Supplementary material for Nokelainen et al. 2019 Functional Ecology

Title: Improved camouflage through ontogenetic colour change confers reduced detection risk in shore crabs

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Contents: Page

Supplementary tables

Table S1 2

Table S2 6

Table S3 7

Supplementary figures

Figure S1 9

Figure S2 10

Figure S3 11

Supplementary references 12

Table S1: Details on photography, vision modelling, pattern analysis and background match estimation.

Photography and vision modelling

Photography, initial image calibration and analysis broadly followed previously used methods (Stevens et al. 2014a). Imaging was undertaken with Samsung NX1000 digital camera converted to full spectrum with no quartz filter to enable UV sensitivity, and fitted with a Nikon EL 80 mm lens. For the human visible photos, we placed a UV and infrared (IR) blocking filter in front of the lens, which transmits wavelengths only between 400 – 680 nm (Baader UV/IR Cut Filter). For the UV images, a UV pass and IR blocking filter was used (Baader U filter), which transmits between 320-380 nm. We have previously characterized the spectral sensitivity of our cameras (Troscianko and Stevens 2015). For calibration purposes, each photograph included a grey reflectance standard, which reflects light equally at 7% and 93% between 300 and 750 nm.

Images were taken in RAW format with manual white balance and a fixed aperture setting. During calibration (in Image J; Troscianko & Stevens 2015), images were converted to uncompressed TIFF files and the images of each crab comprised four bandpass layers corresponding to the long-wavelength (LW), medium-wavelength (MW), short-wavelength (SW), and UV parts of the spectrum. To control for nonlinear responses in image value to changes in light levels, we linearized all images based on quantified camera responses to a set of eight Spectralon grey standards with reflectance values ranging from 2–99% (Stevens et al. 2007, 2009). We controlled for differences in ambient light by standardizing (equalizing) the images to the grey standard, and scaled each image channel to reflectance, where an image value of 255 on an 8-bit scale equals 100%

reflectance (Stevens et al. 2007, Troscianko and Stevens 2015). These images therefore corresponded to the reflectance properties of the crab patterns in four parts of the spectrum and could be used for analysis of coloration and pattern. For each image we measured the entire dorsal side of the crab carapace.

We modelled predator vision according to fish visual sensitivity, because fish are major predators of juvenile shore crabs (Crothers 1968). We used the spectral sensitivities of pollack (*Pollachius pollachius*) adults (Shand et al. 1988) to generate cone catch values for fish predators based on pigment model (Govardovskii et al. 2000) incorporating the lens transmission data (Troscianko and Stevens 2015). The cone mapping procedure converts images from camera reflectance space to animal receptor values and is highly accurate compared to alternative methods using reflectance spectrometry (Cuthill et al. 2006, Pike 2011, Stevens et al. 2014a, Troscianko and Stevens 2015).

Pattern analysis procedure

The pattern analysis technique (a 'granularity' analysis) involves decomposing an image into a series of different spatial frequencies ('granularity bands') using Fourier analysis and band pass filtering, followed by determining the relative contribution of different marking sizes to the overall pattern (Barbosa et al. 2008, Hanlon et al. 2009, Stoddard and Stevens 2010). The filtering into different frequency bands functions like a sieve, capturing information at different spatial scales corresponding to different sized markings. Pattern analysis was conducted in custom files for Image J (Troscianko and Stevens 2015), with analysis based on different pixel sizes. The analysis calculates the amount of information, or energy, corresponding to markings of different sizes, starting

with small markings (few pixels) and increasing in size to larger markings. For each granularity band, we calculated the pattern 'energy', being the sum of the squared pixel values in each image divided by the total number of pixels (Barbosa et al. 2008, Chiao et al. 2009, Stoddard and Stevens 2010). The energy values across all filtered images produce a 'granularity spectrum', being a plot of energy versus pixels (marking size). From each granularity spectrum we obtained crab carapace pattern information as described below (Stoddard and Stevens 2010).

Crab phenotype determination and background match

To characterize crab phenotype, we analysed the data both with normalised camera responses and fish vision modelled data. We calculated five measures of crab appearance based on colour and pattern metrics using previously published methods (Barbosa et al. 2008, Hanlon et al. 2009, Stoddard and Stevens 2010, Stevens et al. 2014b). 1) Luminance: the perceived lightness based on the LW receptor in fish and LW channel in normalised camera responses. 2) Hue: calculated as a ratio of shortwave versus longwave receptor responses (e.g. (SW/ LW) (Komdeur et al. 2005, Spottiswoode and Stevens 2011). 3) Proportion energy (i.e. how much one marking size dominates, or the diversity of marking sizes), being the proportion of the total energy across the entire spectrum corresponding to the maximum energy point, with a high value indicating that the pattern is dominated by one or a few marking sizes. 4) Total energy (i.e. pattern contrast), being the total energy of the spectrum (Chiao et al. 2009), whereby higher values indicate more contrasting markings. 5) Marking size (i.e. carapace marking size), with the maximum energy value at any point in the spectrum corresponding to the dominant marking size.

