 (2.2–8.0) $n = 10$	3.8 (2.1–7.5) $n = 10$	Negative binomial	0.03	1	0.87
Intruder offensive behaviors (# min^{-1})	2.2 (1.6–3.0) $n = 10$	3.6 (2.5–5.0) $n = 10$	Poisson	4.02	1	**0.045**
Intruder offensive behaviors outlier removed (# min^{-1})	2.5 (1.8–3.4) $n = 9$	2.9 (1.9–4.2) $n = 9$	Poisson	0.34	1	0.56
Total offensive behaviors (#)**	2.4 (0.8–5.7) $n = 10$	1.7 (0.5–4.1) $n = 10$	Poisson	1.92	1	0.17
Resident offensive behaviors (# min^{-1})**	0.7 (0.2–1.6) $n = 10$	4.8 (2.2–10.6) $n = 10$	Poisson	11.06	1	**<0.001**
Total resident offensive behaviors (#)**	1.3 (0.5–2.4) $n = 10$	2.3 (1.2–3.9) $n = 10$	Poisson	1.60	1	0.21

Generalized linear models were run and significance of *Treatment* was assessed using marginal hypothesis tests. Recording duration was included as a covariate for *duration outside burrow* to standardize for different trial durations. Bold indicates $P < 0.05$.
*Indicates that stomatopod ID was included as a random effect.
**Indicates a unique identifier for each trial was included as a random effect.

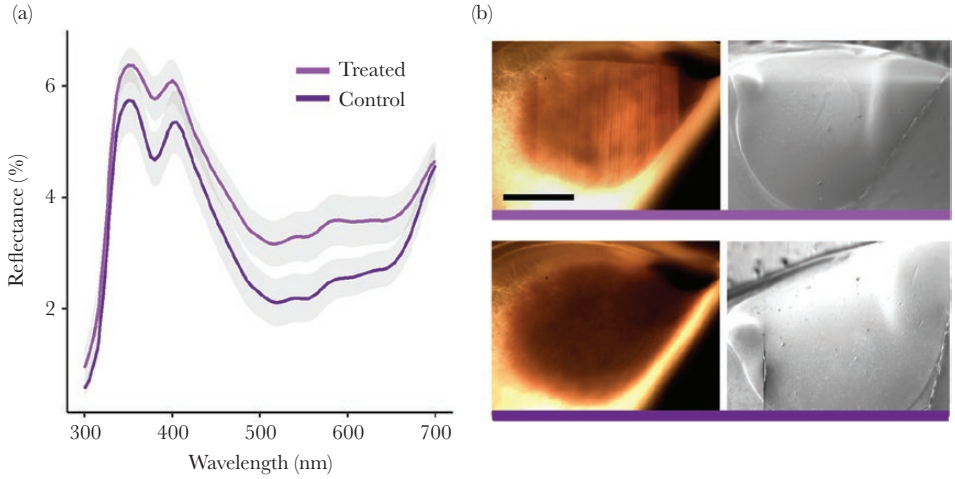

Figure 2
The effect of the ultrafast laser on *Neogonodactylus oerstedii* meral spot brightening. (a) Reflectance spectra of control (dark purple) and treated (light purple) meral spots (mean ± SE). (b) Left: images depicting the visual change in color between control (bottom) and treated (top) meral spots. Right: SEMs of control (bottom) and treated (top) meral spots demonstrate no surface damage to the cuticle. Scale bar: 500 µm.

translation stage (Supplementary Figures S2 and S3b). We could also control the degree of lightening by modifying the speed of the translation stage (Supplementary Figure S3a). It is important to note that slow movement resulted in a larger number of laser pulses which could result in thermal accumulation and associated burning of the cuticle. These complications were avoided by selecting translation speeds and laser powers that would not damage the tissue. Scanning electron micrographs taken subsequent to laser treatment show that the cuticle remained intact (i.e., there was no apparent surface damage from the laser; Figure 2b).

The largest difference between control and treated meral spots was a 25% increase in meral spot total reflectance between 300 nm and 700 nm (mean ± SE: control = 1327 ± 158 [AUC]; treatment = 1657 ± 139 [AUC]; Figure 2a). The spectral response of the treated spot was comparable to the control with both UV peaks at similar wavelengths (control: 350 ± 0.8 nm and 402 ± 1.1 nm; treatment: 351 ± 1.7 nm and 398 ± 1.6 nm) indicating little change in hue. There was only a 5% decrease in spectral saturation of the treated meral spots (control = 3.8 ± 0.3; treatment = 3.6 ± 0.3).

