Animal vision and colour change for camouflage

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I certify that all material in this thesis which is not my own work has been identified and that any material that has previously been submitted and approved for the award of a degree by this or any other University has been acknowledged.

Abstract

Camouflage is a well-studied form of antipredator defence. A key issue is how animals ensure camouflage effectiveness when the visual environments many camouflage strategies rely on vary. Phenotypic plasticity allows animals to adjust coloration to best match such environmental variation. It is assumed that vision is used in identifying this variation and guides changes in colour. However, questions still exist regarding the opportunities and limitations afforded from vision-guided changes for camouflage. *Carcinus maenas*, the green shore crab, already a widely used species to investigate a variety of questions regarding camouflage, was used to test the assumption that vision is directly responsible for guiding (and limiting) colour change for camouflage. In the first chapter, tests of spectral sensitivity and colour discrimination were performed, which were then compared to colour change responses. Following this, crabs' spatial resolution was tested and compared to pattern change responses on uniform and patterned backgrounds. Finally, crabs' brightness change responses to varying illumination and substrate brightness conditions were recorded to examine directional light's role in substrate perception for plasticity. My results indicate that *C. maenas* colour change for camouflage is determined and limited by their vision. First, spectral and colour discrimination results indicate *C. maenas* cannot perceive differences in colour. This aligns with colour change results, with crabs only showing significant achromatic change despite apparently possessing the chromatophores needed for chromatic change. Following this, crab's changed patterning by increasing pattern contrast proportionate to background pattern size, without changing pattern shape or size. This change in patterning is indicative of a shift from uniform background matching to disruptive markings. Finally, *C. maenas* colour change corresponds to the relative reflectance of substrates, accounting for illumination. This indicates some level of assessment of directional light, likely dependent on the differential stimulation of an eye perceiving light from multiple directions at once. These results indicate that while species' vision can limit colour change for camouflage, effective improvements in camouflage are still capable within these limits.

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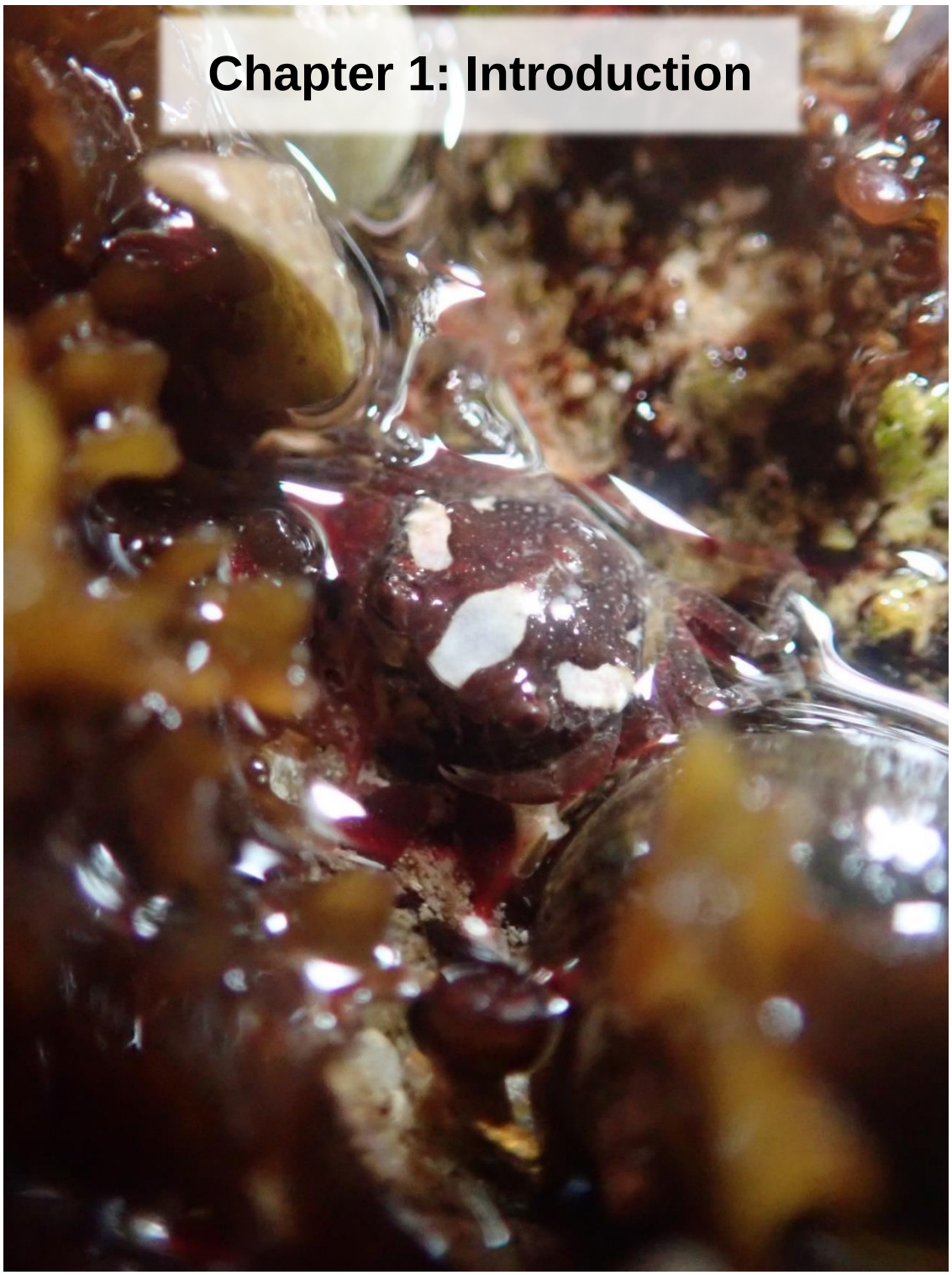
Author’s declaration

The writing, data collection, analysis, and interpretation, in this thesis is my own. My supervisory team has given feedback on the contents of chapters, as well as provided guidance during experimental design and the interpretation of results. This research has had several other researchers contribute to it. Martin How produced and provided the MATLAB code used in the optokinetic experiments in Chapters 1 and 2. Ally Iwrin helped with collection of samples for the spectral sensitivity optomotor experiments while in Bristol. Kathryn Bullough and Claudia Minkin provided practical assistance in the lab with the pilot appetitive colour discrimination trials, and the concurrent colour change experiment (blue and yellow colour change) from Chapter 1. Sharon Mandipe and Beks Hill provided practical assistance during the design and building of the experimental containers and protocol used in Chapter 3. The photographs of natural substrates, both the raw images and multispectral versions, were taken/created by Natasha Price for a separate project also supervised by Professor Martin Stevens, used with his permission. Anna Hughes provided advice on statistical analysis and R coding throughout the project. Anna Hughes, Sara Mynott, and Sam Green all gave practical advice regarding experimental design across this project, with Sara Mynott and Sam Green providing specific discussion and feedback on the experimental set up in marine systems. The Sensory Ecology and Evolution Research Group provided general feedback and insight on interpretation when experimental designs and results of this project were presented to them. Although this piece of work is in the main part an independent investigation, the individuals above contributed to its improvement, for which I am immensely grateful.

Chapter 1: Introduction



Defining camouflage

Animals matching their environment for protection is a concept that has been recognised as far back as Aristotle, who noted the ability of the octopus to change to match its background (Cresswell 1862). More recently it has been foundational in the development of key theories in the biological sciences, such as support for natural selection, used by both Wallace (Wallace 1877, 1867, 1889) and Poulton (Poulton 1890). However, most of the direct investigation and research into animal camouflage occurred in the 20th Century onwards. Thayer (Thayer and Thayer 1909; Thayer 1896) classified "background picturing" (now background matching), countershading and disruptive markings, whose work was expanded upon by Hugh Cott (Cott 1940). Studies that followed became archetypal in the study of protective coloration as well as evolution in action. For example, Henry Kettlewell performed a series of fundamental studies on the coloration of the peppered moth, *Biston betularia* that are now an axiomatic example of evolution in action. Following observations that the industrial revolution caused a shift from the previously dominant *typica* morph, Kettlewell demonstrated selection for *typica* in unpolluted forest where they matched lichen present, while *carbonaria* had the advantage in polluted forests against soot strained trunks without lichen (Kettlewell 1955; Kettlewell and Conn 1977). These are valuable teaching examples of evolution (Cook and Saccheri 2013; Majerus 2009) and have led to the continued study of the system, both in the wake of post industrialisation recovery of forests and the return of *typica* morphs (Cook, Mani, and Varley 1986; Clarke, Mani, and Wynne 1985), as well as the direct linking of coloration and survival, quantified to predator vision (Walton and Stevens 2018). Multiple studies have followed, classifying camouflage and testing its theoretical effectiveness (reviewed in (Cuthill 2019; Merilaita, Scott-Samuel, and Cuthill 2017; Stevens and Merilaita 2011)). Expanding from Thayer's comparisons of animal and background colour based on human vision (Thayer 1896; Thayer and Thayer 1909), it is now standard to account for the vision of relevant observer species (Norris and Lowe 1964; Endler 1978, 2008; Guilford and Dawkins 1991).

Camouflage is a collection of strategies where individuals use coloration to remove or obscure salient cues that would otherwise be used to identify them.

Over time these have been broadly codified into discrete strategies (see Table 1 for a breakdown of the most commonly accepted, adapted from (Galloway et al. 2020), for further definition see (Stevens and Merilaita 2009a)). These discrete definitions help to clarify the mechanisms a species is using to avoid detection and-or recognition. Individuals can match either the generally appearance of the background to avoid detection (background matching of both general coloration and patterning e.g., the improved survival of ground-nesting birds that match their environment (Troscianko, Wilson-Aggarwal, et al. 2016)), or specific objects that may not be of interest to predators to prevent recognition as potential prey (or predator) (masquerade/mimicry, e.g., twig mimicry seen in caterpillars (Skelhorn 2015)). In some extreme examples, species can directly transmit light from the background through their own bodies to match their background (i.e. transparency (Cronin 2016)). Alternatively, coloration can act to remove salient features of the individual without directly matching environmental features, such as obscuring the edges of body parts or whole bodies (disruptive markings (Stevens and Merilaita 2009b)), shadows (countershading (Cuthill et al. 2016; Rowland et al. 2008; Stevens and Merilaita 2009b)), or draw attention away from recognisable features without obscuring them directly (distractive markings (Dimitrova et al. 2009), although they may actually increase detection (Troscianko et al. 2013)).

Most of these strategies are directly related to the visual environment the individual is in, and to the observer visual system the strategies are aimed at (Troscianko et al. 2009; Stevens and Merilaita 2009a). The intended effect of camouflage is often described in terms of signal and noise (Merilaita, Scott-Samuel, and Cuthill 2017; Galloway et al. 2020). The individual is attempting to reduce the difference between the visual cues they produce (the signal) relative to the environmental cues around them (noise), to below the threshold an observer can differentiate between the two. For strategies such as background matching (Merilaita and Stevens 2011; Cuthill et al. 2005), the individual is attempting to reduce the signal: noise ratio of their overall appearance compared to backgrounds in terms of reflectance. Whereas disruptive markings (Cuthill et al. 2005; Espinosa and Cuthill 2014; Stevens and Cuthill 2006; Stevens et al. 2006) act to reduce/eliminate the cues from a specific salient

feature acting as the signal, such as specific body shapes, by matching part of environmental variation to break the continuous outline in question.

Table 1: Table defining the major camouflage terms, adapted from (Galloway et al. 2020).

Strategy	Mechanism
Background matching	General appearance matches the colour, contrast, lightness, and pattern of one (specialist) or multiple (generalist) backgrounds.
Disruptive coloration	Contrasting markings that generate the appearance of false edges within the body surface and/or break up the true body outline to thwart detection or recognition of body shape.
Countershading	Coloration of the body surface facing ambient lighting (usually the dorsal surface) is darker than the opposite body surface. Can either act to reduce cues from shadows or three-dimensional structure
Transparency	Part or all of body tissues rendered colourless owing to lack of pigment expression, preventing detection.
Masquerade	hindering recognition after detection by resembling an uninteresting or inedible object from within the environment, such as a stick or leaf.
Distractive markings	colour patches or patterns that draw the attention of observer away from cues such as body outline that would facilitate object detection

Environmental information is key to the success of these strategies. There is now a growing body of work demonstrating that successful camouflage increases survival, in virtual (Nokelainen et al. 2019; Troscianko et al. 2021; Troscianko et al. 2017), artificial model (Vignieri, Larson, and Hoekstra 2010), and real-world systems (Duarte, Stevens, and Flores 2018; Mynott 2019).

Animal behaviour plays a key role in reducing the signal to noise ratio, ensuring successful camouflage (reviewed in (Stevens and Ruxton 2019)). Research shows animals relying on habitat matching will preferentially chose environments which are a close match for their coloration (Allen, Mäthger, Barbosa, et al. 2010; Eacock et al. 2019; Green et al. 2019; Kang et al. 2014; Kjernsmo and Merilaita 2012; Moles and Norcross 1995; Stevens et al. 2017; Uy et al. 2017), and even at smaller micro-habitat scales, individuals will orient their bodies to maximise crypsis effectiveness (e.g. resting orientation in moths (Kang et al. 2012; Webster et al. 2009) and postural camouflage in caterpillars (Rowland, Burriss, and Skelhorn 2020)). Some species manipulate their environment to improve matching (e.g. in ground nesting birds (Troscianko, Wilson-Aggarwal, et al. 2016)). In some extreme examples, individuals (often those employing masquerade/object mimicry) may move in ways that will better mimic the object they pretend to be (e.g. evidence of stick insects moving to mimic wind movement (Bian, Elgar, and Peters 2016)).

One of the greatest challenges in camouflage strategies is the lack of homogeneity in many environments, across a variety of scales and characteristics. Given the necessity for many species to move, either in search of food, shelter, or conspecifics there is the chance individuals will move from an area where conditions facilitate camouflage, to one where they do not, or risk losing access to resources by staying on matching substrates (Ruxton et al. 2019). A key division in background matching, one of the most commonly studied strategies, is that of specialist versus generalist (Merilaita, Tuomi, and Jormalainen 1999; Hughes, Liggins, and Stevens 2019). In the former individuals are characterised by a close match to a specific and limited number of environments, which while having a commensurately greater survival benefit to individuals under those conditions, limits the effectiveness of camouflage outside them. The latter group adopts a strategy of partial matching across a variety of habitats, allowing for a certain amount of success on a broader range of conditions, while losing effectiveness for any given habitat compared to a specialist of the same location.

Colour change for camouflage

Rather than matching a limited range of backgrounds well or a broad range of backgrounds imperfectly, species can manipulate their own coloration in

response to changes in environmental variation. Plasticity in the features used in camouflage – brightness, colour, and their variation over an individual – allows individuals to improve or at least maintain matching when conditions that camouflage is dependent on change. Individuals can change overall reflectance (e.g. shifts in brightness in crabs (Stevens 2016; Stevens, Rong, and Todd 2013), or colour in caridean shrimp (Duarte, Stevens, and Flores 2018; Green et al. 2019) or both in *B. betularia* larvae (Eacock et al. 2017)) or specific redistribution of patterns (e.g. in cephalopods (Barbosa et al. 2008; Barbosa et al. 2007; Hanlon, Messenger, and Young 1988) and fish (Akkaynak et al. 2017; Healey 1999; Smithers, Wilson, and Stevens 2017; Kelman, Tiptus, and Osorio 2006; Ryer et al. 2008; Sumner 1911)).

Colour change for camouflage can be found across species, both aquatic vertebrates (e.g. colour and pattern change in fish (Akkaynak et al. 2017; Allen et al. 2015; Smithers, Wilson, and Stevens 2017; Stevens, Lown, and Denton 2014; Sumner 1911; Sumner and Keys 1929)) and invertebrates (e.g. in cephalopods (Hanlon et al. 2011; Hanlon et al. 2009; Mäthger et al. 2008; Nakajima et al. 2022) and crustaceans (Brown Jr and Sandeen 1948; Duarte et al. 2020; Hultgren and Stachowicz 2008; Nokelainen et al. 2019; Stevens 2016; Stevens, Lown, and Wood 2014b; Stevens, Rong, and Todd 2013; Duarte, Stevens, and Flores 2018; Green et al. 2019; Bedini 2002)), as well as terrestrial species (e.g., mammals (Nagorsen 1983; Rothschild 1942), reptiles (Stuart-Fox, Moussalli, and Whiting 2008; Stuart-Fox, Whiting, and Moussalli 2006; Fulgione et al. 2014), and arthropods (Bückmann 1979; Burtt 2009; Eacock et al. 2017; Eacock et al. 2019; Filshie, Day, and Mercer 1975; Grayson and Edmunds 1989; Kang, Kim, and Jang 2016; Valverde and Schielzeth 2015). Some species even use colour change for camouflage (combined with other behaviours) to adapt camouflage in response to specific predator types (e.g., dwarf chameleons (Stuart-Fox, Moussalli, and Whiting 2008; Stuart-Fox, Whiting, and Moussalli 2006)).

In addition to adjusting appearance to better match backgrounds, change can also result in shifts between camouflage strategies. Research shows that cuttlefish can alter dorsal patterning in response to background conditions, shifting from background matching to large pattering more akin to disruptive markings (Barbosa et al. 2008; Chiao, Chubb, and Hanlon 2015; Hanlon et al.

2011; Hanlon et al. 2009). Colour change between background matching and disruptive markings has also been proposed as the mechanism for effective camouflage in *Carcinus maenas* across mesoscale habitats (e.g.: mudflats versus rockpools) (Nokelainen et al. 2017; Todd et al. 2012; Price et al. 2019).

Changes in appearance can vary in quality (pattern, brightness, and colour), speed, and magnitude of change. In terms of mechanisms, colour change (including for camouflage) is broadly split into two categories: physiological and morphological (Duarte, Flores, and Stevens 2017; Figon and Casas 2018; Stevens 2016). Physiological colour change is associated with the rapid (seconds to hours) redistribution of existing pigments within chromatophores (pigment-containing cells). By changing the distribution of coloured material within a cell, the likelihood a pigment will be struck by and ultimately reflect light changes, as does the overall colour of the individual/part of the individual. This mechanism is often associated with cephalopod (Barbosa et al. 2008; Hanlon, Messenger, and Young 1988) and rapid vertebrate colour change (Smithers, Wilson, and Stevens 2017; Stevens, Lown, and Denton 2014; Stuart-Fox and Moussalli 2009; Stuart-Fox, Moussalli, and Whiting 2008; Kelman, Tiptus, and Osorio 2006; Ryer et al. 2008; Sumner 1911) but is also found in some other invertebrates (Umbers et al. 2014). Morphological colour change is the production, modifying, or removal of pigments and pigment containing tissues. This process can take longer periods of time (days to months) (Bagnara and Matsumoto 2006), and tends to be found in invertebrates (e.g. the change in colour of caridean shrimp on novel algal substrates (Duarte, Stevens, and Flores 2018; Green et al. 2019), or shifts in caterpillar coloration with ecdysis (Eacock et al. 2017)), but there are multiple examples in vertebrate species (e.g., the seasonal change in plumage of birds and coats of mammals (Zimova et al. 2018)). These mechanisms are not mutually exclusive, with species exhibiting short-term changes, as well as longer changes (e.g., shore crabs, exhibiting some limited short term change (Stevens, Lown, and Wood 2014b), with more significant changes over weeks associated with moulting (Stevens 2016)).

Colour change for camouflage does have limitations, however. It is generally assumed that redistributing, generating, or removing pigments both have metabolic costs (Duarte, Flores, and Stevens 2017; Stevens 2016; Stuart-Fox

and Moussalli 2009), diverting resources from other processes. Another key limitation is the speed at which individuals can respond to environmental change: an individual changing physiologically may be better able to respond to faster, unpredictable changes in environmental conditions than morphological strategies. A prolonged period of compromised matching during change will likely have a survival cost, given the previously mentioned benefit of camouflage for survival. Often colour change for camouflage strategies are associated with the predictable environmental change. Some species (e.g., lagomorphs and small mustelids (Nagorsen 1983; Rothschild 1942)), shift between potential pelages associated with predictable shifts in background conditions, which can have its own drawbacks. In an interesting case study, anthropogenic climate change is altering the times at which habitat conditions shift, increasing the mismatched period of species changing in response to seasonal shifts in backgrounds, highlighting their previous dependence on the predictable nature of these changes (Zimova et al. 2020; Zimova, Mills, and Nowak 2016). Another example of slower change in cryptic colour associated with (potentially) predictable changes in environmental conditions is ontogenetic change (Booth 1990; Duarte et al. 2020; Nokelainen et al. 2019). Many species have changes in traits associated with life history, and as such are likely to encounter shifts in the environmental conditions they encounter over time. Ontogenetic shifts in cryptic coloration have been documented in a variety of species, both vertebrate (pythons shift from red/yellow coloration to green associated with a shift to the green leaf-rich canopy (Wilson, Heinsohn, and Endler 2007)) and invertebrate (smaller green shore crabs shift from high levels of variation across individuals with multiple camouflage strategies, to uniform green background matching, that best corresponds to their respective habitats (Nokelainen et al. 2019), with similar patterns in other shore crab species (Duarte et al. 2020)). These shifts potentially allow for camouflage to be maintained as individuals exhibit adult behaviours or shift to habitats better suited for older life stages. In the example of shore crabs, one proposed reason is adult crabs are more mobile (Edwards 1958), and the uniform green coloration provides a better match across a wider range of habitats, while specific patterns are less likely to match in any given habitat (Nokelainen et al. 2019).

Vision directing colour change for camouflage

A major assumption is that reversible colour change for camouflage is directed by vision (Duarte, Flores, and Stevens 2017; Stevens 2016). Given that camouflage is generally associated with reducing visual cues as a result of differences between the individual and backgrounds (e.g., visible outlines, or differences in brightness, colour, or pattern) (Cuthill 2019; Galloway et al. 2020; Merilaita 2003; Merilaita, Lyytinen, and Mappes 2001; Merilaita, Scott-Samuel, and Cuthill 2017; Stevens and Merilaita 2011; Troscianko et al. 2009; Stevens and Merilaita 2009a), animals should be relying on the same visual cues to direct the change and ensure matching. Animals detect changes in the visual environment (from movement or external factors) and respond to them through colour change. It is crucial that differences are correctly identified so subsequent change is accurate, and camouflage is effective at reducing predation. An exception could be if these specific cues correlate with other aspects of vision (for example colour and brightness, proposed as a potential means of colour matching whilst colourblind in cuttlefish (Marshall and Messenger 1996)), or other sense entirely (e.g., chemosensory cues from algal grazing substrates of caridean shrimp, that also act as backgrounds to be matched (Gamble and Keeble 1900; Green et al. 2019; Keeble, Gamble, and Hickson 1900)).

Assuming vision is used in colour change for camouflage, it carries its own set of factors to consider alongside colour change mechanisms. First, perhaps the key aspect of vision for colour change is spectral sensitivity and colour discrimination. The assumption being that to match chromatic elements of backgrounds, individuals need to be able to perceive difference between body colour and background colour. While a visual system capable of efficient colour discrimination may logically be the most useful if used for guiding colour change, it will potentially come at a cost. By allocating some portion of photoreceptors to different spectral sensitivities, the overall sensitivity of the eye to certain wavelengths of light may be reduced, especially in systems such as compound eyes where photoreceptor numbers (and sensitivity to their respective wavelengths of light) are space limited (Land and Nilsson 2012; Warrant and Nilsson 2006). This gives rise to another generalist versus specialist trade-off, where colour sensitivity is weighed against overall light

sensitivity, both of which may be important to change. Finally, colour change will not be the only behaviour guided by vision, and there for providing pressure for certain sensitivities. There will have to be trade-offs between the selective pressures acting on visual systems. Using the previous issue of the space trade off in colour vision, while sacrificing overall sensitivity for colour discrimination may benefit a species presented with habitats of varying colour it could ultimately disadvantage behaviours requiring low light vision.

Camera type eyes, compound eyes, and simple photoreceptive systems such as ocelli each have their own advantages and disadvantages (Land and Nilsson 2012; Cronin et al. 2014) that can affect colour change. Camera types tend to have greater resolving power at distance compared to compound eyes (Caves, Brandley, and Johnsen 2018), but the latter often allow wider focal ranges (Land and Nilsson 2012) useful for viewing at close range e.g. viewing resting substrates. Individuals attempting to match the background of spatially complex environments, will potentially need to not only match the general brightness and-or colour, but also any patterning present on said background. If vision is the cue, then logically they will also need sufficient acuity to resolve the spatial information to change patterns accordingly. This will also be true when changing to different camouflage strategies, specifically for disruptive coloration.

Most of the studies linking vision to colour change for camouflage involve manipulating the qualities of substrates and recording colour change responses (Akkaynak et al. 2017; Carter, Tregenza, and Stevens 2020; Duarte, Stevens, and Flores 2018; Eacock et al. 2017; Fulgione et al. 2014; Green et al. 2019; Kang, Kim, and Jang 2016; Siegenthaler et al. 2018; Stevens 2016; Stevens, Lown, and Denton 2014; Stevens, Rong, and Todd 2013). This allows for inferences for the role of vision, especially if the characteristics of the vision of the species in question is analysed. In these studies, a “correct” response, i.e., change to match the novel substrate, is indicative of perception of the differences. But the direct linking of the visual characteristics to colour change, to identify causality is limited in the literature, especially outside a few specific case studies (e.g.: cephalopods (Allen, Mäthger, Barbosa, et al. 2010; Allen, Mäthger, Buresch, et al. 2010; Barbosa et al. 2007; Chiao, Chubb, and Hanlon 2015; Chiao and Hanlon 2001a, 2001b; Chiao, Kelman, and Hanlon 2005; Kelman, Osorio, and Baddeley 2008; Mäthger et al. 2006; Zylinski et al. 2011;

Zylinski, Osorio, and Johnsen 2016; Zylinski, Osorio, and Shohet 2009b)) (reviewed in (Chiao, Chubb, and Hanlon 2015)). However, research in these study systems has directly investigated multiple aspects of vision and their roles in colour change for camouflage, especially the visual processing of spatial information (Barbosa et al. 2008; Barbosa et al. 2007; Chiao, Chubb, and Hanlon 2007; Chiao and Hanlon 2001a, 2001b; Chiao, Kelman, and Hanlon 2005; Zylinski, Osorio, and Johnsen 2016; Zylinski, Osorio, and Shohet 2009b). It has also raised questions about the assumptions regarding vision and colour change. For example, behavioural assessments of one species of cuttlefish's vision corresponded to a single photoreceptor type and theoretically no means of colour discrimination (Mäthger et al. 2006). However, on naturally coloured substrates they successfully adjust coloration, not just brightness as would be expected, to improve crypsis (Mäthger et al. 2008). A variety of potential mechanisms could be allowing chromatic colour change responses in cuttlefish despite their lack of colour vision, ranging from approximating chromatic cues from intensity information paired with chromatophores similar to natural colours (Mäthger et al. 2008), to photoreception outside of the eye (recorded in other cephalopods (Ramirez and Oakley 2015)), to novel mechanisms for detecting colour altogether (Stubbs and Stubbs 2016) (although such mechanisms are contested (Gagnon et al. 2016)). Despite this, there still lies the question of role of vision in colour change for camouflage in different contexts: are all species limited by vision in similar ways? For example, does a lack of colour vision inhibit chromatic matching, and are those species that are apparently not inhibited in such away exceptions (e.g.: cuttlefish (Mäthger et al. 2008))?

Outside of the properties of a species visual system, external factors will also affect the perception of their visual environment. One factor that will play a part in substrate perception but has not recently been considered in relation to colour change for camouflage is illumination. Illumination will dictate what light is available to be reflected, and therefore influence what the substrate reflects (Troscianko et al. 2009). Various factors can influence the qualities of substrate illumination, both atmospheric conditions and the physical properties and topography of the environment, and these effects can change in both relatively short and long timescales. Ideally individuals changing colour would match the "actual" reflectance properties of the substrate, rather than the apparent

reflectance that varies with illumination. This would mean individuals would continually match the substrate reflectance regardless of changes in illumination. Perceptual mechanisms like luminance and colour constancy (Foster 2011) allow for the detection of actual reflectance presumably based on apparent substrate radiance relative to illumination, ensuring matching regardless of illumination. This is assumed to be based on the relative stimulation by directional light – illumination from above, reflectance from below the individual (thought to be used in substrate perception for colour change, based on the limited testing to date (Brown Jr and Sandeen 1948; Bückmann 1979; Sumner and Keys 1929)).

With regards to colour change outside of the camera type eye and physiological colour change pairing, a large number of species changing colour potentially for camouflage lie within the arthropods (Umbers et al. 2014). This group is often characterised by a lack of camera eyes, and both physiological and morphological colour change (e.g.: lepidopteran larvae (Eacock et al. 2017; Grayson and Edmunds 1989), crustaceans (Bedini 2002; Duarte, Stevens, and Flores 2018; Green et al. 2019; Siegenthaler et al. 2018; Stevens 2016; Stevens, Lown, and Wood 2014b; Stevens, Rong, and Todd 2013), orthopterans (Tanaka, Harano, and Nishide 2012; Burtt 2009; Filshie, Day, and Mercer 1975; Peralta-Rincon, Escudero, and Edelaar 2017; Valverde and Schielzeth 2015), and phasmids (Bückmann 1979, 1977)). These colour change behaviours vary from changes between discrete phenotypes (e.g., red versus green phenotypes of *Hippolyte varians* based on algal substrate (Green et al. 2019)) or variation along a continuum (e.g., luminance in crabs in response to background intensity (Stevens 2016; Stevens, Lown, and Wood 2014b)). Indeed, recent experiments have highlighted further examples of colour change occurring despite limitations in vision that would be thought to equally limit change. One major example is that larvae of the lepidopteran *Biston betularia* are not only capable of colour change for camouflage (both brightness and chromatic matching) when their ocelli are obscured, but that the tissues containing the chromatophores themselves detect and respond to light (Eacock et al. 2019). It is evident that continued study of vision's role in colour change is needed, and specifically the roles of less complex systems of vision.

While vision is the main sense thought to be used when changing colour, other senses may be used to obtain the information needed for accurate colour change. In many species using camouflage, the backgrounds they rest on are also their main food source, and therefore dietary cues could be used instead of vision. This has been proposed as a mechanism of colour change in *Hippolyte* shrimp (Duarte, Stevens, and Flores 2018; Gamble and Keeble 1900; Green et al. 2019; Keeble, Gamble, and Hickson 1900), marine isopods (Hultgren and Mittelstaedt 2015; Lee 1966), and in certain caterpillars (Greene 1996). In this case, whether dietary substrates provide information that stimulates colour change, or whether these individuals simply express materials or pigments from the substrates directly (as proposed in the colour change in certain spiders (Gillespie 1989)) is less clear. It raises the question of whether vision is even used in certain species changing colour, or if other modalities provide the information needed for matching.

Shore crabs as a model

The green shore crab, *Carcinus maenas* is an ideal candidate for continuing the investigation of vision's role in colour change for camouflage. It is an intertidal decapod crustacean, and while its native range is the west coast of Europe and Africa, it has spread as an invasive species to most coastal regions of the globe (Neal and Pizzolla 2008; Le Roux, Branch, and Joska 1990). Its life history can broadly be grouped into three distinct stages. First, it exists as free floating, planktonic larvae, which are transparent, potentially as a camouflage strategy in the open ocean (Cronin 2016). Following settlement and upon reaching adult morphology, subadults occupy intertidal environments (Crothers 1968; Amaral et al. 2009; Young and Elliott 2020). These are often visually diverse, both within and between environments. Subadult *C. maenas* can be found in spatially and spectrally uniform mudflats, in more spatially complex environments such as sand, kelp, and mussel beds, and the heterogenous rockpool environments (Crothers 1968; Hogarth 1978, 1975; Nokelainen et al. 2017; Price et al. 2019; Stevens, Lown, and Wood 2014a; Todd et al. 2006; Todd et al. 2012). At this stage they exhibit strong phenotype – environment associations. Individuals in more uniform environments tend to be more uniform in coloration, both within and between individuals, while individuals from heterogenous environments tend towards high levels of polymorphism and

individual patterning (Nokelainen et al. 2017; Stevens, Lown, and Wood 2014a; Todd et al. 2006; Todd et al. 2012). Finally, as previously noted, individuals lose a large amount of their variation with age, in an ontogenetic shift to more uniform green coloration (Nokelainen et al. 2019).

C. maenas has been demonstrated to use both background matching and disruptive marking camouflage strategies, with strategies being associated with habitat types: background matching in uniform habitats, and disruptive markings in heterogenous ones (Price et al. 2019). Their camouflage is under pressure from a range of potential predator visual systems (Crothers 1968). Common predators include tetrachromatic avifauna (e.g., gulls (Håstad, Partridge, and Ödeen 2009)), di- and trichromatic fish predators (e.g. gobies (Utne-Palm and Bowmaker 2006) and pollack (Shand et al. 1988)), as well monochromatic species including cephalopods (Mäthger et al. 2006) and elasmobranchs (Gačić et al. 2006). They are also subject to predation from larger conspecifics (Moksnes 2004), and may have to rely on camouflage to deceive their own visual system. Given their free floating planktonic life stage, it is unlikely they have fine control over the habitat type they settle upon, in fact *C. maenas* in the south west of England have been identified as a single genetic population (Silva et al. 2010) indicating a high level of intermixing. If coloration is entirely fixed individuals risk mismatching their eventual habitat. While there is the potential from movement to new habitats following settlement (Nokelainen et al. 2017; Price et al. 2019), there will be a significant risk of predation, both from the initial mismatch (as seen in other crustaceans (Duarte, Stevens, and Flores 2018; Mynott 2019)) and the required movement leading to increased conspicuousness (Rushton, Bradshaw, and Warren 2007). With the unpredictable nature of the habitat, the ability to change colour to match settlement site is likely to provide a significant survival benefit, and this has been proposed as a key mechanism for the phenotype-environment association in the species (Nokelainen et al. 2017; Price et al. 2019; Todd et al. 2012).

In terms of potential colour change *C. maenas* possess at least three chromatophore types, identified by Poulton as red, black, and white, distributed across the sub-carapace tissue (Powell 1962b; Powell 1962a). While some physiological colour change has been documented (Powell 1962b; Stevens, Lown, and Wood 2014b), the most noticeable changes in colour are discrete

shifts associated with moulting (Stevens 2016; Carter, Tregenza, and Stevens 2020; Mynott 2019). Following moulting, crabs can undergo significant changes in colour, most noticeable are changes in brightness although some evidence for chromatic change has been presented (Stevens 2016). The specifics of pigment changes are less clear, although hormone-stimulate changes in chromatophores has been recorded (Alexander et al. 2020). Potentially, short term changes are facilitated by the redistribution of pigments within chromatophores in response to external stimuli (Powell 1962b; Powell 1962a; Stevens, Lown, and Wood 2014b).

The more visible changes in coloration following moulting (Stevens 2016) could be a result of multiple mechanisms. It may be that coloration change is chromogenic, as pigments are generated or lost slowly when backgrounds change, and the pigmentary change is obscured partially or fully by a well-established carapace. These could be incorporated into the newly forming carapace and then become visible when the old carapace is shed. Alternatively, there could be a rapid physiological change immediately following moulting, potentially in a primary response as chromatophores are (more) exposed to direct light. The former may be more likely, given the scale of changes with moulting seem to be beyond recorded physiological changes (e.g., the moulting based change seen in (Carter, Tregenza, and Stevens 2020; Mynott 2019; Stevens 2016), versus the physiological change seen in (Stevens, Lown, and Wood 2014b)). In either case, these subcuticular pigments presumably become part of the developing carapace

While these changes are reversible, especially in smaller/younger crabs, the ability to change colour reduces with age, alongside the ontogenetic shifts towards uniform green coloration (Nokelainen et al. 2019). While there is little evidence that individuals' body markings are plastic, there is some anecdotal evidence that contrast between elements of patterns can change, both from reversible colour change in subadults, as well as the previously mentioned ontogenetic shift (Nokelainen et al. 2019). This shift may also involve changing priorities in antipredator strategies (as seen in other crustaceans (Anderson et al. 2013)): larger adults possess thicker carapaces and larger claws, and may rely more on physical defences, as oppose to camouflage and colour change.

Regarding *C. maenas* vision, certain aspects have already been documented. They possess compound, apposition eyes, with short eye stalks and lacking pronounced acute zones, characteristic of species in less flat habitats (Zeil, Nalbach, and Nalbach 1986). There are two investigations of their spectral sensitivity from the mid-20th century. Both used electroretinography measures to characterise photoreceptor sensitivity, the first finding a single photoreceptor with a λ_{max} of 498 nm (Bruno, Mote, and Goldsmith 1973) while the later paper identifying two photoreceptor classes. The majority possessed a λ_{max} of 508 nm, while a single photoreceptor from a single individual (out of 108 cells measured) recorded a λ_{max} of 440 nm (Martin and Mote 1982). These values follow a trend in intertidal crab species, with many possessing a mediumwave sensitive photoreceptor (Forward, Cronin, and Douglass 1988) (with the potential for other photoreceptor sensitivities e.g., (Bruno and Goldsmith 1974; Martin and Mote 1982)). While this evidence is by no means definitive, *C. maenas* may possess multiple photoreceptors of differing sensitivities, and therefore the potential for colour vision. This, as well as behavioural thresholds of spectral sensitivity, have not yet been identified though. Beyond that, their spatial acuity has also been characterised, with an acuity of 0.51 cycles per degree (CPD), (calculated in (Feller et al. 2021), using data from (Zeil, Nalbach, and Nalbach 1986)). This is low compared to human vision, for example, at around 30 CPD but expected for compound eyes of their size (Caves, Brandley, and Johnsen 2018).

Research Questions

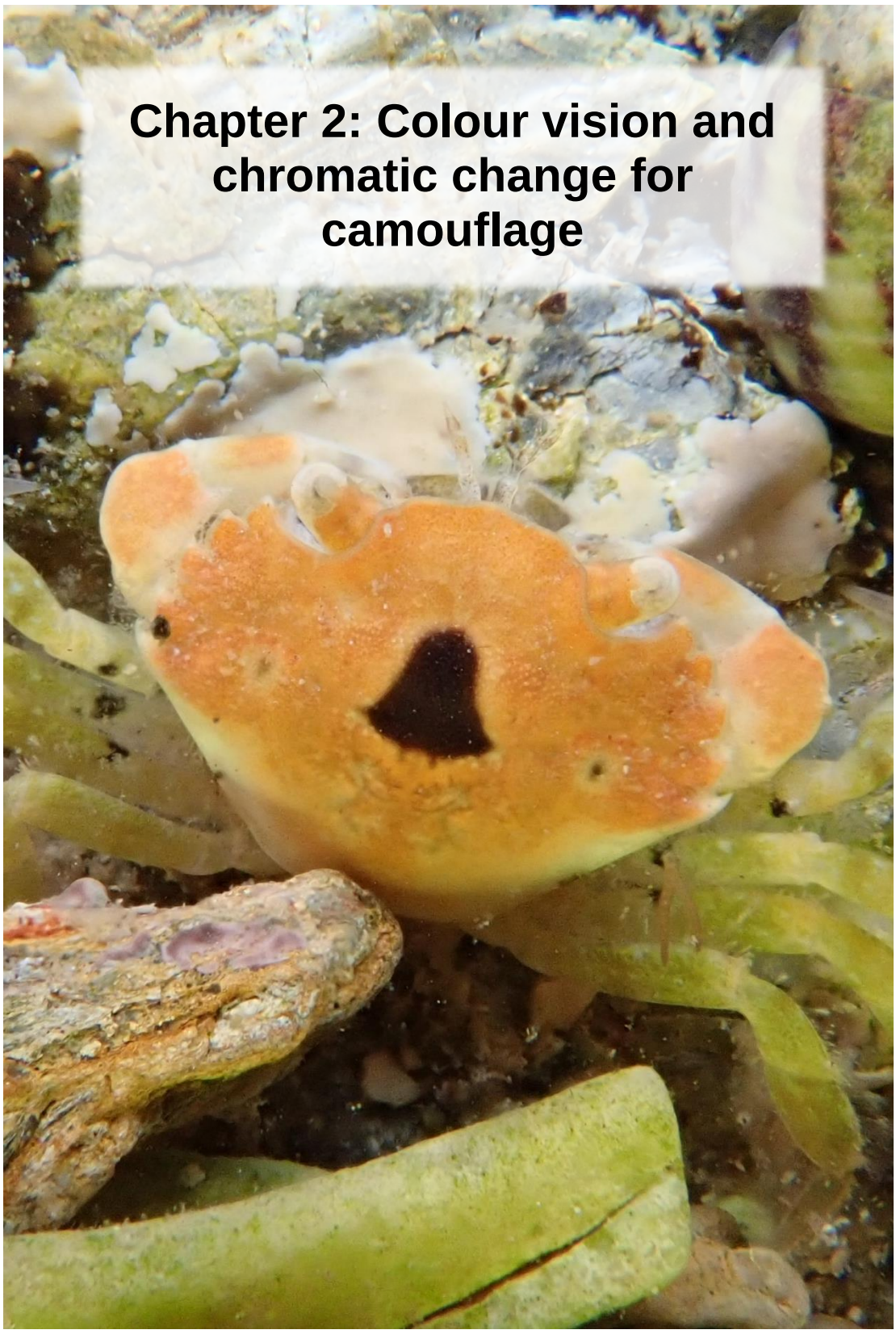
Using *C. maenas* as a model, my thesis investigates the associations between vision and colour change responses, to identify the specific roles vision and perceptual processes play in colour change for camouflage, and whether features of vision lead to relative limitations in colour change. This will help broaden the existing knowledge of colour change for camouflage and the role of vision, to determine whether there are general causal trends in this relationship, or if there is a need to account for case by case variation according to species.

To that end, the following questions will be answered:

1. What roles do intensity and spectral information play in *C. maenas* colour change? Does the presence or absence of colour discrimination transfer similar abilities or constraints on *C. maenas* colour change?
2. Does *C. maenas* use spatial information when changing colour, and if so, does its vision limit its ability to match complex backgrounds?
3. How does *C. maenas* obtain information about substrate appearance under different lighting conditions? Is information being obtained via ocular photoreception, and if so, what information is being used and how?

Answering these questions will provide a greater understanding of vision's role in colour change for camouflage. They will not only show how compound eyes affect colour change compared to camera type eyes, but also provide insight into how animals depend on and are limited by vision, as well as how these limitations can potentially be mitigated.

Chapter 2: Colour vision and chromatic change for camouflage



Abstract

The ability to discriminate colour is thought to be crucial in animals that can change their own appearance to chromatically match backgrounds. When changing to match a substrate, substrate colours are assumed to be identified along with differences between individual and substrate colour. It is less clear if a lack of chromatic vision and colour discrimination limits chromatic change for camouflage. Recent examples show that mechanisms allow colourblind animals to successfully match the colours of natural backgrounds. To test if colour vision can limit chromatic change, I compared the two in *Carcinus maenas*. I developed tests of colour vision based on measurements of the *C. maenas* photoreceptor system. I then compared how individuals performed in these visual tests with their colour change responses. I confirmed the number of photoreceptors with differing spectral sensitivity via optokinetic responses. I used this information to generate stimuli discriminable only via chromatic cues for tests of colour discrimination. Finally, I recorded the species' ability to change colour over eight weeks, both chromatically and achromatically, and related this to visual data. Behavioural measures identified a single group of photoreceptors with a maximum sensitivity of λ_{max} = 496nm, which corresponds well to past research. Crabs showed selection between black and white stimuli, with marked preference for black over white. This preference reduced when black stimuli were associated with an aversive result however, indicating avoidance based on visual cues. When achromatic stimuli were replaced with isoluminant (matching brightness to crab perception) coloured stimuli, with only chromatic cues available, this differentiation was lost. While not definitive, this indicates of a lack of colour discrimination. Finally, while some chromatic colour change occurred, it did not uniformly result in an improvement of camouflage. The greater part of appearance change recorded was achromatic, which significantly improved matching on substrates where crabs initially mismatched. I conclude that while some chromatic change may occur, potentially stimulated outside the eyes, or caused by unrelated metabolic processes, the majority of *C. maenas* colour change for camouflage is achromatic and directed by apparently colour-blind eyes. In this instance, a lack of colour vision seems to limit chromatic change for camouflage.

Introduction

Cryptic coloration is an archetypal example of natural selection driving appearance (Wallace 1889, 1867; Cott 1940). Individuals match features of their habitat, to avoid being detected or recognised as viable prey, or potential predators (Stevens and Merilaita 2009a). Multiple visual characteristics influence the success of cryptic coloration. One of the strategies most associated with camouflage is background matching (Cuthill 2019; Merilaita and Stevens 2011; Stevens and Merilaita 2011). Matching the habitat in brightness, colour, the degree and size of patterning, as well as orientation of patterning have all been identified as influencing detectability (and recognisability) of camouflaged individuals (Cuthill 2019; Galloway et al. 2020; Merilaita, Scott-Samuel, and Cuthill 2017; Skelhorn and Rowe 2015; Troscianko, Skelhorn, and Stevens 2017; Troscianko et al. 2009; Stevens and Merilaita 2009a).

Colour matching is a key parameter when deciding if an individual is camouflaged (Cuthill 2019; Stevens 2007; Stevens and Merilaita 2011; Edmunds 1974; Norris and Lowe 1964; Stevens and Merilaita 2009a). Initially judgements on whether animals matched habitats were made based on human colour sensitivity (Thayer and Thayer 1909; Cott 1940). It is now expected that studies of animal coloration account for the visual systems of observers (Norris and Lowe 1964; Endler 1978, 2008; Guilford and Dawkins 1991). Visual information can be compared from either spectrophotometric measurements (Akkaynak 2014) (used in (Akkaynak et al. 2017; Spottiswoode and Stevens 2010; Stoddard and Stevens 2010) for example), or digital images of subjects of interest (Stevens et al. 2007; Troscianko and Stevens 2015) (used in (Duarte, Stevens, and Flores 2018; Green et al. 2019; Nokelainen et al. 2017; Nokelainen et al. 2019; Price et al. 2019; Smithers, Wilson, and Stevens 2017; Stevens 2016; Stevens, Lown, and Denton 2014; Stevens, Lown, and Wood 2014b, 2014a) for example), converted to account for various spectral sensitivities .This understanding has allowed virtual experiments to apply these variations in sensitivity to virtual prey, to help understand the variation in predation on cryptic species (Fennell et al. 2019; Niu, Stevens, and Sun 2021; Troscianko et al. 2021; Troscianko et al. 2017).

However, camouflage will not always be solely dependent on the visual system of the observer. Cryptic species' habitats are unlikely to be uniform, either

because of variation within habitats, or between various habitat types occupied by the species (Stevens and Merilaita 2009a). These variations can result in mismatching, as individuals may be viewed against backgrounds of a colour more different than their own (Ruxton et al. 2019; Stevens and Merilaita 2009a). Given individuals need to move within and between habitats to access various resources or because of changes in life history (Stevens 2016; Booth 1990), mismatching moves from the possible into the probable. Multiple adaptations have been found that can alleviate this problem. When considering static coloration, generalist coloration (matching an average of the coloration of the habitat) is a potential counter to this, where individuals match common aspects of the habitat that are less likely to be affected by variation in background. This strategy's effectiveness has been repeatedly demonstrated using model experiments (Briolat et al. 2021; Houston, Stevens, and Cuthill 2007; Merilaita, Lyytinen, and Mappes 2001; Walton and Stevens 2018; Toh and Todd 2017). Alternatively, behavioural responses can allow for improvement matching. Background selection (Allen, Mäthger, Barbosa, et al. 2010; Eacock et al. 2019; Kettlewell and Conn 1977; Kjernsmo and Merilaita 2012; Stevens et al. 2017; Uy et al. 2017) and modification (Troscianko, Wilson-Aggarwal, et al. 2016), as well as orientation to align body coloration with that of the habitat (Kang et al. 2014; Kang et al. 2012) have all been demonstrated to improve camouflage. While other sensory cues could guide behaviours that improve (visual) camouflage (e.g. chemical cues, as seems to be the case with colour change in some caridean shrimp (Duarte, Stevens, and Flores 2018) and lepidoptera (Poulton 1903; Noor, Parnell, and Grant 2008; Greene 1996) for example), vision may be the most direct channel. The behavioural change should be directed based on the visual cues, resulting in initial mismatch and ultimate improvement.

Another behaviour where vision is potentially crucial is colour change for camouflage (Stevens and Ruxton 2019). Rather than rely on finding the optimum conditions for matching, individuals adjust part or all their coloration to match changes in the environment (Duarte, Flores, and Stevens 2017; Stevens 2016; Stuart-Fox and Moussalli 2009). Colour change for camouflage can be split into two broad categories: non-reversible and reversible. Non-reversible changes are often associated with ontogenetic changes in ecology, and habitats

specifically. Ontogenetic changes in camouflage are seen in multiple species (Booth 1990) including butterfly larvae changing from crypsis to conspicuousness as adults (Medina et al. 2020) , and shifts in cryptic coloration strategies in snakes (Wilson, Heinsohn, and Endler 2007), frogs (Bueno-Villafañe et al. 2020; Bulbert et al. 2017), fish (Cortesi et al. 2016), crabs (Nokelainen et al. 2019) and other intertidal crustaceans (Lee 1966). These changes could be guided by vision, but given the timescales and life history tending to be pre-determined, changes may be a result of other factors (as seen in the loss of plasticity and tendency to uniform coloration of *C. maenas* with age (Nokelainen et al. 2019)). Changes that are most likely to be guided by vision (or at least sensory information in general) are reversible changes in coloration within an individual's life stage. While some reversible changes in coloration are in response to predictable events (e.g. seasonal changes in coat colour in response to changes in snowpack cover, seen in mammals (Rothschild 1942; Nagorsen 1983; Mills et al. 2013) and birds (Hewson 1973; Jacobsen Jr, White, and Emison 1983)) most are likely to be in response to unpredictable changes (e.g., random movement of individuals by external forces, or shifts in habitat make up from unpredictable events for example a heatwave wiping out a specific of algae used as a background for camouflage) in visual backgrounds.

Non-seasonal reversible colour change for camouflage has been shown across a large array of species; including reptiles (Stuart-Fox, Moussalli, and Whiting 2008; Stuart-Fox, Whiting, and Moussalli 2006), amphibians (Kang, Kim, and Jang 2016), fish (Allen et al. 2015; Kelman, Tiptus, and Osorio 2006; Smithers, Wilson, and Stevens 2017; Stevens, Lown, and Denton 2014; Ramachandran et al. 1996; Tyrie et al. 2015; Akkaynak et al. 2017), cephalopods (Allen, Mäthger, Barbosa, et al. 2010; Barbosa et al. 2008; Hanlon 2007; Hanlon et al. 2009), gastropods (Manríquez et al. 2009), and both terrestrial (Burtt 2009; Eacock et al. 2017; Eacock et al. 2019; Filshie, Day, and Mercer 1975; Peralta-Rincon, Escudero, and Edelaar 2017; Théry and Casas 2009) and marine arthropods (Duarte, Stevens, and Flores 2018; Green et al. 2019; Hultgren and Mittelstaedt 2015; Hultgren and Stachowicz 2008; Lee 1966; Stevens, Lown, and Wood 2014b; Stevens, Rong, and Todd 2013; Keeble, Gamble, and Hickson 1900) (reviewed in (Umbers et al. 2014)). These colour changes tend

to be split based on the mechanisms by which they are achieved, with rapid physiological colour change occurring as existing pigments are redistributed over seconds to hours, and morphological changes as pigments and pigment containing tissues are metabolised or lost over days to months.