To examine the level of background match, we calculated how changes in the crab carapace influenced their level of match to the experimental backgrounds. To do so we used a visual discrimination model (Vorobyev et al. 1998), which is based on differences in colour or luminance based on photo catch values. We used a Weber fraction value of 0.05 for the most abundant cone type (Govardovskii et al. 2000) with receptor cone ratios SW 168 and LW 339 for the pollack vision. The model yields values in 'just noticeable differences' (JNDs), whereby differences between 1 and 3 are interpreted that two stimuli are unlikely to be discriminated by an observer (and hence indicate a good background match), and larger values are increasingly likely to be discriminable (Siddiqi et al. 2004).

Table S2: Linear mixed effects analyses (LMER) testing the luminance and colour change of green shore crabs over time. LMER predicts the luminance and hue changes as a response to crab original appearance (shade), rearing background type (background), time (week) and their interactions. Intercept has crab ID and tank as random variables.

Subject	Estimate	s.e.	DF	t-value	P
Luminance					
(Intercept)°	13.80	1.41	21.7	9.77	<0.001
Background [rock pool]	0.44	1.73	53.9	0.25	0.798
Shade [pale]	7.29	2.08	89.0	3.50	<0.001
Time [week]	-0.32	0.13	531.5	-2.36	0.018
Background * Shade	3.68	2.61	54.8	1.41	0.163
Shade * Week	0.14	0.20	530.9	-5.31	<0.001
Hue					
(Intercept)°	1.17	0.02	76.5	49.01	<0.001
Background [rock pool]	-0.03	0.03	74.3	-1.00	0.319
Shade [pale]	0.03	0.03	58.0	1.08	0.281
Time [week]	0.01	0.01	527.8	9.12	<0.001
Background * Shade	-0.01	0.04	57.0	-0.18	0.853
Background * Week	-0.01	0.01	525.2	-1.00	0.314

Intercept includes factor level: Background [mud] & Shade [dark].

Table S3: Linear mixed effects analyses (LMER) testing the changes in carapace patterning over time. LMER predicts the carapace pattern change in relation to crab original appearance (shade), rearing background type (background), time (week) and their interactions. Intercept has crab ID and rearing tank as random variables.

Subject	Estimate	s.e.	DF	t-value	P
Pattern diversity					
(Intercept)°	1.450e-01	2.984e-02	2.100e+00	4.860	0.036
Background [rock pool]	1.320e-02	7.528e-03	1.124e+02	1.754	0.082
Shade [pale]	2.589e-02	8.029e-03	1.151e+02	3.225	0.001
Time [week]	4.049e-04	5.790e-04	5.264e+02	0.699	0.484
Background * Shade	-1.252e-02	1.131e-02	1.124e+02	-1.107	0.270
Background * Week	-1.517e-03	8.558e-04	5.281e+02	-1.772	0.076
Shade * Week	-4.201e-03	9.806e-04	5.362e+02	-4.285	<0.001
Background * Shade * Week	3.531e-03	1.319e-03	5.308e+02	2.677	0.007
Pattern contrast					
(Intercept)°	3537.47	787.92	2.70	4.49	<0.001
Background [rock pool]	131.61	475.23	54.50	0.27	0.780
Shade [pale]	1170.15	530.67	67.20	2.20	0.030
Time [week]	-112.19	23.28	550.50	-4.81	<0.001
Background * Shade	1201.02	715.42	54.80	1.67	0.090
Background * Week	-285.63	35.31	550.40	-8.08	<0.001

Table S3 continued

Marking size					
(Intercept)°	150.13	27.641	19.30	5.431	<0.001
Background [rock pool]	-44.01	36.31	213.4	-1.21	0.226
Shade [pale]	21.21	38.89	219.3	0.54	0.586
Time [week]	10.31	3.63	534.4	2.836	0.004
Background * Shade	-31.28	54.57	213.0	-0.57	0.567
Background * Week	-1.71	5.37	537.2	-0.31	0.750
Shade * Week	-15.37	6.11	550.5	-2.51	0.012
Background * Shade * Week	17.60	8.26	541.9	2.131	0.033

Intercept includes factor level: Background [mud] & Shade [dark].

Figure S1: Comparison between artificial and natural backgrounds. Here the match of our artificial backgrounds to natural ones was compared using a photographic data. Briefly, we quantified similarity of the backgrounds in trichromatic RGB colour space, based on reflectance data, to quantify brightness (A) and hue (B).

