Different ontogenetic trajectories of body colour, pattern, and crypsis in two sympatric intertidal crab species

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ABSTRACT

Animals frequently exhibit high appearance variation, especially in heterogeneous habitats where individuals may be differentially concealed against backgrounds. While background matching is a common anti-predator strategy, gaps exist in understanding within- and among-species variation. Specifically, the drivers of appearance changes associated with habitat use and occurring through ontogeny are poorly understood. Using image analysis, we tested how individual appearance and camouflage in two intertidal crab species – the mud crab *Panopeus americanus* and the mottled crab *Pachygrapsus transversus* – relates to ontogeny and habitat use. We predicted that both species would change appearance with ontogeny, but resident mud crabs would exhibit higher background similarity than generalist mottled crabs. Both species showed ontogenetic changes, but while mud crabs become darker, mottled crabs turn greener. Small mud crabs were highly variable in colour and pattern, likely stemming from utilising camouflage in heterogeneous habitats during the most vulnerable life-stage. Being habitat specialists, mud crabs concealed better against all backgrounds than mottled crabs. Mottled crabs are motile and generalist, occupying macroalgal-covered rocks when adults, which explain why they are greener and why matches to specific habitats are less valuable. Differential habitat use in crabs can be associated with different coloration and camouflage strategies to avoid predation.

Keywords: Araçá Bay - background matching - camouflage strategies - Crustacea - granularity analysis - habitat use - image analysis – life-history traits - substrate heterogeneity

INTRODUCTION

The remarkable diversity of animal coloration has fascinated evolutionary biologists for centuries, being used as key examples of adaptation and natural selection (Darwin, 1859; Wallace, 1867; Kettlewell, 1955). The colour expressed by an animal can intercede important processes throughout its life, including social signalling during mate choice, thermoregulation, and defence against predators (Cuthill *et al.*, 2017). One of the most common and widespread anti-predator strategies mediated by coloration in nature is camouflage, which works by reducing the probability of detection or recognition of prey by the visual system of predators (Stevens & Merilaita, 2009). Although many different camouflage strategies have already been described and tested, especially in artificial systems, including disruptive coloration (Cuthill *et al.*, 2005), countershading (Rowland *et al.*, 2007), and masquerade (Skelhorn *et al.*, 2010), most of the work to date has focused in the most familiar and intuitive type of camouflage, known as background matching. In this strategy, there is a match between the general appearance of the individual in terms of colour, brightness and/or pattern, and the background (Stevens & Merilaita, 2009). This leads to a reduction in the detection of well-concealed individuals by visual predators, and ultimately increases the survival chances of camouflaged prey (Duarte *et al.*, 2018).

Colour is frequently used as a species-specific trait, but many animal populations exhibit considerable variation among individuals (i.e. intraspecific variation). In such cases, populations can be characterized by discrete or continuous colour variation, which may result in polymorphic (i.e. presenting genotypic and phenotypic variation) or polyphenic populations (i.e. exhibiting only phenotypic variation) (West-Eberhard, 1989). Intraspecific colour variation has been described for many species belonging to different taxa, from invertebrates (Reimchen, 1989; Krause-Nehring *et al.*, 2010; Eacock *et al.*, 2017) to vertebrates (Cheney *et al.*, 2009; Calsbeek *et al.*, 2010; Passarotto *et al.*, 2018), and can be maintained in populations by different selective processes, such as assortative mating, differential niche use, environmental heterogeneity and frequency-dependent (i.e. apostatic) selection, usually guided by visual predation (Bond & Kamil, 2002, 2006; Gray & McKinnon, 2007). Differential coloration among individuals would be particularly important for species associated with heterogeneous backgrounds, and could be driven by a wide range of evolutionary processes, such as differential concealment of colour types against contrasting microhabitats (Stevens *et al.*, 2015; Nokelainen *et al.*, 2017; Duarte *et al.*, 2018). This can include the use of specific camouflage strategies on each background type (Price *et al.*, 2019), or as a means to defeat predator search images (Bond & Kamil, 2002). In the common shore crab *Carcinus maenas*, for example, juveniles are highly variable in terms of colour and brightness (Todd *et al.*, 2006; Stevens *et al.*, 2014). However, crabs inhabiting homogeneous mudflats are more uniform and match the background closely. On the other hand, crabs from rock pools, where substrates are very colourful and heterogeneous, are more variable in brightness and do not match the background well, but instead exhibit much higher levels of disruptive coloration (Price *et al.*, 2019).

Camouflage against spatially or temporally heterogeneous backgrounds can be improved either by oriented behavioural choices towards colour matching substrates or by mechanisms of colour change (Kang *et al.*, 2015; Duarte *et al.*, 2017; Eacock *et al.*, 2017; Green *et al.*, 2019; Stevens & Ruxton, 2019), allowing the individuals to cope with environmental changes occurring over a range of spatial and temporal scales (Caro *et al.*, 2016; Duarte *et al.*, 2017). Besides flexibly altering coloration over multiple timescales for concealment (Duarte *et al.*, 2017), many animal species also change appearance through ontogeny (Reimchen, 1989; Booth, 1990; Wilson *et al.*, 2007; Todd *et al.*, 2009; Hultgren & Stachowicz, 2010; Jensen & Egnotovich, 2015; Nokelainen *et al.*, 2019). A variety of intertidal crab species, for example, exhibit remarkable colour and pattern variation as juveniles but not during the adult phase (Palma *et al.*, 2003; Todd *et al.*, 2009; Caro, 2018; Nokelainen *et al.*, 2019). High colour variability may allow individuals to differentially conceal against specific patches of the heterogeneous habitats on which they reside during this life stage (Palma & Steneck, 2001; Stevens *et al.*, 2014), or to defeat search images in visual predators (Krause-Nehring *et al.*, 2010; Karpestam *et al.*, 2014). Ontogenetic colour change is commonly associated with a shift in habitat use, with small crabs occupying highly heterogeneous backgrounds but moving to less diverse substrates as adults (Palma & Steneck, 2001; Todd *et al.*, 2006). Moreover, in many crab species, adults become more active than juveniles and frequently move across habitat types, potentially adopting a generalist camouflage strategy (i.e. compromise coloration; Hughes *et al.*, 2019). Here, individual concealment is not directed towards a specific background but works as an efficient way to improve camouflage against a wide range of backgrounds (Nokelainen *et al.*, 2019). Not mutually exclusively, ontogenetic colour shifts may also occur due to a reduction in vulnerability to predation in the adult phase, since, after achieving a size refuge, larger crabs would have a reduced need for camouflage (Palma & Steneck, 2001; Krause-Nehring *et al.*, 2010).

In crabs, phenotype-environment associations have been demonstrated in several species, across multiple spatial scales, especially as juveniles (Palma & Steneck, 2001; Palma *et al.*, 2003; Todd *et al.*, 2006; Krause-Nehring *et al.*, 2010; Stevens *et al.*, 2014; Nokelainen *et al.*, 2017). However, whether such associations improve camouflage are still poorly explored (but see Jensen & Egnotovich, 2015; Russell & Dierssen, 2015; Nokelainen *et al.*, 2017; Price *et al.*, 2019). Additionally, most studies have focused on a restricted number of crab species, mainly from temperate areas, and used subjective estimates to measure individual coloration and camouflage (Palma & Steneck, 2001; Palma *et al.*, 2003; Todd *et al.*, 2006; Krause-Nehring *et al.*, 2010). This results in a gap of knowledge about intraspecific colour variation, crypsis and ontogenetic shifts in tropical and subtropical species (but see for example Hemmi *et al.*, 2006; Detto *et al.*, 2008; Stevens *et al.*, 2013; Carvalho-Batista *et al.*, 2015). Therefore, it is necessary to expand our knowledge of animal camouflage to those poorly-known species and diverse ecosystems where predation is intense (Schemske *et al.*, 2009; Roslin *et al.*, 2017), as a way to understand whether the patterns of variation and camouflage, as well as the underlying ecological processes described for temperate species are commonplace in nature.

The Araçá Bay, located in the northern coast of São Paulo, Brazil, is a large tidal flat of high biological diversity (Nucci *et al.*, 2001; Amaral *et al.*, 2010; Dias *et al.*, 2018), composed of different substrate types, ranging from large rocks frequently covered by ephemeral green algae (*Ulva* spp.) to coarse sand and fine silt/clay sediments (Amaral *et al.*, 2010). Two of the most common crab species found at this site is the narrowback mud crab *Panopeus americanus* Saussure, 1857 (Decapoda, Panopeidae) (hereafter "mud crab"; Figure 1A) and the mottled shore crab *Pachygrapsus transversus* Gibbes, 1850 (Decapoda, Grapsidae) (henceforth "mottled crab"; Figure 1B) that exhibit remarkable variation in colour and pattern on their carapace. Both species occupy the intertidal zone of rocky substrates and muddy beaches in estuarine and mangrove areas, exhibiting ontogenetic changes in habitat use, with juveniles occupying different substrate types to adults (Abele *et al.*, 1986; Flores & Negreiros-Fransozo, 1999; Vergamini & Mantelatto, 2008a,b; García *et al.*, 2016). There are marked differences in habitat use and life-history traits between mud and mottled crabs (Table 1), but ontogenetic habitat shifts may broadly affect concealment to background colour and texture in individuals of both species. Here, we used digital image analysis to test whether the appearance and crypsis of mud and mottled crabs inhabiting different microhabitats (Figure 1C) varies according to the size (i.e. age) of the individuals. We predict substantial changes in both species, but high background similarity in mud crabs, as they are less active, not moving far from their shelter (Micheli, 1997; Vergamini & Mantelatto, 2008a; Carvalho-Batista *et al.*, 2015), and low overall concealment in mottled crabs, which move faster and over larger foraging areas, making them habitat generalists (Abele *et al.*, 1986; Christofoletti *et al.*, 2010).