Visual information is the assumed cue for physiological colour change, given the allowance for rapid acquisition and application of information (Duarte, Flores, and Stevens 2017; Stevens 2016). With regards to morphological colour change and slower chromatophore responses, there has been less study relating vision (and other sensory inputs) to colour change for camouflage, despite the diversity and abundance of these changes (see (Umbers et al. 2014) for a review of arthropods alone). These longer changes reduce the value of the immediacy of visual information, but not the value of accurate background identification for matching. Experimental evidence of vision guiding these colour change behaviours is found in the responses to manipulated visual backgrounds and lighting (e.g. in crabs (Hultgren and Stachowicz 2008; Stevens, Lown, and Wood 2014b; Stevens, Rong, and Todd 2013) and other arthropods (Eacock et al. 2017; Eacock et al. 2019; Burtt 2009; Filshie, Day, and Mercer 1975; Peralta-Rincon, Escudero, and Edelaar 2017; Wiklund 1972; Smith 1980)). Direct investigation of the specific roles of visual information and colour sensitivity, as well as ocular/non-ocular photoreception are less common. Outside of rapidly changing species, there is evidence of vision influencing chromatophore responses via the hormonal transmission of information from eye stalks of *Uca* crab species (Fingerman and Yamamoto 1967) and *Carcinus maenas* (Alexander et al. 2020), as well as the control of extraocular hormones via the optic nerve in *Cancer magister* (Shibley 1968). Alongside effecting colour change via information gathered by eyes then relayed to sites of colour change, these sites can respond directly to light. In multiple species, chromatophores respond directly to light (e.g. crabs (Brown Jr and Sandeen 1948; Powell 1962b), cephalopods (Ramirez and Oakley 2015), and lepidoptera (Eacock et al. 2019)), and can even allow for accurate colour matching without colour vision

C. maenas is a model species for camouflage and colour change. It exhibits significant variation in phenotypes (Brian et al. 2006; Hogarth 1978, 1975; Stevens, Lown, and Wood 2014a). There is a strong association between

phenotype and environment, with a focus on pattern and achromatic matching (Nokelainen et al. 2017; Price et al. 2019; Todd et al. 2006; Todd et al. 2012), but also evidence that chromatic matching is still useful (Nokelainen et al. 2019). The crab has been recorded as possessing three types of chromatophore, containing either red, black, or white pigments (Powell 1962b; Powell 1962a). This implies the possibility of some chromatic change, at least in terms of relative longwave reflectance. In addition, early work has highlighted a primary response in chromatophores when eyes were covered (Powell 1962b). Most documented colour change is in terms of brightness however, with some small change immediately post moulting (Stevens, Lown, and Wood 2014b), that cumulatively results in significant change with repeated moulting over weeks and months (Carter, Tregenza, and Stevens 2020; Stevens 2016). Little chromatic change in response to background change has been recorded, although this has focussed primarily on the short term (Stevens, Lown, and Wood 2014b). Whether any significant change occurs over longer periods is less clear.

Shore crab vision has had some characterisation. Mid-20th century studies classified it as possessing at least one mediumwave sensitive photoreceptor, both via microspectrophotometry (Bruno, Mote, and Goldsmith 1973), and electroretinography (Martin and Mote 1982). Of these, the latter identified a second class of photoreceptor maximally sensitive to shortwave light (Martin and Mote 1982), indicating a potential for opponent colour vision (although only one photoreceptor from one individual was identified). Outside of this, little is known about their ability to discriminate colour. Colour discrimination is found in other brachyurans (Takeda 2006; Bursey 1984; Detto 2007), and may be present in *C. maenas* as well (given the second photoreceptor found in (Martin and Mote 1982)). Taking all this into consideration, there is clear value in both characterising *C. maenas* colour discrimination, and subsequently relating it to its colour change abilities.

In this chapter, I had three aims. The first was to confirm the spectral sensitivity of *C. maenas*. This was done using behavioural measures. Given colour change for camouflage is a behavioural response, behavioural thresholds (i.e., stimuli thresholds that generate a behavioural response) may be more relevant, as absolute measures of stimulation/absorbance (while detectable) may not

elicit a response. To that end, I used the well-established technique of the optomotor response (Baldwin and Johnsen 2011; Groeger, Cotton, and Williamson 2005; Krauss and Neumeyer 2003; Schaerer and Neumeyer 1996; Utne-Palm and Bowmaker 2006; Yamaguchi et al. 2008) where eyes or the whole body track features of the environment moving relative to the individual. This can be used to assess whether stimuli are visible under specific conditions, based on its presence or absence. The responses to specific colours at varying intensity were then used to create spectral sensitivity curves based on the intensity of various colours that caused a response. While there are limitations of this method (detailed further in the discussion), the rapid and non-invasive measurements along with the existing electrophysiological data provide a valuable insight into *C. maenas* vision.

Following this, the novel behavioural data along with the combined prior knowledge of *C. maenas* spectral sensitivity was used in the creation of isoluminant stimuli for tests of colour discrimination. Traditionally, alongside inferences from molecular (e.g. (Rajkumar et al. 2010))/electrophysiological measures, behavioural choice tests have been used to identify colour discrimination (as reviewed in (Kelber, Vorobyev, and Osorio 2003)). Preferential selection of a training colour is indicative of colour vision. Presentational studies have demonstrated colour vision in crustaceans (Daly et al. 2017; Detto 2007; Marshall, Jones, and Cronin 1996; Kawamura et al. 2020), and were therefore applied to *C. maenas*. Initial attempts using appetitive stimuli were unsuccessful, and trials swapped to aversive stimuli, as this has been shown to produce a learnt response in the species (based on directional cues (Magee and Elwood 2013)). Initial training was tested using black and white stimuli (that should be discriminable regardless of colour vision) before testing using coloured stimuli. Dominant wavelengths of the stimuli were selected based on equidistance either side of the peak sensitivity of *C. maenas* calculated in the prior experiments, in a patchwork of varying intensities, seen in previous studies (e.g. (Roth and Kelber 2004)) to remove intensity/brightness as a cue.

Finally, crabs' ability to chromatically change colour was tested, using methods previously applied to the species (Carter, Tregenza, and Stevens 2020; Nokelainen et al. 2019; Stevens 2016; Stevens, Lown, and Wood 2014b).

Crabs were placed on various coloured backgrounds, and colour change responses were quantified over six to eight weeks (sufficient time for moulting to occur), both in brightness (luminance to predator vision) and colour (hue, the ratio of longwave and mediumwave to shortwave reflectance), as well as matching success in both. Colour was measured using digital image analysis (Stevens et al. 2007; Troscianko and Stevens 2015), as this has been an effective tool in studying camouflage in this (Nokelainen et al. 2017; Nokelainen et al. 2019; Price et al. 2019; Stevens, Lown, and Wood 2014b, 2014a; Troscianko et al. 2021; Carter, Tregenza, and Stevens 2020), and other species (Duarte, Stevens, and Flores 2018; Green et al. 2019; Smithers, Wilson, and Stevens 2017; Stevens, Lown, and Denton 2014; Stevens, Rong, and Todd 2013; Stevens et al. 2017; Troscianko, Wilson-Aggarwal, et al. 2016).

I predicted that if *C. maenas* was found to be monochromatic (possessing only the mediumwave sensitive photoreceptor), then it should not display any ability to discriminate between stimuli that differ only in dominant wavelength, not perceived brightness. Following this, if colour discrimination is not evident then little to no colour change resulting in improved matching in the colour change experiments is expected. If there was evidence of photoreceptors of differing sensitivity, and subsequent colour discrimination, then I expect some chromatic change in coloration, most likely in the ratio of longwave reflectance (given the red chromatophore).

This experiment contributes to the nascent field of study combining visual and camouflage metrics in the study of animal colour change for camouflage (Eacock et al. 2019; Barbosa et al. 2007; Zylinski, Osorio, and Shohet 2009b; Marshall and Messenger 1996). Specifically, it will be to my knowledge the first to investigate, in the same study, the colour change behaviour and vision of a marine species changing appearance predominantly though morphological colour change and possessing a compound eye. This will also be one of the first studies combining and directly relating visual metrics of both the species changing colour and multiple theoretical predators.

Methods

Identifying spectral sensitivity

Sample Collection

Carcinus maenas were collected from Littleharp Bay, a region of shoreline comprised of mud/silt flats with rocky outcrops and some algal (predominantly *Fucus sp*.) cover, in Clevedon, United Kingdom (Long/Lat: 51.439354, -2.864936). Crabs were collected using collapsible net traps that were left for two tide cycles, then retrieved approximately 24 hours after traps were set. Crabs were transported back to the University of Bristol Life Sciences Building (in individual containers to prevent conflict), and individually housed with constantly cycling salt water and rocks for shelter. The water was regularly tested for salinity and maintained at ~ 32‰. Crabs were fed twice weekly with commercially sourced, frozen, cold-water prawns. For this experiment, fully adult crabs (cephalothorax diameter >50mm) were used, as there were the easiest size classes to collect efficiently.

Optokinetic Experiments

Optokinesis experiments were conducted using an in silico optomotor drum produced using MATLAB software (The MathWorks 2018), with code provided by Dr Martin J How. Optomotor and optokinetic responses have been seen in multiple crustacean species (Baldwin and Johnsen 2011; Barnatan, Tomsic, and Sztarker 2019; Drerup and How 2021), and *C. maenas* specifically (Horridge, Sandeman, and Callan 1964), They have also been used in the assessment of behavioural spectral sensitivity in species outside crustaceans (e.g., (Utne-Palm and Bowmaker 2006)). The drum was constructed using four Samsung LCD screens arranged to form a cube (Figure 1). The grid was produced as a sinusoidal wave between an RGB pixel value of 0, 0, 0 (black) at one extreme and an RGB pixel value of either red, blue, or green (RGB) resulting in a smooth transition between black and coloured stripes. The RGB pixel increased by one value per second, starting at a value of zero (black) and increasing up to 255 (full saturation). Absolute irradiance measurements were taken from the screens (using an Ocean Optics Flame Spectrophotometer and 200µm fibre optic cable) to obtain spectral/wavelength values of the red, green, and blue stimuli. The wavelength values used to quantify stimulus colours were the peak values on the absolute irradiance curves recorded. The grid was then

displayed on each of the four screens with six stripes (three black, three coloured) per screen. Stripes were rotated across each screen, broadening at edges of the screens to create the perspective of a rotating cylinder to the observer.

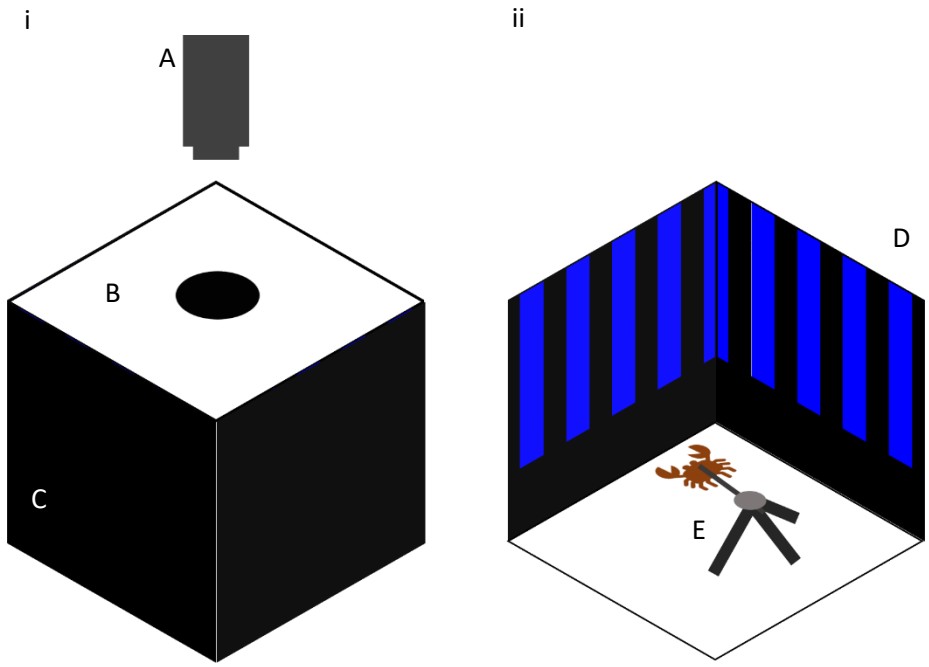


Figure 1: Diagram of the virtual optomotor drum set up.
i: External view. A: the video camera, held directly above the drum in a retort stand. B: the cover placed over the top of the drum to prevent external stimuli from affecting the experiment, with a hole to film through. C: the four LCD screens arrange to form a cube around the crab. ii: Internal view. D: the virtual grid, rotating clockwise with a black horizontal bar used to create a horizon line to aid in stabilization behaviour. E: the tripod with metal support to attach to the crab.

The drum moved in a clockwise direction at a speed of 3.5 degrees per second, and a horizon line of black opaque card was placed at the bottom of the screen to aid in visual stabilisation of the individual in the trial. Crabs had white marks placed on the back of each eye stalk to aid in tracking movement from video, and a third mark placed on the carapace behind and between the eyes. The third point acted as a means of stabilisation to allow any movement by the crab not related to optokinesis to be removed during analysis. Crabs were fixed to a metal harness via cyanoacrylate adhesive (sufficient to fix the crab in place while still allowing safe removal at the end of the experiment) which was then

attached to a tripod behind the individual's field of view. Individuals were placed into the virtual drum with each screen displaying stripe pixel values of 0, 0, 0, and 0, 0, 0 (black screens). A cover was placed over the top of the drum to prevent conditions outside the chamber affecting the experiment, with a hole in the centre to allow filming. The trials were recorded on a Panasonic HC-X900M High-Definition Video Camera in 1080p resolution, and filming was started five seconds prior to the start of each trial and ended five seconds after each trial finished. Once an RGB pixel value of 255 for the coloured stripe was reached, the drum stopped moving and the trial was considered finished. Each crab was run through each colour trial once (red, blue, and green stimuli) in succession, with the order being randomised for each crab. During the experiment, tones were produced at intervals of 40-pixel values starting at zero and increasing to 240 with a final tone at 255. These were transmitted directly to the recorder PC running MATLAB and acted as synchronisation points between the video file and the tabulated file containing intensity values at each time-point generated by the MATLAB program, as well as drum rotation speed and direction. Intensity of the coloured stripe was recorded as the chromatic contrast relative to black, on a scale of zero (black) to one (fully saturated/pixel value = 255) calculated by dividing the stripe's pixel value by 255 (the maximum value achievable). This project was a novel part of a set of experiments being run in the Visual Ecology research group at Bristol, and as such any ethical approval was arranged prior to my arrival via research governance by the Research and Enterprise Division.

Analysis of video files

Once trials were completed, each video file was analysed using a second MATLAB program (also created by Dr Martin J How) that synchronised the video files and the output files from the grid generating software. The video file was then analysed by isolating the eye and stabilisation markers placed on the individual being recorded and tracking the change in position of these markers for each frame of the video. These changes were output directly to the file table in the form of three sets of x-y coordinates (corresponding to the three paint marks on the crab) at each video frame.

Calculation of gain and optokinesis start

Eye tracking data was converted from the MATLAB output file to tab delaminated text and then analysed using R statistical software (ver. 3.5.1 (R

Development Core Team 2020), using RStudio interface (RStudio Team 2020)). The x-y coordinates for each eye marker were adjusted by subtracting the x-y values of the stabilisation marker to remove any movement of the markers not caused by eye movement. A straight vector between the two eye markers at each frame was calculated, and the change in the angle between this vector and the x-axis was used to calculate eye rotation. This was then divided by the rotation speed of the drum, to produce a value of gain used to measure the accuracy of optokinesis. These values of gain at each time frame were then simplified to only record motion relevant to drum movement by taking any values greater than two, and less than zero, and making them equal to zero. This was done to remove the extreme positive values of gain resulting from rapid non-tracking eye movement, or from the saccadic return of eyes to their original position during optokinesis.

To objectively isolate the start of optokinesis, the values of gain for each individual were smoothed using a loess regression via the loess() function with a span of 0.1 in base R (R Development Core Team 2020). This removed much of the noise from the data, whilst preserving the change in gain with increasing intensity, as well as removing any remaining spikes in gain resulting from non-saccadic eye movement. The data was then transformed to a binary on-or-off format, where values of gain greater than 50% of the maximum gain for the individual were assigned a value of one, and those lower being assigned a value of zero. Video frames with a value of one were conservatively assumed to contain optokinesis, and those with zero were not. For the above to occur, the maximum gain of the individual undergoing thresholding had to reach more than 50% of the populations maximum gain (0.478). This was done to isolate samples where optokinesis did not occur. The intensity value of the first video frame with a value of one was considered the start of optokinesis. The maximum gain reached was also recorded. If optokinesis did not occur, a value of 1.1 was assigned to allow these samples to be examined alongside those with optokinesis.

Statistical Analysis

The data was then fitted to spectral sensitivity curves calculated using the template from Stavenga et al. 1993 [36], alongside curves fitted to the λ_{max} values obtained from Bruno et al 1973 (505nm) [31], and Martin and Mote 1982

(508nm) [32]. The relative sensitivities for this experiment were calculated by inverting the colour contrast values recorded for the start of optokinesis. Values of 1.1 (where no optokinetic response was recorded) were converted to zero, as the absolute minimum of sensitivity is the inability to detect stimuli. The data was fitted to mixed effect models using the lme4 package [37] in R, comparing the optokinesis start threshold (lowest value of chromatic contrast where optokinesis was recorded) and maximum gain achieved to fixed effects of drum colour, crab size, and crab sex, with the interaction effects between colour and all other fixed effects to check for the impact of individual size and sex on colour sensitivity. Crab ID was included as random effects to account for the repeat measurements taken from each crab. The two full models were then simplified based on AIC comparison and effect significance.

*Model: lmer(Optokinensis.Start/Max.Gain ~ Colour*Size + Colour*Sex + (1|Crab.ID), na.action = na.omit, data = Opt.Full)*

Following the spectral sensitivity optomotor experiments conducted at Bristol, colour discrimination and colour change experiments were carried out at the University of Exeter's Penryn campus.

Testing for colour discrimination

Sample Collection

Colour discrimination experiments took place at the Sensory Ecology laboratory, at the University of Exeter's Penryn Campus, Cornwall, United Kingdom, TR10 9FE. These followed the basic associative training as those past experiments listed in (Kelber, Vorobyev, and Osorio 2003). Individuals were trained to associate a specific stimulus (in this case a colour) with an outcome (usually positive). Individuals were presented with a choice of the positively associated colour and alternatives that were isoluminant, to remove brightness information as a reliable cue. Noise could be introduced to stimuli's brightness by either presentation of multiple shades of a given wavelength (e.g., (Kelber, Yovanovich, and Olsson 2017)) or presentation of colours alongside grey stimuli of multiple brightness levels (e.g., (Olsson, Lind, and Kelber 2015)). Selection of the positively associated stimulus colour is taken as evidence of discrimination. This general methodology was applied to test *Carcinus maenas* colour discrimination.

Crabs were collected from the mudflats at Penryn, Cornwall (Lat/Long: 50.1697,-5.0989) for both the initial appetitive pilots, and the ultimate aversive trials. This stretch of the Penryn River is characterised by fine silt mud, with some dark gravel cover closer to the town, with significant flotsam from the moored and abandoned boats along the sample site. Individuals were collected by hand by lifting flotsam used as cover along the tideline and stored individually in empty plastic containers (Figure 5) before being transported directly back to the Sensory Ecology and Evolution Research Group (MS) lab. Individuals were preferentially selected based on size, with individuals' size being approximately 15mm to 20mm. This larger size class was used based on the assumption of increased robustness, given the increased handling during experiments (and the shift to aversive stimulus). Once in the lab, crabs were cleaned using freshly made salt water (Aquarium Systems Instant Ocean) to remove any residual mud/detritus. While such material could augment camouflage by creating an exact match between the body of crabs and the environment, it obscured evidence of colour change, and was removed. Crabs were then housed in a 45cm by 120cm tank filled with ~150 litres of freshly made saltwater (to a depth of ~20cm), separated in individual plastic containers (Figure 5) filled with a neutral grey substrate, with adequate water flow and overhead light exposure, and were fed three times a week using Hikari Crab Cuisine pellets. Diet can have an impact on coloration (Duarte, Flores, and Stevens 2017), including in crustaceans (D'Abramo et al. 1983; Hultgren and Mittelstaedt 2015), but given food (both quality and quantity) remained constant across this and all other colour change experiments, it was not deemed as a factor in analysis. Crabs were housed under a 12-hour light regime (light 0700-1900, dark 1900-0700) and placed on a black gravel substrate for burying that was close enough to their habitat to prevent significant colour change. Tank salinity was maintained at 31‰, measured following feeding, water was replaced weekly (50% tank water replaced with freshly mixed salt water), and water quality (nitrite, nitrate, and ammonia content) was recorded weekly. Individuals were housed for a minimum of 48 hours, or until the experiment started.

Discrimination Trials

Initial pilot attempts at colour discrimination trials, conducted across September 2018 to May 2019 using food reward stimuli proved inconclusive both using colour cues (blue and yellow, selected based on rough position on the spectrum relative to *C. maenas* maximum spectral sensitivity assuming monochromacy), and maximally contrasting achromatic stimuli (black and white). In this case, the coloured stimuli had yet to be absolutely matched in terms of brightness, as this was a test of initial methods. In both cases crabs failed to show the ability to discriminate based on visual cues, but this was eventually thought to be due to experimental design. Crabs were originally presented with stimuli in binary, y-choice chamber experiments (shown to be effective in other colour choice experiments in crustaceans (Green et al. 2019)). These ultimately proved unsuccessful, with crabs showing selection only when food rewards were present, and therefor were likely using chemical cues. Details of the initial trials are in Appendix 1A.

Magee and Elwood (Magee and Elwood 2013) found that crabs learned to avoid potential refuges (covered portions in an arena) that delivered a shock upon entry, and this shock avoidance learning appeared to be based on directional cues. Further consideration of *C. maenas* ecology raised the issue of appetitive rewards in training. *C. maenas* is an opportunistic scavenger, likely using chemosensory cues to find food in its habitat, many of which may have poor visibility resulting in olfacto-gustatory cues being more reliable than vision. Their low acuity especially at distance ((Feller et al. 2021), see Chapter 2) also reduces the likelihood of dependence on visual hunting/foraging. As such the likelihood of successfully forming associations about food rewards based solely on vision is potentially low/non-existent. Considering this ecological context, and the success of Magee and Elwood's methodology, colour discrimination trials were redesigned using aversive stimuli. This redesigned methodology was approved via ethical application in 2019 (e-ethics application number: eCORN001701 v2.1)

The novel methodology used the structure of Magee and Elwood's experiment, albeit with several key differences. Stimuli remained as refuges, however, they were coated, both on the exterior and interior, in the relevant colour for the trial. Shelters were constructed using 65mm^2 square piping, with one side removed

to create a lid to the remaining three sides (see Figure 2). Shelters for the black and white trials were made from black and white plastic, respectively. For the chromatic trial, stimuli were generated with isoluminant shades of the two respective colours, as a patchwork of various intensities to create noise reducing the reliability of luminance cues, based on past colour choice experiments (Roth and Kelber 2004). Colours for these trials were generated first by selecting dominant wavelengths equidistant from the λ_{max} calculated in the previous section. The wavelengths chosen were 120nm apart, assuming even if colour discrimination is present, it is likely to be relatively poor assuming dichromacy and a difference of this size should be discriminable based on the potential second shortwave photoreceptor (Martin and Mote 1982). Wavelengths of ~436nm and ~556nm were selected as the benchmarks for the colours (as seen in Figure 2). A cone-catch model of *C. maenas* vision was constructed in the MICA toolbox (Troscianko and Stevens 2015) in imageJ software (Schneider, Rasband, and Eliceiri 2012) using the spectral sensitivity data obtained in the optokinetic experiments. The relative sensitivity values from the fitted Stavenga template were used as an analogue for the relative cone-catch values, and this was then compiled into a cone catch model used to assess the luminance of stimuli. Digital photos were taken of the coloured patches (using the same method detailed further in the colour change experiments, as well as past research quantifying coloration (Duarte, Stevens, and Flores 2018; Green et al. 2019; Nokelainen et al. 2017; Nokelainen et al. 2019; Price et al. 2019; Smithers, Wilson, and Stevens 2017; Stevens 2016; Stevens, Lown, and Wood 2014a)). The *C. maenas* visual model was applied to a scale of 15 patches for each colour to identify pairs (one from each colour) that were isoluminant. The eight candidate patches for each colour were then arranged in eight different four by four patterns comprising two of each patch arranged randomly (Figure 2). This was done to allow for randomisation of the patterns of patches presented to further reduce luminance/spatial cues that could be used to identify aversive stimuli (as seen in (Roth and Kelber 2004)). These were than used to cover the same containers used in black and white,

except only white containers were used as the base to prevent any visible bases providing a cue to stimulus type.

Both the black and white, and blue and green trials were conducted under identical conditions, with the only difference being the stimuli colours. Crabs were tested individually, and upon removal from holding containers, wire electrodes were immediately fitted to the rear most legs at the base of the merus and the crab was placed in a pretrial container to acclimatise for five minutes. This container was covered to reduce stress, with the cover removed 30 seconds prior to transfer into the trial container. During acclimatisation the stimuli were prepared, and their position relative to crab starting point (left or

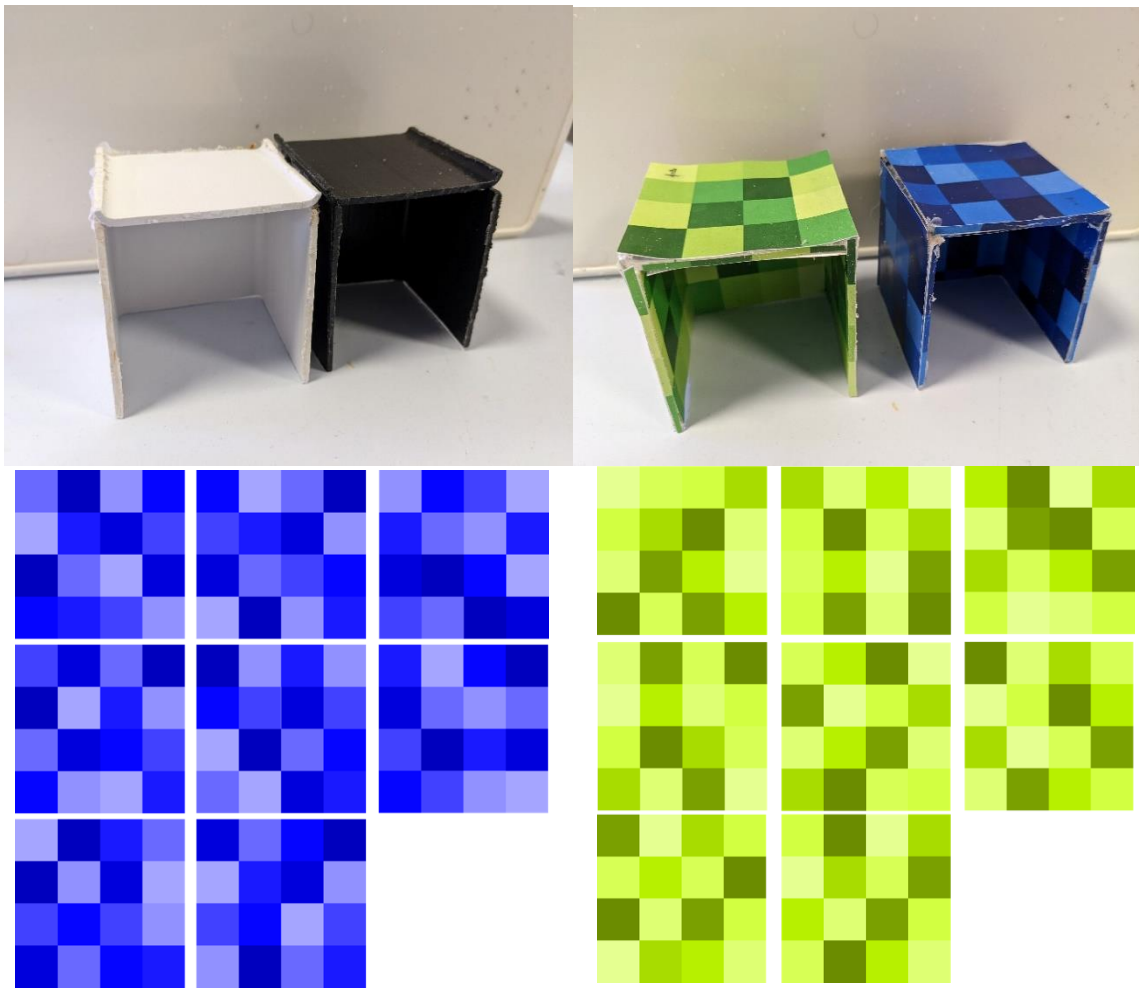


Figure 2: Examples of aversive colour discrimination trial stimuli used. Top left: Photographs of black and white stimuli. Top right: Photographs of the blue (436nm) and green (556nm) stimuli. Bottom: Diagrams of the eight randomised arrangements of the blue and green patches in stimuli.

right) was randomly determined via coin toss and placed in the testing arena: a

30 by 17 cm plastic tray with a y-shaped lining to present the stimuli (Figure 3), filled with freshly made salt water. The sides of the arena were obscured to minimise the effect of outside stimuli on the trial (although observation was required to determine when shocks should be delivered). The trial arena was lit from above and behind the crab starting position using a 70 W, 6500K Iwasaki Colour Arc Lamp with the UV filter, as this was the illumination used for isoluminant stimuli calibration and ensured maximum illumination of stimuli.

Crabs were then placed in the middle of the container, with both stimuli in view and equidistant once the divider was removed. The crab was then allowed to freely roam the arena and interact with the stimuli. Trials ran until crabs had either made an interaction with either stimuli, at which point they were left for two minutes to remain in the chosen stimulus or exit and explore the arena further, or 10 minutes had passed without any interaction (recorded as a null result). A crab was considered to have interacted with a stimulus if it entered the container with at least 50% of its body inside the limits of the container. If the individual entered the non-aversive stimulus, no event happened and the crab was left undisturbed for the remaining two minutes, unless it left the container and interacted with the aversive stimulus. If the aversive stimulus was interacted with, shocks were delivered based on the following parameters either for the two minutes remaining, or until interaction ceased i.e.: the crab left the shelter. Shocks were delivered using the same parameters as Magee et al. (Magee and Elwood 2013) although an alternative pulse stimulator had to be used due lack of access to the exact model. Shock parameters were 10 V at 180 Hz for 200ms, delivered at five second intervals via an A-M Systems Isolated Pulse Stimulator 2100 AS IS. The colour of the aversive stimulus for each crab was designated prior to the trial start and remained constant to simplify learning, with equal numbers of trials for each crab on each colour, with 10 crabs for having one of the two colours as aversive stimulus (20 in total).

Crabs underwent trials back-to-back, with crabs returned to the acclimatisation chamber to recover for five minutes, while the used saltwater was replaced, the stimuli changed for the next set based on random selection of assigned numbers (one through eight, Figure 2) and placed on the predesignated sides. After 10 trials had been completed, the individual had the wire electrodes removed and was returned to its holding container and fed a high value food

item (two grams of bacon) before being returned to the holding tank, and the next crab was collected. The same crabs were used in both the black and white trials as the blue and green trials, after a ~two-week interval as the chromatic stimuli were prepared. Following the black and white trails, crabs were allocated which of the coloured stimuli would be aversive. This was done semi randomly – half the crabs with black as the aversive stimulus had blue as the aversive stimulus and the other half had green, with the same for those where white was the aversive stimulus. This was done to account for any learned biases from earlier trials, based on discriminability between black and white substrates. The chromatic trials followed the same method as the black-white trials.

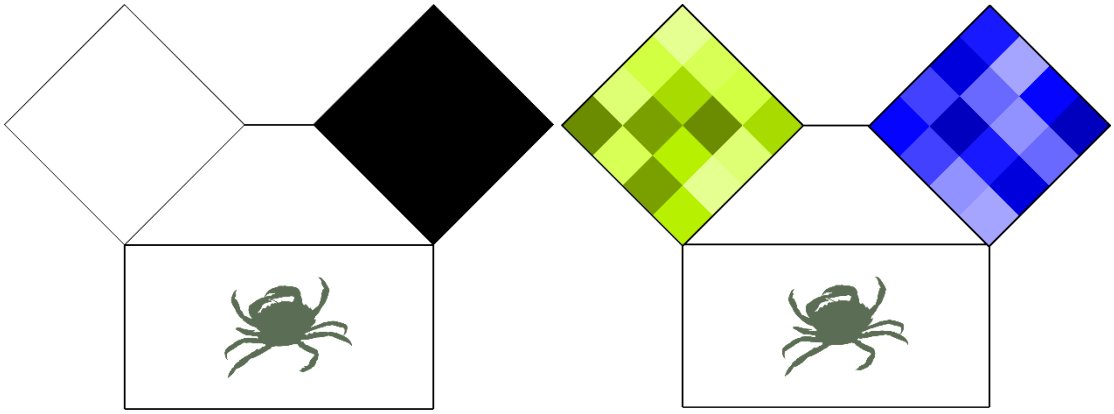


Figure 3: Diagrams of the experimental set up for aversive colour discrimination trials.
Examples correspond to black and white (left) and blue and green (right) stimuli. The divider directly in front of the crab was removed at the start of trial once acclimatisation had finished. The side each stimulus was presented on was randomised for each trail (ten per crab).

Statistical Analysis

Once trials were completed, data were simplified into four key variables – time to first choice, whether the previous choice was successful, whether the previously correct side was chosen, and whether the correct choice was made. This allowed for binomial test to test if stimulus selection deviated from random, and if so, whether individuals correctly chose to avoid the aversive stimulus. In the case of black versus white trials, this was done to determine the success of training methodology, while blue versus green trials were used to test for colour discrimination. Binomial tests were also used to investigate if substrate choice

in relation to stimuli position affected choice, as the results of Magee and Elwood's experiments indicate crabs may selected to avoid stimuli based on directional cues (Magee and Elwood 2013).

Chromatic Change Experiments

Sample Collection and Housing

As with the colour discrimination experiments, animals were collected from Penryn mudflats. This habitat was selected for three reasons. Firstly, the location was efficient for collection and same day return to the laboratory. Secondly, the mudflat has a large population of *C. maenas*, specifically subadults with the potential for maximum colour change, and likely dependency on camouflage to avoid predation. Finally, as previously stated, *C. maenas* exhibits strong phenotype environment associations, and given the habitats uniform appearance, collected crabs were equally uniform (Nokelainen et al. 2017; Price et al. 2019; Todd et al. 2006). This provided a starting sample with relatively low variation, increasing the clarity of any change that would occur. Crabs were preferentially selected for size, with average crab size ranging from 15mm to 20mm. This was done based on the abundance of individuals in this size range at times of sampling (smaller individuals would have been preferable), as well as larger individuals exhibiting slower colour change for a variety of factors (Crothers 1968; Nokelainen et al. 2019; Powell 1962a), in line with past *C. maenas* colour change experiments (Carter, Tregenza, and Stevens 2020; Stevens 2016; Stevens, Lown, and Wood 2014b).

Samples were run in two batches. The first occurred concurrently with initial blue-yellow appetitive stimulus training in the summer of 2019, with crabs on blue and yellow substrates and ran for 56 weeks. The second was run at the start of 2020, with crabs on red and green substrates, running for only 42 weeks due to lab closure in response to the COVID-19 pandemic. Crabs were housed in the same manner as those used in the colour discrimination trials, having been hand collected at the Penryn Mudflats, transported back to the Sensory Ecology lab, washed, assigned ID numbers, and acclimatised for a minimum of 48 hours in a 45cm by 120cm tank filled with freshly made saltwater, cycling through a filter and chiller to keep the temperature at 16°C, and salinity was maintained at ~31‰.

Digital Photography

At the start of the experiment, individuals were randomly allocated a substrate colour (numbers assigned based on their initial position in the holding tank, then randomly assigned to substrate colour (Figure 5), then photographed under standardised conditions to record the baseline that change was compared against. Containers were transported to the darkroom within the MS Lab. Crabs were removed from containers and gently cleaned with a toothbrush to remove any matter (algae growth for example) that could affect coloration. Colour change measures used similar methodology to multiple other colour change experiments both in this species (Carter, Tregenza, and Stevens 2020; Nokelainen et al. 2019; Stevens, Lown, and Wood 2014b) and other intertidal species (Duarte, Stevens, and Flores 2018; Green et al. 2019; Stevens, Lown, and Denton 2014). Individual crabs were then placed inside a PTFE ring that acted as a light diffuser to reduce shadows and carapace shine (Figure 4). A 93% and 7% reflective Spectralon photo standard with attached scale, as well as an identifying number for the specific crab were placed inside as well (see Figure 4). Illumination was provided by a single arc lamp (70 W, 6500K Iwasaki Colour Arc Lamp) with the ultraviolet (UV) filter removed to provide both human-visible (420-680nm) and ultraviolet (320-380nm) light.

Images were taken from directly above the sample using a Nikon D7000 Digital Camera, converted for full spectrum sensitivity by removing the UV and infrared (IR) filter (Advanced Camera Services Limited, Norfolk, UK). Human visible spectrum photos were obtained using a Baader UV–IR blocking filter (Baader Planetarium, Mammendorf, Germany), and UV photos were obtained using a Baader UV pass filter. UV and Visible spectrum photos were taken sequentially, ensuring the subject did not move between photos. All photos were taken in RAW format for maximum information. Digital image analysis was used as it has proven to be a suitable and efficient alternative to spectrometry (Stevens et al. 2007).

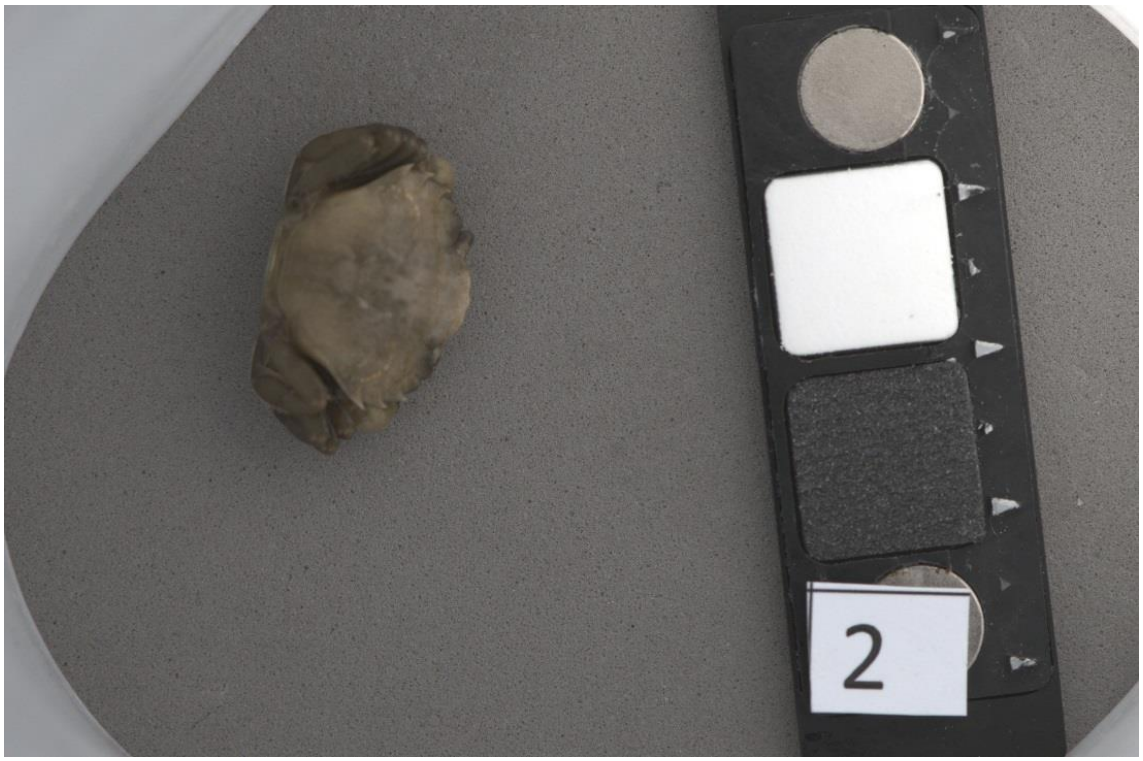


Figure 4: Example image to demonstrate digital photography set up. Includes crab on the left and photostandard with ID number on the right. The edge of the PTFE diffuser can be seen on the left-hand edge of the photo and the corners NB. This is a false colour image created in ImageJ from the linearised multispectral image.

Following initial photographing, individuals were then transferred to identical containers to those used in pre-experiment housing, but with the neutral substrates replaced with treatment substrates (Figure 5). These were course aquarium gravel, with an approximate grain size of 5mm to 7mm in four colours: blue, green, red, and yellow. These colours were selected based on the availability of substrates, as well as maximal chromatic difference (assessed based on human trichromatic vision) to ensure maximal colour change and potential effect sizes. Individuals were then returned to a tank with the same housing conditions as the pre-experiment tank. Individuals were housed for six to eight weeks (depending on experimental run) to allow time for a minimum of one moult cycle to ensure the opportunity for a colour change response. Over this period individuals were regularly photographed every two weeks using the above methodology, to record any trends in colour change behaviour over time. Gravel samples were also photographed under the same conditions at the end of the experiment. Photo data was simplified to the initial and end photographs

to allow for easier analysis of total colour change, and account for the disrupted schedule from the start of the COVID-19 pandemic. Ethical approval was obtained via successful application through the University of Exeter's e-ethics system, 2018 (Application number: eCORN001700 v2.1)

Image Analysis

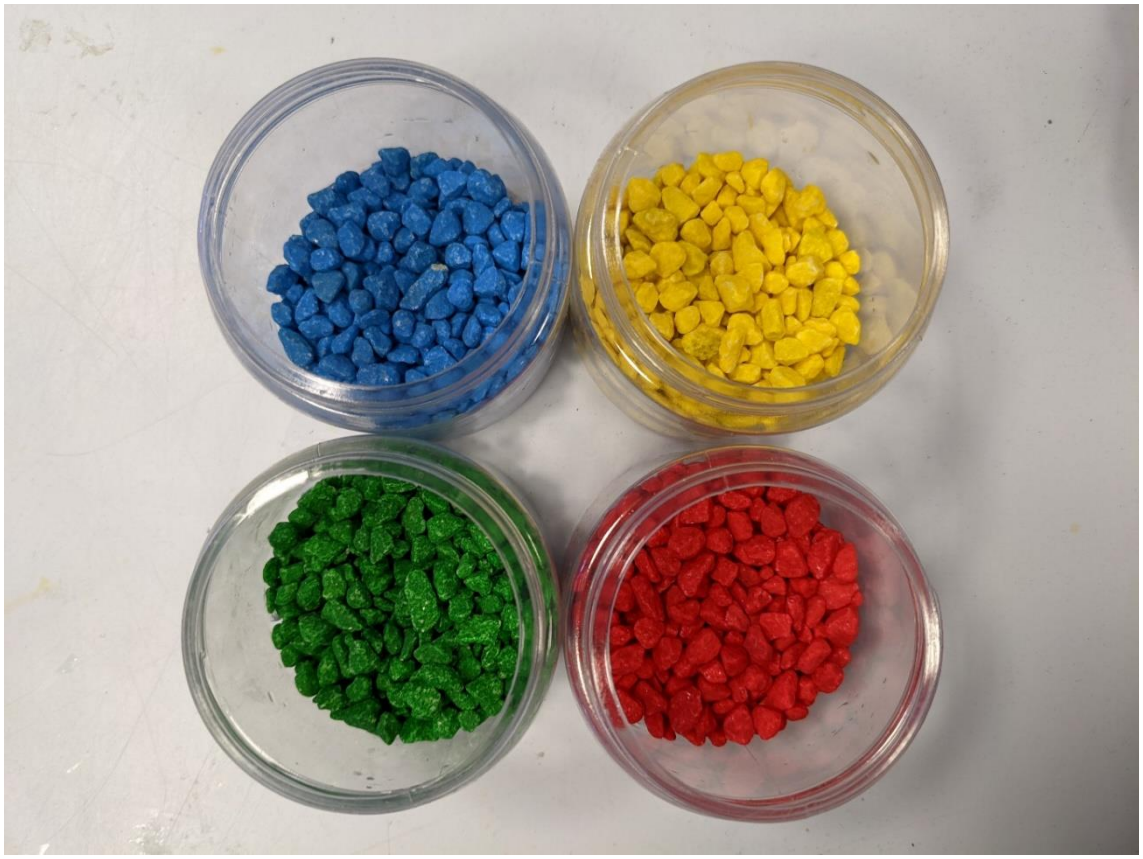


Figure 5: Examples of crab containers for holding samples for colour discrimination and change experiments.
Shows samples of gravel used in colour change experiments. Pots' diameters were 65mm. Screw top lids with an opening covered with 3mm plastic mesh were placed on top to contain crabs but allow light and cycled water to enter the containers.

The photos were imported into ImageJ software (Schneider, Rasband, and Eliceiri 2012), using the Multispectral Image Calibration and Analysis Toolbox (MICAToolbox, V2.2 (Troscianko and Stevens 2015)) to create linearised images due to nonlinear camera sensor responses to light combining the visible and ultraviolet photographs in a single multispectral image, that were also equalised using the photostandard to correct for difference in light conditions between photos (Stevens et al. 2007). Regions of interest (ROIs) were selected,

specifically the scale bar on the photo standard, a straight line (l) across the widest point (between the rear most anterolateral teeth) of the carapace for size measurement, and finally an area of the crab's carapace (specifically the area of the cephalothorax) for colour analysis. Images were then converted from camera colour space (sensitivity of camera setup detailed in ((Troscianko and Stevens 2015; Stevens, Lown, and Wood 2014b)) into relevant predator vision systems via a polynomial mapping function. Predator visual models used dichromatic (Pollack, *Pollachius* (Shand et al. 1988), possessing Longwave (LW) and Mediumwave (MW) sensitive photoreceptors) and trichromatic fish predators (Two Spot Goby, *Gobisculus flavescens* (Utne-Palm and Bowmaker 2006), possessing Longwave (LW), Mediumwave (MW), and Shortwave (SW) sensitive photoreceptors), as well as tetrachromatic avian predators (Common Peafowl, *Pavo cristatus* (Hart 2002) Longwave (LW), Mediumwave (MW), Shortwave (SW), and Very-Shortwave/UV (VSW) sensitive photoreceptors). These species were based on the availability of their spectral sensitivity information, as well as their being predators, or analogues of predators, of *C. maenas*. These include dichromatic fish e.g. *Gadus morhua* (cod), trichromatic rockpool fish e.g. *Gobius paganellus* (rock goby), and tetrachromatic avifauna, e.g. gulls, all of which have evidence of predation on *C. maenas* (Crothers 1968).

Once cone catch values for predators were generated, this data was used in the calculation of hue based on the ratio of cone-catch values of shortwave to longwave. Calculations used the formulae derived from Principle Component Analysis from past research, (Green et al. 2019) for trichromat vision, and (Stevens, Lown, and Wood 2014b) for tetrachromat vision. This identified the main axis of colour variation, and a logical colour channel for each visual model, although in the case of dichromats a simple ratio of short- to longwave was used as these were the only receptors present. The specific formulae for hue were SW/LW for dichromats, SW / MW + LW for trichromats, and SW + VSW (very shortwave or UV) / MW + LW for tetrachromats. Measures of difference between crab and substrate coloration to assess camouflage success relative to varying predator visual systems were also created using the inbuilt tool in the MICA toolbox (Troscianko and Stevens 2015). Vorobyev and Osorio's receptor-noise model for colour discrimination (Vorobyev and Osorio 1998) was used to

produce JND (Just Noticeable Differences) as units of the ability to discriminate between chromatic stimuli. JND values of 1 or less indicate that the stimuli are not discriminable from one another, but values of 3 or less are broadly indicative of reasonable matching. JND values were obtained from comparisons between crab carapace ROIs and photographs of the gravel used in the sample's treatment. General luminance (lightness measures based on predator vision, using the assumed/known luminance channel for each species), and luminance JND values to assess achromatic camouflage were also collected as, if *C. maenas* is monochromatic, achromatic differences and matching may be of greater importance.

Statistical Analysis

CSV data files of the raw spectral data, colour JND values, and luminance JND values calculated for each visual system were imported into R software (ver. 4.1.0 (R Development Core Team 2020), using the RStudio interface (RStudio Team 2020) and the tidyverse package collection for data organisation (Wickham et al. 2019)). Hue values were calculated in R, and data was combined into both longitudinal data of start and end colormetrics and short format for repeated measures of the same individual. Using the shortened dataset, a single measure of colour change was calculated based on the change in either hue, colour JND, or luminance JND from the initial measurement, compared to the measurement after eight weeks. This was done to simplify statistical measurements of overall change in response to substrates by removing repeated measures, while the longitudinal data was preserved to compare patterns of colour change over time between groups.

The total colour change data was tested for normality, then fitted to respective Paired Wilcoxon Signed Rank Tests (using the wilcox_test() function from rstatix, ver. 0.7.0 (Kassambara 2021)) for each of the change measurements (hue, colour JND, and luminance JND) to test for significant change for each substrate. Then the single change in colour values for each crab were fitted linear mixed effect models (used based on only minor deviance in residual fit, and non-transformability of data). For hue and colour JND, three separate models were run for each (the crab visual model was not used as monochromacy was assumed to only allow for luminance perception), one for each visual model's value of hue and colour JND. This was done as values

were calculated differently across visual models, based on the different number of photoreceptor sensitivities. These used the base R's lm() function (R Development Core Team 2020) as no random effect needed to be included as samples were not repeated across fixed effect. Fixed effects of colour and size were included, as well as the interaction effect between the two. for the Un-simplified model outputs can be found in Appendix 1C.

*Model: lm(Hue/Colour JND ~ Colour*Av.Size, na.action = na.omit, data = colchange.df[Visual Model])*

Given luminance and luminance JND were based on a single photoreceptor across visual models, vision was included as a fixed effect, using the lmer() function of the lme4 package (1.1.27.1 (Bates et al. 2015)), with fixed effects of substrate colour, average size of crab over the experiment, and visual model, as well as the interaction between substrate colour and crab size, and substrate colour and visual model. The interaction between substrate colour and size was included to test if crabs of differing sizes were better able to change brightness on different colours, while the interaction between substrate colour and vision was included to see if differing luminance perception A random effect of crab ID was included to account for the repeated use of samples between visual models.

*Model: lmer(Luminance/Luminance JND ~ Colour*Av.Size + Colour*Vision + (1|ID), na.action = na.omit, data = colchange.df)*

Results

Spectral sensitivity via optokinesis

From a sample pool of 54 individuals, 132 of a potential 162 trials (81.48%) resulted in a measured optokinetic response. An initial Chi-squared test found that there was a significant association between the stimulus colour and an optokinetic response occurring ($\chi^2 = 22.336$, $p < 0.0001$). Of the trials without a response, ten (6.17%) were blue, one (0.62%) was green, and the remaining 19 (11.73%) were red.

The chromatic contrast threshold (i.e.: the minimum difference in chromatic contrast between the coloured and black grids where optokinesis started) was consistently lower in blue and green trials in comparison to the red (Figure 6). Green trials had a median threshold of 0.207 (IQR =0.111-0.413) and blue

0.318 (IQR = 0.133-0.612), while red trials had a median threshold of 0.891 (IQR = 0.613- no response at all).