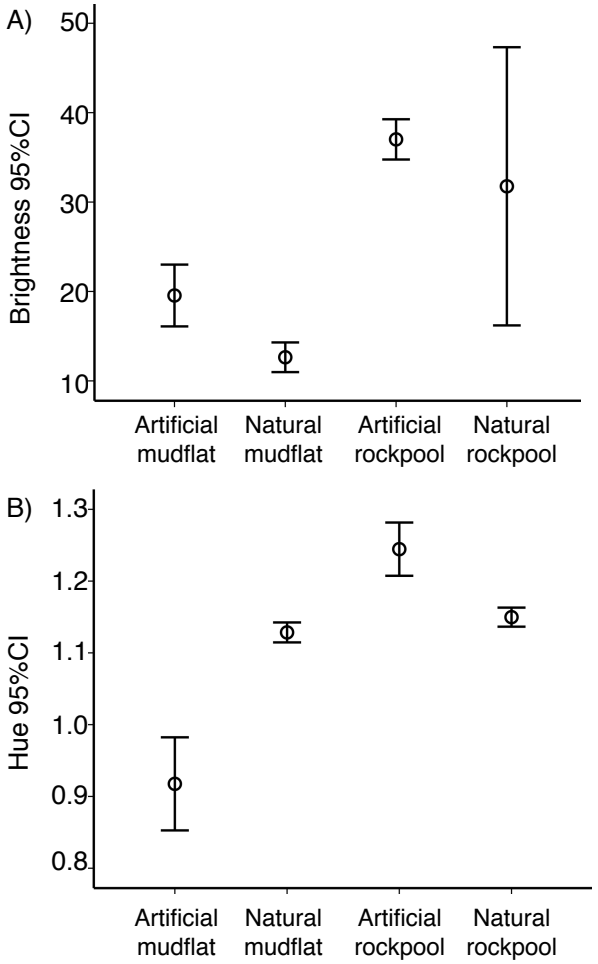


Figure S2: Ontogenetic colour change in the green shore crab (*Carcinus maenas*). The figure shows the change in carapace colour over time obtained from normalised camera responses. The panels show different wavelenght channels: red (A), green (B), blue (C) and UV (D). The time is shown on x-axis and the mean colour responses (i.e. the relative expression of the channel as 0-100) on y-axis. The lines are different treatment groups as follows. Solid green: dark-shaded crabs on mud background (DAMD); Solid blue: dark-shaded crabs on rock pool background (DARO); Dashed green: pale-shaded crabs on mud background (PAMD); Dashed blue: pale-shaded crabs on rock pool background (PARO). Notice that the expression of green wavelengths shows mean increase over time in relation to other wavelengths. The combined influence of predominant red and increases in green channel (as well as decreases in blue and low expression of UV) apparently drives the ontogenetic colour change causing the 'mudflat' phenotype.