MATERIALS AND METHODS

SAMPLING AND PHOTOGRAPHY

Sampling was conducted at Araçá Beach and Ilha Pernambuco at the Araçá Bay, located in Southeast Brazil (23°48'78.1"S 45°24'46.9"W). We concentrated our sampling in a single day in August 2016 during the low tide when we searched for mud crabs (*Panopeus americanus*; Figure 1A) and mottled crabs (*Pachygrapsus transversus*; Figure 1B) in an area of approximately 200 m². Crabs of all sizes were manually collected while removing boulders, pebbles and searching over gravel beds. Collected individuals (61 mud crabs and 53 mottled crabs) were housed in plastic containers filled with seawater and small rock pieces and taken to the laboratory where they were photographed (see details below). In order to quantify the colour range of the different microhabitats used by the two crab species, we obtained at the field 24 photographs of three clearly distinct background types that are found in the study area (Figure 1C): (i) large rock covered by green algae (hereafter "alga", *n* = 8), (ii) muddy areas containing small rocks (hereafter "mud rock", *n* = 8), and (iii) sandy areas covered by small pebbles (hereafter "pebbles", *n* = 8).

We used a Nikon D80 digital camera coupled with a Nikkor 60 mm lens and a UV-blocker filter (62 mm, Tiffen, USA) to photograph backgrounds in the field from a fixed distance of 1.5 m and crabs in an external area of the Centre for Marine Biology (CEBIMar-USP, São Sebastião, Brazil) using a copy stand for photography under natural illumination conditions. Images were taken in RAW format, with manual white balancing and fixed aperture settings to avoid over-exposure (Stevens *et al.*, 2007), and included a ruler and black (7.5%) and white (91%) Spectralon reflectance targets (Labsphere, Congleton, UK) following current standard procedure (Troscianko & Stevens, 2015). Carapace width (CW) was measured using the ruler included in the photographs. All images were first linearized based on curves modelled from eight Spectralon standards ranging from 2 to 99% of reflectance to correct for camera non-linear responses to light intensity (Troscianko & Stevens, 2015). Next, photographs were equalized for changes in light conditions using the black and white standards and saved as 32-bit multispectral images. Image channels were then scaled to reflectance values, where an image value of 255 on an 8-bit scale equals 100% reflectance (Stevens *et al.*, 2007). After all these procedures, images correspond to the physical reflectance properties of crabs and backgrounds in three parts of the spectrum (LW – long-wavelength, MW – medium-wavelength, and SW – short-wavelength) and could be used for analysis of coloration and pattern. All routines were performed using functions from the 'Multispectral Image Calibration and Analysis Toolbox' implemented in the ImageJ software (Rasband, 1997; Troscianko & Stevens, 2015).

MEASURING CRAB COLOUR AND PATTERN

For each multispectral image, we selected regions of interest (ROIs – crab carapace and entire background, avoiding areas of specular reflectance) from where we extracted reflectance values that were used to calculate several metrics of appearance based on brightness, colour and pattern. Similar to other studies on crabs (Detto *et al.*, 2008; Stevens *et al.*, 2013, 2014), we preferred to work with normalised reflectance data instead of using visual modelling because there is a wide range of potential predators from different taxonomic groups (e.g. other crabs, fish and birds) consuming crabs in the Araçá Bay, and existing information about their identity is scarce and mostly anecdotal (Carvalho-Batista *et al.*, 2015). Brightness was calculated as (LW+MW+SW)/3 and is a simple achromatic measure of how dark or bright crabs are across the entire spectrum (Stevens *et al.*, 2014). The colour metrics saturation and hue were also calculated. Saturation is generally assumed as the amount of a given colour to white light (i.e. the colour richness), and was calculated by transforming the standardized LW, MW and SW reflectance values (i.e. proportional value to the summed reflectance across the entire spectrum) to XY coordinates of a trichromatic colour space (i.e. Maxwell triangle). Saturation was considered as the shortest distance from the given colour point to the achromatic centre of the space, with larger values representing greater saturation (Kelber *et al.*, 2003). For hue, we first conducted a principal component analysis (PCA) to define the main axis of colour variation for both crab species, and used this to determine a logical colour channel (as in Green *et al.*, 2019; Nokelainen *et al.*, 2019). PCA was applied to the standardised reflectance values from the three reflectance channels and hue was further defined as the ratio MWS/(SWS + LWS), which is a simple measurement of medium-wavelength versus the two extremes of the light spectrum, broadly analogous to an opponent-style colour channel (Spottiswoode & Stevens, 2011).

Besides colour, mud and mottled crabs also greatly differ in pattern, exhibiting colour spots of different shape and size on their carapace (Figure 1A, B). We employed the widely used and well-established 'granularity' analysis approach to measure pattern in the two crab species. This method is based on the decomposition of an image into a series of different spatial frequencies (i.e. granularity bands) using Fourier analysis and band-pass filtering (Barbosa *et al.*, 2008; Stoddard & Stevens, 2010; Stevens *et al.*, 2014), followed by the determination of the relative contribution of different marking sizes to the overall pattern. In this analysis, the amount of information ('energy') is calculated from markings of different sizes, starting with small markings (i.e. formed by few pixels) and increasing in size to larger markings. We used a log-scale setup with a starting size of two pixels and a log multiplier of 1.414 increment up to a maximum of 4096 pixels, where no pattern energy was further observed. Next, for each granularity band, we calculated the overall pattern ‘energy’ as the sum of the squared pixel values divided by the total number of pixels (Barbosa *et al.*, 2008). Finally, after processing all images, we calculated three different metrics of pattern from each granularity spectrum (i.e. each decomposed image), being: (i) maximum frequency (i.e. the spatial frequency with the highest energy, which corresponds to the dominant marking size); (ii) summed energy (i.e. the energy summed across all scales, which is a measure of pattern contrast); (iii) proportion energy (i.e. the energy at the maximum frequency divided by the summed energy, which is a measure of pattern diversity) (Chiao *et al.*, 2009; Stoddard & Stevens, 2010).

MEASURING CRAB BACKGROUND MATCHING

In order to measure the degree of concealment of both mud and mottled crabs against their main habitats in Araçá Bay, we used the colour values extracted from the carapace and from backgrounds, categorized into the three broad types: “alga”, “mud rock” and “pebble” (Figure 1C). For that, we first converted the camera colour channels values of crabs and backgrounds to XY coordinates of a two dimensional colour space, where each colour is expressed as a single point (Kelber *et al.*, 2003). We then calculated the Euclidean distance between each crab coordinate and the eight replicate values of each of the three backgrounds, which were averaged to a single value. This provides a receiver-independent estimate of the degree of background match of the two crab species across the different habitat types, for which lower distances indicate more similar coloration between crabs and backgrounds.

CLASSIFICATION OF SIZE GROUPS

In order to understand how the appearance and crypsis of crabs of both species change with ontogeny, we classified individuals as small or large based on the size at which individuals spend more time on alternative foraging habitats and, in the case of mottled crabs, become more active. This behavioural change takes place, approximately, when individuals reach 13 mm CW in the case of mud crabs (Carvalho-Batista *et al.*, 2015) and 14 mm in the case of mottled crabs (Abele *et al.*, 1986). We then compared how effectively crabs fell into these categories based on the appearance metrics we measured (e.g. brightness, saturation, hue, maximum frequency, summed energy, and proportion energy) using Discriminant Function Analysis (DFA).