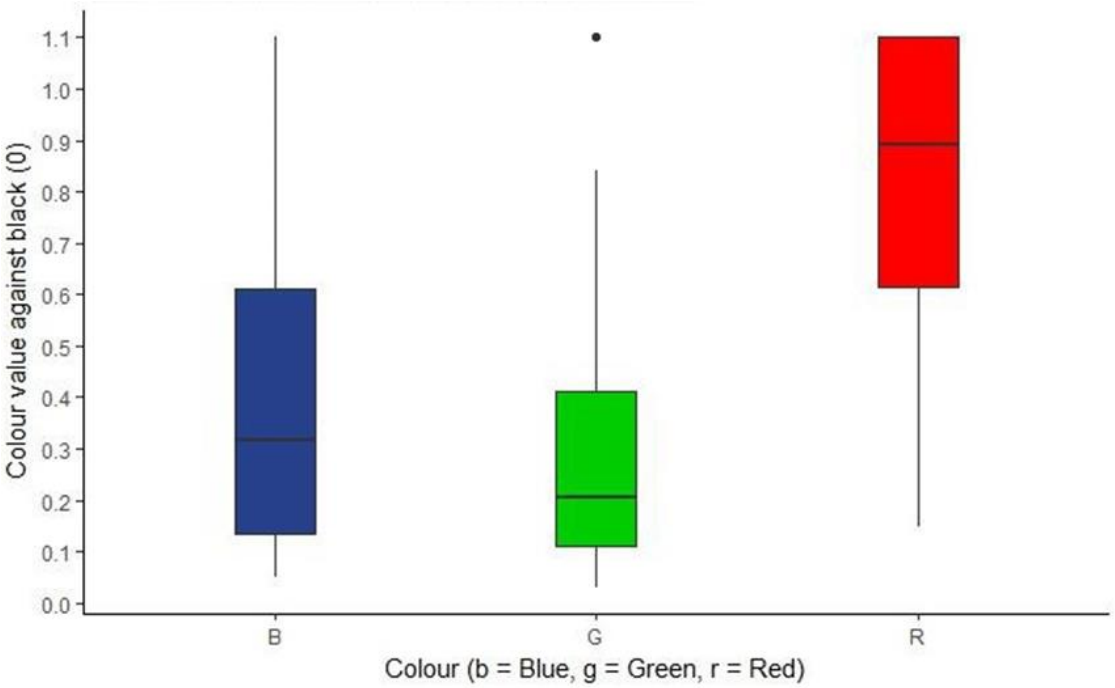


Figure 6: Boxplot showing the earliest point at which optokinesis occurred for each coloured stimulus in optomotor spectral sensitivity experiments.
Point in experiment measured in terms of chromatic contrast to black, on a scale of 0 - Black, to 1 - fully saturated colour. Values of 1.1 indicate that an optokinetic response did not occur.

Gain, the accuracy which the crabs tracked the stimulus, followed a similar pattern to the threshold, albeit on a reversed scale (Figure 7). Green (median = 0.349 IQR = 0.306-0.381) and blue (median = 0.356 IQR = 0.148-0.376) trials resulted in higher average gain than red (median = 0.309, IQR = 0.148-0.376).

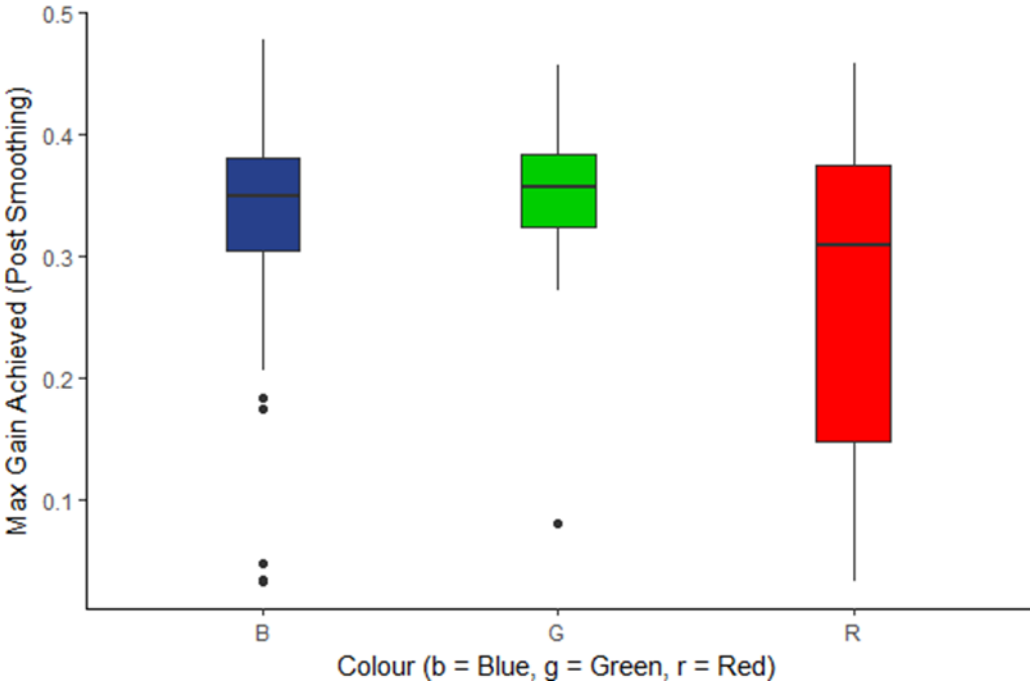


Figure 7: Boxplot showing the maximum gain (success of eye tracking) achieved for each coloured stimulus in optomotor spectral sensitivity experiments.
The data has been smoothed to remove non-saccadic eye movement. 0 = no movement, > 0 <1 = eye movement slower than the drum rotation, 1 = perfect eye tracking, > 1 = eye movement faster than the drum, < 0 = eye movement in the opposite direction to the drum.

Both chromatic contrast and smoothed gain were fitted to linear mixed effect models, despite threshold values' lack of normal distribution and non-transformability. This was done as it resulted in improved model fit (assessed based on AIC values and model homoscedasticity) over a generalised linear mixed effect model with a non-gaussian error structure. Gain data was squared to normalise residuals and improve model fit.

For the chromatic contrast threshold, model simplification removed all the fixed and interaction effects save drum colour. The minimum adequate model found the chromatic contrast threshold varied significantly with drum colour ($F_{2\ 106}$ = 54.248 p < 0.0001). Responses to green were significantly earlier than blue (estimate = -0.139 ± 0.047 t = --2.953 adjusted p = 0.004) and red (estimate = -0.478 ± 0.047 t = -10.127 adjusted p < 0.001), and responses to blue were significantly earlier then red (estimate = -0.338 ± 0.047 t = -7.174 adjusted p < 0.001). See Table 2 and Table 3 for model outputs.

Table 2: ANOVA statistics from the simplified model fitted to chromatic contrast when optokinesis started in optomotor spectral sensitivity experiments. Includes the remaining fixed effect of drum colour. A random effect of crab ID was included to account for repeated measures from individuals. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	**F value**	**DF**	**p**
Colour	54.248	106	< 0.001
Model: lmer(ChromCon ~ Colour + (1|CrabID)), na.action = na.omit, data = dfull)			

Table 3: Summary statistics from the simplified model fitted to chromatic contrast when optokinesis started in optomotor spectral sensitivity experiments. Includes the remaining fixed effect of Drum Colour. A random effect of crab ID was included to account for repeated measures from individuals. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	**Estimate**	**SE**	**t**	**p**
(Intercept)	0.436	0.0378	11.552	< 0.001
Colour = Green	- 0.139	0.0472	-2.953	0.00388
Colour = Red	0.338	0.0472	7.174	< 0.001
Model: lmer(ChromCon ~ Colour + (1|CrabID)), na.action = na.omit, data = dfull)				

The model for maximum gain achieved (squared to improve model fit) produced similar findings, with model simplification resulting in a minimum adequate model containing the fixed effect of drum colour. A significant association was found between drum colour and the maximum gain achieved ($F_{2,\ 106} = 12.681$, $p < 0.001$). Significant differences were found between green and red (estimate = - 0.0405 ± 0.00822, t = -4.930, adjusted p <0.001), and blue and red (estimate = - 0.0276 ± 0.00822, t = -3.355, adjusted p = 0.0011), but not between blue and green (estimate = 0.0129 ± 0.00822, t = 1.575, adjusted p = 0.118).

Table 4: ANOVA statistics from the simplified model of Maximum Gain (tracking efficiency) achieved in optomotor spectral sensitivity experiments. Includes the remaining fixed effect of Drum Colour. A random effect of crab ID was included to account for repeated measures from individuals. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	**F value**	**DF**	**p**
Colour	12.681	106	< 0.001
Model: lmer(MaxGain ~ Colour + (1|CrabID)), na.action = na.omit, data = dfull)			

Table 5: Summary statistics from the simplified model of Maximum Gain (tracking efficiency) achieved in optomotor spectral sensitivity experiments. Includes the remaining fixed effect of Drum Colour. A random effect of crab ID was included to account for repeated measures from individuals. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	**Estimate**	**SE**	**t**	**p**
(Intercept)	0.116	0.00723	16.060	< 0.001
Colour = Green	0.0129	0.00822	1.575	0.118
Colour = Red	- 0.0276	0.00822	- 3.355	0.0011
Model: lmer(MaxGain ~ Colour + (1|CrabID)), na.action = na.omit, data = dfull)				

Data was initially compared to Stavenga templates for invertebrate opsin sensitivity (Stavenga, Smits, and Hoenders 1993) fitted to the λ_{max} values from the electrophysiological measures by Bruno (Bruno, Mote, and Goldsmith 1973) (Figure 8A), and Martin and Mote (Martin and Mote 1982) (Figure 8B), with the assumption of the R8 rhabdomere sensitive to ultraviolet light with a peak absorbance at ~ 350nm (Warrant and Nilsson 2006). My data aligned well with the results (Figure 8C), especially the green measurements, although blue and red responses were higher than those predicted by the template.

To compare the results of my experiment more accurately, the data collected was fitted to their own template via nonlinear least-squares regression, using base R's nls() function (R Development Core Team 2020). This resulted in the curve in Figure 8C, with a best-fit λ_{max} of 496.22 ± 2.09 (t = 237.7 p < 0.001). Compared to previous electrophysiological data, my peak sensitivity is slightly lower than both the single receptor found by Bruno (502-506nm (Bruno, Mote, and Goldsmith 1973)) and the green sensitive receptor found by Martin and Mote (508nm (Martin and Mote 1982)), but still relatively similar to both. In summary, behavioural measures of spectral sensitivity correspond well with past electrophysiological work, with only a 6 – 12 nm difference between the peak sensitivity calculated from my data and those of past work.

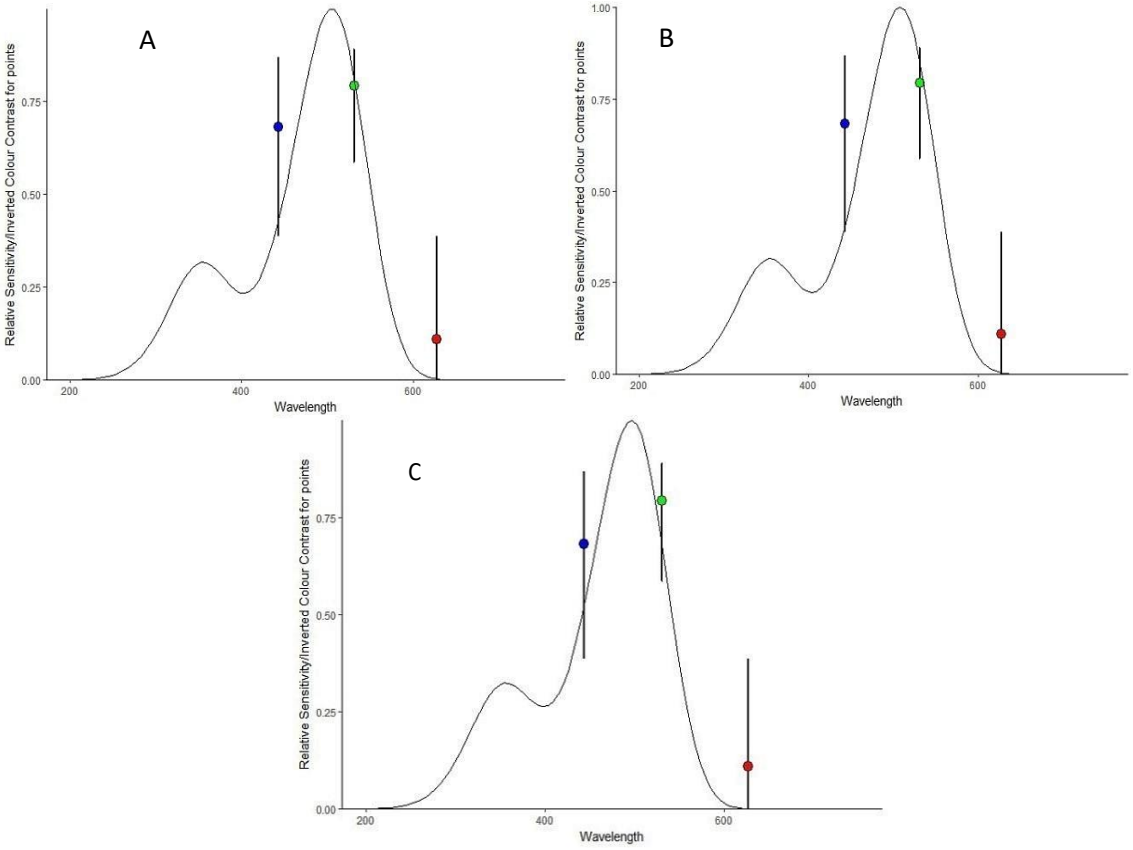


Figure 8: Spectral sensitivity curves of *C. maenas*, derived from optomotor spectral sensitivity data, and past electrophysiological measurements. Curves were produced using the template set out in Stavenga et al. (1993) using λ_{max} values from A: Bruno et al. (1973) = 505nm, B: Martin and Mote (1982) = 508nm, C = Nonlinear Least-Squares Regression based on the data collected from this study = 496.218 ± 2.088. Points on the graph correspond to the median sensitivity values (with IQR bars) obtained for each colour stimulus (point colour corresponds to stimulus colour).

Colour Discrimination

20 crabs were used in both achromatic and chromatic trials, allowing the difference in responses to be compared. Of the two rounds of ten trials for each crab (400 total), 368 trials had a decision made in them, while 32 did not. The 32 trials with no decision made were not used in analysis (unless otherwise stated, although included in Figure 9 as NA). Initial tests with black and white shelters found that when a choice was made, crabs preferentially selected the black shelter regardless of whether it was the aversive stimulus ($b.test_{234, 368}$ = 63.6%, $p < 0.001$). In trials where the black shelter was the aversive stimulus, this fell to 58.3% ($b.test_{105, 180}$, $p = 0.0304$), and rose to 68.6% ($b.test_{129, 188}$, $p < 0.001$) when it was not aversive.

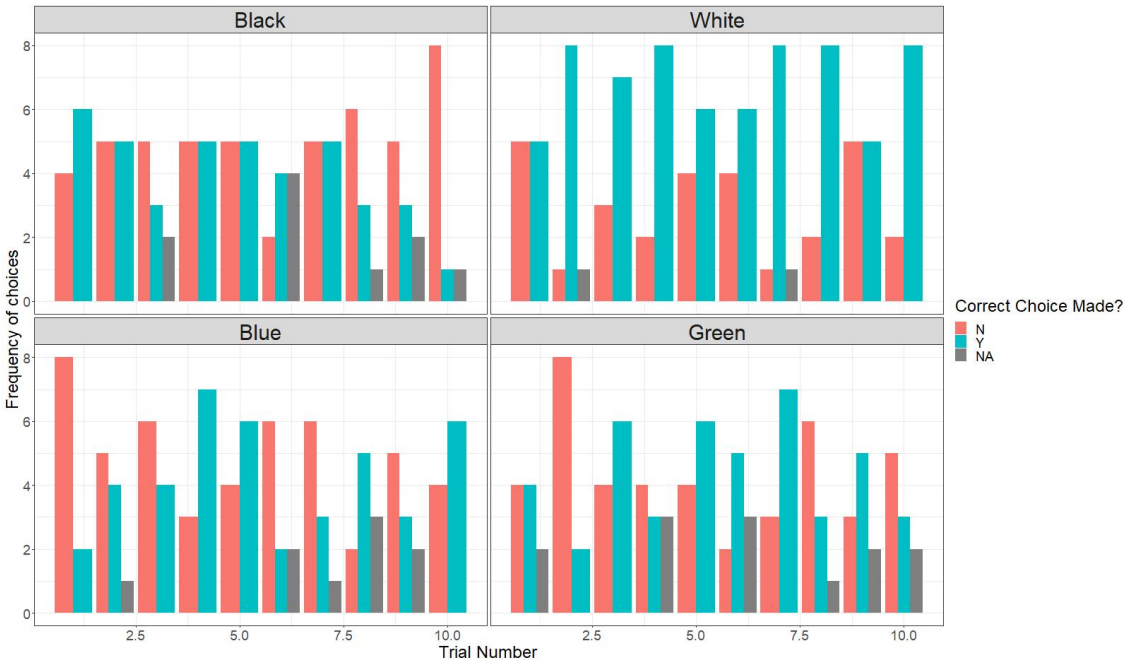


Figure 9: Decisions of crabs in behavioural trials of colour discrimination. Faceted by colour of aversive stimulus. NA indicates no choice was made during the trial period

Analysis of success used all trials where a decision was made minus the first, as crabs had prior no context for what conditions to avoid. Binomial tests of success versus failure (selecting the safe versus shock shelter) found no deviation from randomness when looking at the whole cohort's trials, (55.487% successful, $p = 0.0531$), as well as trials where black was aversive (42.5% successful, $p = 0.219$), but crabs reliably picked the correct stimulus when white was aversive (72.727% successful, $p < 0.001$).

Finally, there was no evidence that crabs selected based on the previously correct side, as expected given the randomisation of the side the aversive stimulus was on. For all trials, crabs chose the previously non-aversive/avoided the previously aversive in 48.809% of trials ($p = 0.817$), rising to 55% when black was the aversive stimulus ($p = 0.434$), and falling to 43.182% when white was the aversive stimulus ($p = 0.241$)

Transitioning to the blue-green trials, any deviation from random decision making was lost as proportions of correct choices fluctuated across trials (Figure 9). Crabs made correct choices 47.568% of the time ($p = 0.5565$), 46.154% when blue was aversive ($p = 0.5296$) and 48.936% when green was aversive ($p = 0.9179$). When checking for any colour bias in decision making, there was no significant difference in colour picked, with crabs choosing blue in 52.247% of trails ($p = 0.5999$). As with black-white trials, there was no evidence of selecting the previously correct colour; crabs selected the previously non-aversive side 50.602% of the time ($p = 0.9382$).

Chromatic Change

In the colour change experiments, 34 crabs survived to the final photographs. Crabs died of presumed natural causes during the experiment, usually found (anecdotally) midway through moulting. Divided amongst substrates this resulted in nine crabs on blue, six on green, 13 on red, and eight on yellow. Average change in colour measurements (hue, colour JND, luminance, and Luminance JND) with standard deviations are recorded in Table 14. Chromatic change was mixed across substrates, with increases in hue recorded on blue and yellow substrates to all visual models, as well as green to tetrachromats, while it decreased on red (Figure 10). Examples of chromatic change can be found in Appendix 1B.

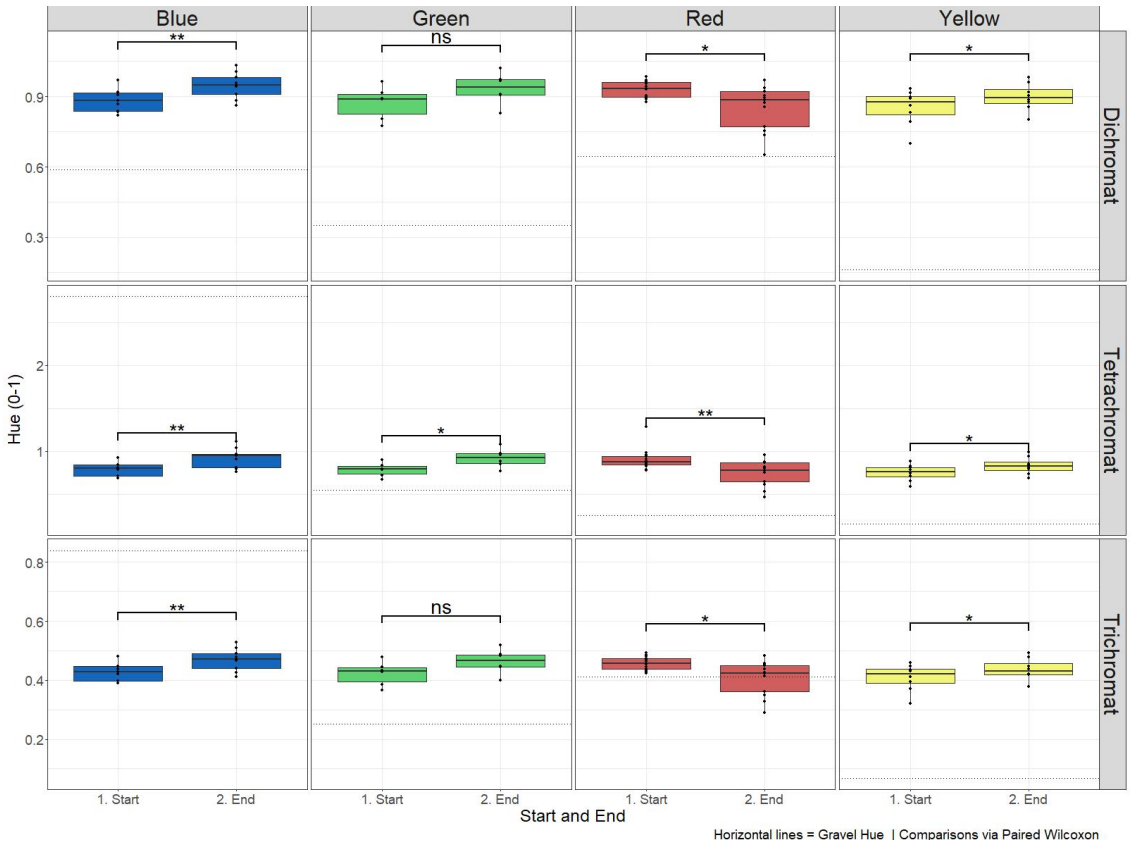


Figure 10: Hue change of crabs on coloured substrates in chromatic change experiments, for each visual model used.
Hue is classified as the ratio of short (and very-short in tetrachromatic) to medium and long wave reflectance. Significance values are derived from Paired Wilcoxon Signed Rank Tests (rstatix ver. 0.7.0 (Kassambara 2021)), and dotted horizontal lines are the hue values of the respective substrates. Y axis scales based on variation in hue respective to each visual model.

Pairwise Wilcoxon testing found significant change in hue on blue, red, and yellow substrates based on all visual models, and as on green substrates to tetrachromat vision. Hue increased (i.e. the ratio of shortwave to longer wave light increased) for crabs on blue (trichromat: + 0.0419, V_0 p = 0.004, tetrachromat: + 0.131, V_0 p = 0.004, dichromat: + 0.0632 V_0 p = 0.004), yellow substrates (trichromat: + 0.0278, V_2 p = 0.023, tetrachromat: + 0.0784, V_3 p = 0.039, dichromat: + 0.0 449, V_1 p = 0.016), and green for tetrachromat vision (+ 0.136, V_0 p = 0.031) but decreased on red in for all visual models (trichromat: -0.0497, V_{77} p = 0.027, tetrachromat: -0.157, V_{77} p < 0.01, dichromat: -0.0786, V_{77} p = 0.027).

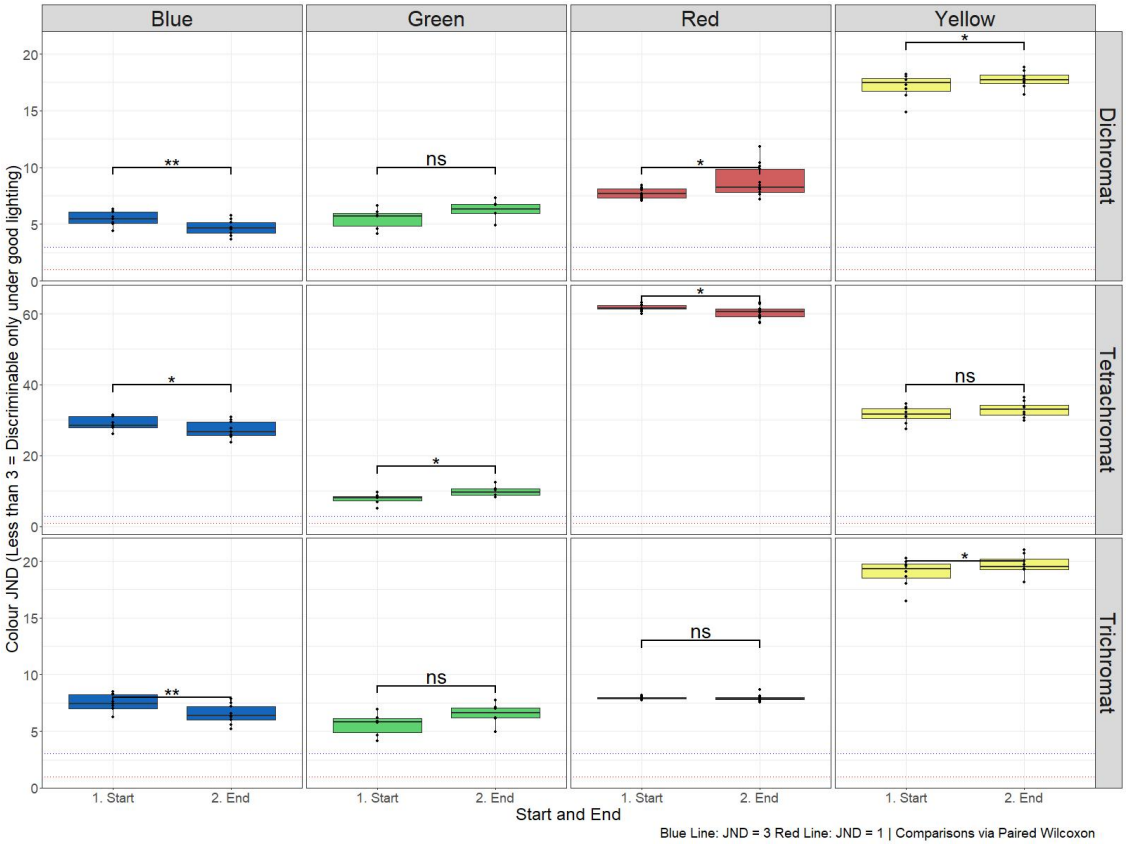


Figure 11: Change in colour JND (just noticeable difference) of crabs on coloured substrates in chromatic change experiments, for each visual model used.

Significance levels are derived from Paired Wilcoxon Signed Rank Tests (rstatix ver. 0.7.0 (Kassambara 2021)). Horizontal lines are blue: JND values of 3, below which crabs are considered discriminable from the substrates only under good lighting conditions, and red: JND value of 1, below the threshold crabs can be discriminated from their substrate. Y axis scales based on variation in colour JND respective to each visual model.

In terms of colour matching, similar patterns of absolute change were found (Figure 11), but these changes did not always result in improved colour matching. Statistically significant improvement (reduction in JND) was found on blue across all predator vision models (trichromat: - 1.000, V_{45} p = 0.004, tetrachromat: -1.791, V_{42} p = 0.02, dichromat: -0.792, V_{45} p =0.004), and on red to tetrachromat (-1.147, V_{78} p = 0.022). There was a statistically significant decrease in matching on yellow to trichromat (+0.727, V_2 p = 0.023) and dichromat vision (+ 1.077, V_1 p = 0.016), as well as on green to tetrachromat (+ 2.127, V_0 p = 0. 031) and red to dichromat (+ 0.612, V_{14} p = 0.027).

Simplification for the overall change in chromatic values reduced all full models (see methods) to just substrate colour (dichromat - Hue: $F_{3, 32} = 10.051$, $p < 0.001$, Colour JND: $F_{3, 32} = 6.798$, $p = 0.0011$, trichromat - Hue: $F_{3, 32} = 10.907$, $p < 0.001$, Colour JND: $F_{3, 32} = 16.79$, $p < 0.001$, tetrachromat - Hue: $F_{3, 32} = 13.774$, $p < 0.001$, Colour JND: $F_{3, 32} = 11.248$, $p < 0.001$,) (See Table 6, Table 7, Table 8, and Table 9).

For the hue model, Tukey post-hoc testing found significant differences in hue change for all visual systems between blue and red (dichromat: estimate = 0.142 $T_{32} = 4.605$ $p < 0.001$, trichromat: estimate = 0.0916 $T_{32} = 4.814$ $p < 0.001$, tetrachromat: estimate = 0.288 $T_{32} = 5.395$ $p < 0.001$), green and red (dichromat: estimate = 0.139 $T_{32} = 3.958$ $p = 0.0021$, trichromat: estimate = 0.0905 $T_{32} = 4.181$ $p = 0.0012$, tetrachromat: estimate = 0.294 $T_{32} = 4.829$ $p < 0.001$), and red and yellow substrates (dichromat : estimate = -0.123 $T_{32} = -3.868$ $p = 0.0027$, trichromat: estimate = -0.0775 $T_{32} = -3.929$ $p = 0.0023$, tetrachromat: estimate = -0.236 $T_{32} = -4.256$ $p < 0.001$).

In the Colour JND model, Tukey post-hoc testing found significant differences in Colour JND change between blue and green substrates (dichromat: estimate = -1.565 $T_{32} = -3.011$ $p = 0.0248$, trichromat: estimate = -1.956 $T_{32} = -6.117$ $p < 0.001$, tetrachromat: estimate = -3.917 $T_{32} = -4.574$ $p < 0.001$) and between blue and yellow for all visual models (dichromat: estimate = -1.404 $T_{32} = -2.931$ $p = 0.0300$, trichromat: estimate = -1.728 $T_{32} = -5.816$ $p < 0.001$, tetrachromat estimate = -3.256 $T_{32} = -4.124$ $p = 0.0013$),), between blue and red for dichromats (estimate = -1.869 $T_{32} = -4.371$ $p < 0.001$) and trichromats (estimate = -1.000 $T_{32} = -3.802$ $p = 0.0033$, between green and red for trichromats (estimate = 0.956 $T_{32} = 3.193$ $p = 0.0159$) and tetrachromats (estimate = 3.274 $T_{32} = 4.082$ $p = 0.0015$), and between red and yellow for tetrachromats (estimate = -2.613 $T_{32} = -3.578$ $p = 0.0059$).

Table 6: ANOVA statistics from simplified models for change in hue of crabs in chromatic change experiments, for each predator visual model.
Includes the remaining fixed effect of substrate colour Included is R software code ((using the lm() function in base R). Model Simplification via AIC comparison.

Vision Model	Effect	F value	DF	p
Dichromat	**Colour**	10.051	3	< 0.001
Trichromat	**Colour**	10.907	3	< 0.001
Tetrachromat	**Colour**	13.774	3	< 0.001

Model: lm (HueChange ~ Colour, na.action = na.omit, data = colchange.df [Visual Model])

Table 7: Summary statistics from simplified models for change in hue of crabs in chromatic change experiments, for each predator visual model.
Includes the remaining fixed effect of substrate colour, and R software code (using the lm() function in base R). Model Simplification via AIC comparison.

Vision Model	Source	Estimate	SE	t	p
Dichromat	(Intercept)	0.0632	0.0237	2.671	0.0118
	Green	-0.00305	0.0374	-0.084	0.936
	Red	-0.142	0.0308	-4.602	< 0.001
	Yellow	-0.0184	0.0345	0.532	0.5984
Trichromat	(Intercept)	0.0419	0.0146	2.864	0.00732
	Green	-0.00107	0.0231	-0.046	0.964
	Red	-0.0916	0.0190	-4.814	< 0.001
	Yellow	-0.0141	0.0213	-0.663	0.512
Tetrachromat	(Intercept)	0.131	0.0411	3.191	0.00317
	Green	0.00543	0.0649	0.084	0.934
	Red	-0.288	0.0534	-5.395	< 0.001
	Yellow	-0.0526	0.0598	-0.879	0.386

Model: lm (HueChange~ Colour, na.action = na.omit, data = colchange.df [Visual Model])

Table 8: ANOVA statistics from simplified models for change in colour JND of crabs in chromatic change experiments, for each predator visual model. Includes the remaining fixed effect of substrate colour, and R software code (using the lm() function in base R). Model Simplification via AIC comparison.

Vision Model	Effect	F value	DF	p
Dichromat	**Colour**	6.798	3	0.00113
Trichromat	**Colour**	16.79	3	< 0.0001
Tetrachromat	**Colour**	11.248	3	< 0.0001

Model: lm (Col.JND.Change ~ Colour, na.action = na.omit, data = colchange.df [Visual Model])

Table 9: Summary statistics from simplified models for change in colour JND of crabs in chromatic change experiments, for each predator visual model. Includes the remaining fixed effect of substrate colour, and R software code (using the lm() function in base R). Model Simplification via AIC comparison.

Vision Model	Source	Estimate	SE	t	p
Dichromat	(Intercept)	-0.792	0.329	-2.410	0.0219
	Green	1.565	0.520	3.011	0.00505
	Red	1.869	0.428	4.371	< 0.001
	Yellow	1.404	0.479	2.931	0.00620
Trichromat	(Intercept)	-1.001	0.202	-4.947	< 0.001
	Green	1.956	0.320	6.117	< 0.001
	Red	1.000	0.263	3.802	< 0.001
	Yellow	1.728	0.295	5.861	< 0.001
Tetrachromat	(Intercept)	-1.791	0.542	-3.306	0.00234
	Green	3.917	0.856	4.574	< 0.001
	Red	0.643	0.705	0.913	0.368
	Yellow	3.256	0.790	4.124	< 0.001

Model: lm(HueChange~ Colour, na.action = na.omit, data = colchange.df [Visual Model])

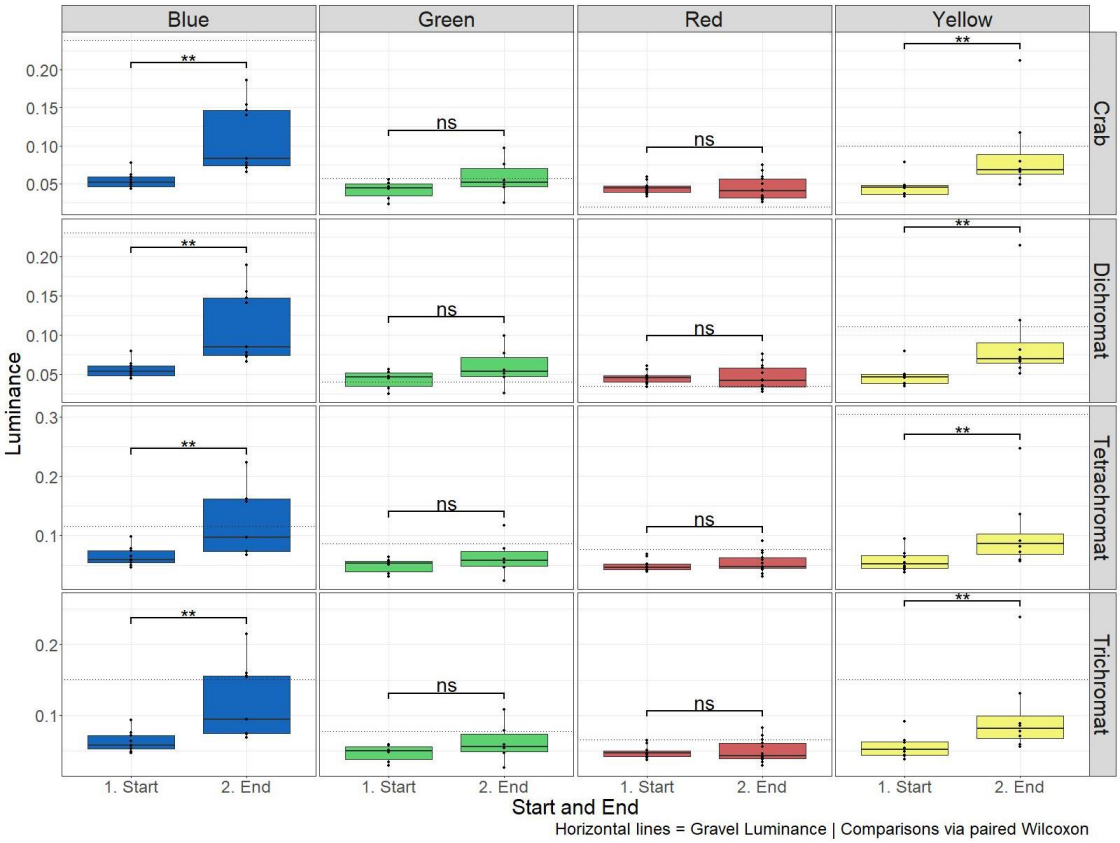


Figure 12: Change in luminance of crabs on coloured substrates in chromatic change experiments, for each visual model used.

Luminance channels derived from single photoreceptor channel for crabs, the longwave channel for dichromat, the medium wave channel for trichromat, and the double cone cells for tetrachromat. Significance values derived from Paired Wilcoxon Signed Rank tests (rstatix ver. 0.7.0 (Kassambara 2021)). Dotted horizontal lines represent the luminance values of the gravel substrates. Y axis scales based on variation in luminance respective to each visual model.

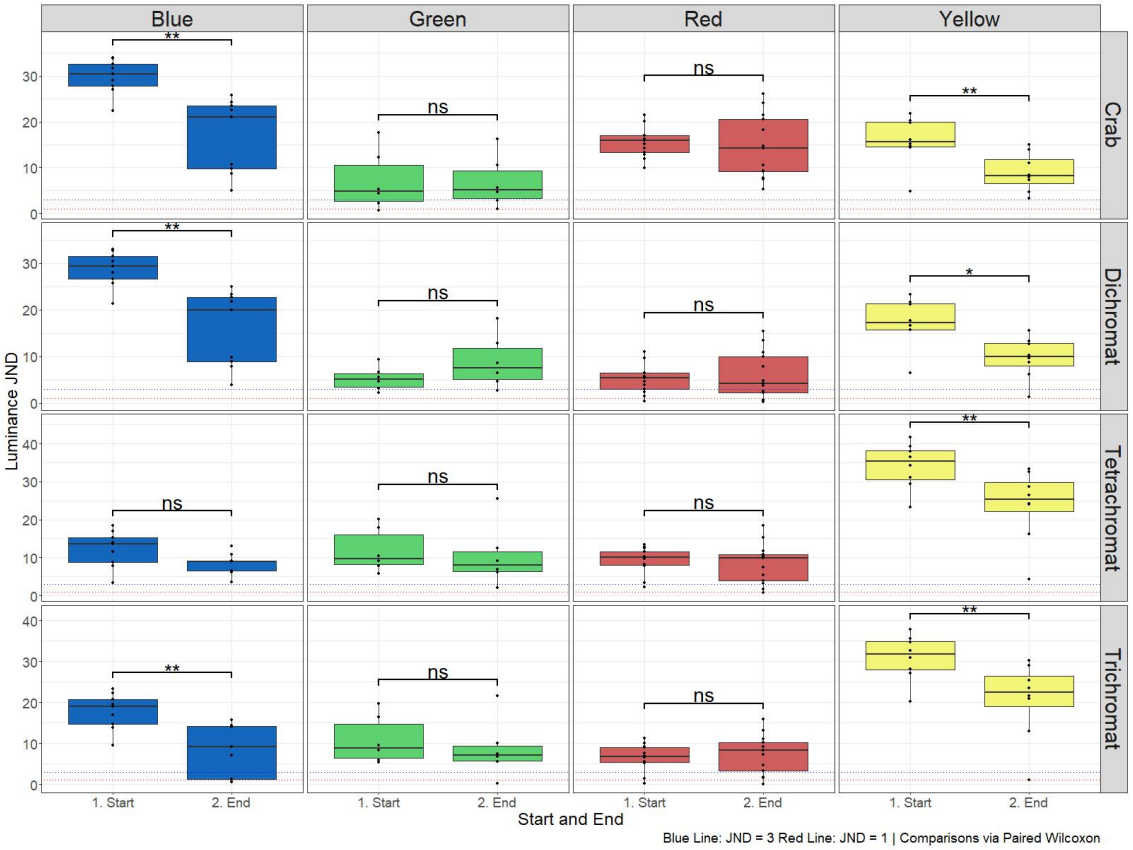


Figure 13: Change in luminance JND values of crabs on coloured substrates in chromatic change experiments, for each visual model used.
Significance levels are derived from Paired Wilcoxon Signed Rank Tests (rstatix ver. 0.7.0 (Kassambara 2021)). Horizontal lines are blue: JND values of 3, below which crabs are considered discriminable from the substrates only under good lighting conditions, and red: JND value of 1, below the threshold crabs can be discriminated from their substrate. Y axis based on variation in luminance JND respective to each visual model.

With regards to luminance, it increased (crabs became brighter) on blue, green, and yellow substrates for all visual systems, and remained the same on red substrates (Figure 12). Significant change was only found on blue and yellow substrates however, with Wilcoxon testing finding significant increases across all visual models on both blue (Crab: + 0.0563, V_0 p = 0.004, trichromat: + 0.0555, V_0 p = 0.004, tetrachromat: + 0.0563, V_1 p = 0.008, dichromat: + 0.0562 V_0 p = 0.004) and yellow substrates (Crab: + 0.0430, V_0 p = 0.008, trichromat + 0.0450, : V_0 p = 0.008, tetrachromat: + 0.0469, V_1 p = 0.008, dichromat: +0.0431, V_0 p = 0.008).

While luminance matching improved (reduced JND) on blue and yellow substrates, it decreased (non-significantly) or at least remained the same on red and green (Figure 13) . All visual models except tetrachromat recorded a significant improvement on blue substrates (Crab: - 13.041, V_{45} p = 0.004, trichromat: - 9.185, V_{45} p = 0.004, dichromat: - -12.763, V_{45} p = 0.004) while all visual models recorded a significant improvement on yellow substrates (Crab: - 6.956, V_{36} p = 0.008, trichromat: - 10.281, V_{36} p = 0.008, dichromat: - 7.562, V_{36} p = 0.008, tetrachromat: -10.438, V_{36} p = 0.008). Crabs on blue substrates also underwent a noticeable increase in matching for the tetrachromat visual model, but this was not found to be statistically significant (- 3.981). A noticeable decrease in matching was found in crabs on green substrates to dichromat vision (+ 3.667), but not to a statistically significant degree.

The luminance model simplified to just substrate colour ($F_{3, 32}$ = 3.8704, p = 0.0181, Table 10 and Table 11), with Tukey post-hoc tests finding significant differences between only blue and red substrates (estimate = 0.0543 T_{32} = 3.085 p = 0.0207). The luminance JND model also simplified to just substrate colour ($F_{3, 32}$ = 12.343, p < 0.001, Table 12 and Table 13). Tukey post-hoc testing found significant differences between blue and green (estimate = -9.6516, T_{32} = -4.019 p = 0.0018), blue and red (estimate = -9.6851, T_{32} = -4.901 p < 0.001), green and yellow (estimate = 8.7185, T_{32} = 3.543 p = 0.0065), and red and yellow substrates (estimate = 8.8521, T_{32} = 4.274 p < 0.001).

Table 10: ANOVA statistics from the simplified model fitted to change in luminance of crabs in chromatic change experiments.

Includes the remaining fixed effect of substrate colour, and a random effect of crab ID was included to account for repeated measures from individuals. Also included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	**F value**	**DF**	**p**
Colour	3.870	32	0.0181
Model: lmer(LumChange ~ Colour + (1|ID), na.action = remove, data = colchange.df)			

Table 11: Summary statistics from the simplified fitted to change in luminance of crabs in chromatic change experiments.

Includes the remaining fixed effect of substrate colour, and a random effect of crab ID was included to account for repeated measures from individuals. Also included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	Estimate	SE	t	p
(Intercept)	0.0561	0.0135	4.144	< 0.001
Colour = Green	-0.0406	0.0214	-1.898	0.0668
Colour = Red	-0.0543	0.0176	-3.085	0.0207
Colour = Yellow	-0.0116	0.0197	-0.587	0.562
Model: lmer(LumChange~ Colour + (1|ID), na.action = remove, data = colchange.df)				

Table 12: ANOVA statistics from the simplified model fitted to change in Luminance JND of crabs in chromatic change experiments.

Includes the remaining fixed effect of substrate colour, and a random effect of crab ID was included to account for repeated measures from individuals. Also included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	F value	DF	p
Colour	12.343	32	< 0.001
Model: lmer(LumJNDChange ~ Colour + (1|ID), na.action = remove, data = colchange.df)			

Table 13: Summary statistics from the simplified model fitted to change in Luminance JND of crabs in chromatic change experiments.
Includes the remaining fixed effect of substrate colour, and a random effect of crab ID was included to account for repeated measures from individuals. Also included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	Estimate	SE	t	p
(Intercept)	-9.742	1.519	-6.414	< 0.001
Colour = Green	9.652	2.402	4.019	0.0018
Colour = Red	9.685	1.976	4.901	< 0.001
Colour = Yellow	0.933	2.214	0.421	0.676
Model: lmer(LumJNDChange ~ Colour + (1|ID), na.action = remove, data = colchange.df)				

Overall, significant chromatic change only occurred on the blue and red substrates, although not to a degree to have a noticeable effect on matching. Predator visual systems with a greater number of different photoreceptor sensitivities were better able to discriminate between crabs and substrate based on chromatic differences (higher JND values, Figure 10 , Figure 11, and Table 14). Far greater change was seen in luminance values, with significant improvement in matching on both the blue and yellow substrates. Discrimination by predators (and assumed monochromatic crabs) was relatively similar based on luminance differences (a similar reduction in JND values, Figure 12, Figure 13, and Table 14).

Table 14: Mean luminance and colour change of crabs on substrates in chromatic change experiments, with std. deviation, based on visual models for relevant species.
Colour metrics only use dichromat, trichromat, and tetrachromat visual information due to polychromacy/ability to discriminate colour. Luminance metrics include crab vision. Luminance based on cone catch values of the luminance channels where the minimum of 0 means no stimulation of photoreceptors, and maximum of 1 means full stimulation. Hue values derived from the ratio of short wave (and UV wave in tetrachromat) cone catch values to medium and long wave cone catch values. Green highlights indicate significant increase, red indicate significant decrease, based on Paired Wilcoxon Signed Rank Tests (rstatix ver. 0.7.0 (Kassambara 2021)).

Vision	Colour	Mean Luminance Change	Luminance SD	Mean Luminance JND Change	Luminance JND Change SD	Mean Hue Change	Hue Change Std. Dev	Mean Colour JND Change	Colour JND Change SD
Crab	Blue	0.0563	0.0434	-13.041	7.442	*NA*	*NA*	*NA*	*NA*
Crab	Green	0.0161	0.0355	-0.255	9.189	*NA*	*NA*	*NA*	*NA*
Crab	Red	-0.000161	0.0196	-0.984	8.726	*NA*	*NA*	*NA*	*NA*
Crab	Yellow	0.0430	0.0540	-6.956	2.925	*NA*	*NA*	*NA*	*NA*
Trichromat	Blue	0.0555	0.0488	-9.1847	5.846	0.0419	0.0272	-1.001	0.623
Trichromat	Green	0.0155	0.0385	-2.293	10.910	0.0408	0.0236	0.956	0.563
Trichromat	Red	0.0022	0.0196	0.755	6.929	-0.0497	0.0614	-0.0004	0.285
Trichromat	Yellow	0.0450	0.0606	-10.281	9.504	0.0278	0.0330	0.727	0.934
Tetrachromat	Blue	0.0563	0.0510	-3.981	4.335	0.131	0.0865	-1.791	1.579
Tetrachromat	Green	0.0142	0.0419	-1.481	11.855	0.136	0.0653	2.127	0.909
Tetrachromat	Red	0.0049	0.0204	-0.828	7.057	-0.157	0.170	-1.147	1.440
Tetrachromat	Yellow	0.0469	0.0626	-10.438	9.470	0.0784	0.0906	1.466	2.253
Dichromat	Blue	0.0562	0.0439	-12.763	7.398	0.0632	0.0398	-0.792	0.485
Dichromat	Green	0.0161	0.0358	3.667	4.857	0.0602	0.0384	0.773	0.499
Dichromat	Red	< 0.0001	0.0194	0.829	6.468	-0.0786	0.102	1.077	1.420
Dichromat	Yellow	0.0431	0.0547	-7.562	1.880	0.0449	0.0497	0.612	0.736

Discussion

Optokinetic experiments of behavioural sensitivity indicate that *C. maenas* has a single photoreceptor class, with a peak sensitivity in the green-blue, or mediumwave, range of the spectrum. The Stavenga template, a generally accepted model for absorbance, fits a peak sensitivity of λ_{max} at 496.12nm. This aligned well with past electrophysiological experiments (Bruno, Mote, and Goldsmith 1973; Martin and Mote 1982), albeit slightly shifted towards the shortwave. Monochromacy was further supported by a lack of any evidence of the ability to discriminate between colours. There was evidence of selection based on visible information when stimuli were black and white, with a preference for black which reduced when it became the aversive stimulus. Any selection based on appearance was lost when the stimuli changed to isoluminant blue and green patterns. Finally, there was some statistically significant chromatic change in coloration, but most improvement was achromatic, especially on blue and yellow substrates. While there were some differences in the chromatic results between visual models, luminance changes (both in general and specific to matching) were notably similar across visual systems. When colour change data were analysed using models of based on *C. maenas* vision, crabs responded most to substrates they least matched based on luminance cues and only when substrates were brighter than themselves.

First, the results of the optokinetic experiments are as expected. Despite the issue of optomotor/optokinetic responses potentially being monochromatic (as motion vision has been found to be monochromatic in various species, such as goldfish (Schaerer and Neumeyer 1996) and *Drosophila* (Yamaguchi et al. 2008)), the behavioural measures aligned well with past electrophysiological data. While the methods could potentially have missed a second photoreceptor type if motion vision is monochromatic in *C. maenas*, the previous studies found the mediumwave photoreceptor to be at least the dominant type (Bruno, Mote, and Goldsmith 1973; Martin and Mote 1982). With regards to the latter, Martin and Mote did find a single *C. maenas* photoreceptor with a significantly lower λ_{max} in the shortwave (Martin and Mote 1982), this was a single sample in a study in combination with another species (*Callinectes sapidus*). Regardless, *C. maenas* spectral sensitivity seems dominated by a photoreceptor type maximally sensitive at ~490-520nm. As stated previously, dichromacy cannot

be discounted given the common shortwave/UV sensitive R8 rhabdomere in crustaceans (Warrant and Nilsson 2006) (e.g. in fiddler crabs (Rajkumar et al. 2010), and stomatopods (Marshall, Kent, and Cronin 1999; Marshall, Cronin, and Frank 2003)), but based solely on the results of these experiments *C. maenas* appears functionally monochromatic.

Correspondingly, the discrimination experiments showed no evidence of selection based on chromatic information. While there is evidence for colour vision in crustaceans (in other crab species (Detto 2007; Baldwin and Johnsen 2012) and in more distantly related species (Daly et al. 2017; Marshall, Jones, and Cronin 1996)), monochromacy is still present in crustaceans (see (Marshall, Kent, and Cronin 1999)). There is the possibility that training was not successful, and crabs failed to reliably differentiate between stimuli not because they lacked the capacity to discriminate the chromatic differences, but because the association between the stimulus and outcome had not adequately been formed. The preference displayed on black could simply be due to a preference for dark refuges as an antipredator strategy (seen in *C. maenas* (Wale, Simpson, and Radford 2013) and other crustaceans (Guerra-Bobo and Brough 2011; Rossong et al. 2011)) or a stress response given the aversive stimulus. Given crabs that received a shock when interacting with the black stimulus chose it ~10% less often than those that did not, this indicates some discrimination is present, although the increased selection of black in the final trial weakens this (Figure 9). When compared to random choices displayed by crabs on both chromatic stimuli, this implies that discrimination is not occurring, most likely due to the loss of reliable cues. Assuming *C. maenas* is dichromatic with a second shortwave sensitive photoreceptor type as found in (Martin and Mote 1982), stimuli should have been discriminable if colour vision is present given the presumed increased shortwave reflectance of the blue stimulus versus the green, and the preference for green over blue (assuming perception as a darker stimuli) should be present. Ultimately my results are not definitive but are at least indicative of a lack of colour discrimination. Molecular examination of opsin expression (as seen in (Rajkumar et al. 2010)) may be the most effective assessment of monochromacy versus dichromacy, and ultimately colour discrimination, given the lack of behavioural responses to colour.