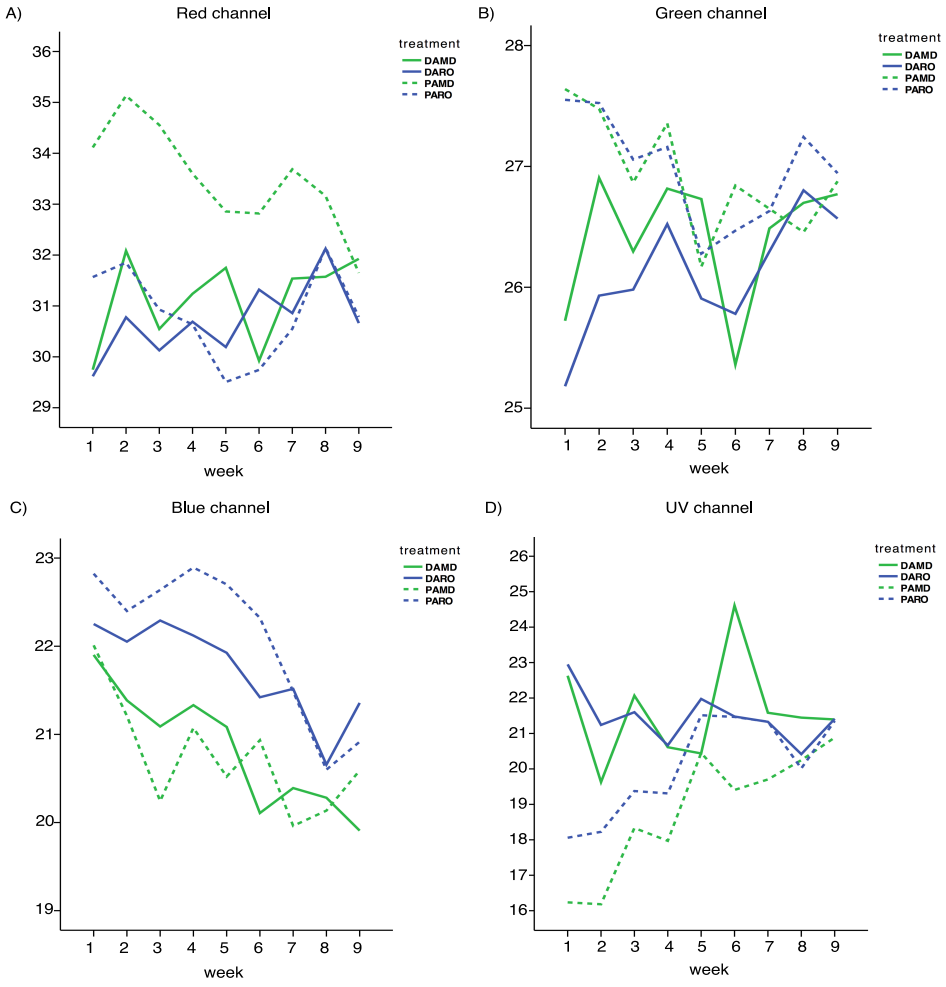
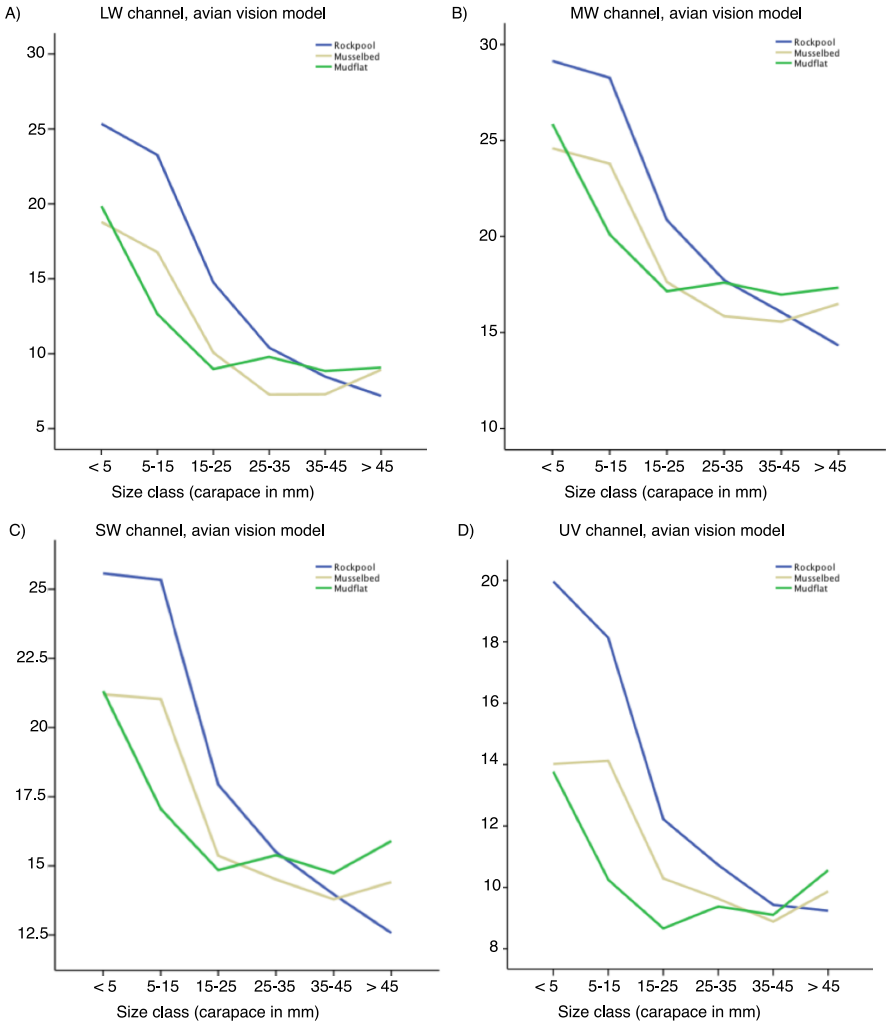


Figure S3: Ontogenetic colour change in the green shore crab (*Carcinus maenas*) in the field. The data is derived from large-scale field monitoring study (Nokelainen et al. 2017). The figure shows the change in carapace colour over time obtained from avian vision model cone catch data in relation to the catching habitat of the crab. The panels show decreases in long wavelengths (A), whereas there is relative increase of medium (B) or short (C) wavelengths and again decrease in UV wavelengths (D) as crabs grow. The same pattern is seen through all habitat types here.



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