STATISTICAL ANALYSES

All statistical analyses were undertaken using the software R [v. 4.0.0] (R Core Team, 2020). We first used the 'lda' function from the 'MASS' package in R (Venables & Ripley, 2002) to run discriminant function analysis (DFA) in order to validate the size categories we previously chosen (i.e. small or large) for both crab species based on individual appearance. Brightness and all colour (saturation and hue) and pattern metrics (maximum frequency, summed energy and proportion energy) were compared individually between crab species (mud or mottled crabs) and size classes (small or large) using a two-way analysis of variance (ANOVA) to test for possible species-specific ontogenetic trajectories of colour and pattern. A linear mixed-effects model was further applied to the estimates of background similarity (i.e. colour distances between crabs and backgrounds) with background types (alga, mud rock or pebble), crab species (mud or mottled crabs) and size classes (small or large) as fixed between-subjects factors, and crab ID as a random factor to control for repeated measurements on the same individual, since each crab was compared to all background types. The ANOVA model was fitted using the 'aov' function in the 'base' package while the mixed-effects model was fitted using the 'lmer' function in the 'lme4' package (Bates *et al.*, 2015) and the associated significance tests through the 'anova' function in the 'lmerTest' package (Kuznetsova *et al.*, 2017). Model residuals were visually checked for normal error distribution using histograms and q-q plots, while the homogeneity of variances was tested using the Bartlett test in R, for which brightness and all pattern metrics needed a log transformation to meet model assumptions. For the estimates of hue and background similarity, variances remained heterogeneous even after log transformation. However, since our sample size is large (minimum sample size = 20), making the models robust to variance heterogeneity (Underwood, 1997), we decided to run both models anyway using the raw data. Finally, in the case of significant effects, the Tukey's post hoc test was applied to compare mean differences between factor levels using the 'emmeans' function from the 'emmeans' package (Lenth, 2019).

RESULTS

DISTINCTIVENESS IN THE APPEARANCE OF SMALL AND LARGE CRABS

The size of mud crabs ranged from 5.8 to 32.2 mm CW (mean ± SD: 14.7 ± 6.2 mm) and the size of mottled crabs from 3.6 to 23.5 mm CW (11.5 ± 4.4 mm). The discriminant function analyses based on appearance metrics validated the classification of crabs of both species into the two size classes previously determined. In the case of mud crabs, the proportion of correct classification was 0.820, with small individuals (n = 32) correctly assigned at a proportion of 0.750, and large crabs (n = 29) at 0.897. For mottled crabs, the proportion of correct classification was 0.755, with small individuals (n = 33) being correctly assigned at a proportion of 0.909, and large crabs (n = 20) at 0.500.

ONTOGENETIC VARIATION IN CRAB COLOUR AND PATTERN

The brightness and colour metrics (saturation and hue) of each crab's carapace were significantly different between size classes and / or species (Table 2). For mud crabs, small individuals were on average brighter than large crabs (mean ± SE: small = 11.22 ± 0.79%, large = 7.07 ± 0.32%; t_{110} = 5.19, p < 0.001; Figure 2A), but for mottled crabs there was no difference in brightness between size classes, with both small and large crabs being equally dark (small = 4.62 ± 0.24%, large = 4.52 ± 0.27%; t_{110} = 0.16, p = 0.871; Figure 2A), resulting in an interaction between species and size class. In addition, small mud crabs exhibited a marked variation in brightness compared to larger individuals (coefficient of variation: 40.09% for small and 24.61% for large crabs), indicating that small individuals of this species are more diverse in brightness compared to larger ones.

Regardless of size, mottled crabs exhibited carapaces with more saturated coloration than mud crabs (mud crab = 0.103 ± 0.004, mottled crab = 0.123 ± 0.005; t_{110} = 3.41, p < 0.001) (Figure 2B; Table 2). On the other hand, differently from brightness, hue was significantly larger for large mottled crabs compared to small individuals (small = 0.519 ± 0.005, large = 0.550 ± 0.009; t_{110} = 4.13, p < 0.001; Figure 2C), indicating that crabs tend to become greener as they grow. For mud crabs, however, there was no difference in hue between size categories, with individuals maintaining similar values over ontogeny (small = 0.511 ± 0.003, large = 0.519 ± 0.002; t_{110} = 1.18, p = 0.239; Figure 2C), explaining the significant interaction between main factors. Therefore, mud crabs become darker as they grow but remain the same colour, whereas mottled crabs remain similarly dark but become greener.

Regarding the pattern metrics, the dominant size of markings on crab's carapace (i.e. the maximum frequency) significantly differed between species and size classes (Table 3), being on average larger for mud crabs (mud crab = 1.986 ± 0.170 mm, mottled crab = 0.641 ± 0.058 mm; t_{110} = 8.52, $p < 0.001$) and larger individuals (small = 0.971 ± 0.100 mm, large = 1.878 ± 0.208 mm; t_{110} = 3.97, $p < 0.001$; Figure 3A). The overall pattern contrast (i.e. the summed energy) varied between size categories but depended on the species (Table 3). Small mud crabs exhibited more contrasting pattern markings than large individuals (small = 10.503 ± 0.582, large = 8.093 ± 0.366; t_{110} = 3.30, p = 0.001), indicating that carapace markings become less contrasting (Figure 3B). Conversely, mottled crabs showed small and similar pattern contrast between size classes (small = 4.858 ± 0.224, large = 5.513 ± 0.308; t_{110} = 1.60, p = 0.112). Finally, although not different between species (Table 3), the diversity of markings (i.e. the proportion energy) was higher for small individuals (small = 0.090 ± 0.001, large = 0.085 ± 0.001; t_{110} = 2.88, p = 0.005), indicating that larger crabs of both species tend to exhibit less diverse pattern markings on their carapace (Figure 3C).

ONTOGENETIC VARIATION IN BACKGROUND MATCHING OF CRABS AGAINST DIFFERENT BACKGROUNDS

The degree of background matching, measured as the colour distance between crabs and substrates, differed between species, size categories and background habitats (Table 4). In mud crabs, regardless of size, crabs were better concealed against mud rock and pebbles compared to algal substrates (Figure 4A). In mottled crabs, however, small individuals were better concealed against mud rock and pebbles, but large crabs showed equally low concealment to all substrates (Figure 4B). Therefore, small and large mud crabs are consistently more cryptic against mud rock and pebbles, while large mottled crabs become generally conspicuous, and thus poorly concealed to background habitats.

DISCUSSION

Here, we show that ontogenetic changes in animal appearance and crypsis can occur through modifications of different colour and pattern metrics, and apparently linked to other species-specific traits. In the less motile mud crab (*Panopeus americanus*), the variability in brightness and the degree of pattern contrast on the carapace of small crabs reduces with ontogeny, resulting in darker and smoother large individuals. These changes probably stem from the adjustment of crabs to the gradual reduction in the chromatic heterogeneity of the backgrounds they are exposed when older. In the more active mottled crab (*Pachygrapsus transversus*), however, ontogenetic changes occur through a modification in carapace colour, with individuals changing from dark to green tones as they grow, which is probably a result of changes in habitat use and behaviour with age. The modification of appearance of mud crabs with ontogeny is not followed by a reduction in background matching, as would be expected in species that can escape predation by achieving a size refuge, when effective camouflage would be less important. On the contrary, both small and large mud crabs show higher background similarity than mottled crabs, in which large individuals present poor camouflage. Therefore, our findings suggest that other life-history traits of the studied species may interfere in the ontogenetic colour and pattern changes. While for more resident species individuals would continuously rely on camouflage to escape predation, for more active species, background similarity is probably less relevant since individuals can move fast and escape from most predators.

High intraspecific colour variability of many animal species is frequently associated with concurrent variation in the chromatic aspects of the backgrounds they are associated with (Caro *et al.*, 2016), resulting in phenotype-environment matching and an increase in camouflage effectiveness (Todd *et al.*, 2006; Stevens *et al.*, 2015; Nokelainen *et al.*, 2017). Therefore, colour diversity of the different backgrounds occurring in our sampling site (e.g. rocks covered by algae, boulder and pebbles on both muddy and sandy substrates), together with the differential use of those by small and large crabs, should select for specific patterns of coloration and camouflage at the different size categories. Indeed, we observed species-specific ontogenetic trajectories in both crab colour and crypsis: in mud crabs, small individuals are very variable in brightness and in the contrast of carapace markings compared to larger crabs, which are darker and less patterned. In mottled crabs, both size classes exhibit low variability in all colour and pattern metrics, but large crabs become distinctly coloured. The ontogenetic change observed in mud crabs is common to many other crab species, in which juveniles are associated to colour heterogeneous backgrounds but inhabit homogeneous substrates when adults (Palma & Steneck, 2001; Palma *et al.*, 2003; Todd *et al.*, 2009; Krause-Nehring *et al.*, 2010; Stevens *et al.*, 2014; Jensen & Egnotovich, 2015). On the other hand, ontogenetic variation in colour but not in the diversity of pattern markings, as observed for mottled crabs, is common to species in which individuals occupy habitats of different coloration as they grow (Booth, 1990; Wilson *et al.*, 2007; Hultgren & Stachowicz, 2010). Therefore, different ecological processes related to changes in habitat use probably underlie the ontogenetic colour and pattern variation in the two crab species we studied here.