Contest trials

During contests, behaviors of intruders and residents differed between the treatment groups. When facing a brightened resident, intruders showed significantly increased frequency of coils ($\chi^2 = 8.66$, df = 1, $P = 0.003$; Figure 3b) but no increase in the total number of coils ($\chi^2 = 0.03$, df = 1, $P = 0.87$). The frequency of offensive behaviors (strikes, lunges, and meral spreads) was greater when facing a brightened resident ($\chi^2 = 4.02$, df = 1, $P = 0.045$; Figure 3b); however, when 1 outlier was removed, this effect was no longer significant ($\chi^2 = 0.34$, df = 1, $P = 0.56$; Table 1). The total number of offensive behaviors did not differ between treatment groups ($\chi^2 = 1.92$, df = 1, $P = 0.17$; Table 1). There may be a difference between the sexes in response to the meral spot treatment; 5 out of 6 females increased their offensive behavior rate, whereas 3 out of 4 males decreased their offensive behavior rate (Figure 3b). However, due to our low sample size, we did not run statistical tests on this observation.

Residents in the brightened treatment group increased their rate of offensive behaviors ($\chi^2 = 11.06$, df = 1, $P < 0.001$); however, there was no increase in total offensive behaviors ($\chi^2 = 1.60$, df = 1, $P = 0.21$). We also detected a difference in contest duration, with contests against brightened residents lasting only 5% as long as contests against control residents ($\chi^2 = 6.18$, df = 1, $P = 0.013$; Table 1). There were no differences in any other recorded behaviors, including proportion of contests that the intruder won, latency until approach, speed of approach, or time that intruder was outside the refuge (Table 1).

DISCUSSION

Here, we have shown for the first time that stomatopods not only can detect small changes in total reflectance, but also that they assess meral spot total reflectance during contests. We achieved this by developing a new technique using an ultrafast laser to precisely increase total reflectance of the meral spot.

This study demonstrated that ultrafast lasers are an effective technique to precisely modify organismal reflectance across the spectrum. Because they can be tuned to match absorption peaks of the tissues of interest, such lasers could be used to modify a wide variety of pigmented colors. This technique could also effectively dim or remove structural colors by disrupting the photonic crystals that generate these colors (Vukusic and Sambles 2003). Many animals, including lizards, fish, birds, insects, and crustaceans, use pigments and/or structural color to create colored patches that are used as courtship signals, aggressive signals, or antipredator signals (Kodric-Brown 1993; Keyser and Hill 2000; Siefferman and Hill 2005; Whiting et al. 2006; Svádová et al. 2009; Baldwin and Johnsen 2012). Both colorblind animals and animals with color vision use achromatic signals to assess opponents or potential mates (e.g., giant cuttlefish, Adamo and Hanlon 1996; blue crabs, Baldwin and Johnsen 2012; preying mantids, Barry et al. 2014; poison frogs, Crothers and Cummings 2015; eagle owls, Penteriani et al. 2007). This novel technique provides a tool for investigating luminance vision and achromatic signaling in many organisms, especially when the organism's size (e.g., jumping spiders), native environment (e.g., aquatic animals such as crustaceans), or visual system (e.g., butterflies) make alterations of color signals challenging.

In this experiment, the laser likely acts by disrupting pigmented molecules in the exoskeleton via multiphoton absorption (Liu et al.