Considering the requirements of *C. maenas* vision outside of potential background matching, it is unsurprising that colour vision is not apparent. Assuming monochromacy, or at least a dominant sensitivity in the mediumwave region, then the position of peak sensitivity aligns well with useful wavelengths in the environment. *C. maenas* is a predominantly aquatic species, and they will be subject to the effects of light transmission through water, even at shallow depths during sub adult stages. Penetration is greatest in the upper-mediumwave region of the visible spectrum, and slightly lower in the open ocean (Clarke and James 1939). Additionally, during twilight and low light level periods, the maximum downwelling irradiance almost exactly aligns with peak sensitivity of *C. maenas*, between 490nm and 520nm (Forward, Cronin, and Douglass 1988), especially in shallower depths. This means that *C. maenas* can take maximal advantage of the most abundant wavelengths in their environment, during periods of higher activity (Almeida, Flores, and Queiroga 2008; Naylor 1958). By focusing sensitivity to this region of the spectrum, *C. maenas* specialises to a reliable source of light information, without the fluctuation of other wavelengths. *C. maenas* is also predominantly nocturnal, active at low light levels. This necessitates maximising the efficiency of vision given the limited amount of light. Theoretical studies imply that monochromacy may allow for maximum absolute sensitivity (Van Hateren 1993; Osorio and Vorobyev 2005). Overall sensitivity is directly dependent on the number and size of photoreceptors, and the size of the eye (Land and Nilsson 2012). To achieve at least traditional colour discrimination, the total number of photoreceptors capable of being housed in one eye must be split between two or more photoreceptor types, also dividing the maximum sensitivity over those types. In vertebrates with multiple receptor-sensitivities, photoreceptor channels are pooled for absolute sensitivity, however in apposition eyes this may be less common (Land and Nilsson 2012) (although not unheard of (Warrant, Porombka, and Kirchner 1996)). If possessing multiple photoreceptor sensitivities means a compromise in overall sensitivity to a key range of wavelengths, by adopting monochromacy crabs can maximise sensitivity during period where activity is highest and available light is lowest.

With regards to other aspects of their ecology, they are omnivorous scavengers and opportunistic predators of other marine invertebrates (Crothers 1968; Neal

and Pizzolla 2008; Rangeley and Thomas 1987; Shelton and Mackie 1971) generally searching for food via chemosensory cues (Shelton and Mackie 1971). This is evidenced by the impact of flow rate on foraging efficiency (Robinson, Smee, and Trussell 2011) (as well as the lack of visual association in early behavioural trials). It is therefore unlikely that they are using vision in foraging, especially at distance, and given the breadth of their diet it is unlikely that colour discrimination is needed in the identification of food resources. In these experiments, initial attempts at colour discrimination trials involved unsuccessful appetitive stimulus training, using a food reward present in the tank, with the aim of forming an association with the colour presented. While there was some evidence of selection associated with the food item, it only lasted if the food reward was presented with the stimulus. Upon the removal of the stimulus the decision making reverted to random, which while not definitive at least implies that vision is not a key cue for foraging.

Finally, an alternative use of vision in *C. maenas* is in predator detection and responses. This is most likely in predators that are out of the water prior to attacks, such as sea birds (Crothers 1968; Dumas and Witman 1993) where other cues may not be transmitted. However simple flight responses to looming stimuli would not require colour vision, especially as predators will often be backlit by the sun, with the silhouetted predator highly contrasting the background in terms of luminance (or polarization, found in *C. maenas* (Shaw 1966) and associated with flight responses in other crab species (How et al. 2015; Smithers, Roberts, and How 2019)). Another aspect of this is the location of and hiding in for refuges (Wale, Simpson, and Radford 2013). This is unlikely to be dependent on colour vision, simple intensity cues are likely to guide these behaviours (as seen in the preference for darker stimuli in initial behavioural choice trials in this experiment), as dark regions as will be a cue of these sheltered regions.

Following the apparent lack of colour vision, only significant change in colour with regards to matching was achromatic (brightness), fitting well with past research on the species (Carter, Tregenza, and Stevens 2020; Stevens 2016; Stevens, Lown, and Wood 2014b). With regards to wider crustacean colour change, restriction to mostly brightness change is also found in other crab species (e.g. (Stevens, Rong, and Todd 2013) and (Hemmi et al. 2006)), as well

as in prawns occupying a similar niche (Siegenthaler et al. 2018). Other marine crustaceans do exhibit significant chromatic change (e.g. kelp-dwelling crabs (Hultgren and Stachowicz 2008), as well as caridean shrimp (Green et al. 2019; Keeble, Gamble, and Hickson 1900; Duarte, Stevens, and Flores 2018) and isopods (Lee 1966) both changing from red to green). These chromatic responses often seem dependent on grazing substrate to achieve matching (although ontogenetic factors may impact isopod change (Hultgren and Mittelstaedt 2015)), which as mentioned could be an alternative/supplementary cue for change. Diet could affect colour change not just as a sensory cue, but also in terms of materials needed for any changes. Crabs were all fed a specific diet, unlikely to match their broad omnivorous diet in the wild (Grosholz and Ruiz 1996; Le Roux, Branch, and Joska 1990; Rangeley and Thomas 1987). It could be that key nutrients needed for noticeable chromatic change were absent from the diet, although the commercial crustacean food used is marketed based on nutrients to promote coloration. In addition, a lack of meaningful chromatic change was seen on all substrates, but significant achromatic change was recorded (reducing mismatch) so the diet provided was at least sufficient for that. Alternatively, stress may have an impact on chromatic change (detailed further in Chapter 5), but as with diet, significant achromatic change still occurred. Achromatic changes were similar across all visual models used. In comparison, chromatic changes, both the degree of change, and the direction overall change between substrate colours varied between visual models. This is to be expected however, as the respective sensitivities of each visual model will affect the quantified measures of colour.

Interestingly, despite the lack of apparent colour vision, there was a “correct” response on both blue and red substrates, where hue (a measure of the ratio of short versus longer wavelengths, loosely corresponding to blue and red) increased on blue and decreased on red. This matched the direction of the hue values of the gravel substrates (Figure 10). This was affirmed in the results of JND analysis, except for dichromat visual systems, where matching decreased. The reason for the latter’s apparent reversal of the trend is unclear, as the difference between crab hue and substrate hue (based on the dichromat visual model) decreased, indicating an increase in matching. This does not seem to be an error in analysis and could be due to the lack of actual red sensitivity in the

dichromat model used. Pollack's "longwave" channel had a peak sensitivity of λ_{max} ~521nm (Shand et al. 1988), so while the hue calculations showed an decrease in the difference between the SW:LW ratio of crabs and substrates, this may not correspond directly to discrimination.

The shift in relative red reflectance is not unexpected given *C. maenas* possesses three recorded chromatophore types with red, black, and white pigments respectively. This would imply there should be some ability to adjust coloration. At the very least crabs should change in terms of "redness" by dispersion and aggregation of the pigments within the red chromatophores. While we found no evidence of colour discrimination, achromatic contrast cues could have stimulated this change. Based on my spectral sensitivity model, *C. maenas* is significantly more sensitive to shortwave than long wave stimuli. This leads to the perception of the red substrate being significantly darker than the blue (Figure 12). An appropriate response if this is the case is an overall dispersal of pigments within the darker chromatophores, and likely the red as well given their similar responses to the melanophores (Powell 1962b). This would result in an increase in the ratio of longwave/red reflectance.

On top of large-scale changes in coloration post moulting or primary response to light, aspects of chromatic change seem to be dependent on ecdysis. Individual coloration is associated with moult cycle, outside of camouflage (Lee and Vespoli 2015; Reid et al. 1997). An integument pigment of *C. maenas* is a blue carotenoprotein (λ_{max} ~625nm (peak absorbance) (Garate et al. 1984)) which denatures to release the red pigment astaxanthin, which is potentially a cause of colour change towards red in crabs with prolonged inter-moult periods, as the carotenoprotein is denatured by heat and light over time (Reid et al. 1997). Moulting will remove these denatured pigments with the old integument, presumably resulting in a reduction of longwave reflectance relative to shortwave. This could mean that crabs that had yet to have a record of colour close to ecdysis at the start of experiment (as there was no sure way of knowing time since last moult) would have moulted during the experimental period, shedding the denatured integumentary carotenoprotein and potentially reducing reflectance of longwave light. Such a reduction in longwave reflectance may ultimately increase hue and improve matching on blue substrates. Considering that the greatest luminance mismatch was on blue, moulting may have occurred

more frequently than red/the other substrates to adjust luminance matching, resulting in a greater/more rapid loss of longwave reflecting astaxanthin. It is worth noting that for this experiment, moulting frequency was not recorded, so this cannot be confirmed directly, only by the change in crab luminance. Additionally, given the previously mentioned similarity in responses of red and black chromatophores (Powell 1962b) the red chromatophores may simply be melanophores with denatured pigments responding with non-denatured chromatophores. Molecular analysis of pigments and direct examination of chromatophore behaviours is needed to confirm this.

Statistically significant changes in coloration aside, the implications of the chromatic changes recorded in relation to camouflage are less meaningful. While the colour change was similar across all crabs on a substrate, the magnitude of that change is not indicative of a significant chromatic change for camouflage, as there was not a uniform trend towards improving camouflage. JND only decreased for crabs on blue substrates for all visual systems, and red to tetrachromat vision (as well as a statistically non-significant amount to trichromat vision). For the rest of the substrates/visual models, colour JND increased indicating a decrease in matching. As well as the lack of uniform colour matching improvement, the degree of change is not likely to be meaningful when considering camouflage success. Given no individual crab achieved a JND score below one, compared to the gravel substrate, it is unlikely colour matching would be significant enough avoid predator discrimination and prey detection. The greatest matching was found in a crab from the cohort on blue substrates, which achieved a final JND score of 3.656 (based on dichromat vision), which lay above even a conservative metric for “matching”.

It is worth noting that the artificial substrates used were unlike those likely to be encountered in natural environments. Primarily treatments were uniform and highly saturated colours. While some highly saturated substrates may be found (e.g., macroalgae or encrusting sponges in rockpools), they are likely to be part of heterogenous environments, and uniform habitats of *C. maenas* tend to be less saturated (e.g., dark mud or pale sand substrates). The rationale behind the use of such unnaturally saturated substrates was to provide the maximum difference between both the treatments themselves, and treatments and the

initial crab coloration (dark green-grey of mudflats). This was thought to allow for maximum discriminability of treatment colour by crabs (if chromatic discrimination is possible) and stimulate maximum change in colour to increase effect size and clarity. In addition, past work on *C. maenas* chromatic change used colours based on natural environments and found little change (Stevens, Lown, and Wood 2014b). In species compared to natural backgrounds, correlations between substrate luminance and colour have been proposed as mechanisms that allow colourblind cephalopod species to still change achieve effective chromatic matching (Duarte, Flores, and Stevens 2017). While this may allow for chromatic matching on natural substrates in *C. maenas*, it has been noted that chromatic matching of *C. maenas* is effective in their mudflat habitats, but less so in the rockpool habitats (Nokelainen et al. 2017; Price et al. 2019; Todd et al. 2006; Todd et al. 2012) (detailed later in this discussion).

Regardless of change in the wavelengths reflected, all crabs showed significant luminance improvement on all substrates except red. The greatest response across all visual models was seen in crabs on the blue substrate, and this corresponds with the greatest initial mismatch (Figure 12 and Figure 13). The greater initial difference requires a commensurately greater brightness change to improve matching. This corresponds to past research on the species (Stevens 2016; Stevens, Lown, and Wood 2014b). Given they must have some luminance perception, it is reasonable to assume the crabs are responding to the information from the background, especially based on the greater change on substrates individuals initially mismatched. Their lack of sensitivity to red/longwave spectra ((Bruno, Mote, and Goldsmith 1973; Martin and Mote 1982), and Figure 6, Figure 7, and Figure 8) means it is likely the red substrate appeared darker to them than the other ones. Crabs came from the Penryn mudflats, one of the darkest habitats (Nokelainen et al. 2019; Price et al. 2019) it is reasonable to assume that they had already achieved the darkest coloration possible.

While tetrachromatic predators seem to be able to significantly discriminate between crab and substrate coloration (the high JND values in Figure 11), the other two species seem well less able to differentiate between the crabs and substrate colour. Initial luminance differences between crabs and substrates tended to be perceived as being greater than chromatic ones (based on JND

scores in Figure 11 versus Figure 13). Additionally, juveniles/subadults are at significant risk of predation by larger conspecifics (Moksnes 2004; Moksnes, Pihl, and Montfrans 1998), and if my results are accurate these would also be monochromats, and even if not they are likely to be dichromats with limited colour discrimination. Brightness differences are likely to be more perceptible to these predators, applying a greater selective pressure on matching that aspect of the habitat, rather than the specific hue of that habitat. Considering *C. maenas*' activity patterns, the times when they may be most exposed to predators and-or camouflage is less valuable are at low-light periods (Almeida, Flores, and Queiroga 2008; Crothers 1968). Even predators with colour discrimination may be relying on brightness cues over chromatic ones at night (reviewed in (Kelber, Yovanovich, and Olsson 2017)), potentially increasing the value of achromatic matching compared to chromatic matching.

As previously mentioned, recent research has shown an association between *C. maenas* camouflage strategies and the habitat types they are in (Price et al. 2019). Background matching is significantly more common in uniform habitats (e.g., mudflats), while disruptive patterns are more common (and more effective) in heterogenous habitats (e.g., rockpools). Crabs tend to match the colour of mudflats, and the variation in rockpools limits colour matching, so change in colour may be of less use. Instead, *C. maenas* seems to switch from background matching (including colour) on mudflats to disruptive coloration on rockpools, that is less dependent on chromatic matching. Research indicates that luminance matching alone can improve the effectiveness of disruptive markings (Stevens et al. 2006), which is within *C. maenas*' ability to change.

Despite some gaps in the data available, the results indicates that *C. maenas* is monochromatic, with a single photoreceptor class in the mediumwave region of the visible light spectrum. Colour discrimination trials while limited, did not indicate any behavioural colour discrimination, even though coloured stimuli should have had both achromatic and chromatic differences if *C. maenas* is dichromatic. Despite possessing chromatophores that should allow chromatic change, there was no meaningful improvement in chromatic matching, while luminance change was more noticeable, in line with past research on this species. When crab colouration was analysed using a model created from the spectral sensitivity data collected in the optokinetic experiments, luminance

change was greatest on backgrounds which crabs significantly differed from, supporting the assumption colour change is guided by monochromatic vision. In terms of *C. maenas* natural habitats, chromatic matching seems only reliable on uniform mudflats and prohibited by the significant variation in heterogenous rockpool habitats. As crabs already tend to match the coloration on mudflats, there is little need to adjust chromatically in these habitats, and with the effectiveness of camouflage strategies other than background matching in heterogenous habitats, chromatic change for matching may have less adaptive value. Ultimately, *C. maenas* colour change for camouflage seems to be dominated by the secondary response from the eyes which, lacking apparent colour discrimination, limit gross colour change to luminance responses. This may not limit *C. maenas* camouflage, as colour matching remains effective on uniform habitats, and the variation in other habitats may limit colour matching's effectiveness. This adds to the growing field examining the sensory processes facilitating camouflage, and provides evidence of lack of chromatic discrimination apparently restricting colour change

Chapter 3: Spatial vision and pattern change for camouflage

Abstract

Carcinus maenas displays significant variation in patterning. This variation is strongly associated with habitat heterogeneity. Crabs on uniform backgrounds tend to have little patterning and uniform coloration, while more patterning and greater variation is found in heterogeneous habitats. C. maenas' ability to adjust brightness may extend into changing the distribution of light and dark areas of the carapace to match these habitats. Given C. maenas' compound eyes are less able to resolve fine detail in the environment, pattern perception may limit pattern change responses as small patterns may not be resolved, detected, and ultimately stimulate a response. To test the relationship of C. maenas spatial vision with pattern change, crab acuity was measured using optokinetic responses to rotating grids of varying sizes. This was used to create backgrounds with patterns either large enough to resolve or too small to resolve. Pattern change was then recorded and compared between crabs on these patterned backgrounds and a uniform control. Acuity measurements aligned well with previous data on similar species, with a strong positive relationship with body size. Larger crabs tended to possess greater acuity. Individual acuity did not affect crab pattern change within treatments, but crab pattern change did vary with background patterning. Pattern contrast significantly increased on the large-patterned backgrounds, with less change on the small-patterned background, and little to no change on the uniform. Only crabs on large backgrounds improved pattern matching, with crabs on uniform backgrounds starting already matching, and crabs on small patterned backgrounds did not improve pattern matching. There was no directional change in pattern size and distribution, with crabs apparently possessing a fixed carapace pattern whose conspicuousness can be changed by adjusting contrast between elements. Ultimately C. maenas pattern change allows for change between a binary of patterned to uniform not limited by acuity, enabling matching effective camouflage strategies to mesoscale habitats.

Introduction

In the previous chapter, *C. maenas* whole-body colour change was found to be almost solely achromatic, corresponding to an apparent lack of chromatic discrimination. However, *C. maenas* appearance is not limited to total achromatic and chromatic reflectance. The spatial variation in these within an individual, i.e., patterning, will also be crucial for successful matching. An individual matching the overall reflectance of a patterned habitat is likely to be conspicuous simply because its uniform coloration will stand out against the background (Hailman 1978), and the outline of the individual will be conspicuous by breaking patterns they are viewed against (Thayer and Thayer 1909). Animals often occupy a range of habitats, and these habitats can vary drastically in the patterning of backgrounds needing to be matched. Ergo, the ability to adjust within body patterns as well as overall reflectance should provide further benefits by allowing camouflage to be tuned to habitats of different brightness, but also habitats where the spatial distribution of brightness varies. Patterning can be split into two elements – the distribution, or shape and size of pattern elements, and the contrast/difference in intensity between pattern elements. Both pattern distribution and pattern contrast can theoretically change, the former resulting in 'new' patterns both in distribution and size, while the latter adjusts the salience of existing patterns.

Colour change, specifically to improve camouflage, is found across multiple taxa (comprehensively reviewed in (Stevens 2016; Duarte, Flores, and Stevens 2017)). Much of the research has focussed on whole body changes in reflectance in various species, including terrestrial (Burtt 2009; Filshie, Day, and Mercer 1975; Peralta-Rincon, Escudero, and Edelaar 2017; Eacock et al. 2017; Eacock et al. 2019) and marine arthropods (Duarte, Stevens, and Flores 2018; Green et al. 2019; Wenner 1972; Stevens 2016; Stevens, Rong, and Todd 2013), reptiles (Stuart-Fox, Moussalli, and Whiting 2008; Stuart-Fox, Whiting, and Moussalli 2006), amphibians (Kang, Kim, and Jang 2016; Polo-Cavia and Gomez-Mestre 2017), and fish (Sumner and Keys 1929; Stevens, Lown, and Denton 2014; Ramachandran et al. 1996). Changes in pattern contrast and distribution should not only allow for tuning of coloration to specific backgrounds, but also shifting between camouflage strategies to maximise effectiveness (Hanlon et al. 2009). While background matching improves overall

survival compared to a lack of matching, detectable features will still be present. Body outlines can be detectable even when individuals match background colour and brightness, and may limit the effectiveness of background matching (Thayer and Thayer 1909; Cott 1940). Disruptive coloration functions to obscure other salient features used in prey identification, specifically outlines of various body parts (Thayer and Thayer 1909; Cott 1940). By creating false edges where part of the body matched parts of the backgrounds, the body outline is disrupted preventing detection based on object recognition (Cuthill et al. 2005). This false edges can often be more salient than the real edges of an individual as a result of higher contrast, further breaking up individual shape to reduce detection (Sharman and Lovell 2019). Disruptive coloration has been demonstrated in multiple species, across multiple taxa

Adjustment of pattern to adjust camouflage strategies has been well documented in cephalopods (Allen, Mäthger, Barbosa, et al. 2010; Barbosa et al. 2008; Barbosa et al. 2007; Hanlon et al. 2009) and those bony fish that have had their colour plasticity investigated (Akkaynak et al. 2017; Kelman, Tiptus, and Osorio 2006; Ramachandran et al. 1996; Allen et al. 2015; Tyrie et al. 2015). There is experimental evidence of cephalopods swapping between camouflage strategies from background matching, with patterning directly comparable to substrate granularity, to disruptive markings that interact with background patterns to break individual outlines (Barbosa et al. 2008; Barbosa et al. 2007; Hanlon et al. 2009). Similar behaviour has also been found in flatfish species (Akkaynak et al. 2017) , which have an even stronger relationship with resting substrates, given their resting proximity to them.

The camera type eyes of both fish and cuttlefish are characterised by greater visual acuity over other eye systems (relative to eye size) (Caves, Brandley, and Johnsen 2018; Cronin et al. 2014). These species are unlikely to face difficulty in resolving fine detail, especially not the information needed for background perception. In fact, cuttlefish have had extensive investigation into the role of their vision in patten change (reviewed in (Chiao, Chubb, and Hanlon 2015)). Multiple aspects of backgrounds are integrated in cuttlefish pattern change responses, including intensity and contrast (Barbosa et al. 2008; Chiao, Chubb, and Hanlon 2007; Chiao and Hanlon 2001a), edge detection (Chiao, Kelman, and Hanlon 2005), object area (Chiao and Hanlon 2001b), and object

depth (Kelman, Osorio, and Baddeley 2008). Compound eyes have received little to no attention regarding their role in pattern change, but their resolution should be at a disadvantage against camera type eyes of the same size. Species with compound eyes tend to be limited given the greater size of individual photoreceptors relative to the eye, increasing the interreceptor angle which is associated with reduced acuity (Land and Nilsson 2012; Land 1997). My question here is: What are the visual and background-matching capacities of species with compound eyes that are also under predation pressure to match the spatial qualities of their environment?

C. maenas possesses two compound eyes, with the relative disadvantage in acuity associated with them. Whether this disadvantage is enough to compromise pattern matching is less certain. *C. maenas* theoretically possess no minimum focal distance, due to the structure of compound eyes allowing for a depth of field that abuts close enough to anything needing resolving (Land and Nilsson 2012; Cronin et al. 2014). Consequently, even small patterns should be resolvable as long as the eye can be brought close enough to them. With an epibenthic lifestyle, its proximity to substrates coupled with the minimal focal distance should compensate for the reduced acuity. Crabs will be close enough to resolve most information at the distance of their background. Regardless of this, *C. maenas* occupies a variety of habitats where pattern matching is crucial. Acuity likely varies between individuals, with most variation resulting from size differences, given the association between compound eye size and acuity (Caves, Brandley, and Johnsen 2018; Feller et al. 2021). Past work on *Callinectes sapidus*, the blue crab, has shown that acuity can vary significantly depending on time to or since the most recent moult (Baldwin and Johnsen 2011). Individuals approaching moulting display a rapid decrease in acuity, as the integument over the eyes separated as the new one forms, creating an interference layer between photoreceptors and external information. This increases as crabs exit the old moult and distance between the new and old ocular integument increases. Once moulting occurs, the previous integument no longer acts as a barrier to light and a subsequent increase in acuity is recorded (Baldwin and Johnsen 2011). In addition to this the previous moult likely accrues damage at various scales through abrasion or other external effects (Greco 2011; Greco et al. 2013) which could also further reduce

acuity. This is important as *C. maenas* colour change is greatest immediately following moulting (Nokelainen et al. 2019; Stevens, Lown, and Wood 2014b; Styrishave, Rewitz, and Andersen 2004; Stevens 2016; Stevens, Rong, and Todd 2013), therefor a marked increase in acuity should coincide with when it would be of most use in pattern perception.

C. maenas is found across spatially varied habitats; from uniform mudflats, algal beds, and mussel beds to rockpool environments where spatial and spectral information varies at multiple scales (Nokelainen et al. 2017; Price et al. 2019; Todd et al. 2006; Todd et al. 2012). Crab phenotype is associated with the visual environment, with patterned crabs more commonly found in patterned environments (Nokelainen et al. 2017; Stevens, Lown, and Wood 2014a; Todd et al. 2006; Todd et al. 2012). There is even more differentiation at the small-scale between microhabitats, and certain pattern elements are conserved across habitat type. There are also associations in phenotype with age. Larger and older crabs tend towards a more generalist camouflage strategy, younger crabs – those occupying the greatest range of visual environments – vary more in degree of patterning and coloration (Nokelainen et al. 2019). Documented examples include overall brightness matching on mudflat habitats, and disruptive coloration in rockpools and other spatially varied habitats (Nokelainen et al. 2017). These strategies have been demonstrated as being the most effective for their respective habitats, with crab coloration being a good match for mudflat environments, while the increased variation of rockpools limits colour matching, but allows effective disruption (Price et al. 2019). A variety of factors could potentially cause this association.

First, the association between crab and habitat patterning could be a result of differential selection by predators in the short term, combined with fixed phenotypes. In this scenario, individuals with various body patterns alight in different habitats during settlement. Individuals that do not match their environment (crabs with large, high contrast patterns in uniform habitats and vice versa) suffer higher predation rates than those that do. There is increasing experimental evidence of the direct adaptive value of camouflage in living systems (Duarte, Stevens, and Flores 2018), prey models (Walton and Stevens 2018; Vignieri, Larson, and Hoekstra 2010), and virtual experiments (Bond and Kamil 2002; Nokelainen et al. 2019). This could lead to a dominant population

that matches the environment they are found in. This has already been called into question however, with past research highlighting the disparity between the abundance of patterned juveniles and the relative scarcity of patterned sexually mature adults (Hogarth 1978). The argument being a patterned adult population should persist in habitats where patterning increases survival (e.g., rock pools). It has also been noted that the degree of predation would need to be especially high to have a significant effect on phenotype abundance, given the overall abundance of *C. maenas* within habitats (Nokelainen et al. 2017). Finally, crabs across habitat types in the south west of England have been shown to be one genetic population (Silva et al. 2010), with indications of intermixing between geographically separate populations, which would not be expected if specific fixed patterns were being selected for in specific locations.

Second, there could be some degree of post-settlement habitat selection by crabs. Selection of backgrounds has been demonstrated to improve camouflage in various species, both terrestrially (e.g. selection of nest site in ground nesting birds (Stevens et al. 2017), and moths selecting and orienting with background features (Kang et al. 2012)), and aquatically (e.g. prawns selecting algal substrates that matched their body colour (Green et al. 2019), ghost crabs choosing backgrounds that improve matching based on body brightness (Uy et al. 2017), killifish choosing background that contained patterns based on orientation of body markings (Kjernsmo and Merilaita 2012)). Habitat selection is already seen in the crab's post-larval stage at settlement (Moksnes 2002), and extends beyond that to the subadult life stages once transitioned to an epibenthic habitat. Other crab species have been shown to select habitats that improve matching (Uy et al. 2017) and it has been suggested as a reason for the phenotype-background association in shore crabs, especially at the small scale (Todd et al. 2012; Nokelainen et al. 2017). While not dependent on colour change, this process would still require identification of substrate patterning. Individuals either recognise similarities between themselves and the habitat and preferentially select those they match. This likely occurs on the very fine scale (see (Nokelainen et al. 2017; Todd et al. 2012)) as crab movement will be limited by size, and distance between different habitat types may be too great for crabs to safely move between. Crabs have been demonstrated to move

kilometres over a period of hours (Ameyaw-Akumfi and Naylor 1987), but individuals doing so simply for background selection seems unlikely.

Finally, as stated above, there is significant evidence of colour change resulting in improved matching in shore crabs (Stevens, Lown, and Wood 2014b, 2014a; Bedini 2002) as well as other crab (Stevens, Rong, and Todd 2013; Hultgren and Stachowicz 2008) and intertidal invertebrate species (Stevens et al. 2015; Keeble, Gamble, and Hickson 1900; Manríquez et al. 2009). Multiple crab species possess circa-tidal rhythms of colour change(e.g. (Stevens, Rong, and Todd 2013; Darnell 2012; Fingerman 1956; Fingerman, Lowe, and Mobberly 1958; Fingerman and Yamamoto 1967)), some examples seeming to improve camouflage (Rao, Fingerman, and Bartell 1967; Brown Jr and Sandeen 1948). *C. maenas* colour change happens in artificial settings when crabs are placed on different backgrounds, seen both in past research (Stevens, Lown, and Wood 2014b, 2014a; Stevens 2016; Mynott 2019) and the previous chapter of this thesis. In natural settings, it should happen both at the site of settlement and when crabs travel to new habitats either deliberately or by outside forces (e.g., wave action). The ability to adjust gradually or grossly to changing habitats permits a wider range of optimal habitats, providing greater access to resources without compromising crypsis. The previously mentioned studies on crabs changing colour have focussed primarily on whole body reflectance changes, rather than within-crab patterning (although some note of change in pattern conspicuousness was made (Stevens, Lown, and Wood 2014b)). What has been less studied is the pattern change for camouflage in these species. Given the variety of habitats that any given *C. maenas* individual could potentially occupy, and the lack of large-scale control habitat they settle in, the ability to tune pattern to match backgrounds (even to a limited degree), should be useful. It is proposed to be the main reason for meso-scale habitat matching in past research (Nokelainen et al. 2017). While the results of the previous chapter indicate significant chromatic change is not present in *C. maenas*, this does not rule out the ability to adjust patterns. These are often highly contrasting, comprised of light and dark elements (Nokelainen et al. 2017; Nokelainen et al. 2019; Price et al. 2019), and disruptive camouflage is effective when luminance is matched to the environment (Stevens et al. 2006), which is within the scope of achromatic colour change. If this is the case, and *C. maenas*

can adjust the distribution and-or contrast of its patterning, then the ability to change these patterns should be limited by its ability to detect them, as well as the pattern change mechanisms themselves. *C. maenas* colour change is primarily morphological, unlike that of other species whose pattern change for camouflage has previously been investigated ((Akkaynak et al. 2017; Barbosa et al. 2008; Barbosa et al. 2007; Smithers, Wilson, and Stevens 2017; Williams et al. 2019; Zylinski, Osorio, and Shohet 2009a; Kelman, Tiptus, and Osorio 2006; Ramachandran et al. 1996; Tyrie et al. 2015; Kang, Kim, and Jang 2016; Chiao and Hanlon 2001a)), lacking the rapid (and potentially fine-scale) control of the physiological changes in, for example, cephalopods and fish.

In this experiment the change in pattern, both contrast and pattern size/distribution of *C. maenas* was measured on backgrounds of varying spatial complexity and related back to acuity. The sample population of crabs had their acuity measured using their innate optokinetic response, based on the minimum size of rotating grid they could track. This was then used to create background pattern sizes above and below the threshold of acuity (based on a limited minimum focal distance). Crabs' patterns change on these backgrounds and a uniform control over eight weeks was then recorded via digital image analysis. Colour change was compared between background patterning, alongside the acuity of individuals, to assess whether acuity limits change in pattern in *C. maenas*. I predicted that if *C. maenas* possess no minimum distance to "focus" then acuity should have little to no impact on their pattern change behaviour, and responses should differ between the small-patterned and uniform treatment. If crabs do possess such a limit, then we would expect to see a threshold in acuity where crabs on sufficiently small-patterned backgrounds change pattern in a similar fashion to those on uniform backgrounds. I assumed the unresolved pattern elements would be blurred, being functionally perceived as lacking patterns.

Methods

Sample collection and housing

Crabs were collected by hand from the mudflats of the River Fal at Penryn (Lat/Long: 50.1697,-5.0989) during low tides at the beginning of September 2021. Mudflats were sampled due to the known uniformity of crab coloration (Nokelainen et al. 2019; Price et al. 2019) in these areas, ensuring a reasonably

homogenous starting population minimising initial variation in the samples across treatments. In addition, crab body size was preferentially selected for, with a cephalothorax width range of 10mm to 25mm, based on the shifts in patterning with ontogeny countered by the abundance of smaller individuals, whilst still being large enough to be physically robust for use in experiments. Crabs were placed into individual plastic containers before being transported directly back to the laboratory where they were transferred to individual containers with a neutral background (black gravel of a similar luminance to the resting substrate they were collected from) and quarantined for one week to ensure health prior to experiment start. Three batches of crabs were collected, two on consecutive days, and a third two weeks later due to changes in low tide times. All crabs were housed for a total of 10 weeks, with one week of quarantine at the start and end of the eight- week experiments. The latter sample was run two weeks behind the initial two samples but were photographed at the same time of day as the rest of the cohort. All crabs were returned to the sample site, excepting those that died (all due to complications during moulting). Fatalities were stored in the laboratory's -20°C freezer, prior to incineration.

Crabs were distributed across two 45cm by 120cm tanks filled with ~150 litres of freshly made saltwater (to a depth of ~20cm using Aquarium Systems Instant Ocean Salt made up to a concentration of ~29-31 ‰). Crabs were individually housed in the same 60mm diameter plastic containers as the chromatic change experiments. Individual housing was necessary for two reasons. Firstly *C. maenas* is known to be cannibalistic (Moksnes 2004; Moksnes, Pihl, and Montfrans 1998). Anecdotal evidence from prior experiments show conflict readily occurs between individuals in proximity, often leading to loss of limbs and other damage. Secondly it allowed for recognition of specific individuals needed for repeated measure of colour for colour change. During initial quarantine, individuals were housed on black aquarium gravel to best mimic the dark uniform mud of the mudflats. During the experiment crabs were randomly assigned one of three background patterns detailed below and remained on it for the rest of the time they were housed in the lab.

Crabs were housed under a 12-hour daylight cycle with 0700-1900 being illuminated with broad spectrum tank lights. The remaining time crabs remained

left in darkness, with a gradually ramping up and down of lights respectively to mimic dawn and sunset. Crabs were fed ad libitum every Monday, Wednesday, and Friday, using Hikari Crab Cuisine Crustacean food (Kyorin Co. LTD).

Acuity measurements

At the end of the initial quarantine, individuals had their behavioural acuity measured. This used the digital optomotor drum developed by Martin J How (University of Bristol), used in the first chapter to assess behavioural spectral sensitivity. It is even better suited to measures of acuity than spectral sensitivity for multiple reasons. First, the issue of spectral tuning is no longer an issue – grids of black and white were used for maximum contrast, with no need to match specific colours as in the spectral sensitivity assessments. Second, the virtual drum allows automatic change in grid size, rather than needing physical grids to be manually replaced to adjust grid size in a physical drum. This markedly sped up data collection, minimising the amount of time individuals spent out of water. Experiments were conducted out of water as mounting individuals to the harness was simpler and presented less risk for the individual outside of water. This allowed for almost total restriction of body movement, while still allowing for eye movement recording without noise from other body motion.

Figure 14: Virtual Optomotor set up.
Four LCD screens arranged in a square, offset with each other to create a drum diameter of 260mm. Top image – arrangement of screens. Bottom image – example of crab position relative to screens. Example grid set to 10 cycles. One cycle consisting of one black bar and one white bar (as defined in (Caves, Brandley, and Johnsen 2018)). NB crab in image is a 3D-printed model, used in lieu of a live crab.

Prior to their acuity measurement each crab was removed from their individual container and measured at the widest point of the cephalothorax (the rearmost set of spines in all cases) using digital callipers. They then had three marks in

white paint applied as with the prior optokinetic experiments (Chapter 1). One mark to the back of each eyestalk to aid in tracking movement, and a third on the cephalothorax to act as a stabiliser against any other movement of the crab that could interfere with eye movement recording. Crabs were attached to a 4mm diameter piece of bamboo dowelling using white tack, which was in turn super-glued to an Amazon Basics desk tripod (amazon.co.uk). This was placed on a black fabric base in the centre of four HP X24c (23.6") FHD Curved Monitors (selected for sized, resolution, and the curved screen aiding in the illusion of a cylindrical arena). Screens were offset (seen in Figure 14) to reduce the arena base size to 260mm x 260mm. Crabs were placed directly over a marker indicating 130mm from each screen to ensure all crabs were viewing stimuli from the same distance (Figure 14).

Once crabs were placed in the drum, the screens were turned on to display a uniform white background and the camera placed above the drum was set to record. The program then ran starting with an additional 10 seconds of white background, before grids appeared. The trial ran from largest grids to smallest, starting at a value of one cycle per screen, a cycle being one grid of each colour was present on each screen (one 130mm diameter white bar, and one 130mm diameter black bar). One cycle was displayed for 30 seconds to ensure optokinesis started. Individuals that did not respond to this size were treated as non-responsive and had their acuity capped at zero cycles for ease of analysis. Following the initial 30 seconds, cycles increased by one (from one black and one white, to two of each) and ran for 10 seconds, then to four cycles for 10 seconds, and followed this pattern for the remainder of the experiment, with one cycle being added, up to a total of 30 cycles. This grid number was chosen as it was lower than, but close to the acuity of a comparable species (*Callinectes sapidus*) measured in a similar fashion (Baldwin and Johnsen 2011). Given the smaller size of individuals sampled (all were subadults <15mm CT (cephalothorax) diameter at the start of experiment) I assumed that the minimum resolvable angle would lie above this point given the relationship between body size and eye size, and eye size and overall acuity (Land and Nilsson 2012; Caves, Brandley, and Johnsen 2018; Warrant and Nilsson 2006). Following initial trials, this was cut to 22 cycles, as no crab responded to cycles

greater than 18, which continued throughout the trials. This was done to reduce the time crabs spent outside of the water.

Grid width at the final full response (eye tracking for the duration of the stimulus period) was used with the fixed viewing distance to calculate the minimum angle crabs responded to which was used as the measure of acuity. Minimum resolvable angle is a commonly used measure of acuity (Caves, Brandley, and Johnsen 2018; Feller et al. 2021; Land 1997; Snyder 1977), and has been used in assessing acuity in other crab species (Baldwin and Johnsen 2011). It allows for a measure of the minimum size of object resolvable, independent of viewing distance, as the latter will affect the former. Once the optomotor sequence had completed, crabs were removed from the drum still attached to the tripod, carefully removed from the white tack, and were then returned to quarantine containers in the holding tank. Acuity was measured this way for all crabs prior to the start of colour change experiments, and then two more times during the experiment, once at the midpoint following week four photographs, and a final time at the end of colour change experiment. An average acuity measure was used as it was assumed acuity would fluctuate within individuals with multiple factors (size (Caves, Brandley, and Johnsen 2018), proximity to moult (Baldwin and Johnsen 2011)).

Pattern Change Experiments

Following initial acuity measurements, the same crabs were used in pattern-change experiments. Crabs were placed on backgrounds of varying pattern for eight weeks, and their colour change was recorded. Crabs were assigned one of three potential backgrounds. These were a uniform grey (RGB 127, 127, 127) at a midpoint between white (RGB 255,255,255) and black (RGB 0, 0, 0), a large-patterned background consisting of 5mm squares of black and white, and a small-patterned background of 0.5mm black and white squares (see Figure 15). The former was used as an analogue of granular background such as rockpool environments. The latter size was used as a mimic of finer backgrounds such as sand substrates and lay at the lower end of measured acuity, at the approximate minimum MRA based on the initial acuity measurements. Based on a viewing distance of 10mm, this pattern should be unresolvable. This distance was selected based on the average size of crabs, and an estimate of the subsequent distance from eyes to the base of the

container. Alternatively, if the assumption regarding a lack of minimum focal distance is correct, then this pattern should be resolvable.

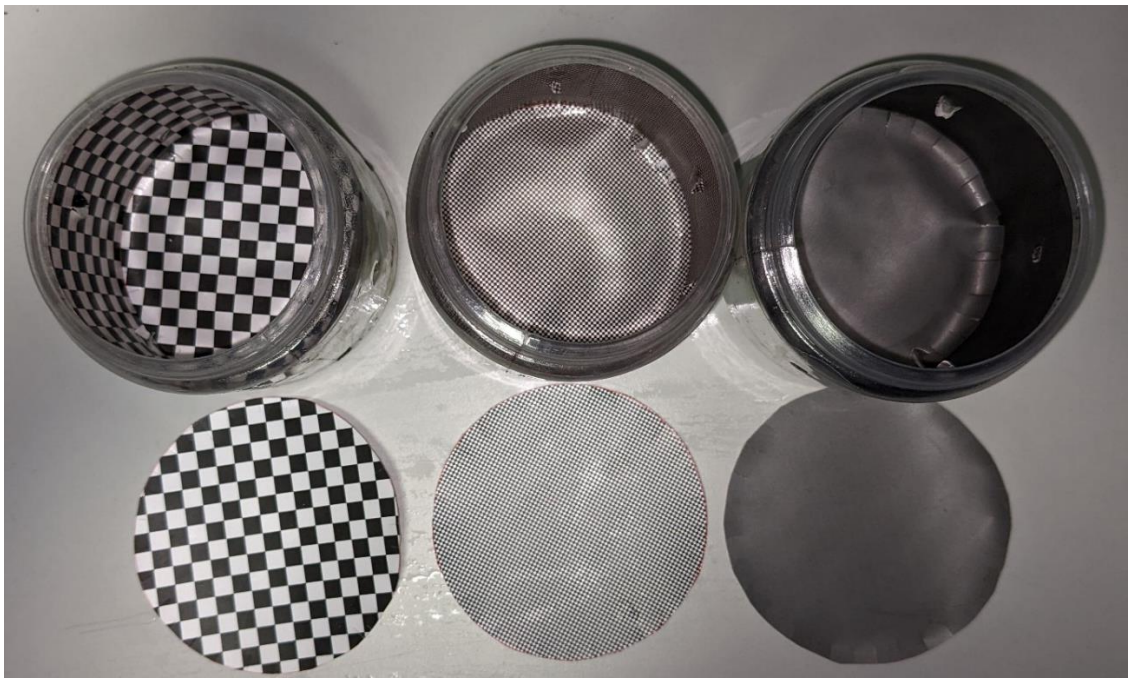


Figure 15: Experimental Containers for pattern change experiments.
Left to right - large treatment (5mm^2), small treatment (0.5mm^2) , uniform treatment (grey set to halfway between black and white elements of patterned backgrounds. Containers measured 6.5mm diameter. Container lids consisted of a screw top with the centre cut out and replaced with plastic mesh, to allow entry of light and fresh saltwater while keeping crabs contained. Holes drilled in the side of containers to aid in fresh saltwater flow.

Crabs were randomly assigned to background types using a random assignment script in R, where an individual's ID number (assigned haphazardly via the order acuity was measured) was paired with a number from one to three corresponding to a background. This was done ensuring even distribution for as balanced an experimental design as possible. Due to sample mortality during the eight weeks, some difference in the sample size of each background occurred. Of the 90 initial samples, 17 died prior to the eight week end-date, and were not included in the final sample. All backgrounds were printed on HP Tough waterproof paper, lining both the base and the walls of the container to a height of 50mm to ensure the pattern was visible regardless of crab orientation. Printed backgrounds, rather than gravel of varying dimensions, were used as they afforded greater control over pattern size and viewing distance. Gravel

would allow crabs to burrow allowing a greater range of viewing distances. Examples of the experimental containers are shown above (Figure 15). Ethical approval for both the optokinetic and pattern change experiments was obtained in the ethical application titled “Acuity and Change in Pattern for Camouflage” using the University of Exeter’s Worktribe ethics system, implemented in 2021 (no reference number).

Digital Photography

Before crabs were placed into their experimental backgrounds, initial photographs were taken to provide a baseline measure of colour and pattern which change was compared to. This was done using standard protocol detailed in previous chapters (Figure 4), but in brief; crabs were cleaned using a natural bristled toothbrush to remove any algal growth and particulate matter that could affect coloration. Crabs were then placed in a PTFE cylinder (both to contain the individual during photography and to diffuse illumination and reduce shadows) on a grey craft foam background (to minimise reflection during photography). A two-brightness grey spectralon standard (96.2% and 4.5% reflectance values) with a built-in scale bar was also included. Crabs were photographed with a Nikon D7000 with the built in UV (ultraviolet) filter removed, under a broad-spectrum arc lamp (70 W, 6500K Iwasaki Colour Arc Lamp, with UV filter removed). Visible and UV photographs were taken using an external filter (Baader Planetarium, Mammendorf, Germany). This is the established protocol for digital image analysis of coloration, especially in small invertebrates (Duarte, Stevens, and Flores 2018; Green et al. 2019; Nokelainen et al. 2017; Nokelainen et al. 2019; Price et al. 2019; Stevens, Lown, and Wood 2014b, 2014a; Troscianko et al. 2021; Walton and Stevens 2018). Photographs took place five times, once at the start of the experiment, and then every two weeks for a total of eight weeks. Samples of the backgrounds were also photographed at the end of the experiment for comparison of matching.

Following initial photography, crabs were transferred from holding containers to their experimental containers and returned to their respective tanks.

Moulting Behaviour

Across the entire experiment, starting at the point of initial acuity measurement, crab moulting behaviour was recorded. This was done to within 12 hours, as moulting that occurred between the hours of 1900-0800 (when the lab was not

occupied) would not be found until the following day. This allowed not only for an assessment of moult frequencies effect on the rate of colour change, both pattern and overall luminance to avian vision, but also to confirm past evidence of ecdysis' effect on acuity in arthropods and especially crustaceans (Baldwin and Johnsen 2011).

Image Analysis

As with previous experiments, images of crabs were loaded into the MICA (Multispectral Image Calibration and Analysis) toolbox in ImageJ software (imageJ version: 1.52 (Schneider, Rasband, and Eliceiri 2012) MICA Toolbox Version: 2.2 (Troscianko and Stevens 2015)). Due to camera misfunction during the initial photographs, a subset (samples 55-82 inclusive) of photos were photographed as JPEG files, rather than RAW files. Given the non-linear nature of JPEG files these photos were linearised using the inbuilt function in the toolbox but were grouped together so any difference in results for JPEG vs RAW photographs could be accounted for during analysis. Regardless of initial file format, all samples had visible and UV photographs compiled into MSPEC (multispectral) file. This aligned images with the speculon standard to control for variation in light conditions, and linearise reflectance (Troscianko and Stevens 2015; Stevens et al. 2007). Crabs' cephalothorax diameter and scale bar were recorded, and the crab cephalothorax (minus any particulate matter and damage which could obscure patterning) was isolated for pattern and luminance analysis. As previously stated, this is due to it being the most visible part of the crab when resting, as limbs are often tucked beneath the cephalothorax. Images were then run through a receptor noise model based on avian vision. The receptor noise model was based on Vorobyev and Osorio's receptor noise model (Vorobyev and Osorio 1998). The visual model used was of the Peafowl (*Pavo cristatis* (Hart 2002)), used as an analogue for other tetrachromatic predators of crabs (e.g. Herring and other gulls). This visual system comprises longwave, mediumwave, shortwave, and very-shortwave ultraviolet cone types. Only avian vision was used due to lack of significant difference in the luminance response of predator visual systems previously investigated in Chapter 1, also seen in past research in the species (Nokelainen et al. 2017) and specifically relating to patterning (Price et al. 2019). Dichromat pattern (Total Pattern

Energy) results are provided in Appendix 3A as an example of the lack of difference.

Samples were then analysed to record both average luminance (relative to peafowl vision) of the area selected, as well as being put through granularity analysis to assess pattern energy of the selected area. This has been used in pattern assessment in other species (e.g. cuttlefish (Barbosa et al. 2008; Chiao et al. 2011), and bird eggs (Stoddard and Stevens 2010)) as well as *C. maenas* patterning (Nokelainen et al. 2017; Nokelainen et al. 2019; Price et al. 2019; Stevens, Lown, and Wood 2014a). Granularity analysis involved the filtering of each image via Fast Fourier bandpass filtering at multiple spatial scales. This filtering started at five pixels, increasing by a factor of 1.1 to a maximum pattern size of 750 pixels (approximately the size of the smallest crab). At each spatial frequency, energy (measured as the standard deviation of the pixel values at each scale (Troscianko and Stevens 2015)) was produced. Larger markings (lower spatial frequencies) were recorded by small filter sizes, and vice versa of smaller markings. Higher values of pattern energy indicate greater pattern contrast at that specific size. Summed pattern energy across all pattern scales (Total Pattern Energy) and the marking size with the highest energy (dominant marking size) were recorded for comparison both within individuals to compare ability to respond to patterns, and between treatments to compare effects of pattern size on these responses. Pattern energy at each spatial scale measured was also recorded to produce pattern energy spectra, used in assessing background pattern matching. Pattern matching was quantified using the MICA toolbox's pattern and luminance difference calculator, based on the difference between crab and background pattern energy at each pattern size measured. I followed the methods of (Price et al. 2019), where the absolute difference between two spectra – in this case each crab carapace compared to the background it was on – was calculated across each spatial scale measured. This produced scores of Pattern Energy Difference (PED), where high scores indicate poorer alignment either in amplitude (amount of energy/contrast level) or shape (the pattern sizes where energy is high or low) and therefore poorer pattern matching, with lower values indicating better pattern matching between crabs and backgrounds. PED has been used in measuring background matching in *C. maenas* (Price et al. 2019), as well as fish (Smithers, Wilson,

and Stevens 2017) and birds (Stevens et al. 2017; Troscianko, Wilson-Aggarwal, et al. 2016). While crabs were primarily compared against experimental backgrounds (checked patterns/uniform grey), a subset of the natural substrate images from (Price et al. 2019) were compared as well. These were only used for qualitative comparisons, as the photo-standards and camera used, as well as distance between camera and subject/standard differed between experiments and could not fully be accounted for in analysis. A sub set of 15 uniform (mudflat) and 14 heterogenous (rockpool) natural substrate photos had their respective pattern data (Figure 22) averaged and used in PED calculations compared to crabs from all three treatments (Figure 20). Finally, MSPECs were scaled based on the 40mm scale bar in all images to ensure comparison of crab and background pattern size was consistent across all images.

Overall luminance was also recorded to compare change in average background matching, not only in crabs on uniform backgrounds, but also those on patterned backgrounds. Luminance has been used in the previous chapter and is a measure of achromatic reflectance based on intensity per unit of area for the dominant achromatic channel for observers. This was done to test the assumption that background patterns below the acuity threshold of individuals should be unresolvable and appear as a uniform background of the average luminance of pattern elements.

Statistical Analysis

Acuity was modelled, specifically the maximum number of grids per screen resolvable. This was used over the MRA values (minimum resolvable angle, calculated based on grid width and the 130mm viewing distance), due to normality of the data, and improved model fit. Fixed effects of crab size, proximity to moult (days moulting took place prior/post acuity measure), the interaction between the two, and the timepoint of measurement (start/middle/end of experiment) were included, with a random effect of individual crab to account for repeated measures.

*lmer(Number of Grids Per Screen ~ Size*ProximityToMoult + Timepoint + (1|ID)*

Image data was extracted from imageJ as .CSV files and transferred to R statistical software (R Development Core Team 2020) for statistical analysis.

Pattern change measures were split into two separate formats, the first using the long data set comparing the effect of time (day of photograph) on pattern to test for colour change. Four models, one for each of dominant marking size, total pattern energy, Pattern Energy Difference (PED the difference between crab and background energy at each pattern size measured), as well as luminance (brightness to predator perception) averaged across the crab were fitted against fixed effects of day, pattern size, average acuity, moult frequency. Interaction effects of day and pattern size were used to measure the effect background pattern on the rate of change in crab pattern metrics/luminance. In addition, two three way interactions were added, combining day, pattern size, and acuity or moult frequency, to determine if crab acuity and-or moult frequency affected rate of change on each of the backgrounds. Also added were random effects of crab ID to account for repeated measures of individuals, and filetype to account for any effects of the difference between RAW and JPEG files because of the error during photography. Crab size was not included as a fixed effect, as it was not retained in models from Chapter 1.

The un-simplified R code (R Development Core Team 2020) for these models was:

*lmer(*Pattern Metric/luminance* ~ Day*Pattern. Size*Av.Acuity + Day*Pattern. Size*MoultFrequecy + (1|ID) + (1|FileType)*

Alongside these models, single measures of change (produced by calculating total change in pattern measures/luminance from the start to the end) were fitted to models to compare the effects of background patterning, average acuity, moult frequency, as well as the interaction effects of all fixed effects. The random effect of Filetype was also included for the same reason as above.

*Lmer(*Change in Pattern Metric/luminance* ~ Pattern. Size*Av.Acuity*MoultFrequecy + (1|FileType)*

All models were fitted using the lmer() function (based on normality, transformability, and model residual fit) from the LME4 package in R (Bates et al. 2015). Model simplification was via assessment of AIC values and effect significance. Un-simplified model output can be found in Appendix 2C.

Results

73 crabs had acuity measures taken, completed the eight-week colour change experiment and moulted at least once (assumed to be needed for significant colour, luminance, and pattern change). This was broken down into 24 crabs on large-patterned backgrounds, 25 on small-patterned backgrounds, and 24 on uniform backgrounds.