The juvenile phase is known to be the most vulnerable life stage for predation risk in many crab species (Palma & Steneck, 2001; Krause-Nehring *et al.*, 2010), and this phase is when stronger selection for camouflage is expected (Caro, 2018). As crabs grow, they may eventually reach a size refuge from predation (Gosselin & Qian, 1997), at which individuals are capable to defend themselves or more rapidly escape from predators, and camouflage become less critical (Todd *et al.*, 2006). The results presented here for mud crabs do not support this hypothesis, since colour distances of small and large individuals against backgrounds are very similar, indicating better camouflage against mud rock and pebble substrates regardless of size. In many cases, prey are exposed to different predators along their ontogeny, with later life predators being sometimes large enough to consume adults at a similar rate to that which juveniles are preyed on, which may result in prey never achieving an escape size (Eggleston *et al.*, 1990; Pessarrodona *et al.*, 2019). The same scenario is expected when predation on juveniles is so intense that it could work as a bottleneck for future ontogenetic stages (Beck, 1995). Therefore, since mud crabs are characterized by lower mobility over their entire lifetime (Micheli, 1997), a continuous need for efficient camouflage over ontogeny is expected (Hughes *et al.*, 2019). Small crabs (juveniles) are found near to the lower limit of the intertidal zone (Vergamini & Mantelatto, 2008a), where desiccation stress is lower, but predation is potentially higher, as exposure to fish increases. Large bird species, such as herons and spoonbills, are frequently seen feeding on the middle and upper part of the intertidal region in the Araçá Bay during low tide (Amaral *et al.*, 2010; Mancini *et al.*, 2018), and could easily prey on even the largest mud crabs. Similar predation pressure is expected over the geographic distribution of mud crabs, and therefore strong selection for crypsis is expected through ontogeny.

An alternative explanation for the differences we observed in mud crab appearance between size categories is that the high variability found on small individuals could result from juveniles using more diverse microhabitats than adults, which would require differential coloration for camouflage, either by means of matching the general appearance of specific background patches or by showing highly disruptive markings (Todd *et al.*, 2006, 2009; Wilson *et al.*, 2007; Nokelainen *et al.*, 2017; Price *et al.*, 2019). Since small mud crabs are associated with highly heterogeneous backgrounds, such as crushed-shell substrates or coarse sandy areas (Carvalho-Batista *et al.*, 2015), it may be challenging for the same crab individual conceal on many backgrounds at once. Therefore, in addition to using background matching for effective concealment against rocky substrates, as we show, it is also possible that small mud crabs could optimize their camouflage efficiency through disruptive coloration (Cuthill *et al.*, 2005). The high contrasting markings located on the carapace of juvenile crabs could contribute to create false edges and boundaries around the body, preventing individual recognition by predators (Webster *et al.*, 2013). Since large adult mud crabs are smoother, exhibiting few and less contrasting markings on their carapace, they probably optimize their concealment only by matching the general appearance of backgrounds. Finally, it is also possible that the highly variable coloration of small mud crabs could contribute to defeating predator search images, resulting in a mechanism of apostatic selection on which predators attack more common colour types disproportionately often, with selection favouring rarer types and maintaining colour variation at the population level (Bond & Kamil, 2002, 2006). Future studies are necessary to understand what camouflage strategies are used by the different life stages of mud crabs, possibly indicating a change from disruptive coloration to background matching along crab's ontogeny. In addition, studies testing whether the high individual variability in brightness and pattern of juvenile mud crabs could effectively defeat predator search images are also needed.

Different to mud crabs, mottled crabs are generally darker and less patterned, exhibiting small variation in all colour and pattern metrics within each size class. Besides possessing more colour saturated carapaces, crabs exhibited ontogenetic variation in colour but not in brightness, with large crabs being greener than small individuals. This differential coloration between size categories is probably a result of specific patterns of habitat use in juvenile and adult mottled crabs (Booth, 1990). During low tide, small crabs are active but remain close to refugees, such as rocky crevices and holes, including the interior of dead mussels and barnacles, where they execute short foraging excursions to feed on detritus (Abele *et al.*, 1986). It is also common to find small crabs hidden under rocks. This would explain their dark coloration and better concealment against mud rock and pebble substrates. As they grow larger, crabs increase mobility and spend significantly more time feeding at flat rocks covered with macroalgae during low tide (Abele *et al.*, 1986; Christofoletti *et al.*, 2010), on which a greenish coloration would increase background matching. However, we found comparable background similarity of adult mottled crabs against all substrates we considered, suggesting a generalist camouflage strategy (Hughes *et al.*, 2019). This strategy is based on the concept of compromise coloration (Merilaita *et al.*, 1999), on which the individual partially matches several background types but matches none perfectly. Considering the prey's perspective, a low dispersal rate of individuals would favour local adaptation and specialization, similar to what we observed for mud crabs, while higher mobility would promote a generalist strategy, as in adult mottled crabs (Hughes *et al.*, 2019). Future work could aim to determine the scales of phenotype-environment matching in juvenile and adult mottled crabs as well as whether the high mobility of individuals allied to a compromise coloration may work together to reduce predation pressure.

It is likely that the colour and pattern differences observed between size categories of the two crab species result from colour changes after moulting events through developmental plasticity

(Duarte *et al.*, 2017), which are common to many crab species (Detto *et al.*, 2008; Hultgren & Stachowicz, 2010; Jensen & Egnotovich, 2015; Nokelainen *et al.*, 2019; Carter *et al.*, 2020). Alternatively, if crab appearance is fixed, it is also possible that such ontogenetic differences result from differential predation on small individuals that are not well concealed to the background they are exposed during ontogenetic habitat changes (Stevens *et al.*, 2014). Considering that the variance of colour distances against backgrounds is similar between small and large individuals of both species, the second hypothesis is less likely to explain ontogenetic changes. However, future studies are necessary to describe the relative importance of these processes. Regardless, differently from other studies with similar species (Freire *et al.*, 2011; Carvalho-Batista *et al.*, 2015; Jensen & Egnotovich, 2015), our study used an objective method to quantify coloration (e.g. standardised reflectance) and considered that mud and mottled crabs did not exist in discrete morphs, but instead exhibited continuous colour variation. Considering the limitations of our study, future work on wider size ranges, and based on larger sample sizes and more prolonged sampling periods are necessary to better elucidate how individuals of both species change their appearance along ontogeny, and how this would affect concealment against different backgrounds. Taking into account the scarcity of studies testing different ecological theories in tropical areas (Martin *et al.*, 2012), especially related to animal coloration and crypsis, which are almost exclusive to temperate regions (but see Hemmi *et al.*, 2006; Detto *et al.*, 2008; Stevens *et al.*, 2013), our work highlights an important avenue of research on evolutionary and behavioural ecology. Intertidal crabs are a highly representative group in tropical regions, where predation pressure is known to be higher than in temperate areas (Schemske *et al.*, 2009; Roslin *et al.*, 2017). Because of that, we expect higher selection for the evolution of anti-predatory strategies in the tropics, where the increased diversity of animal's life history would also contribute for the existence of still unexplored patterns of animal coloration and crypsis in nature.

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REFERENCES

Abele LG, Campanella PJ, Salmon M. **1986**. Natural history and social organization of the semiterrestrial grapsid crab *Pachygrapsus transversus* (Gibbes). *Journal of Experimental Marine Biology and Ecology* **104**: 153–170.

Amaral ACZ, Migotto AE, Turra A, Schaeffer-Novelli Y. **2010**. Araçá: biodiversidade, impactos e ameaças. *Biota Neotropica* **10**: 219–230.

Barbosa A, Mäthger LM, Buresch KC, Kelly J, Chubb C, Chiao CC, Hanlon RT. **2008**. Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. *Vision Research* **48**: 1242–1253.

Bates D, Maechler M, Bolker B, Walker S. **2015**. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software* **67**: 1–48.

Beck MW. **1995**. Size-specific shelter limitation in stone crabs: a test of the demographic bottleneck hypothesis. *Ecology* **76**: 968–980.

Bond AB, Kamil AC. **2002**. Visual predators select for crypticity and polymorphism in virtual prey. *Nature* **415**: 609–613.

Bond AB, Kamil AC. **2006**. Spatial heterogeneity, predator cognition, and the evolution of color polymorphism in virtual prey. *Proceedings of the National Academy of Sciences of the United States of America* **103**: 3214–3219.

Booth CL. **1990**. Evolutionary significance of ontogenetic colour change in animals. *Biological Journal of the Linnean Society* **40**: 125–163.

Calsbeek B, Hasselquist D, Clobert J. **2010**. Multivariate phenotypes and the potential for alternative phenotypic optima in wall lizard (*Podarcis muralis*) ventral colour morphs. *Journal of Evolutionary Biology* **23**: 1138–1147.

Caro T, Sherratt TN, Stevens M. **2016**. The ecology of multiple colour defences. *Evolutionary Ecology* **30**: 797–809.

Caro T. **2018**. The functional significance of coloration in crabs. *Biological Journal of the Linnean Society* **124**: 1–10.

Carter EE, Tregenza T, Stevens M. **2020**. Ship noise inhibits colour change, camouflage, and anti-predator behaviour in shore crabs. *Current Biology* **30**: R211–R212.

Carvalho-Batista A, Pescinelli RA, Garcia JR, Guerra PGM, Pardo LM, Mantelatto FL. **2015**. Crypsis in the mud crab *Panopeus americanus* Saussure, 1857 (Decapoda, Panopeidae): relationship to sexual maturity. *Crustaceana* **88**: 963–977.