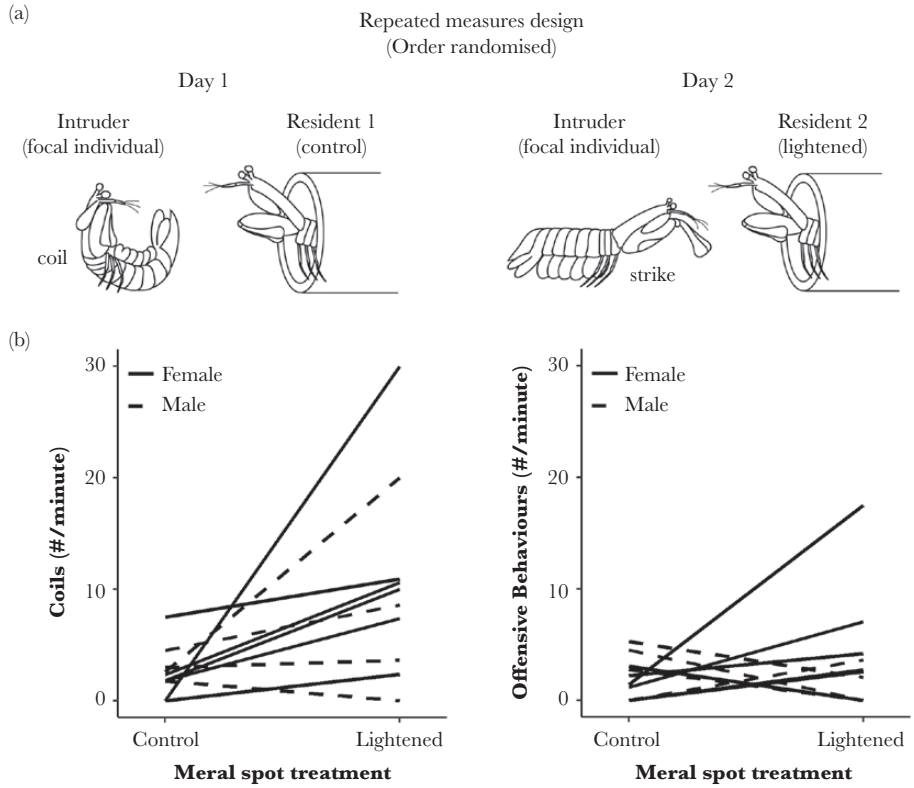

Figure 3
Intruder behaviors in response to increased total reflectance of the resident's meral spot. (a) A schematic of the experimental design. Intruder stomatopods would face both a control and a treated resident on consecutive days (order randomized). (b) Intruder responses to control and treated residents. Each line indicates an individual intruder stomatopod. Dashed lines are males (n = 4) and solid lines are females (n = 6). Graph on left shows rate of intruder coils (depicted in *a* above) and graph on right shows rate of intruder offensive behaviors, which include strikes (depicted in *a* above), lunges and meral spreads (see Figure 1).

1997). It appears to be a nonlinear process because we did not observe any changes to the meral spot when the laser was operated in continuous-wave mode at the same average power as the pulsed mode. The treatment caused no visible damage to the cuticle, even when examined at high magnification, indicating that only pigment molecules deposited within the exoskeleton were disrupted. The increase in total reflectance produced by this procedure closely mimics natural variation in *N. oerstedii* meral spot color (Supplementary Figure S4) and is a similar increase in total reflectance as that observed day 3 after a molt (compared to an intermolt stomatopod; Supplementary Figure S1; Supplementary Table S1). Consequently, we believe these stomatopods perceived the manipulation as a natural meral spot color.

To investigate the role of meral spot total reflectance in stomatopod contests, we staged contests between an intruder and a resident stomatopod. Several behavioral changes were observed when the intruder encountered a resident with an experimentally brightened meral spot. The first behavioral change was an increase in the rate that the intruder stomatopod assumed a coil position. In this behavior, the intruder exposes its hardened telson (tail) to its opponent. In a contest, both stomatopods can alternate between coiling and punching, a behavior known as telson sparring (Green and Patek 2015). Historically, the coil position was considered a defensive posture that protects the abdomen from an opponent's punch (Dingle and Caldwell 1969; Caldwell 1979). More recent evidence, however, suggests that telson sparring could also be a ritualized behavior to assess an opponent with low risk of personal injury (Smith and Price 1973; Immelmann and Beer 1989; Green and Patek 2015). The stomatopod that delivers more strikes during telson sparring is more likely to win (Green and Patek 2015). This suggests that telson sparring might convey information about an opponent's resource holding potential (RHP), such as stamina or aggression.