Acuity Measurements

Acuity measured across the experiment revealed several interesting trends. Firstly, acuity fit well with our predictions based on prior information of crab acuity. All crabs had acuity measures taken at the three timepoints, and the average acuity for each crab (minimum resolvable angle) ranged from 18.3° to 3.6°. There is a noticeable positive relationship between crab size and acuity, with larger crabs possessing greater acuity (smaller minimum resolvable angle: Figure 16) than smaller individuals. With every millimetre increase in crab diameter, the minimum resolvable angle reduced by 0.368 ± 0.09.

We did not find a clear link between acuity and proximity to moult as found in (Baldwin and Johnsen 2011). While there was some evidence of an increase in acuity in crabs measured soon before moulting (based on loess smoothing due to the assumed non-linear relationship between acuity and proximity to moult Figure 17) there is no noticeable trend due to large amounts of noise. The crabs with the lowest MRA (highest acuity) were found to have moulted within the three days prior to their measurement.

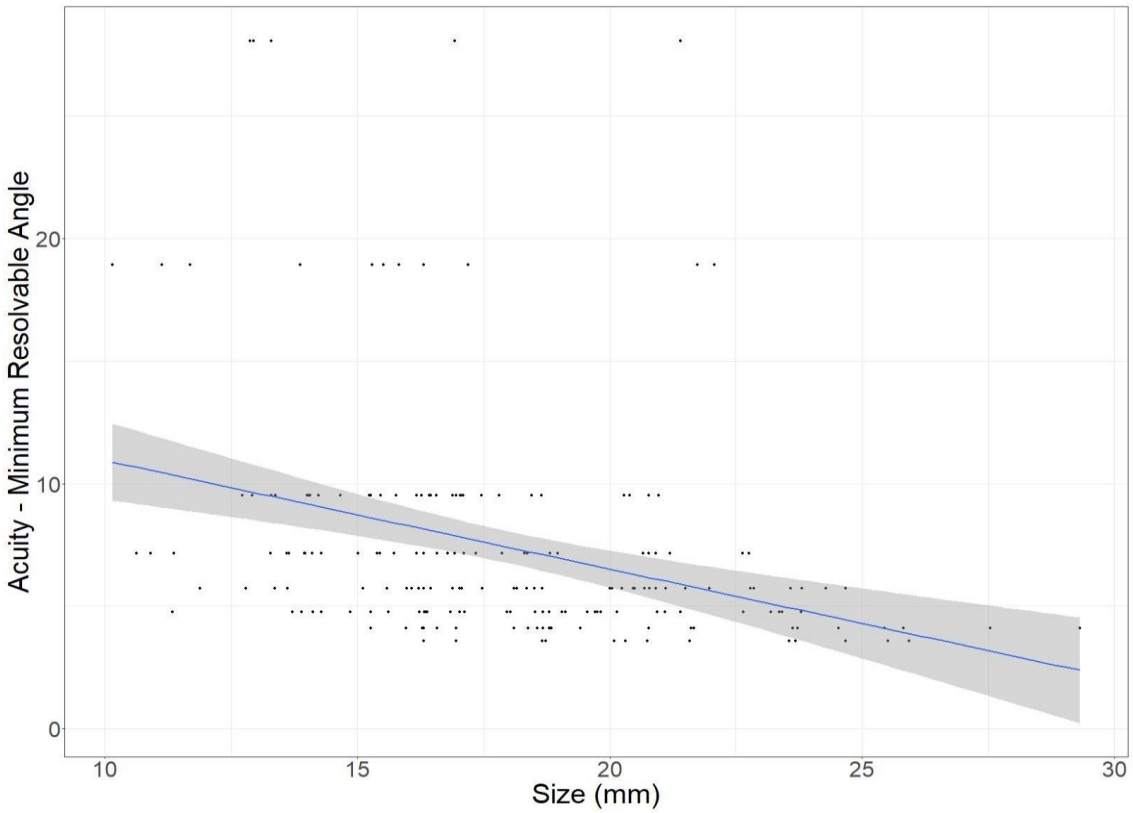


Figure 16: Crab acuity compared against size in pattern change experiments. Acuity metric is the minimum resolvable angle, based on a complete response to minimum resolvable grid. Lower minimum resolvable angle equals greater resolving power. Size is the width of crab at the widest point, from the points of the rearmost rostral spines. Straight line is “y ~ x” linear regression, with shaded area showing 95% confidence intervals. Graph produced using the ggplot2 R package (Wickham 2016).

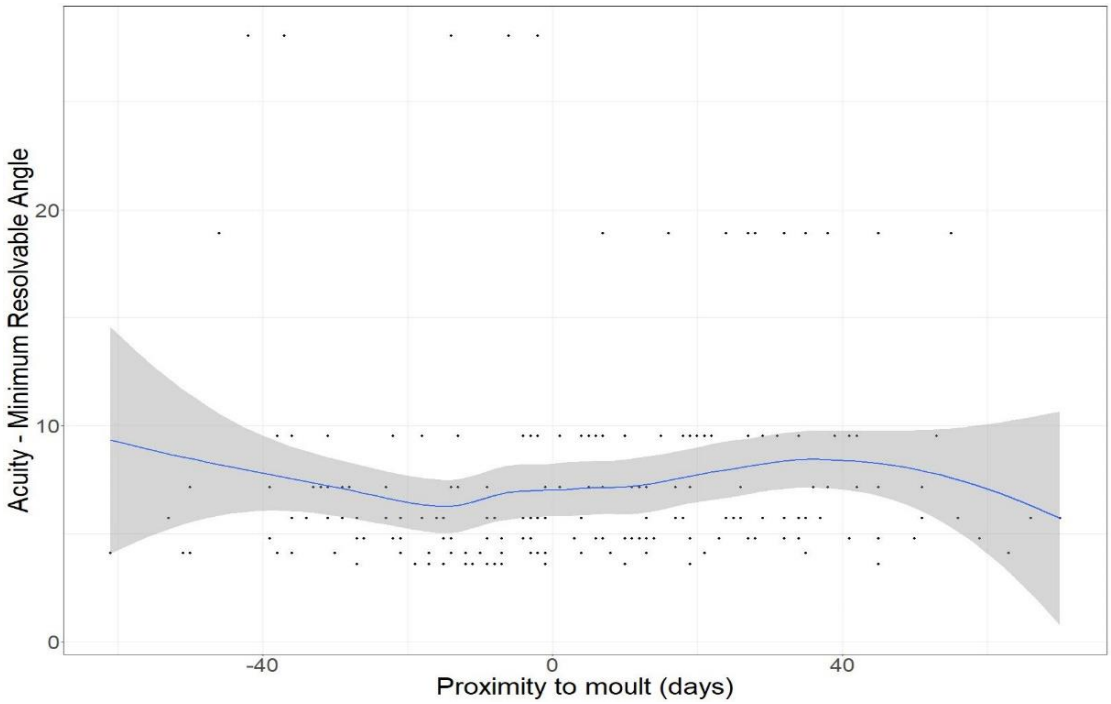


Figure 17: Crab acuity compared against proximity to moult from pattern change experiments.
Acuity metric is the minimum resolvable angle, based on a complete response to minimum resolvable grid. Proximity to moult > 0: acuity measurement taken before nearest recorded moult, <0: measurement taken after nearest moult. Line of fit produced via local regression smoothing, with shaded area showing 95% confidence intervals. Graph produced using the ggplot2 R package (Wickham 2016). Zero on the x-axis indicates date of acuity measure, points below zero indicate the measure was taken after nearest moult, above indicate measure was taken prior to nearest moult.

These results were confirmed in modelling - simplification removed all fixed effects save size (Table 15 and Table 16). Number of optomotor grids present rather than MRA was used in modelling given its linear progression and normal distribution as well as improved residual fit.

Table 15: ANOVA statistics from the simplified model of crab acuity measured in the form of maximum number of grids visible at 130mm (used over MRA due to better model fit) in pattern change experiments.
Includes remaining fixed effect of crab size. A random effect of crab ID was included to account for repeated measures from individuals. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	F value	DF	p
Size	59.738	192.346	< 0.001
Model: lmer(MaxFullOpt ~ Size + (1|ID))			

Table 16: Summary statistics from the simplified model of crab acuity measures in the form of maximum number of grids visible at 130mm (used over MRA due to better model fit) in pattern change experiments.
Includes remaining fixed effect of crab size. A random effect of crab ID was included to account for repeated measures from individuals. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	Estimate	SE	t	p
(Intercept)	1.425	1.053	1.353	0.178
Size	0.450	0.058	7.729	< 0.001
Model: lmer(MaxFullOpt ~ Size + (1|ID))				

Total Pattern Energy change over time

There was a qualitative difference in change in total pattern energy (overall contrast summed across all crab pattern sizes measured) between background pattern sizes. Crabs on both small and large substrates increased overall pattern energy with time, while crabs on uniform changed less (Figure 18). Examples of crab pattern change can be found in Appendix 2B.

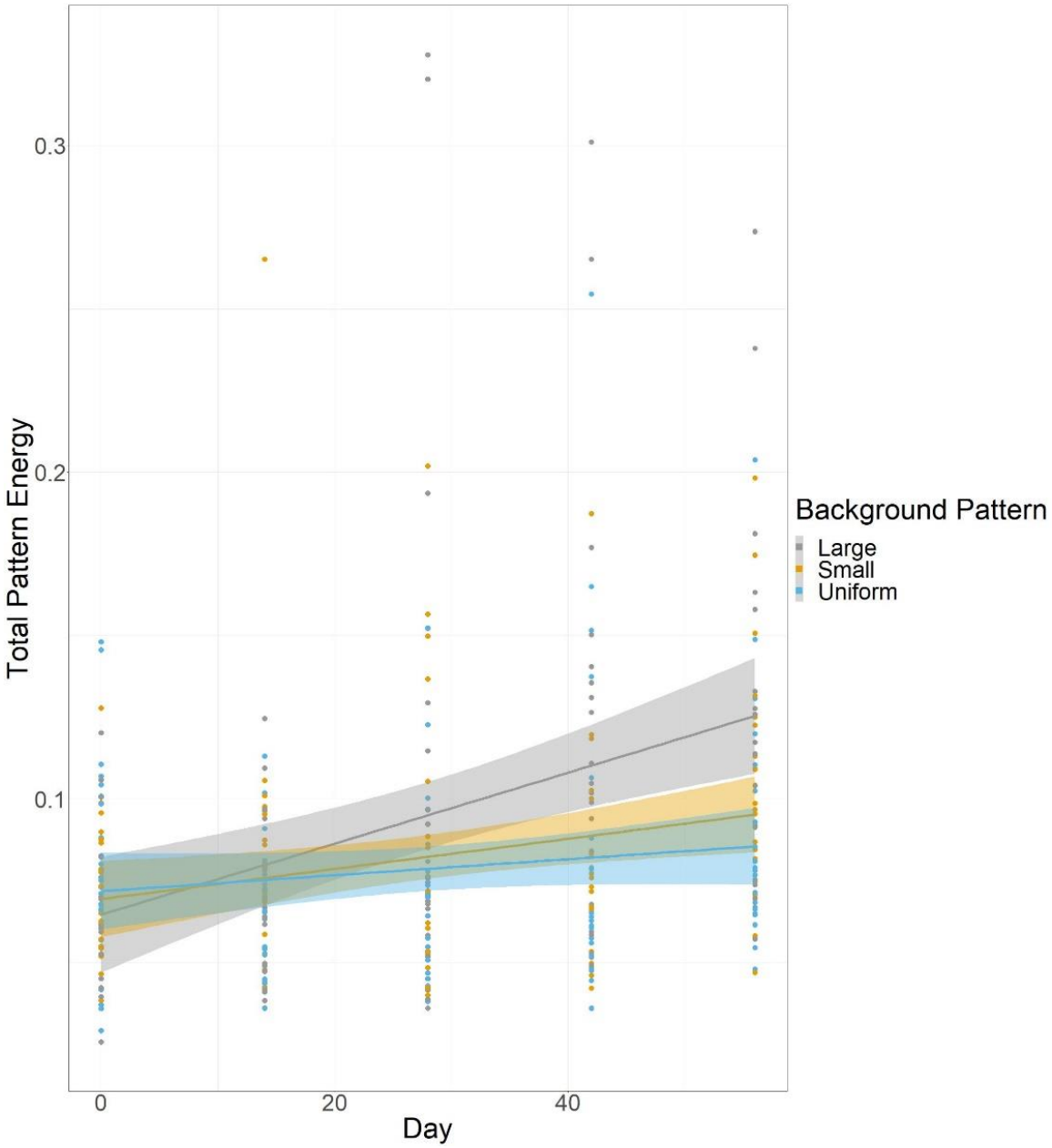


Figure 18: Total Pattern Energy (contrast) change of crabs against time from pattern change experiments.
Lines are linear regressions based on y ~ x. Colours correspond to background pattern. Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Shaded region corresponds to 95% confidence intervals. Graph produced using the ggplot2 R package (Wickham 2016).

Mixed effect models fitted to the total pattern energy measures confirmed the difference in total pattern energy. In the model fitted to longitudinal measures of Total Pattern Energy (log transformed for normality, Table 17 and Table 18), model simplification reduced the model to fixed effects of Day, Pattern Size, and

the interaction effect between the two although only Day and the interaction effect were found to have a significant effect on model fit.

Crabs on both large- and small-patterned backgrounds showed some increase in total pattern energy, while crabs on uniform backgrounds showed a negligible amount of change. For every day passed, total pattern energy increased by 0.00107 on the large-patterned background, 0.000495 on the small-patterned background, and 0.000245 on uniform backgrounds.

Table 17: ANOVA statistics from the simplified model of longitudinal data of log-transformed Total Pattern Energy (the sum of all contrast measurements at each pattern scale - sumPower) in pattern change experiments.
Includes remaining fixed effects of day, background pattern size and the interaction effect of the two. Includes a random effect of individual crab (ID) was included due to repeat sampling, and Filetype (RAW vs JPEG) to remove any variation from Photo file format. Also included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	**F value**	**DF**	**p**
Day	62.171	273	< 0.001
Pattern Size	0.411	126.086	0.664
Day:Pattern Size	8.178	273	< 0.001
Model: lmer(log(sumPower) ~ Day + P.Size + Day:P.Size + (1|ID) + (1|Filetype))			

Table 18: Summary statistics from the simplified model of longitudinal data of log-transformed Total Pattern Energy (the sum of all contrast measurements at each pattern scale - sumPower) in pattern change experiments.
Includes remaining fixed effects of day, background pattern size and the interaction effect of the two. Includes a random effect of individual crab (ID) was included due to repeat sampling, and Filetype (RAW vs JPEG) to remove any variation from Photo file format. Also included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	Estimate	SE	t	p
(Intercept)	-2.799	0.0745	-37.553	< 0.001
Day	0.0109	0.00144	7.648	< 0.001
P.SizeSmall	0.0600	0.104	0.575	0.566
P.SizeUniform	0.0954	0.107	0.895	0.372
Day:P.SizeSmall	-0.00512	0.002	-2.549	0.011
Day:P.SizeUniform	-0.0082	0.002	-3.994	< 0.001
Model: lmer(log(sumPower) ~ Day + P.Size + Day:P.Size + (1|ID) + (1|Filetype))				

Overall change in Total Pattern Energy

When comparing the overall change, crabs on both large and small patterned backgrounds tended to increase total pattern energy, while crabs on uniform substrates increased slightly, remained the same, or decreased pattern energy (Figure 19).

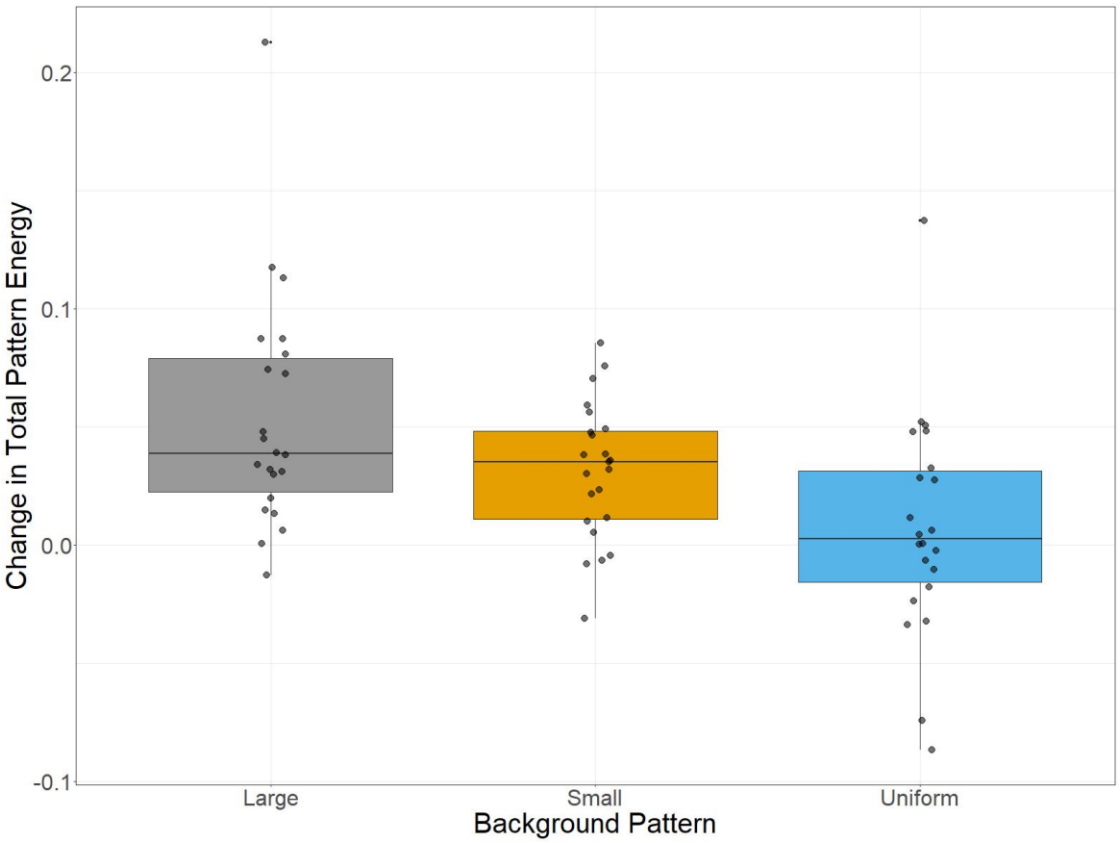


Figure 19: Overall change in Total Pattern Energy (contrast) of crabs over 56 days from pattern change experiments.
Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Graph produced using the ggplot2 R package (Wickham 2016).

When comparing overall change in total pattern energy, the same pattern was seen (minus the effect of day, Table 19 and Table 20). Crabs on large-patterned backgrounds increased pattern energy on average by 0.05010, crabs on small-patterned backgrounds by 0.03220, and crabs on uniform backgrounds by 0.00506. Post-hoc Tukey testing only found significant differences between the large-patterned and uniform treatments (estimate = 0.0461 ± 0.0129, $t_{63.1}$ = 3.585, p = 0.0019). No difference was found between large- and small-patterned treatments (estimate = 0.0214 ± 0.0129, $t_{63.6}$ = 1.660 p = 0.2287), and small-patterned and uniform treatments (estimate = 0.0247 ± 0.0128, $t_{63.3}$ =1.937, p = 0.1368.

Table 19: ANOVA statistics from the simplified model of overall change in Total Pattern Energy pattern in pattern change experiments.
Includes remaining fixed effect of background pattern size. Also, a random effect of Filetype (RAW vs JPEG) in case of effects of filetype on image analysis. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	F value	DF	p
Pattern.Size	6.376	65.287	0.003
Model: lmer(sumPower.chg ~ Pattern.Size + (1|Filetype))			

Table 20: Summary statistics from the simplified model of overall change in Total Pattern Energy pattern in pattern change experiments.
Includes remaining fixed effect of background pattern size. Also, a random effect of Filetype (RAW vs JPEG) in case of effects of filetype on image analysis. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	Estimate	SE	t	p
(Intercept)	0.050	0.011	4.404	0.033
P.SizeSmall	-0.0179	0.0124	-1.438	0.155
P.SizeUniform	-0.0450	0.0127	-3.549	< 0.001
Model: lmer(sumPower.chg ~ Pattern.Size + (1|Filetype))				

Change in Pattern Energy Difference over time

Crabs' background matching success varied significantly with background pattern (Figure 20). With regards to improvement over time, only crabs on large-patterned background showed any improvement in matching, while crabs on small-patterned backgrounds and crabs on uniform backgrounds showed little change, although some crabs in the latter increased pattern difference (reduced matching) from the background. Crabs on patterned treatment backgrounds matched significantly worse than those on uniform, regardless of timepoint. When compared to natural substrates (data derived from multispectral images from (Price et al. 2019), averaged over multiple photographs of each natural

substrate type), all crabs were a close match for the mudflat habitats (Figure 20). While crab matching was poorer (PED higher) on natural rockpool substrates, it was still noticeably better than matching on artificial patterns.

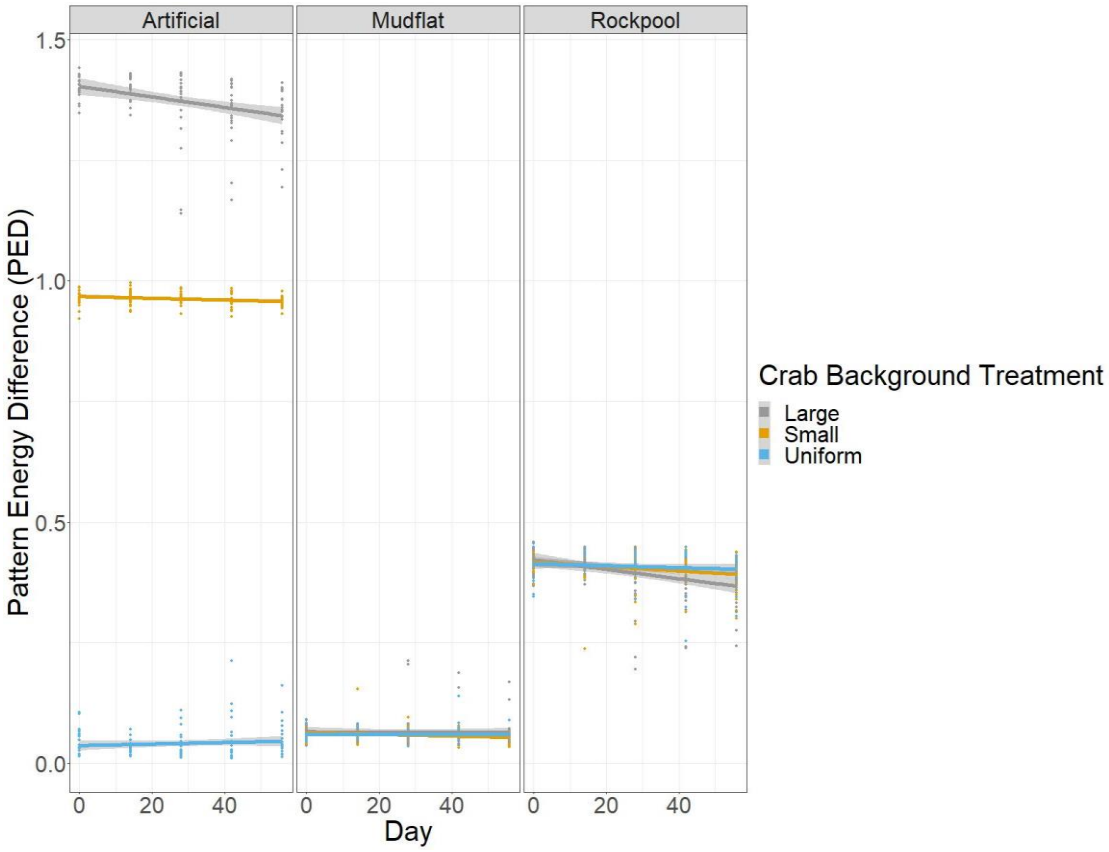


Figure 20: Change in Pattern Energy Difference of crabs compared to artificial and natural substrates against time from pattern change experiments. PED is the difference in pattern energy between crabs and a given background – higher values (e.g., Crabs on the large artificial background) indicate a poorer match between crab and background patterning. Data facetted into three groups. Artificial indicates crabs compared to their corresponding artificial substrate (i.e.: crabs placed on large backgrounds only compared to large background etc.) used in colour change experiments. All crabs from all treatments from this experiment were also compared to both natural substrates; mudflats (uniform) and rockpools (heterogenous) collected in past experiments. Colour corresponds to crab background treatment. Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Straight lines are basic linear regressions based on "y ~ x". Shaded areas correspond to 95% confidence intervals. Graph produced using the ggplot2 R package (Wickham 2016).

When longitudinal PED data was modelled (Table 21 and Table 22), pattern size regardless of day was found to have a significant effect on model fit alongside day and the interaction effect of Day and Pattern Size. Crab pattern matching was significantly worse on large-patterned backgrounds (Mean PED = 1.373 ± 0.058) than small-patterned and uniform, and worse on small-patterned backgrounds (Mean PED = 0.963 ± 0.014) than uniform (Mean PED = 0.041 ± 0.032).

Additionally, the change in matching over time varied between treatments (Table 21 and Table 22). Only crabs on large-patterned backgrounds showed any significant improvement, with PED reducing by approximately 0.00109 ± 0.00014 per day. In the other two treatments pattern matching changed only a small amount, with crabs on small-patterned backgrounds improving matching (PED decreases by 0.000188 ± 0.00019 per day), and crabs on uniform worsened matching (PED increasing by 0.000156 ± 0.0002 per day).

Table 21: ANOVA statistics from the simplified model of longitudinal data of Pattern Energy Difference in pattern change experiments.
Includes remaining fixed effects of Day, Background (P.Size) and the interaction effect of the two. A random effect of individual crab (ID) was included due to repeat sampling, and Filetype (RAW vs JPEG) to remove any variation from Photo file format. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	**F value**	**DF**	**p**
Day	21.802	265.000	< 0.001
P.Size	10147.990	128.296	< 0.001
Day:P.Size	21.781	265.000	< 0.001
Model: lmer(PED ~ Day*P.Size + (1|ID))			

Table 22: Summary statistics from the simplified model of longitudinal data of Pattern Energy Difference in pattern change experiments.

Includes remaining fixed effects of Day, Background (P.Size) and the interaction effect of the two. A random effect of individual crab (ID) was included due to repeat sampling, and Filetype (RAW vs JPEG) to remove any variation from Photo file format. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	Estimate	SE	t	p
(Intercept)	1.403	0.00694	202.343	< 0.001
Day	-0.001	0.000138	-7.865	< 0.001
P.SizeSmall	-0.436	0.00970	-44.893	< 0.001
P.SizeUniform	-1.367	0.00981	-139.352	< 0.001
Day:P.SizeSmall	0.000902	0.000193	4.675	< 0.001
Day:P.SizeUniform	0.00125	0.000195	6.383	< 0.001
Model: lmer(PED ~ Day*P.Size + (1|ID))				

Overall change in Pattern Energy Difference

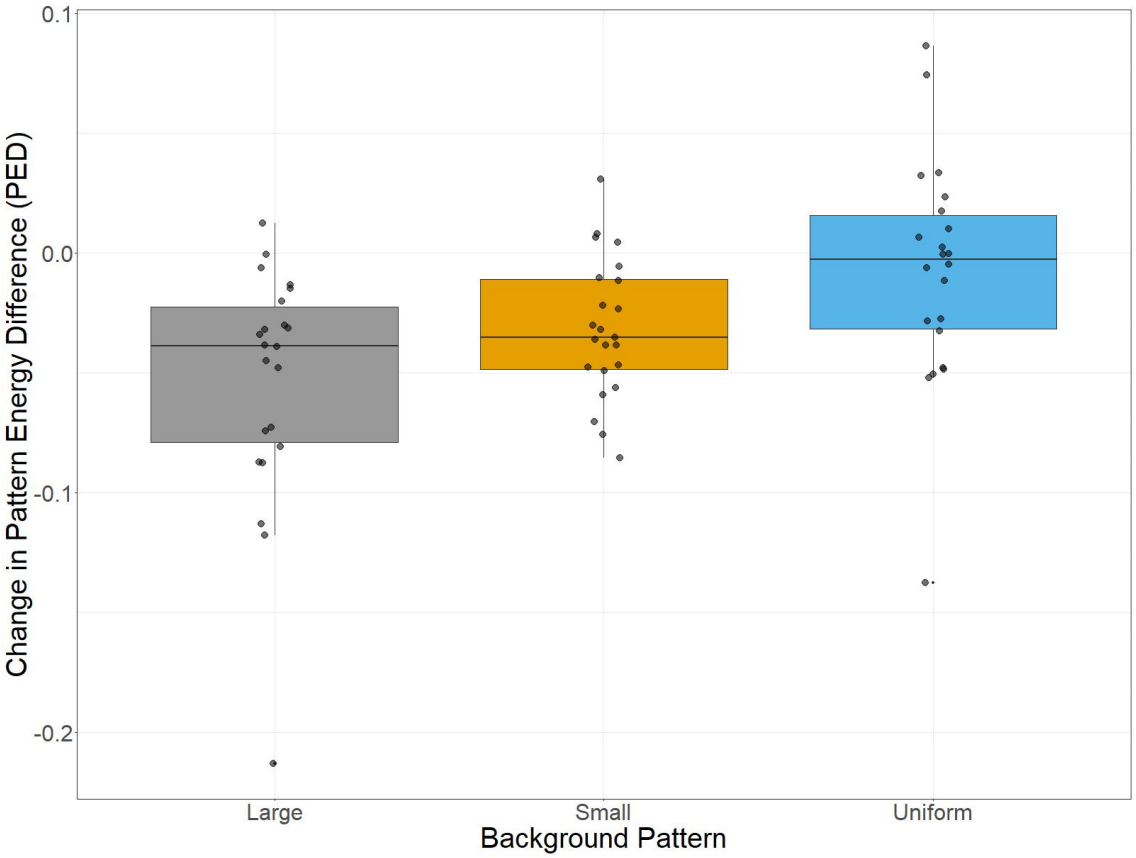


Figure 21: Total change in Pattern Energy Difference of crabs over 56 days from pattern change experiments.
Used to measure Pattern Matching (between crab and respective treatment background), as opposed to Figure 19 showing pattern contrast. Colour corresponds to background pattern. Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Graph produced using the ggplot2 R package (Wickham 2016).

Crabs on both large- and small patterned backgrounds showed improvement in pattern matching, while uniform showed little change (indicated by a decrease in PED, Figure 21). When modelling overall matching change, only background type remained after simplification (Table 23 and Table 24). Both crabs on large- and small patterned backgrounds improved pattern-matching overall, while crabs on uniform backgrounds worsened pattern-matching, but only by a negligible amount. Significant differences were only found between the large- and small-patterned treatment (estimate = -0.044 ± 0.0121, t_{64} = -3.619, $p < 0.001$) and the large-patterned and uniform treatment (estimate = - 0. 0569 ± 0.012, $t_{63.2}$ = -4.742, $p < 0.001$) via post-hoc Tukey testing. No significant

difference was found between crabs on small-patterned backgrounds and uniform backgrounds (estimate = -0.013 ± 0.012, $t_{63.5}$ = -1.092, p = 0.522). Crabs on large-patterned backgrounds reduced PED by -0.054 ± 0.050 on average, while crabs on small-patterned backgrounds reduced PED by -0.010 ± 0.016. Crabs on uniform backgrounds increased PED by 0.003 ± 0.04.

Table 23: ANOVA statistics from the simplified model of overall change in PED in pattern change experiments.
Includes remaining fixed effect of Background. A random effect of Filetype (RAW vs JPEG) in case of effects of filetype on image analysis. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	F value	DF	p
Pattern.Size	12.458	64	< 0.001
Model: lmer(PED.chg ~ Pattern.Size + (1|Filetype))			

Table 24: Summary statistics from the simplified model of overall change in PED in pattern change experiments.
Includes remaining fixed effect of Background. A random effect of Filetype (RAW vs JPEG) in case of effects of filetype on image analysis. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	Estimate	SE	t	p
(Intercept)	-0.0539	0.00846	-6.373	< 0.001
P.SizeSmall	0.0439	0.01183	3.708	< 0.001
P.SizeUniform	0.0569	0.0120	4.758	< 0.001
Model: lmer(sumPower.chg ~ Pattern.Size + (1|Filetype))				

Pattern size and distribution

When plotting pattern energy spectra (Figure 22, and Figure 23 with examples of crab coloration and spectra) energy at specific pattern sizes increased, but the pattern sizes that changed remained the same across all days. There is a significant difference between the average crab pattern energy at the start and the end of the experiment, and this difference varies between background types. This is most evident on the large backgrounds where several crabs increased patterning at close to, albeit slight smaller than, the dominant marking size of the background. However, the pattern sizes with peak energy did not change across the experimental period.

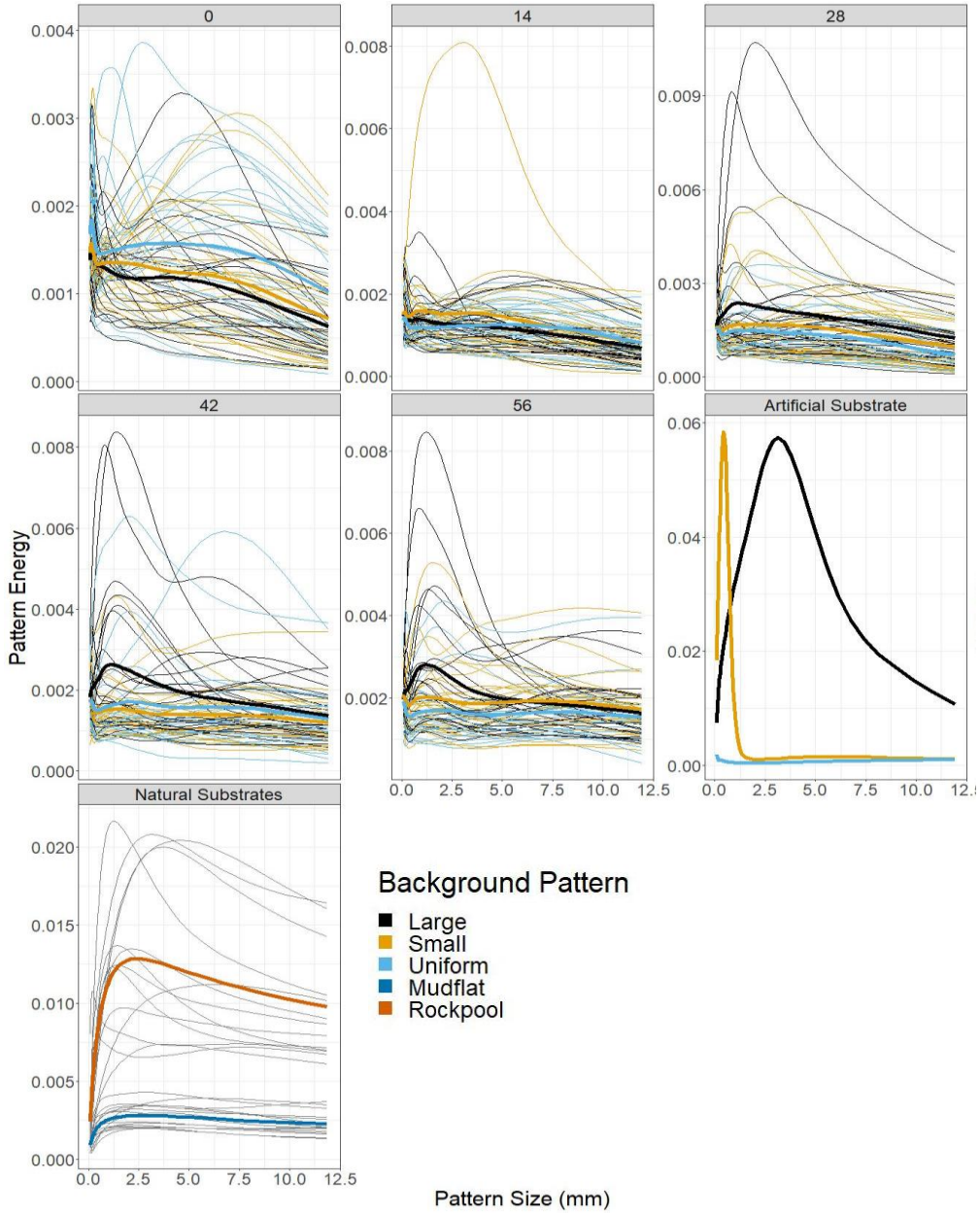


Figure 22: Crab Pattern Energy spectra faceted by day from pattern change experiments, with background pattern spectra of artificial substrates used in colour change experiments and a sample of both rockpool (patterned) and mudflat (uniform) substrates.

Natural background spectra derived from those used in (Price et al. 2019). Artificial Substrate sizes correspond to Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. NB.Y axis scales are free - peak energy of backgrounds is significantly higher than that of crabs and natural substrates. Bold lines are averages for each pattern at crabs at each timepoint or sample natural substrates. Graph produced using the ggplot2 R package (Wickham 2016).

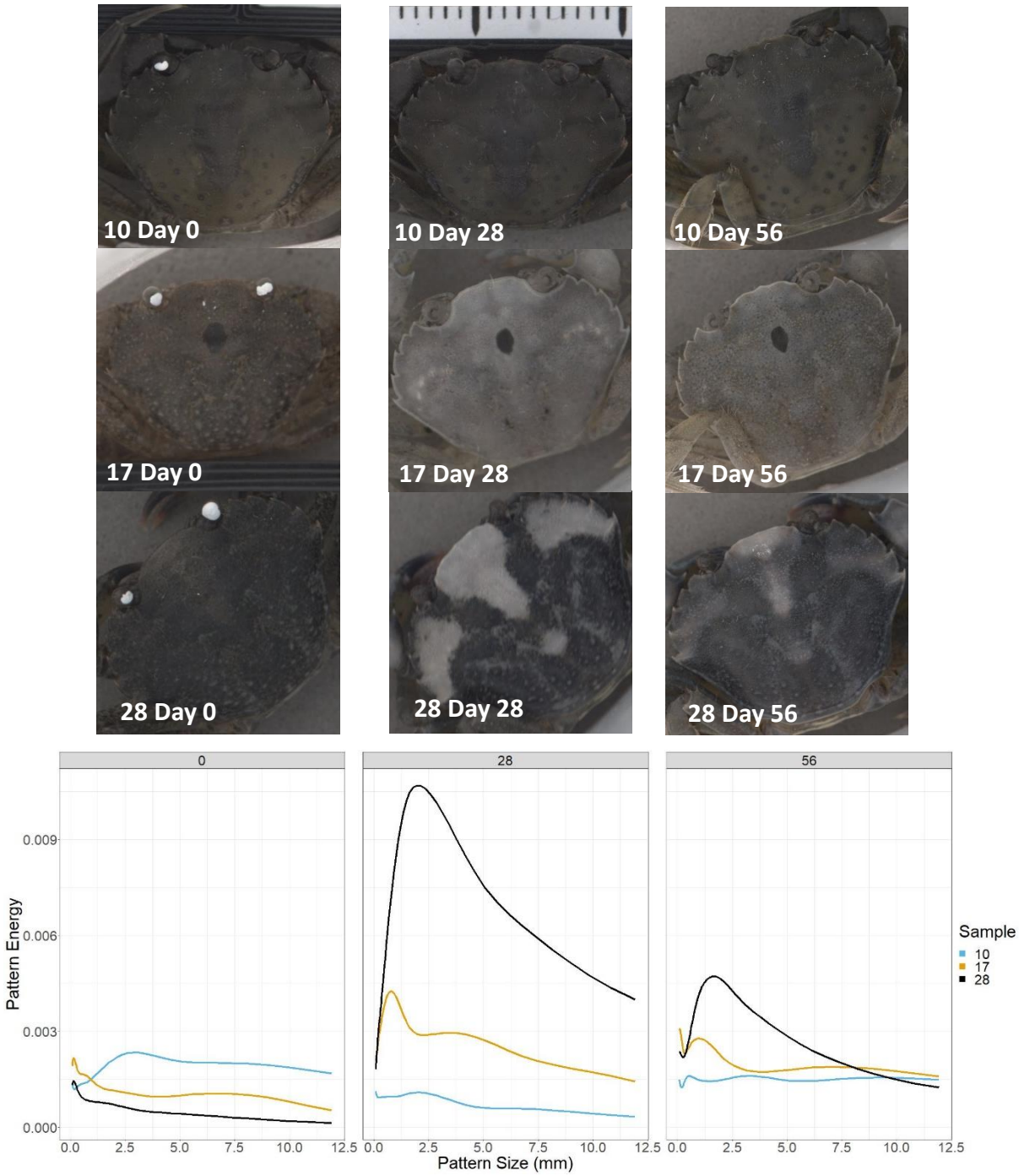


Figure 23: Examples of individual crabs' pattern energy spectra from pattern change experiments, with presentation images of the crabs from each treatment.

Samples used were: 28 from the Large-patterned background, 17 from the Small-patterned treatment, and 49 from the Uniform treatment. Spectra and images the start (Day 0), midpoint (Day 28), and end of the experiment (Day 56). Presentation images not to scale.

There was no discernible trend in the change in dominant pattern size. While individual crab's dominant pattern size changed between measurements, it seemed to do so at random. The models fitted against dominant pattern size and overall change in pattern size both simplified to a completely empty model, with none of the fixed effects explaining a significant amount of variation in pattern size. While individuals changed dominant pattern size, it appeared to be at random (Figure 24 and Figure 25).

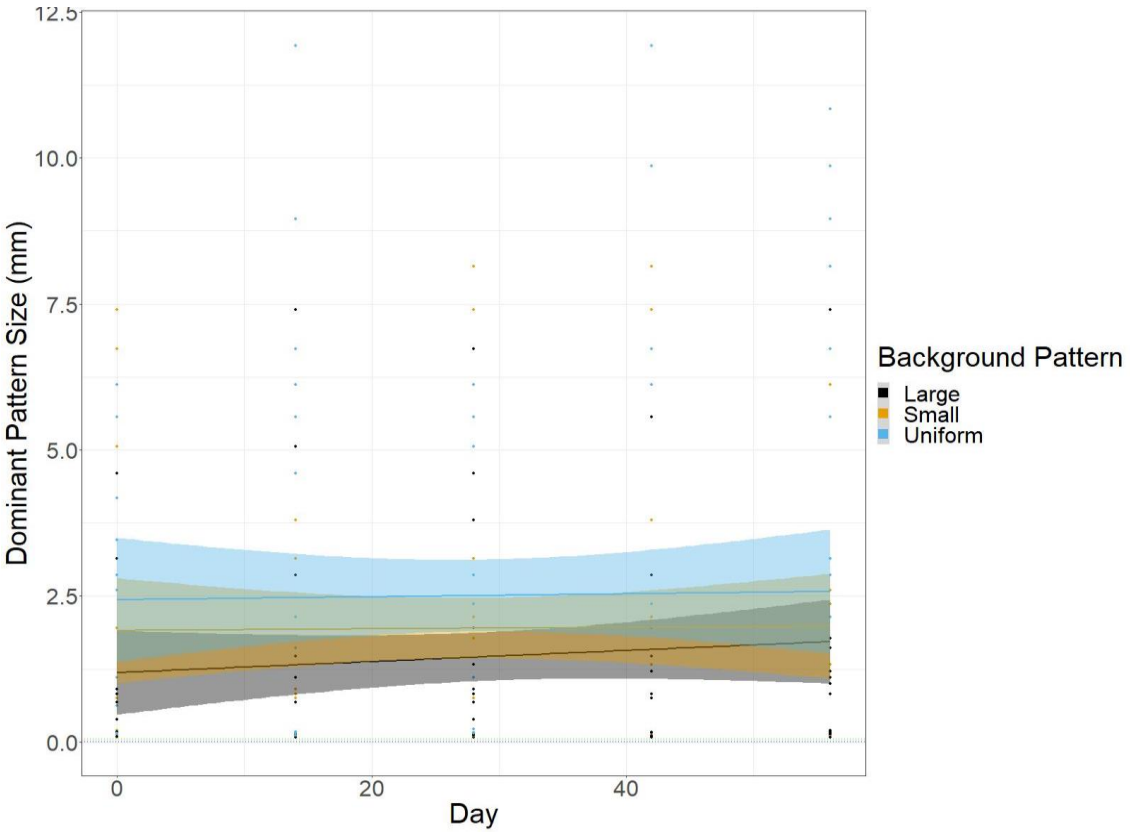


Figure 24: Change in dominant pattern size of crabs against time from pattern change experiments.

Dominant pattern size characterised as the pattern size with the highest energy (contrast) in mm. Colours correspond to background pattern. Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Lines are linear regressions with the formulae "y~x", and shaded regions are 95% confidence intervals. Graph produced using the ggplot2 R package (Wickham 2016).

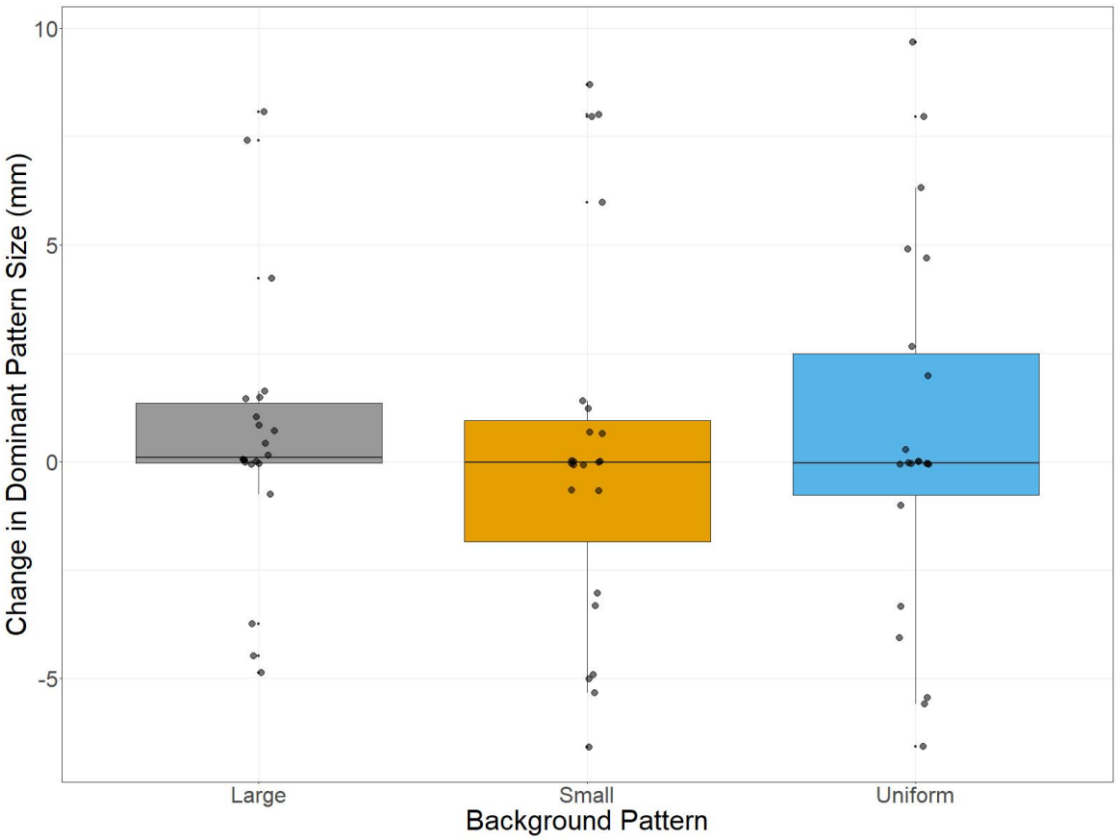


Figure 25: Total change in dominant pattern size of crabs over 56 days from pattern change experiments.
Dominant pattern size characterised as the pattern size with the highest energy (contrast) in mm. Colours correspond to background pattern. Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Graph produced using the ggplot2 R package (Wickham 2016).

While there was no noticeable association between the change in pattern size and the background crabs were placed on, there were noticeable trends in the patterning crabs developed. Of those which developed a distinct pattern, multiple crabs developed extremely similar patterns. For example, 11 crabs developed patterns (to a certain degree of contrast) comprising a pale triangular mark with the base of the triangle at the rostrum, coupled with white markings running below the rostral spines (see Figure 26 for examples).

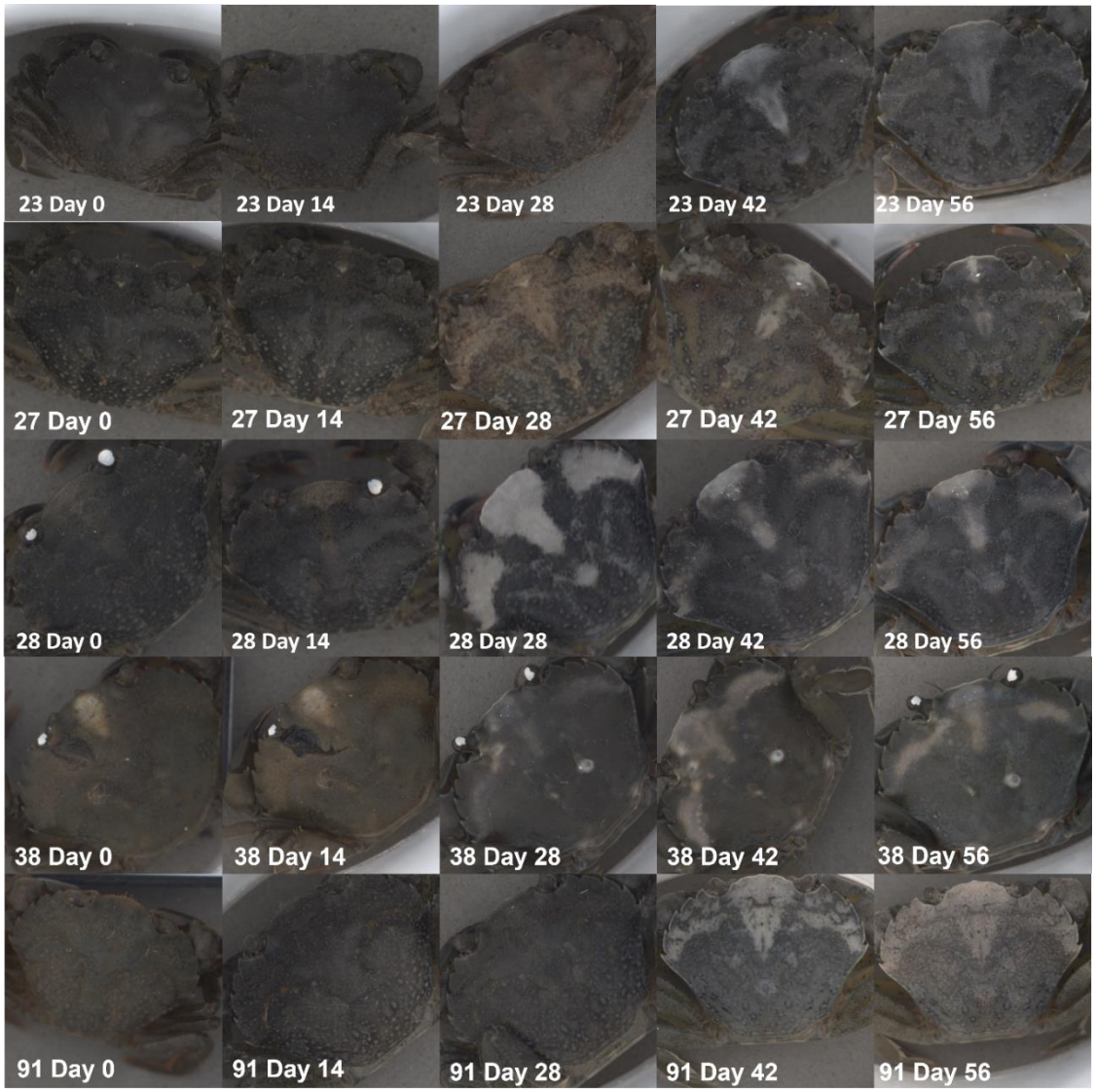


Figure 26: Examples of crab pattern similarity in pattern change experiments. Pattern characterised by a white triangular mark at the rostrum and white ridges either side.