Cheney KL, Skogh C, Hart NS, Marshall NJ. **2009**. Mimicry, colour forms and spectral sensitivity of the bluestriped fangblenny, *Plagiotremus rhinorhynchos*. *Proceedings of the Royal Society B: Biological Sciences* **276**: 1565–1573.

Chiao CC, Chubb C, Buresch K, Siemann L, Hanlon RT. **2009**. The scaling effects of substrate texture on camouflage patterning in cuttlefish. *Vision Research* **49**: 1647–1656.

Christofoletti RA, Murakami VA, Oliveira DN, Barreto RE, Flores AAV. **2010**. Foraging by the omnivorous crab *Pachygrapsus transversus* affects the structure of assemblages on sub-tropical rocky shores. *Marine Ecology Progress Series* **420**: 125–134.

Cuthill IC, Stevens M, Sheppard J, Maddocks T, Párraga CA, Troscianko TS. **2005**. Disruptive coloration and background pattern matching. *Nature* **434**: 72–74.

Cuthill IC, Allen WL, Arbuckle K, Caspers B, Chaplin G, Hauber ME, Hill GE, Jablonski NG, Jiggins CD, Kelber A, Mappes J, Marshall J, Merrill R, Osorio D, Prum R, Roberts NW, Roulin A, Rowland HM, Sherratt TN, Skelhorn J, Speed MP, Stevens M, Stoddard MC, Stuart-Fox D, Talas L, Tibbetts E, Caro T. **2017**. The biology of color. *Science* **357**.

Darwin C. **1859**. *On the origin of species by means of natural selection, or the preservation of favoured races in the struggle for life.* London.

Detto T, Hemmi JM, Backwell PRY. **2008**. Colouration and colour changes of the fiddler crab, *Uca capricornis*: a descriptive study. *PLoS ONE* **3**: e1629.

Dias GM, Christofoletti RA, Kitazawa K, Jenkins SR. **2018**. Environmental heterogeneity at small spatial scales affects population and community dynamics on intertidal rocky shores of a threatened bay system. *Ocean and Coastal Management* **164**: 52–59.

Duarte RC, Flores AA V, Stevens M. **2017**. Camouflage through colour change: mechanisms, adaptive value and ecological significance. *Philosophical Transactions of the Royal Society B: Biological Sciences* **372**: 20160342.

Duarte RC, Stevens M, Flores AAV. **2018**. The adaptive value of camouflage and colour change in a polymorphic prawn. *Scientific Reports* **8**: 16028.

Eacock A, Rowland HM, Edmonds N, Saccheri IJ. **2017**. Colour change of twig-mimicking peppered moth larvae is a continuous reaction norm that increases camouflage against avian predators. *PeerJ* **5**: e3999.

Eggleston D, Lipcius R, Miller D, Coba-Cetina L. **1990**. Shelter scaling regulates survival of juvenile Caribbean spiny lobster *Panulirus argus*. *Marine Ecology Progress Series* **62**: 79–88.

Flores AAV, Negreiros-Fransozo ML. **1999**. On the population biology of the mottled shore crab *Pachygrapsus transversus* (Gibbes, 1850) (Brachyura, Grapsidae) in a subtropical area. *Bulletin of Marine Science* **65**: 59–73.

Freire AS, Pinheiro MAA, Karam-Silva H, Teschima MM. **2011**. Biology of *Grapsus grapsus* (Linnaeus, 1758) (Brachyura, Grapsidae) in the Saint Peter and Saint Paul Archipelago, Equatorial Atlantic Ocean. *Helgoland Marine Research* **65**: 263–273.

García AF, Bueno M, Leite FPP. **2016**. The Bostrychietum community of pneumatophores in Araçá Bay: an analysis of the diversity of macrofauna. *Journal of the Marine Biological Association of the United Kingdom* **96**: 1617–1624.

Gosselin LA, Qian PY. **1997**. Juvenile mortality in benthic marine invertebrates. *Marine Ecology Progress Series* **146**: 265–282.

Gray SM, McKinnon JS. **2007**. Linking color polymorphism maintenance and speciation. *Trends in Ecology and Evolution* **22**: 71–9.

Green SD, Duarte RC, Kellett E, Alagaratnam N, Stevens M. **2019**. Colour change and behavioural choice facilitate chameleon prawn camouflage against different seaweed backgrounds. *Communications Biology* **2**: 230.

Hemmi JM, Marshall J, Pix W, Vorobyev M, Zeil J. **2006**. The variable colours of the fiddler crab *Uca vomeris* and their relation to background and predation. *Journal of Experimental Biology* **209**: 4140–4153.

Hughes A, Liggins E, Stevens M. **2019**. Imperfect camouflage: how to hide in a variable world? *Proceedings of the Royal Society B: Biological Sciences* **286**: 20190646.

Hultgren KM, Stachowicz JJ. **2010**. Size-related habitat shifts facilitated by positive preference induction in a marine kelp crab. *Behavioral Ecology* **21**: 329–336.

Jensen GC, Egnotovich MS. **2015**. A whiter shade of male: color background matching as a function of size and sex in the yellow shore crab *Hemigrapsus oregonensis* (Dana, 1851). *Current Zoology* **61**: 729–738.

Kang C, Stevens M, Moon JY, Lee SI, Jablonski P. **2015**. Camouflage through behavior in moths: the role of background matching and disruptive coloration. *Behavioral Ecology* **26**: 45–54.

Karpestam E, Merilaita S, Forsman A. **2014**. Natural levels of colour polymorphism reduce performance of visual predators searching for camouflaged prey. *Biological Journal of the Linnean Society* **112**: 546–555.

Kelber A, Vorobyev M, Osorio D. **2003**. Animal colour vision **-** behavioural tests and physiological concepts. *Biological Reviews* **78**: 81–118.

Kettlewell H. **1955**. Selection experiments on industrial melanism in the Lepidoptera. *Heredity* **9**: 323–342.

Krause-Nehring J, Matthias Starck J, Richard Palmer A. **2010**. Juvenile colour polymorphism in the red rock crab, *Cancer productus*: patterns, causes, and possible adaptive significance. *Zoology* **113**: 131–139.

Kuznetsova A, Brockhoff PB, Christensen RHB. **2017**. lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software* **82**: 1–26.

Lenth R. **2019**. emmeans: Estimated Marginal Means, aka Least-Squares Means.

Mancini PL, Reis-neto AS, Fischer LG, Silveira LF, Schaeffer-Novelli Y. **2018**. Differences in diversity and habitat use of avifauna in distinct mangrove areas in São Sebastião, São Paulo, Brazil. *Ocean and Coastal Management* **164**: 79–91.

Martin LJ, Blossey B, Ellis E. **2012**. Mapping where ecologists work: biases in the global distribution of terrestrial ecological observations. *Frontiers in Ecology and the Environment* **10**: 195–201.

Merilaita S, Tuomi J, Jormalainen V. **1999**. Optimization of cryptic coloration in heterogeneous habitats. *Biological Journal of the Linnean Society* **67**: 151–161.

Micheli F. **1997**. Effects of experience on crab foraging in a mobile and a sedentary species. *Animal Behaviour* **53**: 1149–1159.

Nokelainen O, Hubbard N, Lown AE, Wood LE, Stevens M. **2017**. Through predators' eyes: phenotype-environment associations in shore crab coloration at different spatial scales. *Biological Journal of the Linnean Society* **122**: 738–751.

Nokelainen O, Maynes R, Mynott S, Price N, Stevens M. **2019**. Improved camouflage through ontogenetic colour change confers reduced detection risk in shore crabs. *Functional Ecology* **33**: 654–669.

Nucci PR, Turra A, Morgado EH. **2001**. Diversity and distribution of crustaceans from 13 sheltered sandy beaches along São Sebastião Channel, south-eastern Brazil. *Journal of the Marine Biological Association of the United Kingdom* **81**: 475–484.

Palma AT, Orrego C, Arriagada M. **2003**. Crypsis in early benthic phases of Brachyuran Decapod crustaceans in central Chile. *Revista Chilena de Historia Natural* **76**: 149–156.

Palma AT, Steneck RS. **2001**. Does variable coloration in juvenile marine crabs reduce risk of visual predation? *Ecology* **82**: 2961–2967.

Passarotto A, Parejo D, Penteriani V, Avilés JM. **2018**. Colour polymorphism in owls is linked to light variability. *Oecologia* **187**: 61–73.

Pessarrodona A, Boada J, Pagès JF, Arthur R, Alcoverro T. **2019**. Consumptive and non-consumptive effects of predators vary with the ontogeny of their prey. *Ecology* **100**: e02649.

Price N, Green S, Troscianko J, Tregenza T, Stevens M. **2019**. Background matching and disruptive coloration as habitat-specific strategies for camouflage. *Scientific Reports* **9**: 7840.

R Core Team. **2020**. R: A Language and Environment for Statistical Computing.

Rasband W. **1997**. ImageJ [online].