We propose that the increase in intruders' coil rate observed in our experiment, functions in assessing the resident's RHP, rather than as a defensive behavior. We observed that most often, stomatopods coiled immediately after punching or lunging (48 out of 79 coils; per trial range (n_{trials} = 20): coils after punch/lunge = 0–13, total coils = 0–13). Additionally, there were 9 coils performed without a prior punch or lunge that positioned the telson over the refuge entrance. In both of these scenarios, the coil may be an attempt to elicit a response from the resident to determine resident's RHP. Indeed, intruder coils frequently (25% of coils) elicited an aggressive response from the resident. We also detected an increase in resident stomatopods' aggressive behavior rate in the brightened treatment group, which could be in response to the intruder's increased coil rate. This behavioral difference is unlikely due to the laser treatment, because all of the control residents also experienced the same procedure (albeit on the carapace). The alternate hypothesis is that coils are solely a defensive strategy. An increase in defensive behaviors might be expected if increased reflectance is perceived as a signal of increased RHP. However, current evidence suggests smaller stomatopods have brighter meral spots (Franklin et al. 2016) and weaker strike force than larger stomatopods (Claverie et al. 2011). Meral spot reflectance also increases after the molt, when

stomatopods are unable to fight (Supplementary Figure S1; Reaka 1975). Therefore, increased total reflectance is more likely to indicate lower RHP. Based on this evidence, intruder coil rate would be expected to increase when facing a resident with brighter meral spots to determine if they have recently molted and/or are prepared to fight.

Territorial contests involving residents with brightened meral spots were much shorter and more active than those with unmanipulated residents. There was no difference in total number of intruder defensive behaviors or resident offensive behaviors, despite the increase in rate of both behaviors. This suggests that a threshold number of behaviors may be required for an intruder to adequately assess the RHP of its opponent. The increase in rate may be a tactic to signal increased motivation when an intruder perceives it has a greater chance of winning. Contests can then end more quickly because sufficient information about an opponent has been received.

An increase in offensive behaviors by an intruder may be expected if increased meral spot total reflectance indicates lower RHP; a stomatopod will perceive it has a greater chance of winning and increase investment into the contest. While we did find a statistically significant increase in the rate of intruder offensive behaviors, this was driven by a single individual and may not accurately reflect a response to increased meral spot reflectance. We also noticed that males and females may respond differently to an opponent's increased meral spot total reflectance. Five out of 6 females increased rate of offensive behaviors while 3 out of 4 males decreased offensive behavior rate. Female *N. oerstedii* tend to have meral spots with greater total reflectance than male *N. oerstedii* (Franklin et al. 2016). It is possible that our treatment caused males to incorrectly classify their sex-matched opponents as female. In this case, they may have decreased their offensive behaviors as an attempt to court the "female" (Dingle and Caldwell 1972). This potential sex difference in behavior indicates that more research is needed into the role of the meral spot in mate recognition.

There were no other behavioral changes in response to increased reflectance of the resident's meral spot. We did not expect increased reflectance to alter latency to approach or speed of approach. In *N. oerstedii*, individuals tend to perform the meral spread after an opponent has approached the refuge, not beforehand (Franklin et al. 2016). Thus, the meral spot is likely to be assessed during contests and is not expected to influence approach behavior. A previous study in *N. oerstedii* that manipulated meral spot color also found no effect on approach behavior (Franklin et al. 2016). We also did not expect the winner of the contest to vary between treatment groups because we modified meral spot reflectance, not resident's fighting ability. Consequently, resident fighting behavior is unlikely to change due to the manipulation. Intruders would likely use other resident behaviors (e.g., strikes and lunges) to further assess resident RHP, after initial assessment of the meral spot. Instead, the winner of the fight is more likely to be related to other factors such as size difference (Caldwell and Dingle 1979) and role in the fight (residents have an advantage; Dingle and Caldwell 1975; Steger and Caldwell 1983).

Beyond *N. oerstedii*, it is likely that other stomatopods can also detect small changes in achromatic cues and may also use meral spot total reflectance as a signal or a cue. Stomatopods in the 2 superfamilies Gonodactyloidea and Lysioquilloidea, have a similar eye structure to *N. oerstedii* and achromatic visual information is likely processed in a similar manner (Marshall 1988; Marshall et al. 2007). Furthermore, many stomatopod species possess colored meral spots that they display in contests. It is common for these spots to lose color and have greater reflectance immediately after molting (Reaka 1975; Supplementary Figure S1). Thus, these species may not be able to bluff RHP after molting, as has previously been documented in a stomatopod with a white meral spot (Steger and Caldwell 1983). Instead, a temporarily brightened meral spot may act as an unwanted cue of low RHP. Further research investigating contests with molted individuals are necessary to determine if stomatopods with colored meral spots can bluff RHP or if the meral spots indicate a recent molt.

SUPPLEMENTARY MATERIAL

Supplementary data are available at *Behavioral Ecology* online.

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