All crabs came from the same starting location. Samples 23, 27, 28, and 38 were on large-patterned backgrounds, 91 was on the uniform background. Photos not scaled to one another. Presentation images (not raw files) made in imageJ (Schneider, Rasband, and Eliceiri 2012), in the MICA Toolbox (Troscianko and Stevens 2015).

Luminance change

Luminance increased over time for all pattern types (Figure 27 and Figure 28). It is worth noting that substrate luminance was not directly comparable when modelled under predator vision. While the patterned substrates both had a luminance ~0.3, the uniform substrate had a luminance closer to 0.15 despite being based on 50% brightness, equal A and B LAB colour generation, while the black and white pattern elements were based on 0% and 100% brightness respectively.

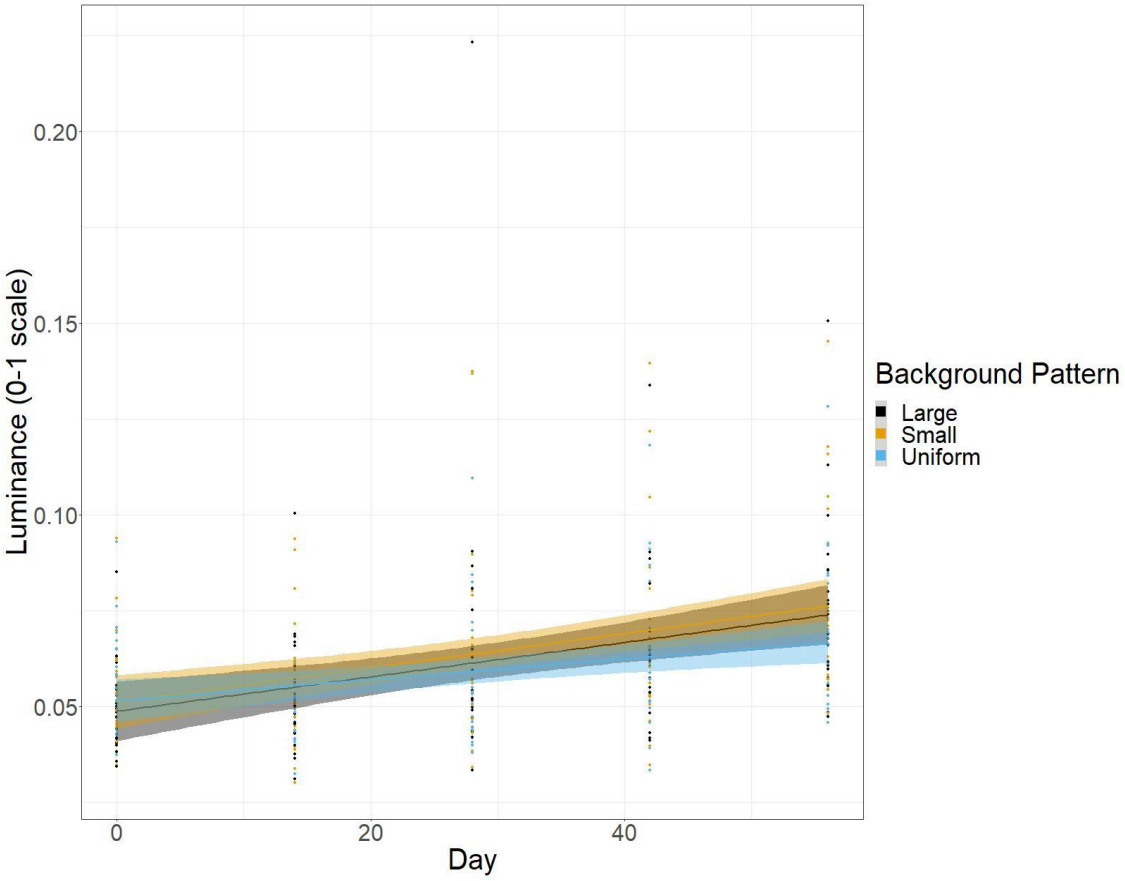


Figure 27: Crab luminance change over time from pattern change experiments, split by background pattern.

Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Lines are "y~x" linear regressions, shaded areas are 95% confidence intervals. Graph produced using the ggplot2 R package (Wickham 2016).

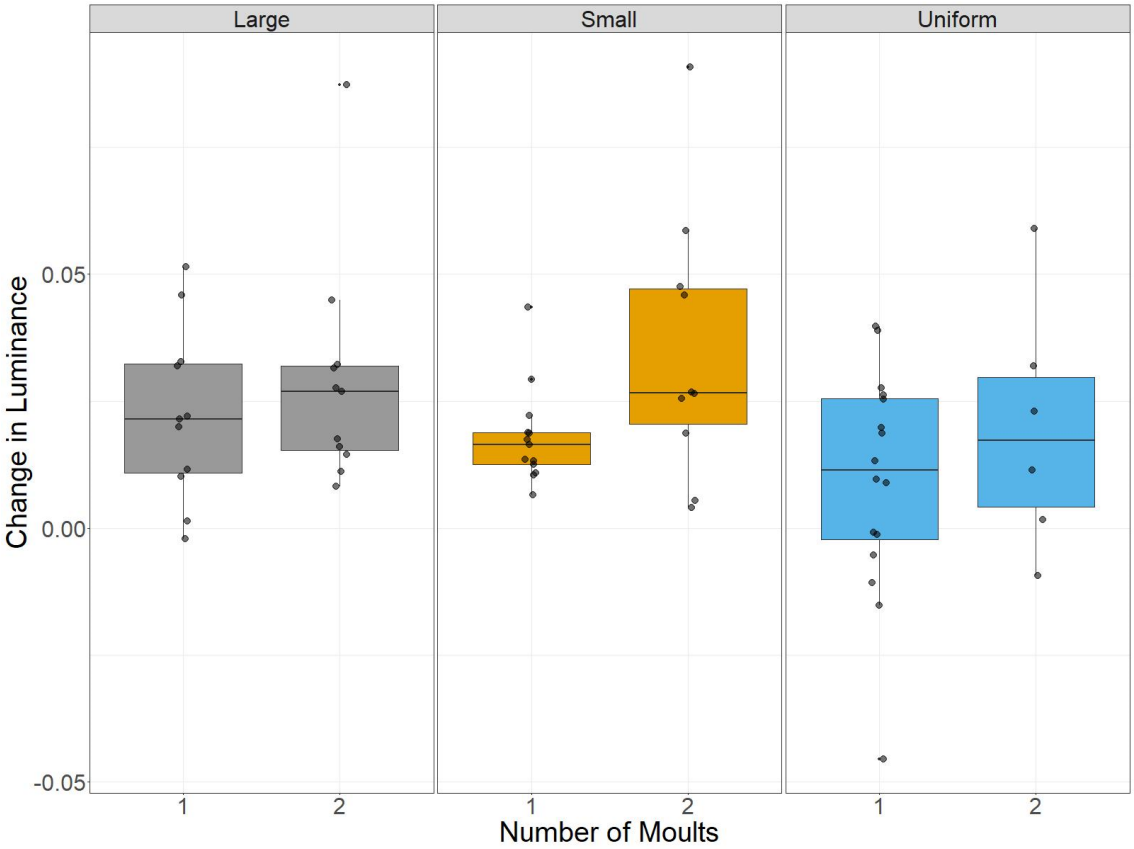


Figure 28: Total luminance change over 56 days based on number of times moulted from pattern change experiments.
Faceted by background type. Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Graph produced using the ggplot2 R package (Wickham 2016).

Regardless of this, no difference was found in luminance change between background patterns. Modelled longitudinally, the only factors that remained in the model were time, moult frequency and the interaction effect of the two. Moult frequency on its own was not significant, however. Luminance increased by 3.1 x 10 $^{-4}$ per day for crabs that moulted once, and this increased to 5.2 x 10 $^{-4}$ if the crab moulted twice (Table 25 and Table 26).

Table 25: ANOVA statistics from the simplified model of longitudinal data of luminance in pattern change experiments.
Includes remaining fixed effects of Day, moult frequency (moultcount) and the interaction effect of the two. A random effect of individual crab (ID) was included due to repeat sampling, and Filetype (RAW vs JPEG) to remove any variation from Photo file format. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	**F value**	**DF**	**p**
Day	97.118	266	< 0.001
moultcount	0.574	115.712	0.450
Day:moultcount	6.275	266	0.013
Model: lmer(Luminance ~ Day*moultcount + (1|ID) + (1|Filetype)			

Table 26: Summary statistics from the simplified model of longitudinal data of luminance in pattern change experiments.
Includes remaining fixed effects of Day, moult frequency (moultcount) and the interaction effect of the two. A random effect of individual crab (ID) was included due to repeat sampling, and Filetype (RAW vs JPEG) to remove any variation from Photo file format. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	**Estimate**	**SE**	**t**	**p**
(Intercept)	0.0491	0.00290	16.978	< 0.001
Day	0.000307	0.00005	5.789	< 0.001
moultcount = 2	0.00346	0.00457	0.758	0.450
Day:moultcount = 2	0.000209	8.34E-05	2.504965	0.013
Model: lmer(Luminance ~ Day*moultcount + (1|ID))				

Modelling total luminance change confirmed this, with model simplification removing all fixed effects save moult frequency. The average luminance increase for crabs that moulted once was 0.01570455 ± 0.01795689, while the average increase for crabs that moulted twice was 0.02907787 ± 0.02406707 (Table 27 and Table 28).

Table 27: ANOVA statistics from the simplified model of overall change in crab luminance in pattern change experiments.
Includes remaining fixed effect of moult frequency (moultcount). A random effect of Filetype (RAW vs JPEG) to remove any variation from Photo file format. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	**F value**	**DF**	**p**
moultcount	6.781	65	0.011
Model: lmer(Lum.chg ~ moultcount + (1|Filetype))			

Table 28: Summary statistics from the simplified of overall change in crab luminance in pattern change experiments.
Includes remaining fixed effect of moult frequency (moultcount). A random effect of Filetype (RAW vs JPEG) to remove any variation from Photo file format. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Source	**Estimate**	**SE**	**t**	**p**
(Intercept)	0.0157	0.00326	4.817	< 0.001
Moultcount = 2	0.0134	0.00514	2.604	0.011
Model: lmer(Lum.chg ~ moultcount + (1|Filetype))				

Discussion

Crab pattern change varied with background pattern size. Crabs on patterns large enough to be reliably resolved, increased their pattern energy (and therefor pattern contrast) the most, with some increase on smaller patterns at the limit of resolution, and little to no change on uniform substrates. Individual acuity had little to no effect on pattern change, with most variation in pattern

energy change being associated with background pattern size. Acuity did vary with size, with larger individuals possessing greater acuity, but no association between proximity to moult and acuity was found. There was no directional change in dominant pattern size, with crabs having fixed patterns that only changed in contrast between elements. Finally, luminance increased across all treatments with little difference between them. Greater moult frequency was found to increase the degree of luminance change.

While acuity did not seem to impact pattern change, it did follow the predicted trend of increasing with body size (Caves, Brandley, and Johnsen 2018; Feller et al. 2021; Snyder 1977). This raises an interesting question in relation to acuity, patterning, and body size. Patterning is most common and functional in smaller crabs, which are more dependent on overall camouflage, as well as specific pattern matching. Larger crabs, with higher acuity, should be less dependent on pattern perception, given their trend to a uniform/generalist camouflage strategy (Nokelainen et al. 2019). While larger eyes and increased acuity will likely provide a benefit in terms of distance resolution, smaller crabs will still be able to resolve patterns at proximity (i.e., those patterns in the background being matched), given the likelihood of small minimum focal distances. Smaller crabs will likely have smaller viewing distances than larger conspecifics simply due to their smaller limb size positioning them closer to substrates and widening the angle of resolution for background patterns. Therefor it seems as if acuity, while a potential limiter in other aspects of *C. maenas* visual ecology (e.g., distance viewing), has a reduced effect on pattern change.

No association between moult proximity and acuity measured was found, however. While some decrease in MRA was recorded immediately prior to moult, the relationship appears to be nonlinear. A key issue was lack of knowledge of prior moulting at the initial measurement. Each crab's nearest moult had to be taken as the first moult after initial measurement. This means that crabs could have potentially moulted immediately prior to collection, and subsequently had a higher acuity that would be linked to a later moult. Additionally, the time period investigated in (Baldwin and Johnsen 2011) is significantly smaller than that of this experiment, so any effect may be more clear when examined at that scale.

Change in total pattern energy corresponded to the size of patterns, rather than individual acuity. The greatest increase occurred on the large-patterned background, with some increase (or at least little to no decrease in patterning) on the small-patterned background, and either no change, or a decrease in energy on the uniform background. This is strong evidence crabs could resolve and respond to the differences in the patterned backgrounds, in the direction that reduced the difference between the crab's pattern and the background. The large-patterned background had the highest total pattern energy (1.468) and crabs on it increased the most, while the small patterned had less (1.026) with a smaller increase in crab pattern energy. Uniform backgrounds had little to no pattern (0.043) with little change in crab patterning. The lack of difference between crabs on small-patterned backgrounds and either of the other treatments makes it harder to infer the relationship between pattern change and acuity. The predicted outcome was that the crabs on the small pattern would respond in a similar fashion to the large pattern if they perceived the pattern, and the uniform background if not. However, given the difference in contrast between the treatments corresponding to the difference crabs' responses and the general increase in pattern energy of crabs on small-patterned backgrounds, it seems most likely that change (and the lack of significant difference between the small-patterned and other treatments) is a proportional response, rather than perception of the small, patterned background as uniform.

The use of Gabor filters is a novel method for analysis of the effectiveness of disruptive coloration by calculating the ratio of false edges (created by patterning) to real edges (that could be used in recognition/detection) (AKA GabRat (Troscianko, Skelhorn, and Stevens 2017)). While we did not conduct GabRat analysis (as used to analyse *C. maenas* disruptive patterns in the past (Price et al. 2019)), this has shown disruptive markings are more common in crabs from habitats of greater patterning (e.g. rockpools (Price et al. 2019)). There could be a threshold degree of background patterning, in contrast and-or size, that stimulates change from uniform to patterning (as seen in cuttlefish (Barbosa et al. 2008; Chiao and Hanlon 2001a; Chiao, Kelman, and Hanlon 2005)). These markings will likely have higher contrast (Cuthill et al. 2005; Schaefer and Stobbe 2006; Stevens and Merilaita 2009b; Stevens et al. 2009), raising pattern energy scores more than simple pattern matching (although

effectiveness may be reduced if levels of contrast exceed that of the habitat (Stevens et al. 2006)). Given crab pattern energy change was greatest on more patterned backgrounds (those similar to rockpool habitats based on the pattern energy spectra seen in Figure 22), it could be that crabs adopted disruptive markings (e.g. those patterns seen on Figure 26). The change between camouflage strategies should be confirmed by future GabRat analysis of the individuals from this experiment, however. Given the decrease in PED on both patterned treatments, and especially in the large-patterned treatment, background/pattern matching could be confounded with disruptive markings. Larger and more contrasting crab patterns would both increase disruption and be a better match for the larger high contrast background patterns. However, when crabs from this experiment were compared to natural backgrounds (Figure 20), pattern matching was significantly worse on rockpools than when crabs were compared to mudflats. The increase in pattern energy of the crabs on large-patterned backgrounds only improved pattern matching a small amount compared to natural rockpools backgrounds. In addition, past work examining locally adapted animals in mudflats and rock pools found crab patterns were a better pattern match compared to mudflats regardless of whether crabs were from rockpools or mudflats (Price et al. 2019). In addition, disruptive markings were more common (higher GabRat values) in rockpool crabs than mudflat samples, indicating that disruption is favoured in rockpool habitats versus background matching on mudflats.

While pattern energy change occurred on both the large- and small-patterned backgrounds, it was nowhere near enough to match the energy of the backgrounds themselves. Pattern energy difference remained high for both treatments, despite increasing overall contrast. Considering the background patterns were aimed at generating as high a contrast as possible, when compared to the spectra of natural backgrounds it is unsurprising that they exceed those of even the most patterned natural backgrounds. The difference in pattern matching between patterned treatments and the uniform treatment, regardless of time, is expected. Given crabs were collected from a uniform habitat, both the initial matching and lack of change on uniform backgrounds, as well as the overall mismatch of crabs on both patterned backgrounds is as expected (based on past work on *C. maenas* camouflage (Nokelainen et al.

2017; Stevens, Lown, and Wood 2014a; Price et al. 2019)). This was confirmed when crabs were compared to the pattern energy of natural spectra – crab-substrate PED was close to zero compared to mudflats, and significantly lower on rockpools than artificial substrates, with crabs on large-patterned backgrounds decreasing PED compared to rockpools. Improvement in pattern matching was minimal on small-patterned backgrounds, at odds with the overall increase in pattern contrast. It could be the result of an inability to perceive the pattern, instead perceiving it as a uniform grey (as with the uniform treatment). This is more likely because pattern elements that increased in contrast seemed to be larger than the grid size of the background. PED is based on the difference between crabs and backgrounds at all pattern sizes measured. The pattern sizes with the highest contrast of the small-patterned background ranged from 0.7mm upwards, noticeable higher than that of the crabs' patterns at the same size. There is a noticeable mismatch between the range of dominant marking sizes in crabs, and the dominant marking size of the small-patterned background. The mismatch between dominant pattern sizes of crabs and backgrounds is more likely to be the reason for the lack of matching improvements. As previously noted, crabs on the small-patterned backgrounds still increased contrast overall, and to a greater degree than those on the uniform backgrounds (albeit not to a statistically significant degree).

With regards to the distribution and size of pattern, there was no discernible directional change in the size of pattern (dominant marking size). Based on the pattern energy spectra (Figure 22 & Figure 23) mean energy increased without a change in distribution. If patterning changed beyond increasing energy, the distribution shape would be expected to change. What the spectra indicate is that crabs increased contrast of fixed patterns. Interestingly, the distribution of pattern energy for crabs on small patterned and unform backgrounds became noticeably similar (albeit with crabs on small-patterned backgrounds having a higher overall contrast). Crabs on large-patterned backgrounds tended to develop contrast most in pattern sizes ~1.25mm, but still increasing pattern contrast across all pattern sizes. There is little evidence that *C. maenas* can change patterns, simply changing to increase pattern contrast/conspicuous. This is theoretically less taxing in terms of resolution, even accounting for the loss of scene contrast with reduced acuity. If crabs can only change pattern

contrast (and presumably conspicuousness), they only need to detect pattern presence (including perhaps at a threshold contrast-to-size ratio). Future work could investigate for such a threshold by comparing responses on a finer scale of pattern sizes, with the addition of varying pattern contrasts. In species capable of rapidly redistributing pattern elements to cause drastic change in patterning, it is often a result of neuromuscular redistribution of pigment (e.g.: cuttlefish (Allen, Mäthger, Barbosa, et al. 2010; Barbosa et al. 2008; Hanlon et al. 2009; Williams et al. 2019) and flatfish (Kelman, Tiptus, and Osorio 2006; Akkaynak et al. 2017; Ramachandran et al. 1996)), outside the capacity of the crabs' more rigid colour change mechanisms. These pattern changes not only rely on background pattern size, but also size proportional to individuals (Barbosa et al. 2007). However, even in these more plastic species, there seem to be limitations the in variety of patterns that can be produced (e.g. flatfish are limited to certain patterns, augmented by background choice (Tyrie et al. 2015)).

This is unlikely to be as much of a disadvantage for *C. maenas* in nature as it was in this experiment. *C. maenas's* patterned habitats are not dominated by a specific pattern size, as shown by the lack of sharp peaks in the pattern energy spectra of the natural substrates (Figure 22). This links with past research showing that *C. maenas* has strong associations between camouflage strategies and habitat types (Price et al. 2019). In uniform habitats crabs rely on background matching, while disruptive markings are more common in more patterned habitats (e.g., rockpools) rather than pattern matching, which may be less effective given the high level of variation. If phenotypic plasticity is the mechanism of achieving differing camouflage strategies at these mesoscale habitat levels (as predicted in (Nokelainen et al. 2017; Price et al. 2019; Todd et al. 2012)), then crabs are unlikely to need to identify specific pattern sizes, just the presence or absence of background patterns, then adjust contrast to increase the conspicuousness of fixed patterns. Any microscale mismatch because of limitations in pattern change could likely be adjusted by background choice (as proposed in *C. maenas* by (Price et al. 2019; Nokelainen et al. 2017; Todd et al. 2012), and in other species changing pattern for camouflage, e.g. flatfish (Tyrie et al. 2015)).

Interestingly, there were trends of certain pattern distributions repeatedly arising on multiple crabs. These were most notable in the large-patterned treatments, although patterns were found across treatments (e.g., sample 91 in Figure 26). These patterns seem conserved over time – the example shown in Figure 26 was notably similar to those found in juvenile *C. maenas* in research from over 40 years ago (Hogarth 1978). Given the repeated expression of these patterns, both within populations and across generations, specific pattern distributions may be genetically coded. There has been some genetic characterisation of *C. maenas* morphology (Brian et al. 2006), although the amount of phenotypic variation linked to genetic variation was relatively small (~20%). However, this 20% could easily comprise the distribution of chromatophore types in specific patterns, with colour change simply raising or lowering the contrast between pattern elements, either through concentration/expansion of pigments with a given cell (physiological), or the generation and loss of pigments (morphological) . This links with the extensive research on polymorphism and crypsis. Prominent levels of polymorphism reduce predator success when hunting a cryptic population, with experimental evidence that visual predation favours both crypsis, and polymorphism in cryptic coloration (Bond and Kamil 2002). One of the key mechanisms is the prevention, or at least reduction, of predator search image formation is by reducing the frequency specific prey phenotypes and specific features are encountered (reviewed in (Bond 2007)). If crabs form the same pattern repeatedly then predators will repeatedly encounter it. These repeated encounters allow for learning of features of the patterns that allow for improved recognition and a decrease in camouflage effectiveness. Some genetic differentiation in patterning could help prevent this. If crabs are limited in the end pattern they can achieve, it could prevent them converging to an optimum but homogenous phenotype within a habitat, one which allows for easier predator learning. *C. maenas*' natural polymorphism has recently been shown to prevent search image formation, and improve crypsis effectiveness (Troscianko et al. 2021). Whether the polymorphism is the result of predators reliance on search images or searching on heterogenous backgrounds, or simply natural selection favouring a variety of cryptic colorations i.e.: disruptive markings in heterogenous habitats (Price et al. 2019) is less certain.

A second unexpected result was the development, but then loss of pattern contrast in multiple crabs. My expectation was that crabs would reach the maximum pattern contrast possible (given the maximally contrasting backgrounds), then maintain this to maintain matching. In our experiment, 23 out of 50 (46%) of the crabs on small-/large-patterned backgrounds, were found to initially increase pattern contrast, but subsequently decreased from peak contrast. This most likely relates to the demonstrated ontogenetic changes in crab coloration and colour change. Larger (and presumably older) crabs tend towards uniform coloration as it is the most effective camouflage strategy later in life (Nokelainen et al. 2019). Loss of pattern should (and does) happen regardless of background patterning, as crabs will not be remaining on patterned backgrounds throughout their life. This is the most likely explanation for the increase then decrease in pattern energy – starting crab size ranged from 10.4mm to 24.9mm, within the size range where pattern energy and the variation in energy rapidly falls (Nokelainen et al. 2019). It is therefore unlikely this has anything to do with visual cues, as ontogenetic changes in colour are non-reversible, often associated with predictable changes in conditions (or the result of phylogenetic inertia) (e.g. (Bueno-Villafañe et al. 2020; Nokelainen et al. 2019; Wilson, Heinsohn, and Endler 2007; Bulbert et al. 2017), reviewed in (Booth 1990)).

Luminance responses followed the exact predicted pattern – luminance increased towards the luminance of the substrate, and crabs that moulted more frequently improved the most. This is the ideal in terms of camouflage. Even if crabs could not successfully match the patterning of the background, or adopt a more useful strategy, they reduce the difference between the average luminance of the background they were placed on. While crabs failed to reach the luminance of artificial backgrounds, there are some potential reasons this is not the case. One is that crabs had not completed changing when the experiment ceased. Crabs will continue to moult beyond a period of eight weeks in the wild. There was no sign of a plateau in the increase in luminance, and crabs may simply have not achieved the maximum luminance possible. The alternative is that crabs were not capable of achieving the luminance of the backgrounds, at least not the brighter, patterned backgrounds. As we have previously stated, this chapter was not focussed on luminance contrast, simply

spatial acuity. We selected maximally contrasting patterns to ensure spatial information was the only factor affecting perception. Either may explain the difference, however artificial backgrounds were likely to be significantly brighter than natural ones (Price et al. 2019). As previously stated, natural *C. maenas* populations are unlikely to need to match the conditions of the artificial patterned backgrounds.

Moulting, despite having a significant effect on the change in luminance, was found to have no significant effect on *C. maenas*' pattern change. Crabs that moulted twice showed little difference in pattern change to those that moulted once. The specific reasons for this are unclear, although the shift towards uniformity both within and between individual crabs with size/age could explain this. Crabs that moulted twice increased size by a greater degree (average size change = 4.95mm) than those that moulted once (average size change = 3.69mm). Following (Nokelainen et al. 2019), these crabs would then tend to be less patterned, given the decrease in contrast with size. The decrease in contrast could counter the increase in contrast of other, smaller crabs moulting multiple times resulting in little difference in average patterning between crabs moulting once versus those moulting twice. Confirmation of this will require specific testing.

In conclusion, *C. maenas* pattern change is limited to adjusting the contrast of existing, apparently fixed pattern elements. These changes seem to be a response to differences in background patterning, with large patterns stimulating the greatest increase in contrast, while small patterns of similar contrast cause less change. While individual acuity was found to have no effect on pattern change, previous relationships between crab morphology and acuity were found. Acuity did increase with crabs' size as predicted, and while there was no linear relationship between acuity and the proximity to moult, crab acuity tended to be higher if it was measured soon after moulting. Change in patterning was not sufficient to significantly improve matching on artificial patterned substrates, but the change could be of use in less contrasting natural habitats. Additionally, the variability within the more heterogeneous natural habitats will mean a given crabs patterning is likely to fall within the habitat's own pattern range. Crabs on large-patterned backgrounds improved pattern matching significantly more than those on small-patterned and uniform

backgrounds. Although crabs on small-patterned backgrounds did not improve matching enough to be distinct from those on the uniform backgrounds, this was most likely due to crabs apparently fixed pattern elements being larger than those of the background leading to mismatch, rather than an attempt to match a uniform background. Overall change in contrast was not significantly different from crabs on large-patterned backgrounds nor from uniform backgrounds. It appears acuity does not limit pattern change, likely due to the mechanics of compound eye vision, and the close viewing distance of backgrounds. Based on natural associations of crab camouflage and environmental variation, it seems likely that *C. maenas* used phenotypic plasticity to shift between background matching on uniform substrates, and disruptive markings on varied substrates. This seems to only require the identification of the presence of background patterns, not the identification of pattern size or shape. *C. maenas*' acuity is sufficient for this, allowing effective camouflage responses despite the limitations of *C. maenas'* ability to change pattern and compound eye resolution.

Chapter 4: Illumination and substrate perception for colour change

Abstract

To effectively match backgrounds, animals should benefit from detecting differences between themselves and the backgrounds. This could be facilitated by other, indirectly related information, but often is the result of direct visual detection of differences While the sensory capabilities of visual organs are key in substrate perception, external factors will influence what is perceived. Illumination affects the light reflected by substrates, and ultimately what light reaches photoreceptors. A key question is: how do animals perceive substrates when changing colour for camouflage, especially when illumination affects substrate appearance? One suggestion is there is some comparison of illumination (downwelling light) to radiance (upwelling light) to assess substrate reflectance. I tested the roles of illumination and substrate reflectance on brightness change for camouflage in shore crabs, by comparing change across controlled levels of downwelling and upwelling light. Crabs were placed in one of three treatments and any colour change responses were recorded via digital image analysis. The first treatment consisted of unrestricted illumination and black gravel, resulting in high overall intensity and illumination, but low relative reflectance. The second treatment reversed this, restricting illumination by half and using white gravel to produce a treatment of lower overall intensity and radiance, but high relative reflectance. Finally, I created a third treatment where a highly reflective substrate and reduced illumination was mimicked by using illumination of matching intensity from above and below through a transparent substrate. This resulted in an overall light intensity equal to that of the high intensity treatment, while maintaining a ratio of upwelling to downwelling light comparable to the white gravel. While the power of the experiment was limited by high mortality, several trends were noted. Save one outlier, all crabs experiencing high intensity and low reflectance darkened, indicating overall illumination intensity does not affect coloration (in line with past experiments). Those crabs on the other two treatments increased brightness significantly, when change occurred. Patterns of brightness change were similar in these two treatments, indicating the shared high relative reflectance influenced brightness change, as the greater radiance in the upwelling light treatment caused no difference between them.

Introduction

For many species, accurately matching backgrounds is key for camouflage (Dimitrova and Merilaita 2014; Merilaita 2003; Merilaita and Stevens 2011; Michalis et al. 2017; Stevens and Merilaita 2011; Troscianko, Skelhorn, and Stevens 2018, 2017). Effective background matching has been experimentally shown to have a direct role in survival (Duarte, Stevens, and Flores 2018; Mynott 2019; Hultgren and Mittelstaedt 2015). While accurately perceiving these substrates can be particularly important for species that can change their appearance, many animals employing camouflage should benefit from the same, to position themselves on substrates that match body coloration. Given variation within habitat types, individuals should find and position themselves on sections of the habitat that best match their brightness, colour, and-or pattern (Kang et al. 2014; Kang et al. 2012; Stevens and Ruxton 2019; Eacock et al. 2019; Green et al. 2019; Stevens et al. 2017; Uy et al. 2017). This is often dependent on accurate perception of the substrate, and this perception is based at least partially on the light reflected from substrates. The characteristics of this reflected light are dictated primarily by two factors; the illumination generating the reflected light, and the properties of the substrate that affect reflection. The former provides the total gamut for what can be reflected by the substrate and the latter how much of the illumination is reflected (both wavelengths and intensity). Illumination can therefore cause the light reflected by substrates to vary both spatially (e.g. dappling and caustics (Cuthill, Matchette, and Scott-Samuel 2019)) and temporally (day-night, seasonal shift etc. (Nilsson and Smolka 2021)). A key question is how do animals correctly perceive different substrates for matching, when illumination affects the light they reflect?

For the purposes of this chapter, the light stimuli involved can be split into three distinct, but interrelated parts. First, is the overall light intensity of the environment. This is a direct function of illumination and determines the radiance of a substrate. The radiance of a substrate is the amount of energy (light) from the substrate, striking a surface (in this case the eye of the observer), from a given direction at a given overall light intensity. Finally, is the relative reflectance. This is the proportion of light that a substrate reflects, also known as the albedo. While the radiance of a substrate will fluctuate with illumination intensity (Stevens et al. 2007), the relative reflectance of substrates,

i.e., the fraction of illumination radiating from the substrate, should remain constant across illumination. The assumption is that animals are responding to relative reflectance of substrates, over the substrate radiance or overall environmental light intensity (Duarte, Flores, and Stevens 2017; Stevens 2016; Brown Jr and Sandeen 1948; Gamble and Keeble 1900). This is because of the constant nature of relative reflectance ensuring matching across illumination will result in matching regardless of changes in illumination.

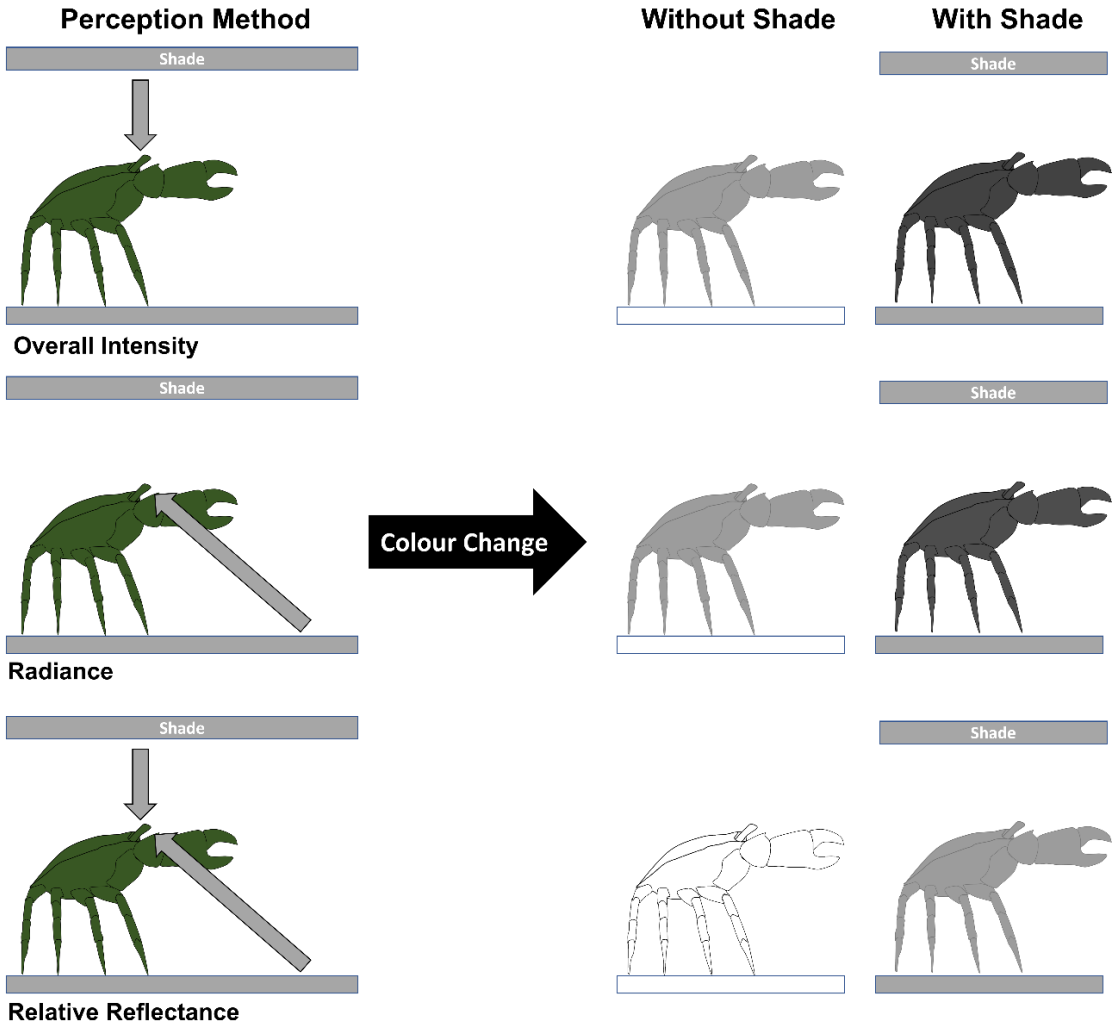


Figure 29: Diagrams of the predicted results of colour change based on different substrate perception methods.
Example is based on a white substrate (bar below crab model) which is shaded in the left hand panel. Arrows represent direction and source(s) of light information used in substrate perception.

If an individual were to change in response to overall light intensity, or only to the absolute intensity of light from the substrate, then camouflage may not be effective. In the former, while background substrates will appear darker or

lighter with illumination intensity, there is no guarantee of specific matching, especially if substrates are at extreme ends of brightness (black versus white). If individuals respond to the radiance of a substrate (especially without self-assessment), there is the risk of the subsequent effects of illumination altering individual radiance beyond that of substrates (see Figure 29 for diagrams of each of these effects).

The broad results of colour change experiments support this assumption, with change only occurring when substrate and body coloration are different, resulting in matching when illumination and reflectance are standardised (Akkaynak et al. 2017; Bedini 2002; Duarte, Stevens, and Flores 2018; Eacock et al. 2019; Green et al. 2019; Stevens 2016; Stevens, Lown, and Denton 2014; Stevens, Rong, and Todd 2013; Stuart-Fox, Whiting, and Moussalli 2006). In direct comparisons of environmental light and substrate reflectance, species only respond when there is a mismatch between substrate reflectance and themselves, not in response to changing light levels (e.g., as demonstrated in grasshoppers (Burtt 2009)). Additionally, cephalopod species seem capable of successful colour change for camouflage across varying light conditions (e.g. camouflage success in *Sepia apama* at day (Zylinski et al. 2011; Hanlon et al. 2011) and at night: (Allen, Mäthger, Buresch, et al. 2010)).

The mechanisms of assessing the appearance substrates that are used by animals changing colour are less clear. Luminance (also referred to as lightness) constancy is the process by which species reliably perceive equally reflective substrates as such, regardless of the effects of illumination on the quantity of light they reflect. There is evidence of species other than humans a affected by illusions relating to brightness perception, where animals misinterpret the brightness of a stimulus based on lighting or shadow cues (e.g., triggerfish and the lightness cube illusion (Simpson, Marshall, and Cheney 2016)). These situations, where substrate coloration and brightness are not reliable cues, may be where colour/lightness constancy is useful in accurate perception and subsequent colour change responses. While little research into the processing allowing luminance constancy outside of humans has been conducted (although there is some evidence of constancy manifesting in early parts of visual cortex (V1) processing e.g., in cats (Macevoy and Paradiso 2001)), specific environmental information should still be needed for species

accurately judging substrates reflectance. There is evidence that colour constancy is achieved in invertebrate systems by receptors adapting to the mean intensity of light at any given time ("von Kries constancy" e.g., in stomatopod crustaceans (Osorio, Marshall, and Cronin 1997)). Perhaps this adaptation allows for luminance constancy as well, as all receptors as a whole adapt to overall intensity, as opposed to each receptor type adapting to wavelengths of light they are maximally sensitive to. Alternatively, the intensities of directional light could be used to infer substrate reflectance, especially in monochromatic species where only a single photoreceptor sensitivity is in use. In simple situations, assuming light from above is illumination and reflected light comes from the substrate below, then an understanding of how illumination itself has changed is not needed. Simply the difference in intensities of directional light (how much less light is upwelling than downwelling) should be enough to judge relative reflectance and provide some element of luminance constancy. With regards to both colour change, and compound eye vision, there is evidence that differential stimulation in different regions of the eye could provide accurate information on substrate brightness for colour change, for examples restricting light to the lower hemisphere of the eye (by covering it) in stick insects causes a reduction in brightness (Bückmann 1979).

One investigation of the effect of directional light on colour change for camouflage is that of Sumner and Keys into the colour change behaviour of marine flatfish (Sumner and Keys 1929). Two experimental treatments were used – one where a darker substrate was brightly lit from below the resting point of the fish with weak incident light, and the other with a paler substrate lit from above but angled away from the light source to appear darker. This resulted in fish becoming paler of on the dark substrate with a higher ratio of upwelling to downwelling light, and fish becoming darker in the treatment with a lower ratio of upwelling to downwelling light. What is needed is confirmation of the phenomena, or whether alternative strategies are used in ensuring substrate matching regardless of illumination. One such alternative may be self-assessment and comparison of individual reflectance to that of the substrate, rather than responding only to light from substrates. A difference between body and substrate could be perceived, and colour change could act in the direction that reduces this difference till perceived matching occurs. This is limited by the

individual's field of view – part or all the camouflaged body regions may be out of sight of the individual.

As previously stated, shore crabs are already a valuable species in the study of camouflage (Nokelainen et al. 2017; Price et al. 2019; Stevens, Lown, and Wood 2014a; Todd et al. 2006; Todd et al. 2012; Troscianko et al. 2021), and colour change to achieve concealment (Carter, Tregenza, and Stevens 2020; Nokelainen et al. 2019; Stevens 2016; Stevens, Lown, and Wood 2014b). While they apparently lack colour vision (Chapter 1), correct perception of substrate brightness is still needed, especially at the subadult stage where camouflage and colour change is crucial (Nokelainen et al. 2019). Even in rockpools when disruptive camouflage is used instead of background matching (Price et al. 2019), luminance matching is still important as it can improve the effectiveness of disruptive markings (Stevens et al. 2006). Given the intertidal habitats crabs occupy, there will be significant variation in illumination (both wavelength and intensity), with a subsequent effect on substrates. Previously mentioned shifts in illumination with seasons, time of day, and weather, or other effects including predictable changes in tides (and subsequently depths of substrates) can occur. There are also less predictable changes, such as saturation of suspended material (e.g. plankton and dead material in the water column) and algal cover, which can affect the light environment (Cummings and Johnsen 2007) and subsequently the appearance of backgrounds. Their requirements for colour change for camouflage are similar to those of species already examined with regards to illumination and substrate perception, both in the slow/morphological change of the ghost crab and grasshopper models (Stevens, Rong, and Todd 2013; Burtt 2009), as well as the intertidal and epibenthic habitat (and associated illumination) of the flatfish (Sumner and Keys 1929). Additionally, while the structure of their compound eyes allows for a wide field of vision, it is possible they lack an adequate view of the carapace on their cephalothorax. Given this is the predominant region visible and in need of camouflage, self-assessment may be less useful, and accurate substrate assessment more important, as crabs may be unable to see how change affects matching.

In this experiment, I tested the relative importance of overall light intensity versus directional light cues in substrate perception for colour change for camouflage. To do this I created three experimental treatments:

1. Unrestricted illumination, with black gravel, resulting in high overall intensity and low substrate radiance and relative reflectance
2. Restricted light, with white gravel, resulting in low overall intensity and lower substrate radiance, but higher relative reflectance
3. Restricted illumination, with matched upwelling light through a transparent substrate, resulting in high overall intensity and substrate radiance, with matched upwelling : downwelling light to mimic a high substrate relative reflectance.

Given *C. maenas*' lack of significant colour change (based on (Stevens, Lown, and Wood 2014b) and the results of Chapter 1), I focussed on achromatic change and illumination/reflectance intensity over colour. Crab brightness change on each of these treatments was recorded and compared using digital image analysis. My prediction being that if *C. maenas* changes in response to overall intensity then brightness increase should be greatest on treatments one and three. If substrate radiance is used, then crabs in treatment two should change to less than relative reflectance of the white gravel, and crabs on treatment three should reach a similar brightness to the upwelling light alone. If relative reflectance is being used then crabs in treatment two should increase brightness to match the substrate under any illumination, and crabs on the upwelling light treatment should increase brightness significantly as well.

Methods

Sample Collection

Samples were collected from the mudflats in Penryn, UK (Lat: 50.169, Long: -5.099). Individual crabs were collected by directly searching beneath flotsam along the mudflats, then hand collecting crabs and placing them in individual containers to prevent intraspecific conflict. Crabs were preferentially sampled based on size, with cephalothorax width ranging from 10-15mm. This was chosen as this size class was large enough to house in the lab, whilst being small enough to retain plasticity in coloration (Nokelainen et al. 2019)). Samples were then immediately transported back to the Sensory Ecology Laboratory at the University of Exeter's Penryn Campus. Crabs were then transferred into saltwater tanks made up with Aquarium Systems Instant Ocean salt at a salinity of 31 ‰. Crabs were individually housed in the 65mm diameter screw top containers used in Chapters 1 and 2, on a dark substrate (black aquarium

gravel) with a similar brightness as the mudflats they were collected from till initial photographs were taken and the colour change experiment began. The water was maintained at 18°C, and monitored for salinity and nitrogen (nitrate, nitrite, and ammonia) content. Water was changed weekly to maintain water quality, and animals were fed two Hikari Crab Cuisine pellets once every two days.

Two batches of crabs were run through the experiment, one in June to September of 2020 and again in January to March of 2021. This was done due to COVID-19 control measures restricting lab access, limiting hours available at any one time as well as restructuring of planned timelines. The two batches were run through the same containers, tank, lights (arranged in the same way), and protocol.

Experimental Set Up

The lighting regime remained at 12-hour light-dark intervals with light from 0700 to 1900, however underwater LEDs were also placed along the base of the tank to provide upwelling illumination. These LEDs were controlled via a timer to match the overhead lighting regime. While the over-tank lighting ramped up and down illumination, gradually brightening and darkening to mimic sunrise and -set, the under-tank lighting was on a simple on-off timer. 0.3 ND filters were used to restrict light by 50%. This allowed matching of overall intensity between the black (100% incident illumination) and light (50% incident and 50% upwelling light). Above tank lights were run at 78% of the maximum output, set via the lighting systems built in controls. This was done as it was shown to be the best intensity match for the waterproof LEDs used for upwelling lighting at full power (upwelling: luminance: 375 cd/m^2 and radiance: 1.83 W/(sr*sqm), downwelling: luminance: 375 cd/m^2 and radiance: 1.21 W/(sr*sqm)). Measurements were taken using a Jeti specbos 1211-2, at the distance each light source would be from the crabs' position in the container. Different in-tank lighting was used instead of matching the specific overhead light models below the tank due to concerns over suspending the tank over lights, both due to tank weight and stability, as well as the positioning of non-protected electrical equipment below a water source.

Unlike the other colour change experiments, this experiment used specially made containers (Figure 30). These were constructed using two 60mm lengths

of 75mm by 75mm square black PVC tubing placed on top of each other, separated by a 75mm by 75mm piece of transparent acrylic which the substrate rested on. For the black treatment, the same size transparent acrylic was placed on top to act as a lid to contain crabs. For the white and light treatments, a piece of acrylic was also used, but coated with a film of ND filter to reduce incident light by half consistently. Each container also had a base on bottom surface of the container. For the white and black gravels these were the same transparent acrylic used for the resting surfaces, with white and black waterproof paper coatings respectively to block any upwelling light. The light treatment had an acrylic square with ND filter which allowed light from underwater LEDs running over the base of the tank to up-well into the container. All acrylic lids and bases had a translucent white polyurethane film to diffuse light, especially important in the upwelling light treatment to minimise specular diffraction through the transparent substrate. Every container had 1mm holes drilled into the tubing, either side of the acrylic divider, to allow for water flow in the absence of a mesh top. Photographs and diagrams of the experimental containers can be found in Figure 30.

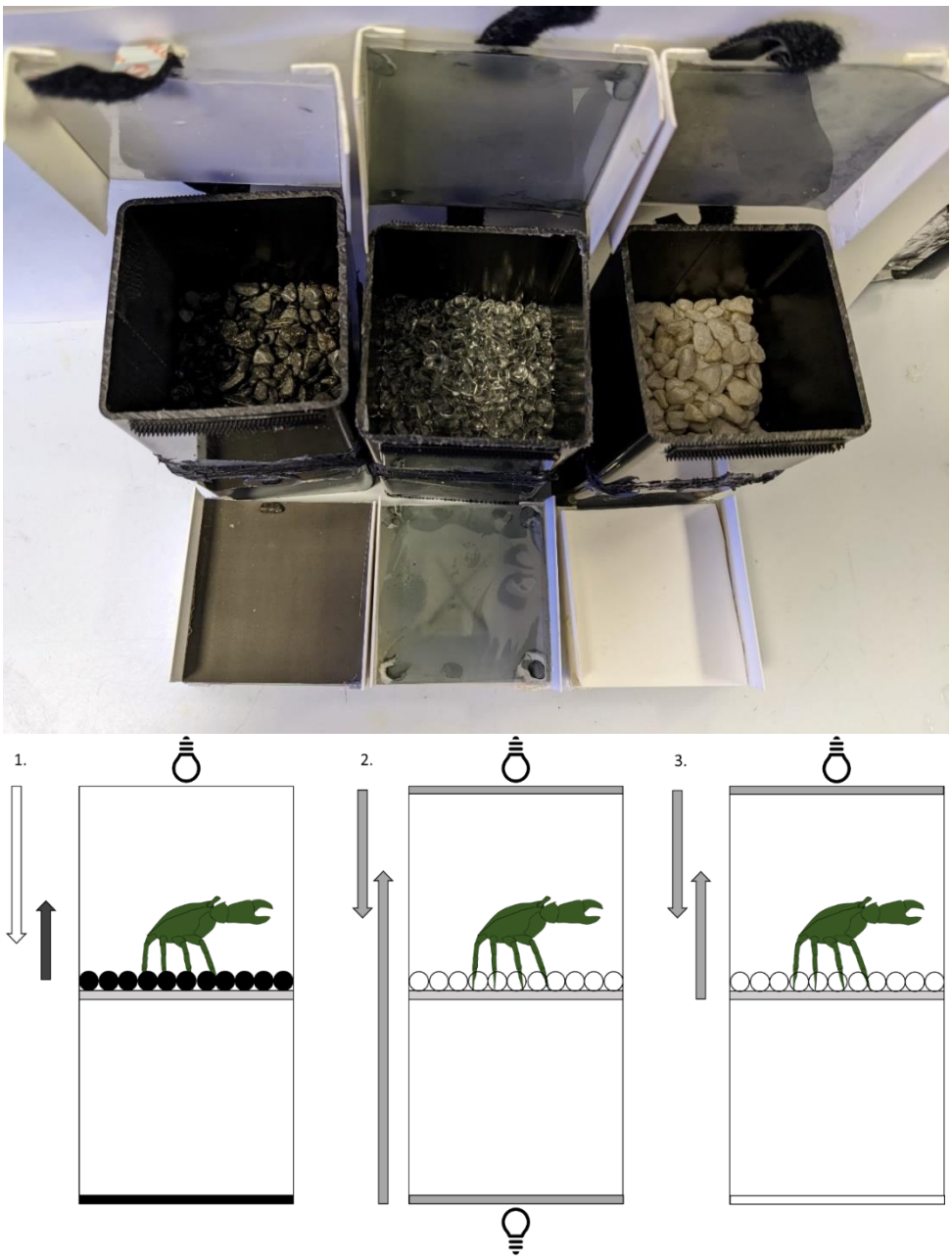


Figure 30: Photographs and diagrams of containers for substrate perception experiments.

Top: Photograph examples of sample containers, with lids and bases exposed. From left to right: Left - the high intensity - low reflectance treatment, with black gravel substrate, only the polyurethane diffuser on the lid, and an opaque black base (in case individuals burrowed to the acrylic divider. Middle; the high intensity – high reflectance treatment, with transparent glass substrate, and the translucent polyurethane diffuser and neutral density filter on both the lid and base. Right, low intensity-high reflectance treatment, with white gravel as substrate, the lid has the same polyurethane diffusor and ND filter as the light based treatment, but an opaque white base for the same reason as the black treatment. ND filters were placed on the inside surface of the diffuser, directly

against it for both lids and bases where used. The three bases have an “X” marked on the bottom for this photo, to demonstrate the transmission of light is only present in the in the upwelling light treatment. Bottom: Cross section diagrams of the containers. Positions correspond to photograph. Lines indicate directional light, with shading representing approximate intensity.

Once photographed, crabs were placed into the top half of the container, pre-lined with the treatment’s gravel (black, white, and transparent respectively). Crabs remained in these containers for the duration of the ten weeks, with the lids only being removed during feeding, cleaning (which took place as the same time as photography), and for photography. This was done to minimise the time individuals were exposed to non-treatment lighting conditions.

Crabs were housed in these containers for 10 weeks, to allow for sufficient colour change. Given the unusual stimuli, an extra two weeks (one additional photo) was added to the experimental protocol compared to previous experiments to maximise any potential change. This deviation from prior experiment duration was done to account for any atypical colour change behaviour under the novel lighting conditions.

Digital Photography

Beyond the previously mentioned extended duration (and the cleaning protocol mentioned below), the experiment followed the protocol of the previous two chapters (Figure 4, as well as past work on *C. maenas* camouflage using digital image analysis (Carter, Tregenza, and Stevens 2020; Stevens 2016; Stevens, Lown, and Wood 2014b)). At the start of the experiment and every two weeks after, crabs were removed from containers in the MS lab darkroom and placed inside a PTFE ring that acted as a light diffuser. Illumination, camera, and filters were the same as those used in prior experiments, along with a 97% and 3% reflective standard plus scalebar placed alongside the crab. A total of six sets (one visible and one UV every two weeks for 10 weeks including the experimental start date) of photos were taken for each crab. Digital image analysis was used as it has proven to be a suitable and efficient alternative to spectrometry. Photographs of the two naturally reflective substrates were taken in the same fashion.