Reimchen TE. **1989**. Shell colour ontogeny and tubeworm mimicry in a marine gastropod *Littorina mariae*. *Biological Journal of the Linnean Society* **36**: 97–109.

Roslin T, Hardwick B, Novotny V, Petry WK, Andrew NR, Asmus A, Barrio IC, Basset Y, Boesing AL, Bonebrake TC, Cameron EK, Dáttilo W, Donoso DA, Drozd P, Gray CL, Hik DS, Hill SJ, Hopkins T, Huang S, Koane B, Laird-Hopkins B, Laukkanen L, Lewis OT, Milne S, Mwesige I, Nakamura A, Nell CS, Nichols E, Prokurat A, Sam K, Schmidt NM, Slade A, Slade V, Suchanková A, Teder T, van Nouhuys S, Vandvik V, Weissflog A, Zhukovich V, Slade EM. **2017**. Higher predation risk for insect prey at low latitudes and elevations. *Science* **356**: 742–744.

Rowland HM, Speed MP, Ruxton GD, Edmunds M, Stevens M, Harvey IF. **2007**. Countershading enhances cryptic protection: an experiment with wild birds and artificial prey. *Animal Behaviour* **74**: 1249–1258.

Russell BJ, Dierssen HM. **2015**. Use of hyperspectral imagery to assess cryptic color matching in *Sargassum* associated crabs. *PLoS ONE* **10**: e0136260.

Schemske DW, Mittelbach GG, Cornell H V., Sobel JM, Roy K. **2009**. Is there a latitudinal gradient in the importance of biotic interactions? *Annual Review of Ecology, Evolution, and Systematics* **40**: 245–269.

Skelhorn J, Rowland HM, Speed MP, Ruxton GD. **2010**. Masquerade: camouflage without crypsis. *Science* **327**: 51.

Spottiswoode CN, Stevens M. **2011**. How to evade a coevolving brood parasite: egg discrimination versus egg variability as host defences. *Proceedings of the Royal Society B: Biological Sciences* **278**: 3566–3573.

Stevens M, Párraga CA, Cuthill IC, Partridge JC, Troscianko TS. **2007**. Using digital photography to study animal coloration. *Biological Journal of the Linnean Society* **90**: 211–237.

Stevens M, Rong CP, Todd PA. **2013**. Colour change and camouflage in the horned ghost crab *Ocypode ceratophthalmus*. *Biological Journal of the Linnean Society* **109**: 257–270.

Stevens M, Lown AE, Wood LE. **2014**. Camouflage and individual variation in shore crabs (*Carcinus maenas*) from different habitats. *PLoS ONE* **9**: e115586.

Stevens M, Broderick AC, Godley BJ, Lown AE, Troscianko J, Weber N, Weber SB. **2015**. Phenotype-environment matching in sand fleas. *Biology letters* **11**: 20150494.

Stevens M, Merilaita S. **2009**. Animal camouflage: current issues and new perspectives. *Philosophical Transactions of the Royal Society B: Biological Sciences* **364**: 423–427.

Stevens M, Ruxton GD. **2019**. The key role of behaviour in animal camouflage. *Biological Reviews* **94**: 116–134.

Stoddard MC, Stevens M. **2010**. Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proceedings of the Royal Society B: Biological Sciences* **277**: 1387–1393.

Todd P, Briers R, Ladle R, Middleton F. **2006**. Phenotype-environment matching in the shore crab (*Carcinus maenas*). *Marine Biology* **148**: 1357–1367.

Todd PA, Qiu W, Chong KY. **2009**. Ontogenetic shifts in carapace patterning and/or colouration in intertidal and subtidal brachyuran crabs. *Raffles Bulletin of Zoology* **57**: 543–550.

Troscianko J, Stevens M. **2015**. Image Calibration and Analysis Toolbox - a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution* **6**: 1320–1331.

Underwood A. **1997**. *Experiments in ecology: their logical design and interpretation using analysis of variance.* Cambridge: Cambridge University Press.

Venables W, Ripley B. **2002**. *Modern Applied Statistics with S*. New York: Springer.

Vergamini FG, Mantelatto FL. **2008a**. Microdistribution of juveniles and adults of the mud crab *Panopeus americanus* (Brachyura, Panopeidae) in a remnant mangrove area in the southwest Atlantic. *Journal of Natural History* **42**: 1581–1589.

Vergamini FG, Mantelatto FL. **2008b**. Continuous reproduction and recruitment in the narrowback mud crab *Panopeus americanus* (Brachyura, Panopeidae) in a remnant human-impacted mangrove area. *Invertebrate Reproduction and Development* **51**: 1–10.

Wallace A. **1867**. Mimicry and other protective resemblances among animals. *Westminster Review (London edition)* **1 July**: 1–43.

Webster RJ, Hassall C, Herdman CM, Godin JGJ, Sherratt TN. **2013**. Disruptive camouflage impairs object recognition. *Biology Letters* **9**: 20130501.

West-Eberhard MJ. **1989**. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* **20**: 249–278.

Wilson D, Heinsohn R, Endler JA. **2007**. The adaptive significance of ontogenetic colour change in a tropical python. *Biology Letters* **3**: 40–43.

Table 1. Main differences in the overall distribution and in the ontogenetic patterns of habitat use and carapace coloration of the narrowback mud crab *Panopeus americanus* and the mottled shore crab *Pachygrapsus transversus*.

Crab species	Overall distribution	Juvenile habitat and coloration	Adult habitat and coloration
Panopeus americanus narrowback mud crab	Intertidal and subtidal (up to 25m) regions along muddy beaches in estuarine and mangrove areas in the western Atlantic coast.	**Habitat:** hidden under rocks and on sandy, muddy or shell-covered substrates, near to the lower limit of the intertidal zone. **Carapace colour**: highly variable in appearance, including white, tan and brown individuals, frequently exhibiting coloured spots.	**Habitat:** wide range of substrates, frequently hidden under rocks, in the median and upper parts of the intertidal zone. **Carapace colour**: homogeneous greenish-grey.
Pachygrapsus transversus mottled shore crab	Intertidal zone of hard substrates on rocky shores and estuarine muddy areas, in both sides of the Atlantic coast and in the Eastern side of the Pacific Ocean.	**Habitat:** hidden under crevices or between spaces created by live or dead barnacles and mussels on rocky substrates. **Carapace colour**: dark brown, tan and black.	**Habitat:** wide range of substrates, frequently on large rocks covered by macroalgae. **Carapace colour**: dark brown containing greenish or yellowish spots and stripes.

Table 2. Summary results of the two-way analysis of variance (ANOVA) testing the effects of crab species (mud crabs - *Panopeus americanus* or mottled crabs - *Pachygrapsus transversus*) and size class (small or large) in the carapace brightness and two different colour metrics (e.g. saturation and hue) measured from digital photographs of individuals sampled in the Araçá Bay, Brazil.

		Brightness			**Saturation**			**Hue**		
Source of variation	*df*	*MS*	*F*	*p*	*MS*	*F*	*p*	*MS*	*F*	*p*
Species	1	12.202	128.36	< 0.001	0.012	10.47	0.002	0.007	10.83	0.001
Size class	1	1.489	15.67	< 0.001	0.000	0.19	0.666	0.009	13.33	< 0.001
Species * Size class	1	1.075	11.31	0.001	0.003	3.02	0.085	0.003	5.15	0.025
Residuals	110	0.095			0.001			0.001		
		Log-transformed data			*Raw data*			*Raw data*		

Table 3. Summary results of the two-way analysis of variance (ANOVA) testing the effects of crab species (mud crabs - *Panopeus americanus* or mottled crabs - *Pachygrapsus transversus*) and size class (small or large) in three different pattern metrics (e.g. maximum frequency, summed energy and proportion energy) measured from digital photographs of the carapace of crabs sampled in the Araçá Bay, Brazil. Data for all metrics were log-transformed to attend ANOVA assumptions.

		Maximum frequency (marking size)			**Summed energy (pattern contrast)**			**Proportion energy (pattern diversity)**		
Source of variation	*df*	*MS*	*F*	*p*	*MS*	*F*	*p*	*MS*	*F*	*p*
Species	1	36.459	79.83	< 0.001	9.877	122.79	< 0.001	0.006	0.59	0.445
Size class	1	7.601	16.64	< 0.001	0.152	1.89	0.172	0.102	9.51	0.003
Species * Size class	1	0.342	0.75	0.388	0.933	11.60	< 0.001	0.011	3.49	0.064
Residuals	110	0.457			0.080			0.011		

Table 4. Summary results of the ANOVA applied to the linear mixed effects model (lmer) testing whether crabs of two different species (mud crabs - *Panopeus americanus* or mottled crabs - *Pachygrapsus transversus*) from different size classes (small or large individuals) exhibit differential camouflage against varied background types (alga, pebble or mud rock). Model intercept includes 'crab ID' as a random factor.