Container cleaning happened immediately after photography, individuals were placed in an unoccupied container which was identical to their treatment within the experimental tank, whilst the original container was cleaned of any algal build up. Following this both the container and the substrate were rinsed, before the substrate was returned to the container, the container to the tank, and the crab to the container (along with food if photography happened on feeding days). This was done to prevent any algae build up obscuring the container lid (or base/resting substrate for the upwelling treatment). Ethical approval for the experiment was obtained via successful application to The University of Exeter's e-ethics system in 2020 (e-ethics application number: eCORN002250 v2.1).

Image Analysis

As with the digital photography, the image analysis followed the methods of the previous chapters. The photos were imported into imageJ software (Schneider, Rasband, and Eliceiri 2012), using the Multispectral Image Calibration and Analysis (MICA) Toolbox to create linearised images due to nonlinear camera sensor responses to light (Troscianko and Stevens 2015). Images were also equalised using the photostandard to correct for difference in light conditions between photographs taken at different times. Regions of interest were selected, specifically the scale bar on the photo standard, a straight line across the widest point of the carapace (between the rear most anterolateral teeth) for size measurement, and finally an area of the crab's carapace (specifically the cephalothorax) for colour analysis. Images were then converted from camera colour space (sensitivity of camera setup detailed in (Stevens, Lown, and Wood 2014b; Troscianko and Stevens 2015)) into relevant predator vision systems via a polynomial mapping function, in this case a tetrachromatic avian predator (Common Peafowl, *Pavo cristatus* -LW, MW, SW, and VS/UV, along with a double-cone (DBL) luminance channel (Hart 2002)) as an analogue for gull predators (Crothers 1968). Only avian predators (specifically the luminance channel) were used as past research (Nokelainen et al. 2019; Price et al. 2019) (as well as the first chapter of this thesis) has shown that the differences in brightness perception (luminance) of the visual models used in this project are negligible. As such only tetrachromatic predators were used to simplify analysis. Graphs of results from alternative visual models can be found in the Appendix

3A . Luminance values were produced on a scale of 0-1 and were compared to the luminance values of substrates.

Statistical Analysis

Data was transferred from imageJ as Comma Separated Value files into R Statistical Software (ver. 3.5.2). For ease of analysis data was converted from bi-weekly photometric data to a single value of luminance change, obtained by subtracting the starting colour values from the final colour value collected. All data manipulation used either base R code, or commands from the tidyverse package (Wickham et al. 2019). Raw luminance values were used for simplicity and both longitudinal data (for comparison of rate of change) as well as single values of overall change were fitted into mixed effect models (using the LME4 package (Bates et al. 2015), with post hoc test values derived from lmerTest (Kuznetsova, Brockhoff, and Christensen 2017)), with fixed effects of day (only in the longitudinal model), treatment, moult frequency, and the associated interaction effects . Finally crab identity (only in the longitudinal model) and repeat number (only in the total change model) were included as random effects, the former to control for repeated measures across the experiment, the latter to control for any small variations in environmental factors between batches sampled at different times of year. While transformation and alternative error structures were tested, none seemed to improve model fit, and therefore a gaussian error structure was used with untransformed data. Un-simplified model output can be found in Appendix 3C.

Candidate model for longitudinal data: *lmer(Luminance ~ Day*Treatment*Moult.Freq + (1|ID), na.action = na.omit, data = Long.DF)*

Candidate model for overall change: *lmer(Luminance.Change ~ Treatment*Moult.Freq + (1|Runthtough), na.action = na.omit, data = Change.DF)*

During experiments, higher than normal levels of crab mortality occurred. During experiments numerous sterilisations of tanks did not reduce mortality levels, and as such disease was lowered as a suspected cause. Most deaths seemed to occur in the two high reflectance treatments (white and light). Survival analysis was conducted to assess if experimental treatment had an impact on the rate of mortality.

Results

Of the 60 crabs sampled over the two experimental periods, 42 survived the full 10 weeks, with the remaining 18 apparently dying mid moult (with at least separation of the rear of the cephalothorax, more commonly partial or full emergence from the old carapace). No evidence of disease or tank failure was found. Of those that survived, 15 were from the black gravel treatment, 12 from the light treatment, and 15 from the white gravel treatment. Of these, 22 moulted once, 16 moulted twice, and four (all from the light treatment) did not moult at all. Examples of brightness change can be found in Appendix 3B

Luminance change over time

Significant differences in luminance change responses were found between white and black gravel treatments. Crabs on the black gravel treatment tended to decrease luminance slightly, by 0.00016 per day, while those on the upwelling light treatment increased luminance slightly, by 0.00029 per day. The greatest response was seen on crabs on the white gravel treatment, which tended to increase luminance by 0.00079 per day (Figure 31), more than twice the rate of change of crabs on the light treatment. In addition, these crabs on white increased brightness the most out of any experiment in this thesis (caveated with the differences in experimental runtime and as well as the different substrates used, barring one outlier crab in the black treatment detailed below). There were some deviations from this, however. A single crab on the black treatment moulted once, dramatically increasing its luminance to the highest value achieved by any crab in this experiment. Additionally, multiple crabs on the light treatment increased brightness, significantly more than other crabs in both their own treatment and the white gravel treatment.

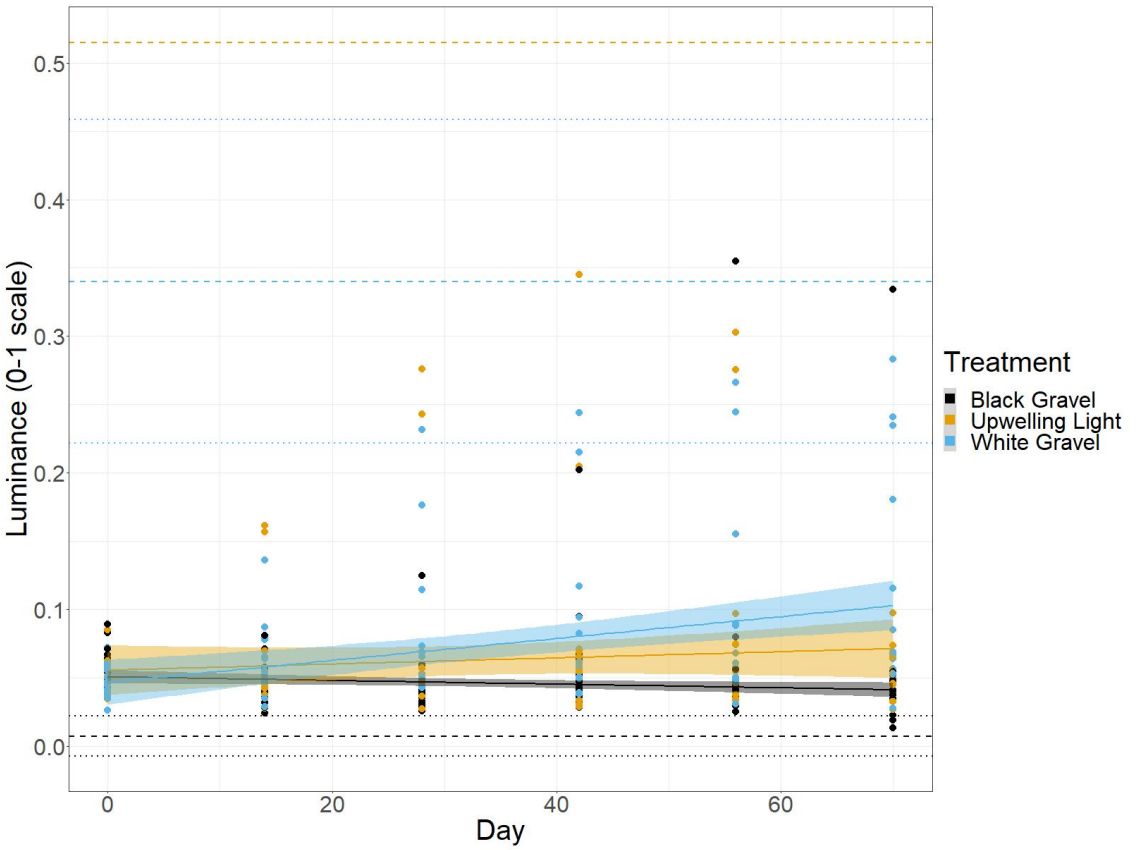


Figure 31: Luminance values for crabs over time from substrate perception experiments.

Colours correspond to lighting treatment. Straight lines produced via luminance ~ time linear models, with 95% confidence intervals. Lines and confidence intervals do not include one sample - B2, isolated as an outlier. B2 was included on the plot and can be seen as the highest black point on days 28 and 42, and the highest point on days 56 and 70. Horizontal lines indicate luminance values for white (blue lines) and black gravel (black lines) respectively. Dashed lines equal the mean treatment luminance, dotted lines ± std. deviation. NB Black gravel deviation cannot be below 0, as 0 indicative of complete absorbance (no reflectance). The upwelling light treatment only had the mean plotted as the std. deviation spanned beyond the limits of luminance scores. It is included simply to demonstrate that even when the white is well lit (via arc lamp, significantly brighter than over than lights even without restriction), the light treatment will be perceived significantly brighter, given radiance will be constant regardless of incident light. Graphs plotted with ggplot2 (Wickham 2016).

Modelling confirmed this, once the single crab on black that changed brightness significantly was removed as an outlier. The longitudinal model (including day as a fixed effect, see methods) retained all fixed effects and both two way interactions post simplification. Of the fixed effects, only Day ($F_{1, 268.922} = 4.56$, $p = 0.0339$), and the interactions between day and treatment ($F_{2, 263.439} = 14.13$, $p < 0.001$) and day and moult count ($F_{1, 267.476} = 11.56$, $p < 0.001$) were found to significantly effect model fit. Crabs on black gravel tended to decrease brightness, compared to upwelling light (estimate = 0.00067, se = 0.00019, $t_{263} = 3.45$, $p < 0.001$) and white gravel treatments (estimate = 000092, se = 0.00018, $t_{261.9} = 5.17$, $p < 0.001$) which both increased. There was no difference between the upwelling light and white gravel treatments (estimate = 0.00025, se = 0.00019, $t_{265.8} = 1.29$, $p = 0.1998$). Increased moulting increased the rate of brightness change (regardless of direction) (estimate = 0.00043, se = 0.00013 , $t_{267.5} = 3.4$, $p < 0.001$) (Table 29 and Table 30).

Table 29: ANOVA statistics from the simplified model of change in luminance over time in substrate perception experiments.
Includes remaining fixed effects of day, treatment and moult frequency, as well as the interactions of day and treatment, and day and moultcount.A random effect of crab ID was used to account for repeated measures. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison.

Effect	**F value**	**DF**	**p**
Day	4.5457	268.922	0.0339
Treatment	0.0959	82.920	0.909
Moult Frequency	0.0469	83.363	0.829
Day:Treatment	14.132	263.452	< 0.001
Day:Moult Frequency	11.559	267.476	< 0.001
Model: lmer(Luminance ~ Day*Treatment + Day*MoultCount + (1|ID)			

Table 30: Summary statistics from the simplified model of change in luminance in substrate perception experiments.
Includes remaining fixed effects of day, treatment and moult frequency, as well as the interactions of day and treatment, and day and moultcount. A random effect of crab ID was used to account for repeated measures. Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison. Light was automatically set as the reference level.

Source	Estimate	SE	t	p
(Intercept)	0.0568	0.0188	3.018	0.0034
Day	-0.000495	0.000273	-1.816	0.0705
Black Gravel	-0.00153	0.0139	-0.110	0.912
White Gravel	-0.00566	0.0136	-0.417	0.678
MoultCount	-0.00193	0.00890	-0.217	0.829
Day:TrtBlack	-0.00066	0.000193	-3.448	< 0.001
Day:TrtWhite	0.000250	0.000194	1.285	0.200
Day:Moultcount	0.000434	0.000128	3.400	< 0.001
Model: lmer(Luminance ~ Day*Treatment + Day*MoultCount + (1|ID)				

Luminance change and moult frequency

Because of high mortality, overall colour change sample numbers are low. As a result, I used data from discrete changes in colour following moulting, as well as time. This allowed individuals which successfully moulted within the experiment, but did not survive until the end, to be included in the experiment. Average change on the high intensity and low reflectance (black gravel) was a decrease in brightness of 0.012, on the low intensity and high reflectance (white gravel) was an increase of 0.056 , and on the high intensity and reflectance (light through glass substrate) was an increase of 0.021. There was significant variation in change in both the white gravel and upwelling light treatments. Overall luminance change in the white treatment ranged from -0.017 to 0.23,

and from -0.047 to 0.26 in the upwelling light treatment (negative values indicating a decrease in brightness, and vice versa). There was significantly less variation luminance change in crabs on the black gravel treatment, which ranged from -0.049 to 0.017 (excluding the outlier which increased luminance by 0.26).

Those crabs that moulted more (once or more for the white treatment, and more than once for black gravel and upwelling light treatments), showed significantly greater change (Figure 32). Black and white treatments showed consistent directional change with moult frequency. Crabs on black gravel that moulted once decreased luminance by 0.010 on average, while those that moulted twice decreased by 0.014 on average. Crabs on white showed little to no change without moulting; decreasing by (0.0031 on average), those that moulted once increased luminance by 0.062 on average, and those that moulted twice increased by 0.061 on average. The upwelling treatment also showed little change without moulting, decreasing luminance by 0.005 on average. Those that moulted once also showed little change, increasing by 0.0096, but the three crabs that moulted twice increased luminance by 0.12 on average. One individual changed ended as brightest crab in the experiment barring the outlier crab from the black treatment, (final luminance = 0.30, total change = 0.26), significantly higher than the brightest crab on white (final luminance = 0.28, total change = 0.23), although the crab in the light treatment died prior to the final measurement.

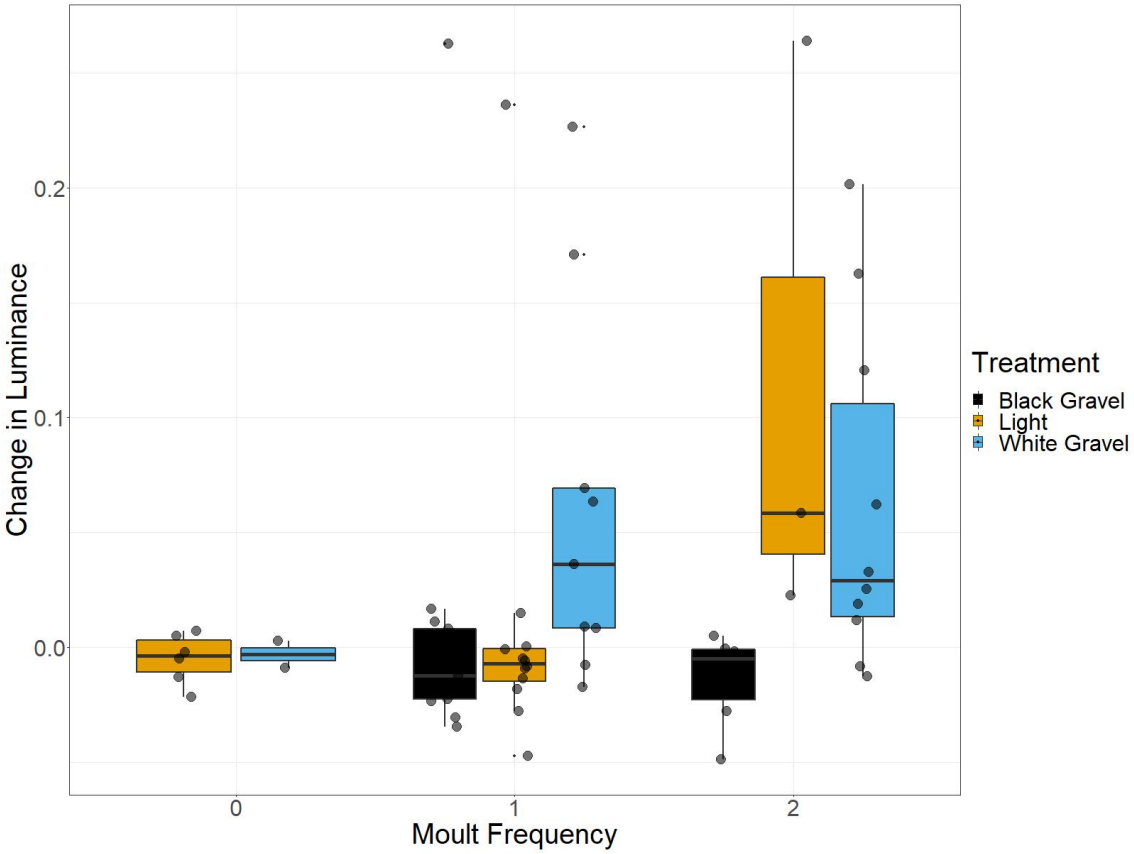


Figure 32: Change in luminance of crabs on each of the three treatments from substrate perception experiments, split by moult frequency.
Fill colour corresponds to experimental treatment. All crabs were included that survived at least 14 days, as this allowed for a minimum of two digital image collections, and potential colour change to be recorded. All 19 black crabs that survived at least 14 days moulted once, while multiple crabs on light and white did not. Graphs plotted with ggplot2 (Wickham 2016).

The model of overall change simplified to just the fixed effect of treatment ($F_{2, 56.01} = 5.571$, $p = 0.0062$). Significant differences were only found between the black gravel and white gravel treatments, with crabs on the white gravel increasing luminance by 0.068 more on average than those on black gravel (se = 0.020, $t_{56.01} = 3.33$, $p = 0.0015$). There was no significant difference between luminance change on the upwelling treatment and either the black (average difference = 0.033 brighter on light, se = 0.020, $t_{56.01} = 1.61$, $p = 0.1127$) or white treatments (average difference = 0.035 brighter on white, se = 0.020, $t_{56} = 1.79$, $p = 0.0788$). Interestingly, moult frequency was not retained in the model. The inclusion of the outlier crab in the black treatment resulted in the removal of

all fixed effects from the model, causing treatment to no longer have a significant effect on model fit (Table 31 and Table 32).

Table 31: ANOVA statistics from the simplified model of total change in luminance in substrate perception experiments.
Includes remaining fixed effect of treatment. A random effect of run-through to account for differences between run-throughs (not included in previous model this was accounted for by the ID random effect). Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison

Effect	**F value**	**DF**	**p**
Treatment	5.5714	56.01	0.0062
Model: lmer(Luminance.chg ~ Treatment + (1|runthrough))			

Table 32: Summary statistics from the simplified model of total change in luminance in substrate perception experiments.
Includes remaining fixed effect of treatment. A random effect of run-through to account for differences between run-throughs (not included in previous model this was accounted for by the ID random effect). Included is R software code (dependent on the LME4 package (Bates et al. 2015)). Model Simplification via AIC comparison

Source	**Estimate**	**SE**	**t**	**p**
(Intercept)	-0.0131	0.0221	-0.591	0.611
Light	0.0328	0.0204	1.611	0.113
White Gravel	0.0679	0.0204	3.331	0.0015
Model: lmer(Lumiance.chg ~ Pattern.Size + (1|Filetype))				

When considering survival on each of the treatments, crabs suffered significantly higher mortality in the light treatment than on either of the other two treatments. Only four crabs died on the black treatment (21.1%, three before day 56, and one before day 70). Six crabs died on the white treatment (28%, one before day 28, three before day 56, and two before day 70). Nine crabs died on the light treatment (42.9%, two before day 28, four before day 56, and

three before day 70). While sample sizes are small enough to limit what can be inferred, a Cox Survival Analysis was conducted to assess treatment's effect on survival (as well as run-through in case season influenced crab mortality). Comparing the three treatments, only the light treatment was found to result in significantly greater mortality when compared to black. Being on the light treatment carried an increased risk of death (Hazard Ratio: 2.475, 95% confidence interval: 1.03 to 5.93, p = 0.0419), compared to the black treatment. There was no significant difference in survival between the white and black gravel treatments (Hazard Ratio: 1.590, 95% confidence interval: 0.626 to 4.039, p = 0.3295), or the light and white gravel treatments (Hazard Ratio: 1.557, 95% confidence interval: 0.309 to 1.334, p = 0.2351).

Discussion

Of the crabs analysed in this experiment, only those on treatments with high reflectance relative to the incidental light showed significant brightness increase, with crabs on a naturally highly reflective substrate (white gravel) increasing significantly more and requiring fewer moults to do so. While five crabs on the light treatment did increase brightness, four of these required two moults to do so. Crabs on the black gravel treatment slightly decreased in brightness, save one crab that rapidly increased brightness with a single moult early in the experiment and remained that way until the end, which was removed from analysis as an outlier.

As with other brightness change experiments in shore crabs ((Nokelainen et al. 2019; Stevens 2016; Stevens, Lown, and Wood 2014b)) and other species ((Stevens, Rong, and Todd 2013; Eacock et al. 2019)), there was significant variation in colour change within treatments, especially those where brightness change was most expected (upwelling illumination and white gravel). While some individuals significantly changed luminance, many did not. (Figure 31 & Figure 32). This reduced the clarity of results; however, several trends were noticed.

The clearest individual result is that overall illumination/environmental light intensity does not apparently dictate colour change for camouflage. Crabs on the high intensity treatment with black gravel changed less, and even tended to decrease brightness, compared to the crabs on white gravel, with a 50% reduction in intensity significantly increasing brightness. While one crab on

black did increase brightness, ultimately becoming the brightest crab in the entire study sample, this is more likely to be an anomalous individual (and was removed as an outlier). What specifically caused this shift is unclear but given that all the rest of the treatment responded in the entirely opposite direction, this individual's change being a response to the greater illumination is unlikely. Additionally, when comparing the light treatment (with ~100% intensity and a similarly high ratio of reflectance to the crabs on white), while there was no statistically significant difference from the black treatment, but there was also no difference between the white and light treatments. In fact, there was a qualitative similarity between the white and light treatments, both resulting in similar ranges of luminance change and maximum luminance achieved. Those crabs that did change significantly on the light treatment (those that moulted twice: Figure 32) increased brightness to a similar degree to those on white. If overall intensity did dictate brightness change in *C. maenas* we would expect crabs on both high intensity treatments to increase brightness significantly more than the low intensity. Instead, results are in accordance with crabs responding to substrates themselves, rather than intensity of light. As it stands, this result supports similar findings in similar species (ghost crabs do not become darker when placed in dark environments (Stevens, Rong, and Todd 2013), and fiddler crab melanophores disperse more on black substrates, even at greater light intensities (Brown Jr and Sandeen 1948)), other crustaceans (*Hippolyte* prawns change to match seaweed in both dark and light conditions (Gamble and Keeble 1900)), as well as other arthropods (grasshoppers only respond to differences in substrate brightness, not illumination levels (Burtt 2009)). Outside of research directly examining camouflage, background colour seems to be a greater determinant of colour change that light intensity in rock lobsters (Melville-Smith, Cheng, and Thomson 2003).

The lack of a response relative to overall intensity also confirms the assumptions from past research (Powell 1962b) and the first chapter, that any colour change in *C. maenas* as a result of a primary response (direct response of chromatophores to incident light) is overridden by a secondary response (control based on stimulation of eyes). If the primary response was responsible for colour change, an increase in luminance on both the high intensity treatments would be expected. Increased light on chromatophores would

stimulate a redistribution of pigments resulting in increased brightness (as seen when eyes are obscured in *C. maenas* (Powell 1962b), and in response to illumination in other crustaceans (Aoto 1963; Brown Jr and Sandeen 1948)). This is especially important considering the chromatophores most needing to change are on the dorsum of the cephalothorax. This is the area most exposed to predators and in need of matching background reflectance (Carter, Tregenza, and Stevens 2020; Nokelainen et al. 2017; Nokelainen et al. 2019; Price et al. 2019; Stevens 2016; Stevens, Lown, and Wood 2014b, 2014a; Troscianko et al. 2021), and is directly facing up towards illumination. Past experiments in extraocular colour change and primary responses have shown only a response to background brightness or colour, but these tended to control illumination intensity ((Eacock et al. 2019; Fulgione et al. 2014), so the direct impact of illumination and light on primary colour change responses needs further investigation.

When examining the use of radiance (upwelling light) versus reflectance (upwelling light relative illumination) for substrate perception, the result is less clear, but inferences can be drawn. The mean final luminance of crabs on black (including those who died prior to the experiment end) was 0.043 (0.058 when including the outlier crab). This was somewhat higher than the black gravel's luminance of 0.0072. As mentioned above, crabs were collected from one of their darker habitats (mudflats (Nokelainen et al. 2017; Price et al. 2019; Stevens, Lown, and Wood 2014a; Todd et al. 2006; Todd et al. 2012)) and may not be able to significantly reduce brightness further, and multiple crabs changed within the range of the standard deviation of black gravel. The mean final luminance of those crabs on white was 0.11, and this was further from the raw luminance of the white gravel: 0.34. Factoring the effect of the ND filter, which is assumed to halve substrate luminance (as it halved incident light), the average final luminance of crabs is closer adjusted gravel luminance of 0.17, which is somewhat closer but still above the average luminance of crabs in the treatment. Considering the variation in crab colour change, many individuals showed little to no colour change. Five crabs on white gravel did increase brightness significantly beyond the adjusted luminance of white gravel (final luminance range: 0.18-0.28, Figure 31). While the majority of crabs did not reach the luminance of the white gravel, the final luminance values of those

crabs that did change significantly were in line with other individuals changing on the same white substrate in past experiments in the species (Mynott 2019). Finally, there was little indication that crabs had ceased changing, and so could have continued to increase in luminance if left in those conditions, potentially reaching the substrate luminance.

The responses of crabs on the upwelling light treatment seemed to follow the same pattern as crabs on white gravel, albeit to a lesser degree. I predicted that if *C. maenas* uses the upwelling versus downwelling light to gauge the "true" substrate brightness, regardless of overall intensity, then colour change should be the same on the two substrates with high reflectance relative to illumination (upwelling light and white gravel). The radiance of the upwelling light in tank conditions was significantly greater than that of the white gravel (as evident by the significantly greater luminance of the upwelling light versus white gravel, even under full illumination via arc lamp for image analysis Figure 31). If *C. maenas* is using radiance of substrates, then we would expect to see a significantly greater increase in brightness compared to the white gravel. Given the illumination intensity was matched in both directions, it should create the illusion of a nearly fully reflective substrate (allowing for some variation between the two because of differences in the spectral output of the different lights and sensitivity of the crabs). As previously mentioned, the range of responses on the light treatment is similar to those on the white gravel treatment, although fewer crabs changed significantly, and those that did matched the brightness achieved by crabs on the white gravel (Figure 32, barring the outlier from the black gravel treatment).

Regardless, there are qualitative similarities between the light and white treatment – both had multiple crabs that significantly increased brightness post moult, and the range of colour change achieved on both treatments was similar. This is apparent when compared to the more uniform decrease seen on crabs on black gravel. Given the common element was the high ratio of upwelling to downwelling light, this seems to indicate that the relative reflectance is responsible for judging substrate brightness, as seen in other species changing colour for camouflage (Sumner and Keys 1929). While high variation and low sample size hinder inferences, this experiment can function as a pilot for more direct testing of the use of radiance versus relative reflectance in substrate

perception. The optimum solution may be to have multiple treatments where the upwelling illumination is maintained at a constant intensity, while the intensity of incident light is adjusted via restrictive filters of increasing strength. This would have the effect of maintaining radiance, whilst adjusting the (apparent) reflectance (ratio of downwelling to upwelling light). The expected result being a greater brightness increase as the intensities of incident and upwelling illumination became closer, mimicking a more reflective and therefor brighter substrate. If brightness change were to be matched across these treatments, it would indicate a direct response to radiance over reflectance.

How the difference in directional light is detected is less clear but is likely a function of relative stimulation of different parts of the eye. While *C. maenas*' field of view has not been characterised, evidence regarding motion perception indicates they have a large visual field (Horseman, MacAuley, and Barnes 2011), and other crabs species display a large field of view with regional specialisation within the eye (Smolka and Hemmi 2009). Parts of the eye are likely directly exposed to both illumination and substrate radiance simultaneously, which should allow for the differentiation in light intensity between the two. This has been proposed as the reason for the difference in black chromatophore behaviours of fiddler crabs in response to background and illumination. Crabs on black backgrounds consistently had a greater dispersion of pigments in black chromatophores than crabs on white, regardless of illumination levels (Brown Jr and Sandeen 1948). The direct impact of differential stimulation of eye regions on colour change has also been demonstrated in other species undergoing morphological change. Obscuring or ablating the lower part of the eyes of *Carausius* stick insect nymphs results in significant darkening via increased epidermal ommochrome expression (Bückmann 1979). Alternatively, the presumably large field of view of *C. maenas* could allow for some level of self-assessment in terms of matching, but this is not determinable from the results of this chapter. There is little evidence of self-assessment for matching when changing colour in the literature (although see (Stevens and Ruxton 2019) for a review of self-assessment in background selection for camouflage).

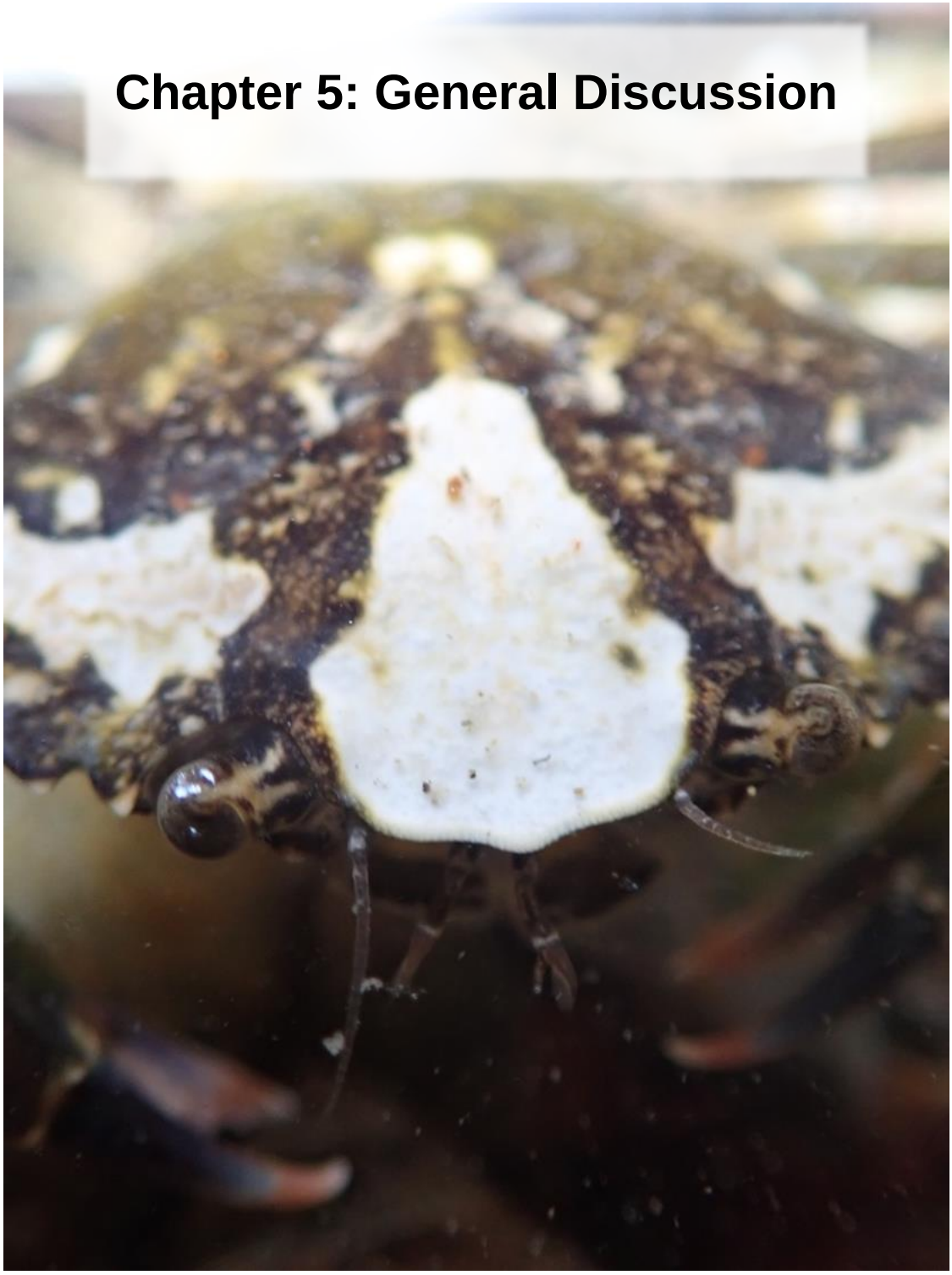
Regarding the energetic cost of colour change, my sample sizes limits the potential for inference, but survival analysis did highlight a potential effect of the

light treatment on mortality. This finding should be treated with care because we only carried out the analysis having already observed a difference so the p value cannot be interpreted in the same way as if we had carried out an experiment blind to its outcome. Nevertheless, as a way marker for future research, we know that gross colour change in *C. maenas* is facilitated by moulting (Stevens 2016; Stevens, Lown, and Wood 2014a; Todd et al. 2006). This process is likely highly stressful for the animal, as it involves both a prolonged period without food and a softened carapace, as well as the loss and necessary regrowth of the various organs (including those needed for senses and oxygen uptake (Phlippen et al. 2000)). The energetic cost of colour change has been repeatedly raised as an area in need of study, both in this species (Mynott 2019) and generally across species (Duarte, Flores, and Stevens 2017; Stevens 2016). The need to change colour in *C. maenas* could increase the frequency of moulting (with an associated cost in energy and physical stress) or changing colour during moulting adding energetic cost on top of the base cost of moulting could divert resources away from other process needed for survival. Given that all crabs on black moulted at least once, and mortality was lower (at least compared to the light treatment), the latter seems more likely, given the reduced need for change in the black gravel treatment. Further testing of the metabolic costs of colour change are needed, especially as they contribute to its value as an antipredator strategy. Over-investment in colour change could have a negative impact on survival, potentially negating, or at least limiting its benefits.

In conclusion, *C. maenas* colour change appears mostly independent of overall light intensity and substrate radiance, instead seeming to combine the two to assess substrate reflectance. This study indicates that colour change is in response to the ratio of reflectance to illumination, as crabs' responses were similar when this matched across treatments, despite differences in overall intensity and radiance. High levels of variation reduced the clarity of results, but the variation was similar across treatments with similar responses. While ideal matching was not achieved, all treatments at least tended to change luminance in a direction that reduced difference in their coloration from the reflectance/upwelling light. Further testing with regards to the ability to account for illumination when judging substrate coloration for colour change, the role of

differential stimulation of eye regions, as well as the ability to self-assess to ensure matching, would be useful in the future. Additionally, the results of this experiment highlight the need for investigation into the metabolic and fitness costs of colour change for camouflage. Regardless, *C. maenas* substrate perception seems most likely to be a response to the difference in incident and reflected light.

Chapter 5: General Discussion



Summary of results

The results of this project demonstrate several key aspects of the relationship between *C. maenas*'s vision and colour change. Firstly, *C. maenas* vision appears to be dominated by a single mediumwave sensitive photoreceptor type. This fits well with past research in the species itself (Martin and Mote 1982; Bruno, Mote, and Goldsmith 1973), and the possession of at least mediumwave sensitive photoreceptors seems to be common across multiple other crab species (Bruno and Goldsmith 1974; Forward, Cronin, and Douglass 1988; Lall and Cronin 1987; Martin and Mote 1982). While dichromacy could not be disproven, behavioural tests for colour discrimination found no evidence of colour vision either. In addition, no significant improvement in chromatic matching occurred over the long-term (in line with past research on short term changes (Stevens, Lown, and Wood 2014b)), with only significant achromatic improvement recorded. This aligns with the *C. maenas*' assumed monochromacy.

Crabs were also capable of changing patterning in response to different background pattern sizes. These changes were limited to increases and decreases in pattern contrast, enabling crabs to adjust degree of patterning (on a scale of uniform to highly contrasting patterns) but not the distribution and size of those patterns. Pattern change, while not able to respond to the scale of patterning, seemed to correspond to the characteristics of *C. maenas* spatial vision. An increase in overall patterning (based on overall contrast between pattern elements) was found on patterns both large enough to see at a normal viewing distance, and a smaller pattern that would be below the threshold of discriminability except at very close viewing distances. This response was only expected if the crabs did not possess a minimum focal distance, or at least a particularly small one (Land and Nilsson 2012), based on the viewing distance needed to reach the minimum resolvable angle calculated.

Finally, shore crabs' responses to directional light correspond to using reflectance compensating for illumination rather than overall intensity or light from substrates alone. Crabs did not increase luminance when overall intensity was greater, even when there were high levels of upwelling light. Instead, the responses were matched across treatments when the relative reflectance was similar (matched upwelling and downwelling light). This, combined with the

results of the first chapter and the past research on *C. maenas*' direct chromatophore responses (Powell 1962b), suggests crabs colour change responses seem to be dominated by directional cues based on information collected by the eyes.

The direct links between vision and slow colour change for camouflage

It ultimately seems that *C. maenas* vision dictates colour change responses for camouflage. This enables responses beyond direct changes in chromatophores to light (primary responses), such as pattern adjustment to spatial cues, and responses to directional light information. It does limit colour change as well, however. By lacking the apparent ability to discriminate colour, *C. maenas* seems to be unable to adjust hue to significantly improve chromatic matching. Such mismatching has been shown to have a significant effect on survival in other crustacean species (Duarte, Stevens, and Flores 2018; Mynott 2019). Across decapod crustaceans, and especially true crab species, achromatic colour change is often the only significant form of plasticity, with occasion limited chromatic change (Atkins 1926; Duarte et al. 2020; Powell 1962a; Stevens 2016; Stevens, Rong, and Todd 2013; Bedini 2002).

Firstly, the clearest link between vision and colour change, is spectral sensitivity and chromatic change. The lack of colour vision and significant chromatic change, despite the apparent presence of coloured chromatophores, implies the individuals are unable to perceive the differences in hue and saturation. This inability to perceive these differences seemingly restricts the response of *C. maenas* to achromatic changes, with small chromatic changes potentially being a by-product of the former achromatic changes (potentially in addition to other factors such as ontogenetic changes (Nokelainen et al. 2019)). These improvements in achromatic matching tended to be proportional to the initial difference between crabs and backgrounds, with crabs initially mismatching to a greater degree changing more than those that started matching. In other species, being unable to perceive chromatic information also limits colour change responses, for example the inability of cuttlefish to respond to isoluminant blue and yellow squares that would otherwise evoke a pattern-change response (Mäthger et al. 2006). In other species undergoing colour change, colour vision is associated with chromatic change, both physiological e.g.: *Hyla* treefrogs (Nielsen and Dyck 1978; Kang, Kim, and Jang 2016), and

morphological e.g.: in crab spiders (Defrize et al. 2011) or locusts (Tanaka, Harano, and Nishide 2012).

Crabs are capable of pattern change in response to background patterning, but only in terms of contrast between pattern elements, without apparent changes in pattern shape or size. Qualitatively different responses were recorded between uniform backgrounds and patterns that should be discriminable, accounting for the ability to resolve information at close distances. These responses were relative to pattern size, with the greatest increase in pattern energy on the large-patterned background, a lesser increase on the small-patterned background, and little to no change on the uniform background. However, the only statistical differences were found between the large-patterned and the uniform backgrounds. The degree of patterning and the patterns recorded aligned well with past research (Nokelainen et al. 2017; Nokelainen et al. 2019; Price et al. 2019; Todd et al. 2006; Todd et al. 2012), and numerous patterns seemed conserved over multiple generations of crabs (Figure 10 versus patterns shown over 40 years prior in (Hogarth 1978, 1975)). The next question is the reason why crabs are only able to adjust overall contrast and not pattern size? In other species, adjusting patterning for camouflage, there is a considerable degree of control over pattern sizes (Akkaynak et al. 2017; Allen, Mäthger, Barbosa, et al. 2010; Barbosa et al. 2008; Hanlon et al. 2011; Hanlon et al. 2009; Hanlon, Forsythe, and Joneschild 2008; Healey 1999; Kelman, Tiptus, and Osorio 2006; Mäthger et al. 2008). There is experimental evidence for direct matching of body patterns to background patterns (i.e., background matching), shifting to disruptive markings on large enough background patterns (Allen, Mäthger, Barbosa, et al. 2010; Barbosa et al. 2008; Hanlon et al. 2009). The key difference between the groups with this fine scale control over pattern distribution and the limited pattern change in crabs, is that the former combines camera type eyes (Land and Nilsson 2012) and rapid physiological change.

In Chapter 2, I highlighted the variation in *C. maenas* appearance between habitats (Nokelainen et al. 2017; Todd et al. 2006; Todd et al. 2012), and the assumption that colour change is responsible for phenotype-environment associations at the mesoscale level (Nokelainen et al. 2017). These mesoscale differences amount to the differences between habitat types such as rockpools

and mudflats, with high levels of variation in variation (background patterns) in the former and general uniformity in the latter. In this experiment, the most conspicuous patterns developed on the more patterned backgrounds and appeared disruptive in nature ,while uniformity was maintained on the uniform background. If these patterns are in fact disruptive, crabs may change camouflage strategies between disruptive markings in rockpool habitats versus background matching in uniform habitats, rather than changing patterning to match that of backgrounds. This is follows the association recorded in natural substrates (disruption in rockpools and background matching in mudflats), where phenotypic plasticity has been proposed as the key reason for the difference between habitat types (Price et al. 2019). If *C. maenas* needs to adjust between discrete camouflage strategies associated with habitat types, resolution of specific pattern sizes may not be needed. This shift may only require the recognition of uniformity or variation (either in general or a specific threshold size/contrast), which seems within the capacity of *C. maenas* acuity. While analysis of disruptive coloration was not conducted, higher total pattern contrast corresponds to higher edge disruption in past research (Nokelainen et al. 2017; Price et al. 2019). Additionally, the qualities of patterns expressed in this experiment indicate that disruptive patterning tended to be expressed more on the larger patterned background. Our results indicate *C. maenas* can shift from background matching to disruptive markings in response to sufficient background patterning. This confirms proposals from past research on the species (Nokelainen et al. 2017; Price et al. 2019; Todd et al. 2006; Todd et al. 2012), and follows trends in disruptive patterning in a wider context (Allen, Mäthger, Barbosa, et al. 2010; Barbosa et al. 2008; Chiao and Hanlon 2001b; Cuthill et al. 2005; Smithers, Wilson, and Stevens 2017; Chiao and Hanlon 2001a).

Finally, differential stimulation of the eyes specifically seems to be responsible for substrate perception for colour change. To accurately detect substrate brightness and coloration independent of illumination, then light information from both the substrate and illumination must be obtained simultaneously. This is so illumination can be accounted for (presumably based on light from above) against the reflected light from the substrate (light from below). *C. maenas*' responses seemed independent of overall intensity or upwelling light alone,

appearing to be in response to directional light cues. Without directional light sensitivity from eyes the difference in downwelling and upwelling light cannot be detected, and illumination cannot be accounted for. This response is in line with other species. Research in other arthropods found colour change only occurred on backgrounds that were comparatively different from the individuals coloration, not in response to overall light intensity (Burtt 2009). In other crabs, responses to illumination were the opposite to those expected if overall light intensity directs colour change, ghost crabs become brighter when placed in dark conditions and vice versa, while changes in background colour caused matching responses in crab colour (Stevens, Rong, and Todd 2013). More directly relating to directional light responses, investigations of stick insect colour change found that covering only the bottom hemisphere of their compound eyes resulted in an increase in dark pigment dispersal, presumably because the covering mimicked reduced upwelling light relative to downwelling (Bückmann 1979).

Factors limiting colour change for camouflage

The key limitation imposed by *C. maenas'* reliance on vision for appearance change is the lack of ability to detect chromatic differences whilst possessing the apparent mechanisms needed to improve chromatic matching. As previously stated, the potential benefit of colour vision outside of colour change for camouflage in this species is limited. In other similar species known to possess colour vision and which also show some chromatic change e.g., fiddler crabs (Brown Jr and Sandeen 1948; Hemmi et al. 2006; Horch, Salmon, and Forward 2002; Rajkumar et al. 2010)) behaviours are known that could be dependent on colour vision e.g., courtship behaviours (Takeda 2006) which adds value to the ability to discriminate colour. It may simply be the potential costs of colour vision (e.g., the potential loss of overall sensitivity (Land and Nilsson 2012)) needed for chromatic change outweighs the benefit of the matching achieved. The comparative benefits of chromatic versus achromatic matching are less clear. In *C. maenas*, chromatic matching is only reliable on mudflats, where the crabs tend to be a good colour match for substrates. In more variable habitats, the increased diversity of potential backgrounds reduces the likelihood a specific background colour will match. In these cases, disruptive markings may be a more reliable means of avoiding detection (Price et al. 2019)

Across all experiments, significant variation in change was seen, with some crabs undergoing significant changes (in brightness, pattern contrast, or both), while many showed no change at all. Despite individuals not changing when it would improve camouflage, very few individuals changed in a way that significantly worsened matching. This variation is generally in line with other morphological changes in this (Stevens, Lown, and Wood 2014b; Hogarth 1983) and other crabs species (Stevens, Rong, and Todd 2013), as well as general trends in arthropod morphological colour change, where significant variation in change achieved by individuals has been recorded (see (Tanaka, Harano, and Nishide 2012; Eacock et al. 2017; Anderson and Dodson 2015)). Additionally, other studies comparing brightness change using black and white have found that individuals tend to better match dark substrates, often failing to reach the brightness of very light/white substrates (e.g. the achromatic change of *Biston betularia* in (Eacock et al. 2019) Fig. 2b).

While this project focussed on the visual characteristics of *C. maenas*, the role of colour change mechanisms – be they physiological or morphological – will also be important in enabling and limiting colour change for camouflage. While the ability of eyes to detect cues abouts substrates can potentially limit colour change behaviours, the range of plasticity afford by a species' colour change mechanisms will limit what changes in coloration can potentially be achieved. The mechanisms for colour change camouflage have been studied and reviewed in a variety of taxa, including in arthropods (Umbers et al. 2014). My working basis for *C. maenas'* chromatophores was the initial research conducted by Powell, which described *C. maenas* as possessing red, white, and black chromatophores types (Powell 1962b; Powell 1962a). Based on this I have also assumed that *C. maenas* possesses the mechanisms needed to adjust chromatically, with changes in red pigments affecting longwave reflectance. Whether *C. maenas*' red chromatophores are used in colour change for camouflage is not confirmed, however.

A key question raised by the project is why does *C. maenas* possess a red chromatophore type, when its visual system only allows for the discrimination of achromatic differences, and colour change mirrors this? Logically, only white and or black chromatophores are necessary for the achromatic changes seen in *C. maenas*, but they still possess red chromatophores. It could be some level of

fixed red coloration useful for crypsis or some other function. One interesting point in the literature is the relationship between the melanophores and the red chromatophores, combined with the denaturing of crustacyanin into astaxanthin (Lee and Vespoli 2015; Reid et al. 1997). A similarity in responses of red and black chromatophores in *C. maenas* has been noted in past research (Powell 1962b; Powell 1962a), and in my own results the responses seemed to align with similar responses in the two. This similarity in response of red and black chromatophores is found in other crab species changing colour, specifically *Uca* fiddler crabs (Brown Jr and Sandeen 1948). Red chromatophores mirror the responses of melanophores, both in background responses, as well as circadian rhythms. Red/longwave sensitivity is generally lower than shortwave to mediumwave sensitivity in *C. maenas*' (Bruno, Mote, and Goldsmith 1973; Martin and Mote 1982) as confirmed by the luminance scores of substrates obtained in Chapter 1. "Correct" chromatic change on the red substrate may be an unintentional result of crabs reducing brightness, by increasing pigments/dispersing existing pigments in the red as well as black chromatophores, in response to the perceived darkness of red substrates.

Contrasting the potential lack of independent control of red versus black chromatophores are specific hormones associated with red pigment dispersal. These hormones are found across crustaceans (Brown Jr 1950; Darnell 2012) including *C. maenas* (Alexander et al. 2020). Evidence suggests they have a direct role in the dispersal of red pigments in chromatophores, without an apparent effect on melanophores (Alexander et al. 2020). Their impact on long term colour change, especially factoring the potential photo-denaturation of integumentary pigments, is less clear. Beyond that, there is no guarantee red chromatophores have a function in crypsis. Arguments have been made that red pigment change in polar crustaceans in response to light (Auerswald et al. 2008; Fuhrmann et al. 2011) could be used in protecting from UV radiation (Fuhrmann et al. 2011). Some combined investigation into sensory ecology, endocrinology, and ontogenetics of species undergoing morphological colour change is needed to untangle specific roles of chromatophore change.

While not necessarily a limit, colour change could be affected by external stressors. I mentioned in Chapter 3 that colour change could be stressful on the individual changing. This comes from both the necessity of moulting to facilitate

gross changes in colour, as well as the presumed cost of pigment movement or generation and degradation. Beyond colour change, crabs are likely to face other stressors in natural environments. These can range from the direct impact of predators (both physical damage and the stress of their presence), as well as unfavourable conditions (for example: extremes of temperature). It has been demonstrated that shore crab colour change is reduced in the presence of shipping noise (Carter, Tregenza, and Stevens 2020). Interestingly, there is evidence that higher temperatures could increase the speed of colour change in crabs, perhaps due to their ectothermic biology (Mynott 2019). Across experiments, the magnitude of colour change was relatively small. It could be that while efforts were made to minimise stressors for samples (initial quarantine to acclimatise individuals to laboratory conditions, minimal handling, ensuring optimum environmental conditions) what stressors there were limited colour change (as shown in past treatments based on sensory stressors (Carter, Tregenza, and Stevens 2020)). With the further acknowledgement of crustacean nociception and sentience, considerations should made not just for the impact of colour change itself, but also the impact of unconsidered stressors on colour change behaviours of interest.

Alternative information pathways for colour change for camouflage

Multiple apparently monochromatic species still maintain a level of chromatic plasticity, often allowing for improvements in colour matching. Whatever mechanisms allow this occur, be it approximations based on luminance cues combined with similarities in chromatophore and background colours (as potentially seen in cuttlefish (Mäthger et al. 2008)), or extraocular photoreception (direct chromatophore responses occur in response to light as seen in *B. betularia* (Eacock et al. 2019)), *C. maenas* seems unable to match this ability.

While extraocular colour change has been demonstrated in other species, and to a limited degree in *C. maenas* (Powell 1962b), the crabs' morphology could significantly limit this. Those species which readily change colour via extraocular photoreception share one key aspect: an epidermis significantly more transmissive than that of *C. maenas*. Cephalopods (Ramirez and Oakley 2015) and lepidopteran larvae (e.g. *Biston betularia* (Eacock et al. 2019))both possess chromatophores with significant access to light, which should facilitate

primary responses by chromatophores. The carapace of *C. maenas* could act as an interference layer, blocking light (as seen to have an effect on acuity in other crabs (Baldwin and Johnsen 2011)), especially on the cephalothorax of older crabs. However, it will likely be thin enough in juvenile crabs, especially on the limbs (Powell 1962b) to allow light information to directly stimulate changes in chromatophores. In addition, discrete changes in coloration are associated with moulting in this and other species (Jensen and Egnotovich 2015). Presumably shore crabs deposit pigmentary material in the new integument during moulting, but in the immediate period post moulting chromatophores could be exposed to light and primary responses may occur. However, were a primary response dictating the colour change responses of *C. maenas*, a greater response on the chromatic backgrounds of Chapter 1 might be expected. If red chromatophores responded to light directly(as seen in (Eacock et al. 2019)), then chromatophores should theoretically respond to the intensity of longwave light, with greater intensity of longwave light on red (and perhaps yellow substrates) leading to greater dispersion of corresponding pigment, and lesser intensity on blue resulting in aggregation.