Subject	*SS*	df_{num} / df_{den}	*MS*	*F*	*p*
Species	0.0004	1 / 110	0.0004	9.78	0.002
Size class	0.0000	1 / 110	0.0000	0.13	0.720
Background	0.0122	2 / 220	0.0061	147.48	< 0.001
Species * Size class	0.0001	1 / 110	0.0001	2.32	0.131
Species * Background	0.0031	2 / 220	0.0015	37.03	< 0.001
Size class * Background	0.0003	2 / 220	0.0002	4.00	0.020
Species * Size class * Background	0.0004	2 / 220	0.0002	5.32	0.006

FIGURE CAPTIONS

Figure 1. Ontogenetic variation in the carapace appearance of the mud crab *Panopeus americanus* (A) and the mottled shore crab *Pachygrapsus transversus* (B) from the Araçá Bay (São Paulo, Brazil). Left and right images represent small and large crabs respectively. At this site, crabs may use different background types (C), including rocks covered by ephemeral algae, and muddy or sand substrates containing small pebbles to medium-size boulder rocks. Crab photographs are not scaled to represent the real size of animals. Images by RCD.

Figure 2. Ontogenetic differences in brightness and colour of the carapace of the mud crab *Panopeus americanus* and the mottled crab *Pachygrapsus transversus* from the Araçá Bay. Brightness (A) is the overall reflectance of the carapace (expressed as %), saturation (B) is the amount of a given colour to white light, on which larger values indicate richer colour, and hue (C) is the colour type, expressed as a ratio among colour channel values (see main text for more details), on which larger hue values indicate greener carapace. Here and in the next figures, boxes display medians and inter- quartile ranges (IQRs), whiskers represent lowest and highest values

within 1.5∗IQRs and circles represent raw data, on which a random noise was added to avoid overlap. The asterisks and ns point to significant ($p < 0.001$) and non-significant differences between size classes, while the different letters indicate significant differences between crab species ($p < 0.05$).

Figure 3. Ontogenetic differences in pattern metrics of the carapace of the mud crab *Panopeus americanus* and the mottled crab *Pachygrapsus transversus* from the Araçá Bay. Marking size (A) is the predominant marking size found on crab's carapace (in mm), pattern contrast (B) is the summed energy across all scales of pattern variation, on which higher values mean more contrasting markings, and pattern diversity (C) is how much one marking size dominates the overall crab pattern, on which larger values indicates that one or a few markings are prevalent. The asterisks and ns point to significant ($p < 0.001$) and non-significant differences between size classes, while the different letters indicate significant differences between crab species ($p < 0.05$).

Figure 4. Colour distances (as a proxy of background matching) of small and large crabs of the mud crab *Panopeus americanus* (light brown boxes; A) and the mottled crab *Pachygrapsus transversus* (light green boxes; B) against different background types that are characteristic of the Araçá Bay (see Figure 1C for more details). The different letters indicate significant differences among background types ($p < 0.05$), while ns means non-significant.

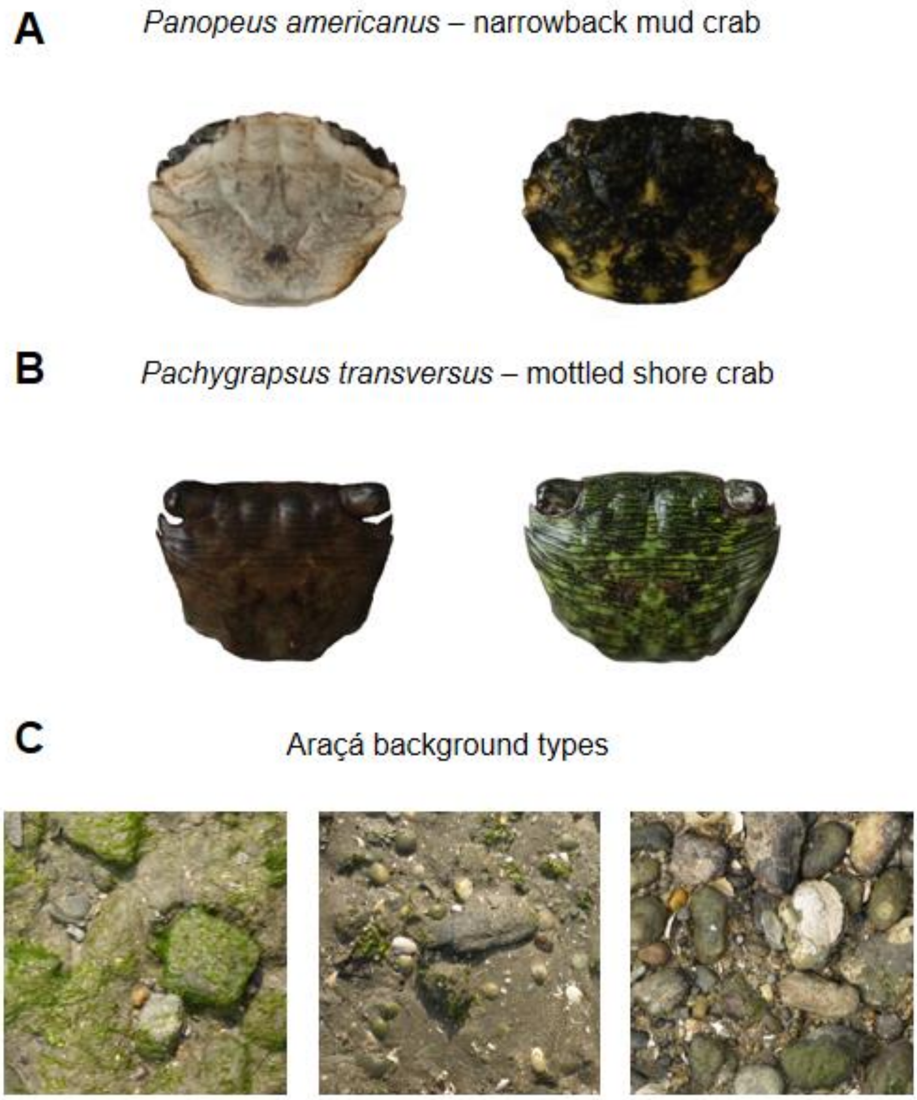
A
Panopeus americanus – narrowback mud crab
B
Pachygrapsus transversus – mottled shore crab
C
Araçá background types

Figure 1

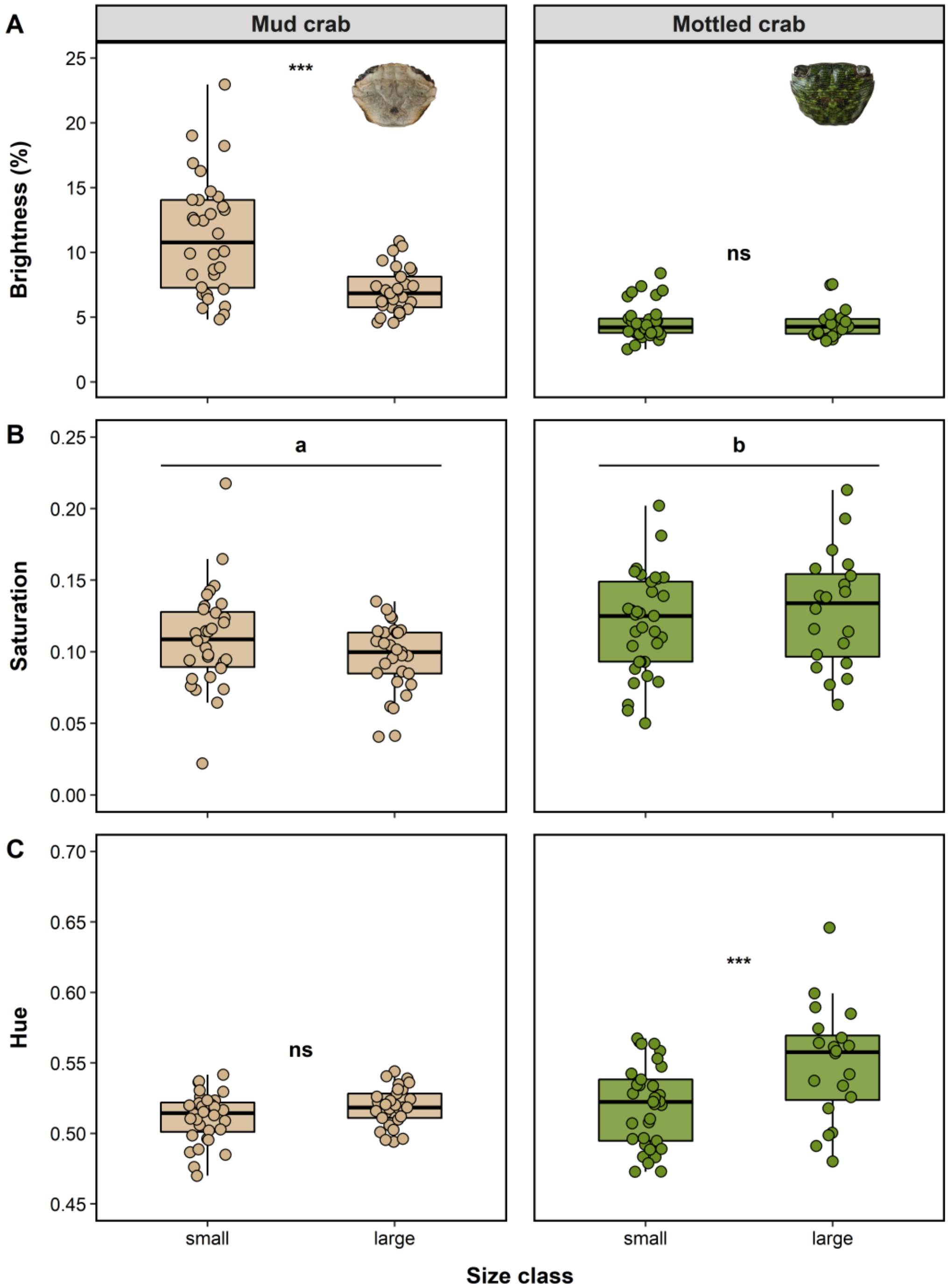
A
Mud crab
Mottled crab
Brightness (%)
25
20
15
10
5
0
***
ns
B
0.25
0.20
0.15
0.10
0.05
0.00
Saturation
a
b
C
0.70
0.65
0.60
0.55
0.50
0.45
Hue
ns
***
small
large
small
large
Size class

Figure 2

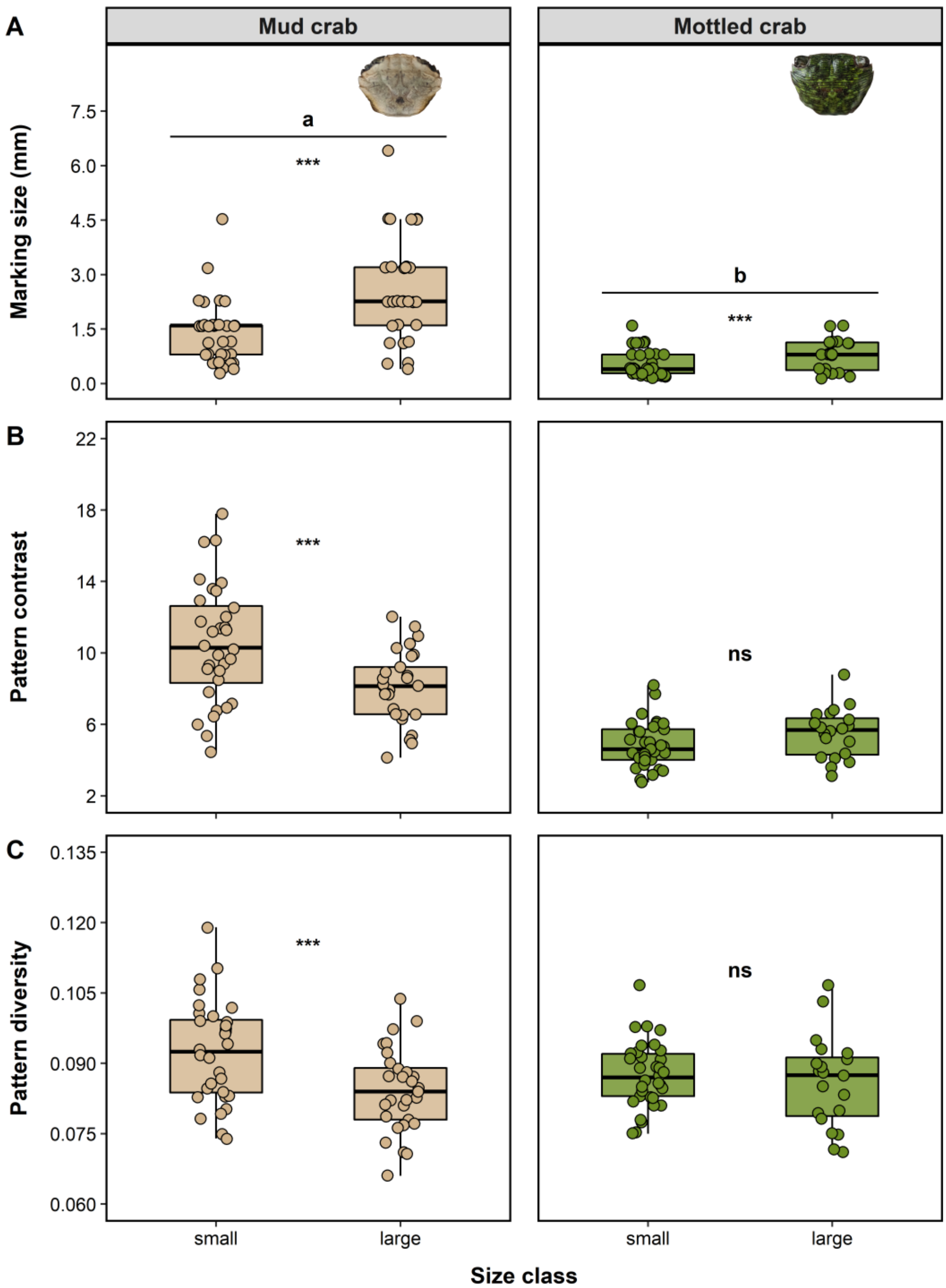
A
Mud crab
Mottled crab
Marking size (mm)
7.5
6.0
4.5
3.0
1.5
0.0
a
***
b
***
B
Pattern contrast
22
18
14
10
6
2
***
ns
C
Pattern diversity
0.135
0.120
0.105
0.090
0.075
0.060
***
ns
small
large
small
large
Size class

Figure 3

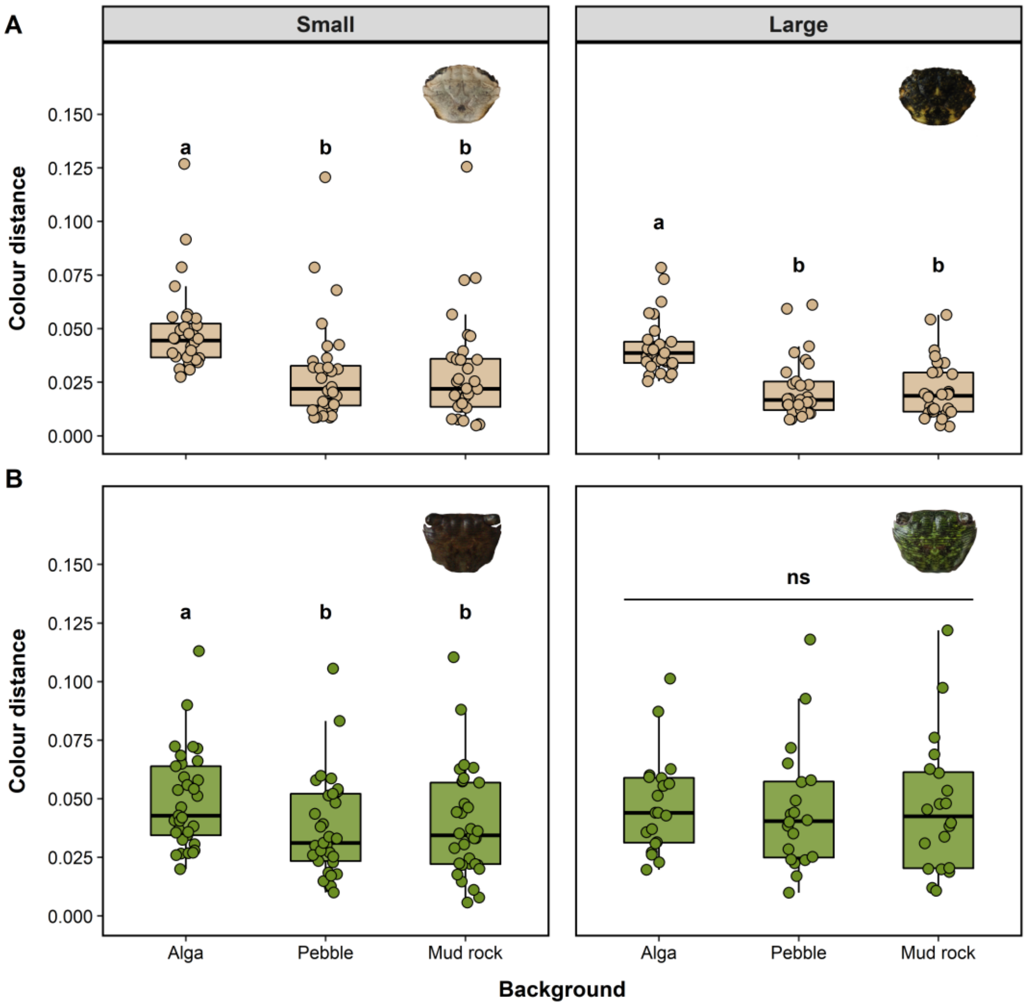
A
B
Small
Large
Colour distance
0.150
0.125
0.100
0.075
0.050
0.025
0.000
a
b
ns
Alga
Pebble
Mud rock
Background

Figure 4