There is little to no evidence for the role in other sensory modalities beyond photoreception guiding colour change for camouflage in *C. maenas*. All substrates used gave no other reliable cues to identify their coloration/appearance other than visual, and I assume crabs matched based on those visual cues in accordance their capability to perceive them. While the direct link between vision and visual camouflage logically means the best information to use when matching is visual, other cues may be used. While multiple other cues are demonstrated to be used in defences against detection through other senses (see (Ruxton 2011) for a review), cues other than vision could provide the information about substrates which is needed to change colour for camouflage. Within the crustaceans, certain caridean shrimp change colour when transferred to novel macroalgae that function as both food source and background substrate for camouflage (Duarte, Stevens, and Flores 2018; Gamble and Keeble 1900; Green et al. 2019; Keeble, Gamble, and Hickson 1900). Similar pairings of food sources and camouflage backgrounds are seen in other marine crustaceans e.g., marine isopods (Hultgren and Mittelstaedt 2015; Lee 1966), as well as multiple lepidopteran larvae (Greene 1996; Noor,

Parnell, and Grant 2008; Poulton 1903). In these circumstances, information about substrate identity could be provided via chemical cues, rather than visual. By using cues other than vision, species may not be limited by deficiencies related to colour change. For example, spectral sensitivity has not been examined in *H. varians*, but similar species are often monochromatic (Johnson, Gaten, and Shelton 2002). If they lack colour discrimination, they still maintain the ability to improve chromatic matching on red, green, and brown/yellow algae (Gamble and Keeble 1900; Green et al. 2019; Keeble, Gamble, and Hickson 1900). Chemical differences could be providing information about seaweed type, removing the limitation of colour vision and enabling accurate colour matching. Even in species without a direct link between dietary cues and their substrate, food and resources obtained from it are likely to be important. Diet has been shown to affect the body coloration of spiders (Gillespie 1989), and outside of potential crypsis functions, pigments from diet are responsible for colour change in a variety of other species (e.g. in other crustaceans (Yamada et al. 1990; D'Abramo et al. 1983) and birds (Fox 1955; Ratcliffe 1936; Johnson, Cézilly, and Boy 1993)). The importance of diet, both in informing colour change as well as providing the materials for it, should be investigated to identify its importance especially in relation to visual cues.

Augmenting imperfect colour change for camouflage.

While appearance change for camouflage is the focus of this thesis, colour change, and camouflage in general, are not the limit of *C. maenas* antipredator defences. *C. maenas* appearance change for camouflage was found to be limited in two key areas: chromatic change and change in pattern distribution. Both have a significant impact on overall matching, which will have a subsequent impact on survival (as demonstrated by previously mentioned predation experiments (Corl et al. 2018; Troscianko, Wilson-Aggarwal, et al. 2016; Duarte, Stevens, and Flores 2018; Mynott 2019)). However, *C. maenas* and other species can reduce detection in addition to changing their own appearance.

Firstly, although *C. maenas* cannot change to match the pattern distribution and chromatic elements of habitats, it still maintains strong phenotype-environment associations resulting in successful camouflage across habitats (Hogarth 1975; Nokelainen et al. 2017; Price et al. 2019; Stevens, Lown, and Wood 2014a;

Todd et al. 2006; Todd et al. 2012). When considering the habitats *C. maenas* needs to camouflage amongst, noticeable trends are observed. Generally uniform habitats often seem to have low saturation of any colour and often lie at either end of a scale of brightness (based on personal observations of sample sites and inferences from past research (Price et al. 2019; Stevens, Lown, and Wood 2014a; Todd et al. 2006; Todd et al. 2012; Nokelainen et al. 2017)). Alternatively, the broader variety of background conditions in rockpools (Nokelainen et al. 2017; Todd et al. 2006; Todd et al. 2012; Price et al. 2019) seem to increase the difficulty of matching backgrounds. There will be a greater variety in backgrounds (both in patterning and colour) that *C. maenas* cannot match, compared to mudflats where matching (both brightness and colour) is consistently effective (Price et al. 2019). As such, changing to disruptive markings seems to be the optimum camouflage strategy rather than background matching. Additionally, the habitat complexity may also reduce predation independently of camouflage (Merilaita 2003), as seen in recent model experiments (Rowe et al. 2021). Habitats of greater heterogeneity seem to favour greater variation in prey coloration (Bond and Kamil 2006, 2002). This seems to be the case in *C. maenas* as well, as heterogenous rockpools seem to favour greater carapace variation specifically for disruptive markings, over attempts at background matching (Price et al. 2019). Following this, the variation in crab coloration in these habitats seems to impede predator search image formation, especially in crabs with disruptive markings (Troscianko et al. 2021).

While colour change is thought be responsible for mesoscale matching, behavioural choice in substrates has been proposed as a mechanism for improving microscale matching (Todd et al. 2012; Nokelainen et al. 2017). Choice of background facilitates matching in other species with fixed coloration (e.g. moths (Kang et al. 2012), reptiles (Nafus et al. 2015), and ground nesting birds (Stevens et al. 2017)), as well as augmenting camouflage in other species capable of colour change (e.g. in marine (Green et al. 2019; Uy et al. 2017) and terrestrial arthropods (Eacock et al. 2019)). Selection of matching backgrounds might allow for the correction of chromatic mismatch within a habitat, although this should be dependent on colour discrimination (identifying matching background colours) which is not evident in *C. maenas*. Beyond selecting

specifically matching backgrounds, other behaviours can also improve camouflage (e.g. orientation in moths (Kang et al. 2012), or posturing in caterpillars (Rowland, Burriss, and Skelhorn 2020), reviewed in (Stevens and Ruxton 2019)).

It is crucial to not consider camouflage as the only antipredator strategy. Crabs are a valuable species for multiple antipredator strategies both at a given timepoint and with ontogenetic change in these strategies (Carter, Tregenza, and Stevens 2020; Moksnes, Pihl, and Montfrans 1998; Nokelainen et al. 2019; Price et al. 2019; Stevens, Lown, and Wood 2014b, 2014a; Troscianko et al. 2021; Wale, Simpson, and Radford 2013). *C. maenas* shifts antipredator strategies with age. Juveniles adopt a specialist strategy to the habitat they are in aided by colour change, then adults appear to transition to a generalist uniform green strategy found to be optimal in the environments they typically inhabit (Nokelainen et al. 2019). This is combined with an increase in overall size and physical defences (integument strength and claw size and strength), as well as a decrease in moult frequency (Styrishave, Rewitz, and Andersen 2004) (and therefor the rapidity of colour change) ultimately means colour change for camouflage may be of less use in larger (and presumably older) individuals. Given that the period of their life history when colour change is a useful antipredator strategy is relatively short, the potential benefits for colour discrimination, especially for colour change, are limited.

Crabs are often found in habitats comprised of sediment of various sizes (e.g., silt in mudflats, and sand or shingle in rockpools (Nokelainen et al. 2017; Stevens, Lown, and Wood 2014a; Todd et al. 2006; Todd et al. 2012)). During the day crabs are often fossorial, burying themselves in sediment or burrows which can improve survival (Coverdale et al. 2013) potentially augmenting any existing camouflage (Bellwood 2002). Burying is also seen in other epibenthic species also using camouflage (e.g.: cuttlefish (Allen, Mäthger, Barbosa, et al. 2010; Boletzky 1996), flatfish (Ellis, Hoowell, and Hughes 1997; Moles and Norcross 1995; Ryer et al. 2008) and rays (Youn, Okinaka, and Mäthger 2019)). By burying themselves in the substrate or hiding in refuges, the individual can immediately adopt the characteristics of the background they must match. This removes the reliance on visual cues needed for colour change or background

selection. Subsequently it could allow for the mitigation of limited colour vision or colour change mechanisms restricting chromatic matching.

Caveats of experimental methods

While this project has identified the links between vision and colour change for camouflage in *C. maenas*, certain assumptions had to be made, and methodologies compromised. One key issue in the assessment of vision was the use of the optomotor response as a behavioural measure. A significant drawback of the optomotor methods in general is that they rely on a moving stimulus, and motion vision has the potential to be achromatic (Krauss and Neumeyer 2003; Schaerer and Neumeyer 1996; Yamaguchi et al. 2008). This could obscure the presence of photoreceptor sensitivities outside those used in motion vision. In addition to this, while the LCD screen virtual drum is a useful and easy to apply method for optokinetic measurements, when examining spectral sensitivity it has another key limitation. The three-colour generation limits the method to three distinct points of measurement, specifical red, green, and blue pixel intensity. While a vast gamut of colours can be produced to human perception, they are not single curve (spectra) stimuli, rather the combination of multiple curves of differing intensity. These stimuli are also very broad, each extending across broad sections of the visible spectrum, potentially explaining the response to red, which is greater than that predicted by the fitted Stavenga template (Figure 8). Crabs may be detecting the irradiance from the shorter wavelengths of the stimulus curve, rather than the peak used to identify it. While this significantly reduces how well any potential sensitivity template can be fitted to the data, it is mitigated based on the visual template. The distribution of sensitivity across the spectrum is standardised across photoreceptor sensitivities, while the point of maximum sensitivity varies (Stavenga and Schwemer 1984; Stavenga, Smits, and Hoenders 1993). This means the curve, while complex, is merely being aligned along the x-axis. This is still dependent on the assumption of monochromacy, as the lack of precision means a second peak could not be resolved. Originally, pre-pandemic plans involved reconducting this experiment with a broad-spectrum light source and narrow band pass filters, which would allow for a greater number of points and more precise measurement along the spectrum, meaning both precision and accuracy of assessment will increase. This could potentially capture any

variation missed by the broader LCD screen method, including potential multiple peaks in sensitivity indicative of polychromacy. Unfortunately changes in the thesis plan because of the COVID 19 pandemic meant there was not time for this.

Additionally, there are limitations in the training experiments. While there was evidence of a preference in the black and white trials, this was assumed to be a sign of a learned association but could simply have been an innate photokinetic response – a preference for darkness and refuges. If this were the case then crabs may not have formed the association between the blue and green stimuli, whilst still being able to perceive them. This could result in individuals making random decisions, not through an inability to differentiate between stimuli but from a lack of context from learning. *C. maenas* colour vision cannot be ruled out, but our results still question colour's specific role in camouflage.

Future Research

Perhaps the next biggest question regarding colour change for camouflage is the direct and indirect costs (Duarte, Flores, and Stevens 2017; Stevens 2016; Stuart-Fox and Moussalli 2009). Many are partially or theoretically answered: presumably a major cost of changing between coloration is mismatching between old and new coloration and the associated increase in predation (as seen in the reduced survival of mismatched individuals in multiple predation trials (Hultgren and Mittelstaedt 2015; Duarte, Stevens, and Flores 2018; Mynott 2019; Vignieri, Larson, and Hoekstra 2010)). Often it is assumed there is some energetic cost of physiological colour change, in effecting neuromuscular redistribution of pigments within chromatophores (as noted by Hanlon et al. with regards to octopus colour change (Hanlon, Forsythe, and Joneschild 2008)). Alternative costs may be related to slower morphological changes, beyond the increased time to achieve matching (and the interstitial period of mismatching). There should be some cost associated in the generation and removal of pigments. In *C. maenas'* case, and likely others, the pigments or precursor molecules will likely be derived from their diets. This will likely offset the costs somewhat by providing fuel for responses, particularly if pigments derived from diets are directedly expressed and-or dietary substrate also act as backgrounds for camouflage. Even so will the subsequent conversion and expression of pigments should divert energy away from other process (perhaps leading to

adverse effects – e.g., increased mortality during moulting as seen in Chapter 3). Experiments measuring respiratory rate of individuals actively changing versus maintaining coloration would be useful. Alternatively, in species changing colour morphologically, comparisons of colour change when food is restricted versus being provided ad libitum could also provide insights. The expectation being restricting food will limit colour change as both resources and energy for potential post-consumption changes to pigments will be reduced. There is evidence from guppies changing colour that individuals consume more food when changing colour (Rodgers et al. 2013) but this was not directly linked to camouflage, and the need to obtain materials may be more important for morphological change.

There is also the specific issue of the spectral sensitivity of *C. maenas*. While my behavioural measures fit the previous electrophysiological recordings, they did not identify, and potentially could have overlooked, a second class of photoreceptor. One of the current methods of directly investigating visual properties is opsin sequencing. Opsin types are directly linked to the spectral sensitivities of the photoreceptors they are expressed in. The use of opsin sequencing has been used in assessing the spectral sensitivity of multiple species and allows for direct qualitative assessment of receptor sensitivities. Beyond that, opsin expression has been used with colorimetric studies to show extraocular colour change is facilitated by dermal opsin expression. This use of opsin sequencing has already proven valuable in the case study of *Biston betularia* (Eacock et al. 2019) larvae previously mentioned, as well as cephalopods (Ramirez and Oakley 2015) and reptiles (Fulgione et al. 2014). Broader investigation of opsin expression both at and outside of the eyes, can provide valuable information about the relationship between species spectral sensitivity and ability to chromatically change.

Historical studies of colour change in *C. maenas* have already manipulated animals eyes through covering and ablation/removal (Powell 1962b). These did demonstrate some short-term responses of chromatophores to light intensity, at least when eyes were covered non-invasively. When eyes were directly manipulated (ablation and removal) results were less clear, perhaps due to the confounding effects on hormone transport pathways. These experiments only examined colour change in the short term. Whether long-term change tends to

be dependent on vision or are facilitated by extraocular photoreception (as seen in long-term change in *B. betularia*) is unknown. A similar approach as used in past experiments ((Powell 1962b; Oguro 1962; Fulgione et al. 2014; Eacock et al. 2019)) is warranted to test this. Such experiments were attempted on *C. maenas* during this thesis. This was among the experiments cut for time. A significant challenge was in maintaining a permanent barrier to light that was safe enough to allow long term survival of crabs when used. This was particularly challenging to maintain due to moulting and the difficulties in predicting its occurrence. These manipulations would still be useful to test if long-term mechanisms, which is required for significant colour change in *C. maenas* and other species, are dependent on feedback from eyes.

Concluding remarks

Vision is, at least in shore crabs, the primary director and limiter of phenotypic plasticity. In the collection of species that have had their colour change for camouflage examined, *Carcinus maenas* seems to be exceptional in its simplicity. Colour change behaviours correspond to the extent and apparent limitations of vision. While they are capable of significant and noticeable achromatic change and can adjust at least pattern contrast to tune matching on backgrounds of varying complexity, they do not show any significant chromatic change to improve matching, despite possessing chromatophores capable of adjusting chroma (at least in terms of relative redness). This tracks with their apparent lack of colour vision. Their limited spatial acuity at distance does not appear to impact their ability to resolve small background patterns, or at least differentiate between these and uniform backgrounds. Finally, evidence suggests they follow the assumption that species judge substrate appearance via directional light cues. Change in brightness accorded with a response the ratio of incidental light a substrate is reflecting, rather than the light reflected from the substrate alone or the overall light intensity. This likely is due to differential stimulation of photoreceptors in the upper and lower hemispheres of their compound eyes, based on the responses to directional light intensity, and experiments in similar species. These achromatic changes function to significantly improve matching in terms of brightness, and allowed for shifts between camouflage strategies that were the optimum for differing habitat types. These shifts in camouflage strategies seem within the limits of *C.*

maenas vision, not requiring chromatic cues and only requiring the recognition of pattern in backgrounds rather than resolving specific pattern sizes. As such, *C. maenas* vision, while potentially limiting the absolute range of colour change, still allows for effective changes in camouflage. What is most needed now is further research into the processes between the initial detection of cues and ultimate colour change, to understand the individual roles of specific information transmission pathways used for visually mediated colour change for camouflage.

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Appendices

Appendix 1A – Methods and results of appetitive discrimination trials in Chapter 1

Prior to the aversive training regime used in Chapter 1, initial testing of appetitive stimuli was used, concurrently with the colour change experiments. Training trials involved an appetitive reward (five grams of smoked bacon, selected for its strong olfactory cue and higher value than regular food items) being placed in the correct choice during training, then removed in test trials to remove the chemosensory cue. Trials involved an open arena, with the colour stimuli – vertical 30mm x 20 mm pieces of waterproof paper printed with the respective colours, with a flat 50mm x 50mm piece underneath – at the opposite end of the container to the crab starting point at equal distances on either side (with colours swapping sides randomly). Bacon pieces were placed on the flat coloured surface of whichever of the colour was being trained for association, behind the vertical surface so crabs had to approach and interact with the stimuli to access them. Crabs started the trial contained within a clear container (an overturned container normally used for housing in other experiments, placed in the arena after the setup of all other elements) and allowed two minutes to acclimatise to tank containers. The container was then removed, and crabs were allowed to freely roam for 5 minutes, with interactions being recorded in real time. These were the first colour interacted with (ultimately used as the measure of a correct choice), number of times each colour was interacted with, and the time to first decision. These were all listed as NA if not choice was made. Results can be seen below in Figure 33. Lack of success in the blue-yellow trials could potentially have been due to a lack of colour discrimination, and to test this the stimuli were swapped to black and white for maximum contrast that should be discriminable even accounting for a monochromatic visual system. Trial methods were also refined, given the potential issues of retention, or lack thereof, of reward information. Trials were conducted back-to-back on the same day, with initial trials containing the food reward (reduced to prevent satiation/maintain appetite) which was then removed, and performance was measured solely based on visual cues. Although a limited sample of individuals was tested (n = four, with two crabs

receiving rewards with black, two with white), there was no evidence training was successful.

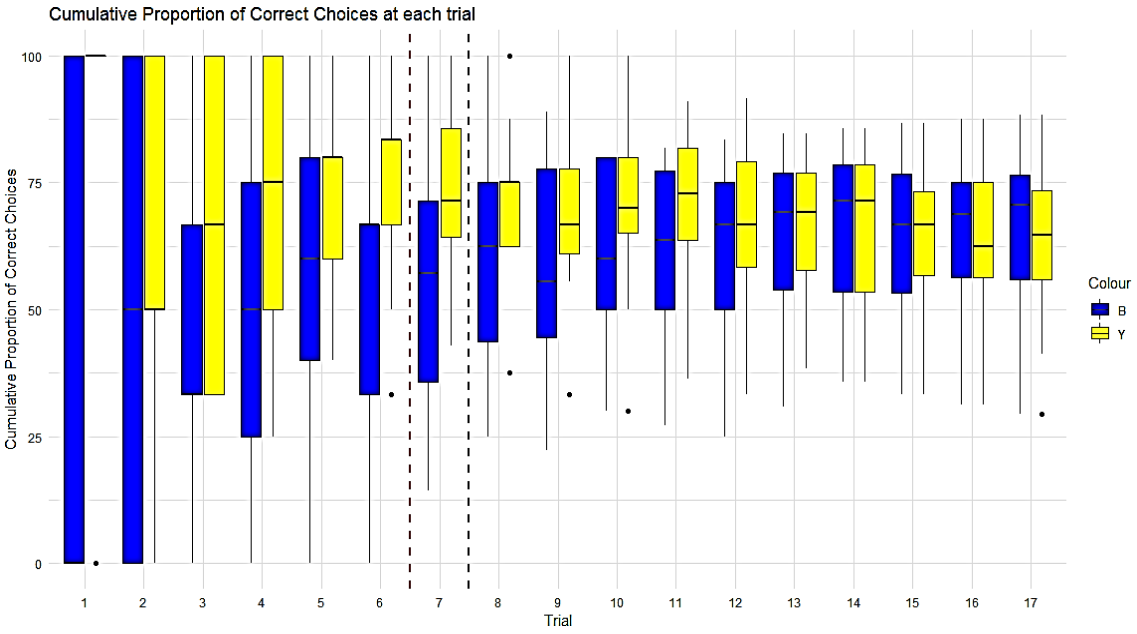

Figure 33: Change in the proportion of correct choice made by crabs at each trial in initial attempts at appetitive colour discrimination trials.
Crabs divided by colour associated with the positive stimulus: B = Blue, Y = Yellow. Vertical line between 6 & 7 - food reward no longer present in chamber. Increasing cumulative proportion would indicate crabs constantly making correct choices, however the levelling off and slight decrease indicates a return to random choice.

Appendix 1B – Examples of crab colour change from colour change experiments in Chapter 1

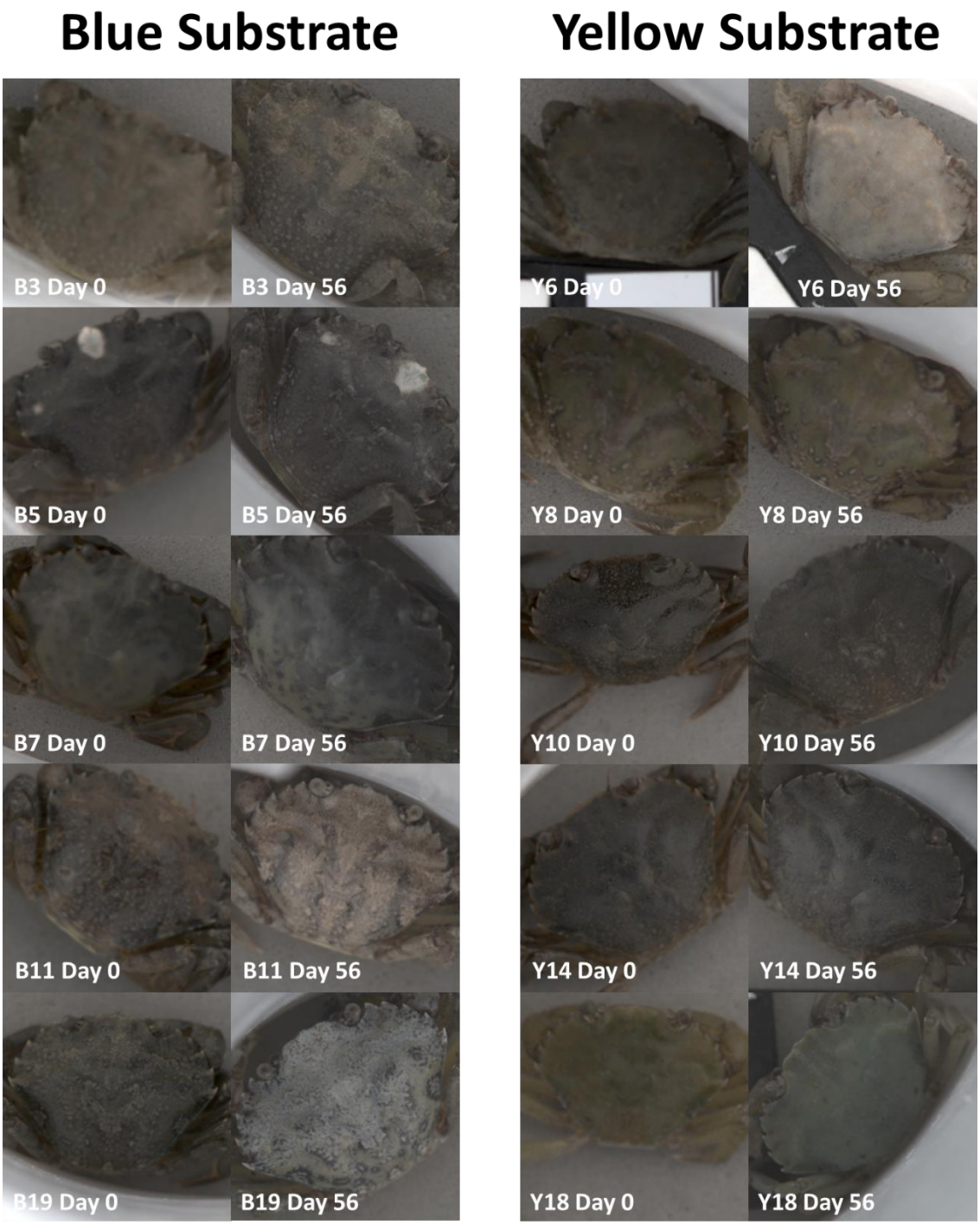

Figure 34: Presentation images of a subsample of crabs from Chapter 1's chromatic change experiments from the treatment groups on blue and yellow substrates.

Photos are from the start (Day 0) and end (Day 56) of experiments. Images are false colour RGB images produced from multispectral images generated by the MICA Toolbox in imageJ. Photographs are not scaled.

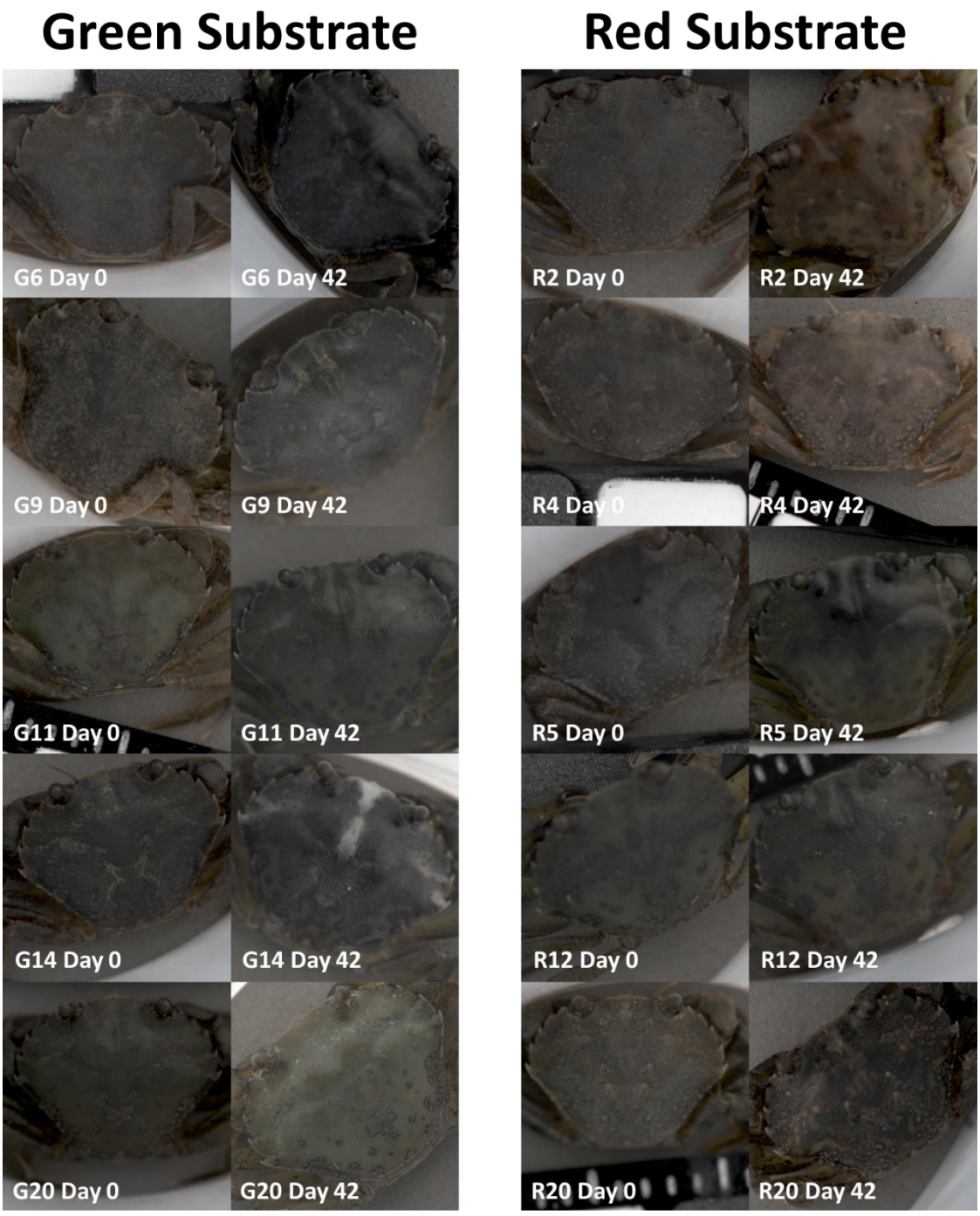

Figure 35: Presentation images of a subsample of crabs from Chapter 1's chromatic change experiments from the treatment groups on red and green substrates.

Photos are from the start (Day 0) and end (Day 42) of experiments. The reduced experiment time was a result of lab closure due to the start of the COVID19 pandemic. Images are false colour RGB images produced from multispectral images generated by the MICA Toolbox in imageJ. Photographs are not scaled.

Appendix 1C – Un-simplified Model ANOVA outputs from optomotor and colour change experiment analysis in Chapter 1

Table 33: Un-simplified model ANOVA output for chromatic contrast at start of optokinesis from Chapter 1 optomotor experiments.
Models produced using the lmer() function in the LME4 package.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Colour	0.501098	0.250549	2	102	4.263969	0.016655
Size	0.001432	0.001432	1	51	0.02437	0.876563
Sex	0.020255	0.020255	1	51	0.344712	0.559712
Colour:Size	0.141455	0.070728	2	102	1.203681	0.304314
Colour:Sex	0.242403	0.121202	2	102	2.062672	0.132384

Table 34: Un-simplified model ANOVA output for tracking efficiency data (Gain) from Chapter 1 optomotor experiments.
Models produced using the lmer() function in the LME4 package.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Colour	0.000525	0.000263	2	102	0.14091	0.868737
Size	0.004584	0.004584	1	51	2.459872	0.122973
Sex	0.00035	0.00035	1	51	0.187947	0.666459
Colour:Size	0.000669	0.000334	2	102	0.17949	0.835959
Colour:Sex	0.001973	0.000987	2	102	0.529502	0.590508

Table 35: Un-simplified model ANOVA output for change in Hue data from Chapter 1 colour change experiments.
Split by visual model. Models produced using the lm() function in base R

Visual Model	Effect	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Dichromat	**Colour**	3	0.152063184	0.050687728	8.998727942	0.000246747
	Size	1	0.002793587	0.002793587	0.49595304	0.487100068
	Colour: Size	3	0.000868676	0.000289559	0.051406109	0.984285808
Trichromat	**Colour**	3	0.062995781	0.020998594	9.764455431	0.00014165
	Size	1	0.001241314	0.001241314	0.57721769	0.45375465
	Colour: Size	3	0.000154082	5.13605E-05	0.023882908	0.994886806
Tetrachromat	**Colour**	3	0.62670184	0.208900613	12.25630547	2.6648E-05
	Size	1	0.003671846	0.003671846	0.215429104	0.646133571
	Colour: Size	3	0.004396565	0.001465522	0.085982896	0.96713777

Table 36: Un-simplified model ANOVA output for change in colour JND data from Chapter 1 colour change experiments.
Split by visual model. Models produced using the lm () function in base R.

Visual Model	Effect	DF	Sum Sq	Mean Sq	F value	Pr(>F)
Dichromat	**Colour**	3	19.82449799	6.608165997	6.085825536	0.002531805
	Size	1	0.302235895	0.302235895	0.278345751	0.601943607
	Colour: Size	3	0.402839872	0.134279957	0.123665839	0.945349144
Trichromat	**Colour**	3	18.53902661	6.179675537	15.14866435	4.76402E-06
	Size	1	0.011367546	0.011367546	0.027866049	0.868623846
	Colour: Size	3	0.344332974	0.114777658	0.28136238	0.838385052
Tetrachromat	**Colour**	3	89.09037756	29.69679252	10.24253232	0.000101229
	Size	1	0.419978116	0.419978116	0.144851988	0.706376562
	Colour: Size	3	2.887939507	0.962646502	0.332020299	0.802238036

Table 37*:* Un-simplified model ANOVA output for change in luminance data from Chapter 1 colour change experiments.
Models produced using the lmer() function in the LME4 package.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Colour	8.23E-05	2.74E-05	3	28.00008	1.855261	0.160145
Size	0.000106	0.000106	1	28.00007	7.200076	0.012098
Vision	0.00017	5.67E-05	3	84	3.836167	0.012573
Colour:Size	7.13E-05	2.38E-05	3	28.00009	1.608232	0.209751
Colour:	9.13E-05	1.01E-05	9	84	0.686663	0.718974
Vision						
Size:Vision	0.000193	6.43E-05	3	84	4.349207	0.006731
Colour:	0.000147	1.63E-05	9	84	1.105005	0.368345
Size:Vision						

Table 38: Un-simplified model ANOVA output for change in luminance JND data from Chapter 1 colour change experiments.
Models produced using the lmer() function in the LME4 package.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Colour	401.1065	133.7022	3	28	3.081735	0.043476
Size	432.7333	432.7333	1	28	9.974178	0.003784
Vision	315.2939	105.098	3	84	2.42243	0.07155
Colour:Size	340.865	113.6217	3	28	2.618894	0.070495
Colour:	758.1361	84.23734	9	84	1.941608	0.056798
Vision						
Size:Vision	271.2526	90.41752	3	84	2.084056	0.108446
Colour:	643.7245	71.52494	9	84	1.648596	0.114746
Size:Vision						

Appendix 2A – Dichromat Total Patten Change Results from Chapter 2

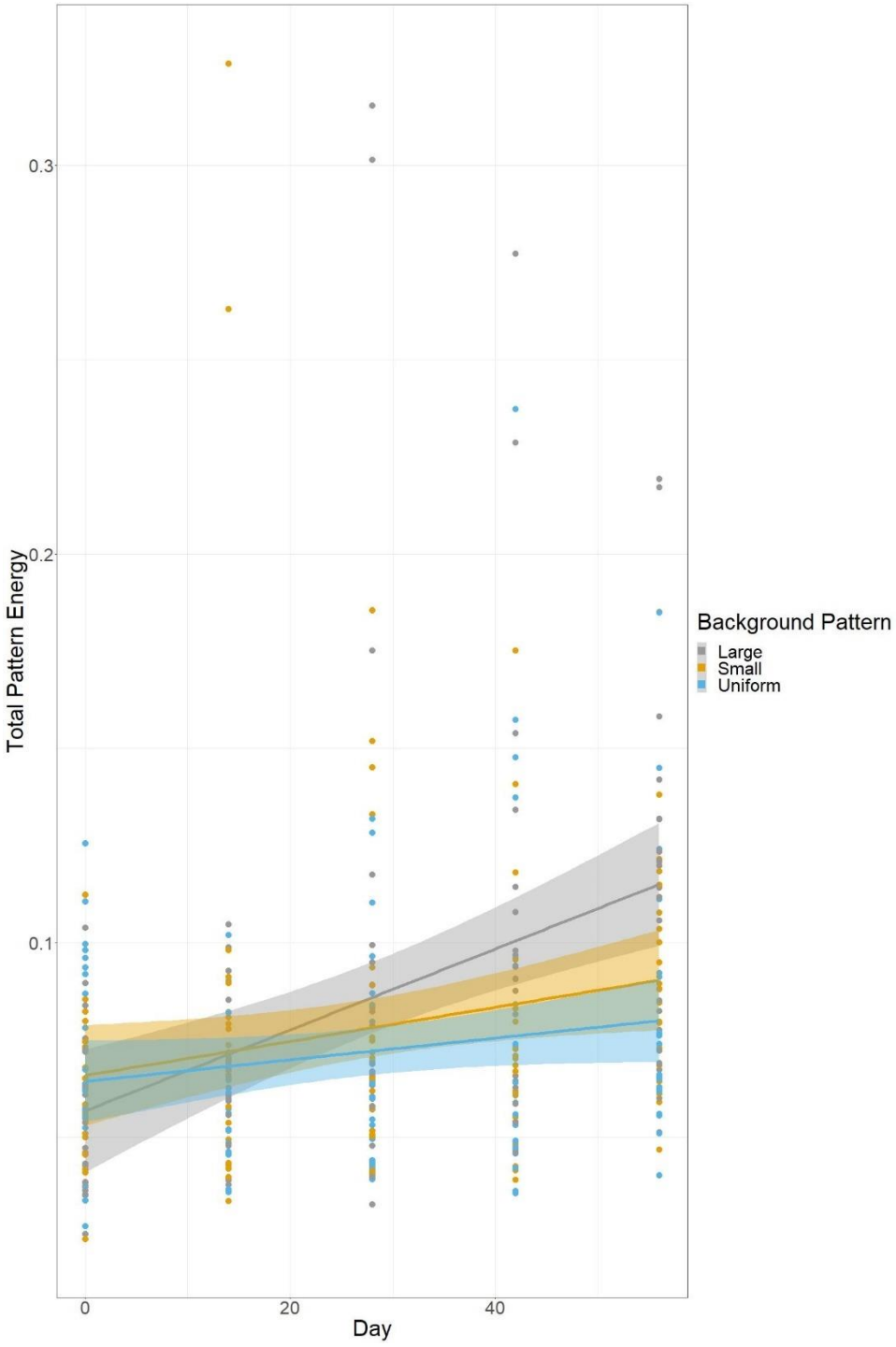

Figure 36: Crab total pattern energy (contrast) change over time, using luminance values based on dichromat vision (Pollack (Shand et al. 1988)) from pattern change experiments.

Lines are linear regressions based on y ~ x. Colours correspond to background pattern. Large = 5mm^2 black and white grid, small = 0.75 mm^2 black and white grid, uniform = neutral grey. Shaded region corresponds to 95% confidence intervals. Graph produced using the ggplot2 R package (Wickham 2016)

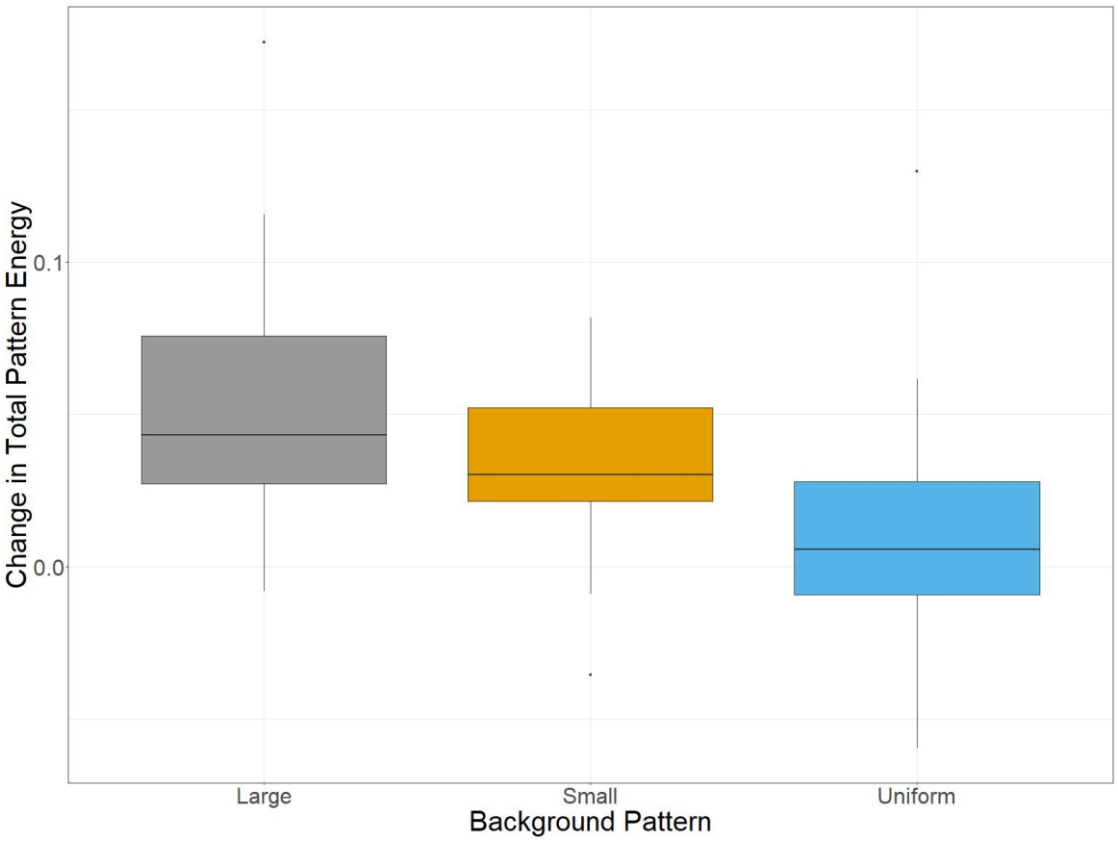

Figure 37: Change in Total Pattern Energy, using luminance values based on dichromat vision (Pollack (Shand et al. 1988)) from pattern change experiments. Backgrounds Large (5mm^2), small (0.75mm^2) patterned backgrounds, or a uniform grey background. Graph produced using the ggplot2 package in R.

Only dichromat visual models were used as a confirmation of the lack of difference in outputs derived from differing visual models. This is based on the results of Chapter 2, as well as the results of the Chapter 4, and the dichromat and trichromat visual models of Chapter 3 data, reported in Appendix 3A (Figure 37 and Figure 38, completed prior to pattern analysis experiments).

Appendix 2B – Examples of pattern change of crabs in spatial change experiments

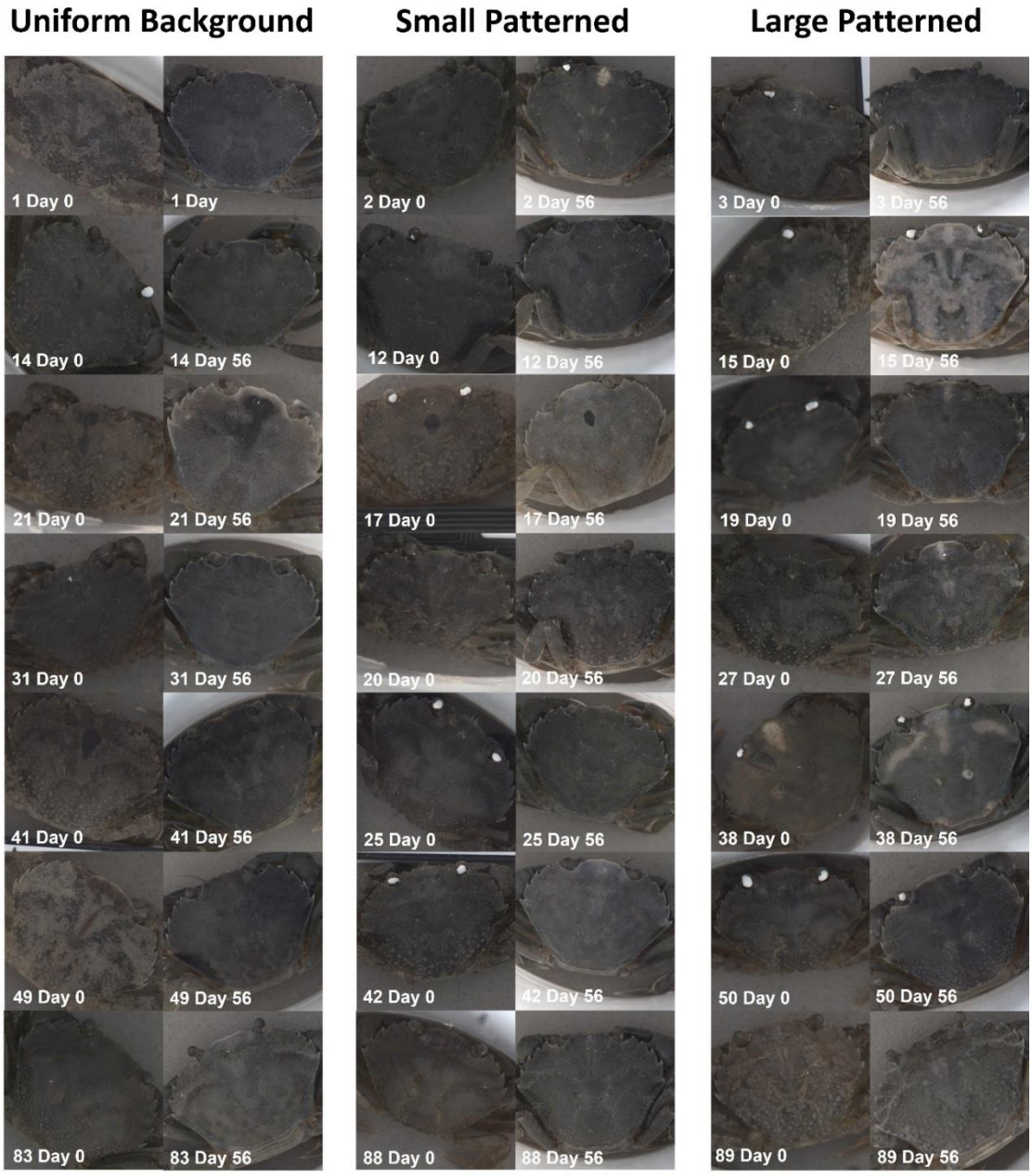

Figure 38: Presentation images of a subsample of crabs from Chapter 2's pattern change experiments.

Photos are from the start (Day 0) and end (Day 56) of experiments. White dots on eyes of crabs were white paint markers used to track eyes in optomotor trials to measure acuity. Images are false colour RGB images produced from multispectral images generated by the MICA Toolbox in imageJ. Photographs are not scaled.

Appendix 2C – Un-simplified model output from pattern change experiments in Chapter 2.

Table 39: Un-simplified model ANOVA output for acuity analysis from Chapter 2.

Models produced using the lmer() function in the LME4 package.

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Size	374.0311	374.0311	1	101.6309	40.16255	6.48E-09
Moult Proximity	6.082644	6.082644	1	184.0205	0.65314	0.420036
Timepoint	27.22812	27.22812	1	192.4571	2.923689	0.0889
Size: Moult Proximity	16.76041	16.76041	1	185.1181	1.799692	0.181393

Table 40: Un-simplified model ANOVA output of longitudinal Total Pattern Energy data from pattern analysis in Chapter 2.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Day	1.415179	1.415179	1	259	15.21807	0.000122
Background	0.276866	0.138433	2	109.6858	1.488632	0.230205
Average Acuity	0.04288	0.04288	1	109.5837	0.461111	0.498537
Moult Count	0.001155	0.001155	1	109.495	0.012415	0.911484
Day: Background	0.569229	0.284615	2	259	3.060591	0.048559
Day: Average Acuity	0.261768	0.261768	1	259	2.814915	0.094598
Background: Average Acuity	0.223837	0.111919	2	109.6924	1.203513	0.30407
Day: Moult Count	0.206082	0.206082	1	259	2.216098	0.137794
Background: Moult Count	0.224525	0.112263	2	108.0607	1.207213	0.30303
Day: Background: Acuity	0.257407	0.128703	2	259	1.384007	0.252419
Day: Background: Moult Count	0.193287	0.096644	2	259	1.039254	0.355189

Table 41: Un-simplified model ANOVA output of longitudinal Dominant Marking Size data from pattern analysis in Chapter 2.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Day	7.076016	7.076016	1	259.0000	2.8176175	0.0944408
Background	3.924450	1.962225	2	197.8040	0.7813436	0.4591982
Average Acuity	0.558415	0.558415	1	197.8040	0.2223568	0.6377697
Moult Count	0.139666	0.139666	1	197.8040	0.0556138	0.8138118
Day: Background	7.812416	3.906208	2	259.0000	1.5554233	0.2130653
Day: Average Acuity	8.415533	8.415533	1	259.0000	3.3510036	0.0683131
Background: Average Acuity	3.351264	1.675632	2	197.8040	0.6672243	0.5142819
Day: Moult Count	0.077999	0.077999	1	259.0000	0.0310587	0.8602472
Background: Moult Count	7.384829	3.692414	2	197.8040	1.4702923	0.2323592
Day: Background: Acuity	5.509461	2.754731	2	259.0000	1.0969135	0.3354461
Day: Background: Moult Count	0.781076	0.390538	2	259.0000	0.1555094	0.8560589

Table 42: Un-simplified model ANOVA output of longitudinal Pattern Energy Difference (PED) data from pattern analysis in Chapter 2.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Day	0.001178	0.001178	1	259.0002	1.447516	0.230026
Background	0.985356	0.492678	2	116.7775	605.2633	2.31E-62
Average Acuity	0.001384	0.001384	1	116.7775	1.699826	0.194875
Moult Count	0.000147	0.000147	1	116.7775	0.180506	0.671721
Day: Background	0.000402	0.000201	2	259.0002	0.246911	0.781394
Day: Average Acuity	0.000624	0.000624	1	259.0002	0.766063	0.382249
Background: Average Acuity	0.001217	0.000608	2	116.7775	0.747401	0.475847
Day: Moult Count	0.000291	0.000291	1	259.0002	0.357243	0.550564
Background: Moult Count	0.000219	0.00011	2	116.7775	0.134682	0.874129
Day: Background: Acuity	0.001343	0.000671	2	259.0002	0.824849	0.439449
Day: Background: Moult Count	0.006016	0.003008	2	259.0002	3.695557	0.026153

Table 43: Un-simplified model ANOVA output of longitudinal luminance data from pattern analysis in Chapter 2.
Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Day	0.82141	0.82141	1	259	20.66244	8.41E-06
Background	0.046842	0.023421	2	96.75428	0.589157	0.556773
Average Acuity	0.033835	0.033835	1	97.47275	0.85111	0.358516
Moult Count	0.01722	0.01722	1	97.61103	0.433155	0.511994
Day: Background	0.069797	0.034899	2	259	0.87787	0.4169
Day: Average Acuity	0.067656	0.067656	1	259	1.701879	0.1932
Background: Average Acuity	0.032954	0.016477	2	96.67921	0.414478	0.661854
Day: Moult Count	0.124967	0.124967	1	259	3.143531	0.077404
Background: Moult Count	0.048248	0.024124	2	90.86115	0.606836	0.547267
Day: Background: Acuity	0.056472	0.028236	2	259	0.710274	0.492464
Day: Background: Moult Count	0.006989	0.003495	2	259	0.087906	0.915874

Overall Change Models

Table 44: Un-simplified model ANOVA output of overall change in Total Pattern Energy data from pattern analysis in Chapter 2.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Background	0.004408	0.002204	2	54.43612	1.146689	0.32524
Average Acuity	0.004404	0.004404	1	54.31036	2.29115	0.135912
Moult Count	0.000762	0.000762	1	54.35355	0.396205	0.531693
Background: Average Acuity	0.003578	0.001789	2	54.44923	0.930799	0.40042
Background: Moult Count	0.001876	0.000938	2	54.41673	0.487931	0.616555
Average Acuity: Moult Count	0.002543	0.002543	1	54.10499	1.32311	0.255093
Background: Average Acuity: Moult Count	0.002375	0.001187	2	54.46923	0.61771	0.542911

Table 45: Un-simplified model ANOVA output of overall change in Dominant Marking Size data from pattern analysis in Chapter 2.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Background	16832.77	8416.387	2	54.00636	0.143332	0.866795
Average Acuity	1018.896	1018.896	1	54.39559	0.017352	0.895687
Moult Count	5191.699	5191.699	1	54.44843	0.088415	0.767333
Background: Average Acuity	45427.29	22713.64	2	54.54033	0.386815	0.681065
Background: Moult Count	23150.5	11575.25	2	53.90564	0.197127	0.821675
Average Acuity: Moult Count	7042.874	7042.874	1	54.13642	0.119941	0.730441
Background: Average Acuity: Moult Count	52280.91	26140.46	2	54.56764	0.445174	0.643019

Table 46: Un-simplified model ANOVA output of overall change in Pattern Energy Difference (PED) data from pattern analysis in Chapter 2.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Background	0.004367	0.002183	2	55	1.326593	0.273737
Average Acuity	0.00277	0.00277	1	55	1.68275	0.199975
Moult Count	0.001382	0.001382	1	55	0.839487	0.363544
Background: Average Acuity	0.004563	0.002281	2	55	1.386105	0.258644
Background: Moult Count	0.002327	0.001163	2	55	0.706767	0.49766
Average Acuity: Moult Count	0.001755	0.001755	1	55	1.066471	0.306264
Background: Average Acuity: Moult Count	0.003649	0.001824	2	55	1.10847	0.337324

Table 47: Un-simplified model ANOVA output of overall change in luminance data from pattern analysis in Chapter 2.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Background	0.004367	0.002183	2	55	1.326593	0.273737
Average Acuity	0.00277	0.00277	1	55	1.68275	0.199975
Moult Count	0.001382	0.001382	1	55	0.839487	0.363544
Background: Average Acuity	0.004563	0.002281	2	55	1.386105	0.258644
Background: Moult Count	0.002327	0.001163	2	55	0.706767	0.49766
Average Acuity: Moult Count	0.001755	0.001755	1	55	1.066471	0.306264
Background: Average Acuity: Moult Count	0.003649	0.001824	2	55	1.10847	0.337324

Appendix 3A – Dichromat and trichromat image analysis results of substrate perception experiments

Dichromat results

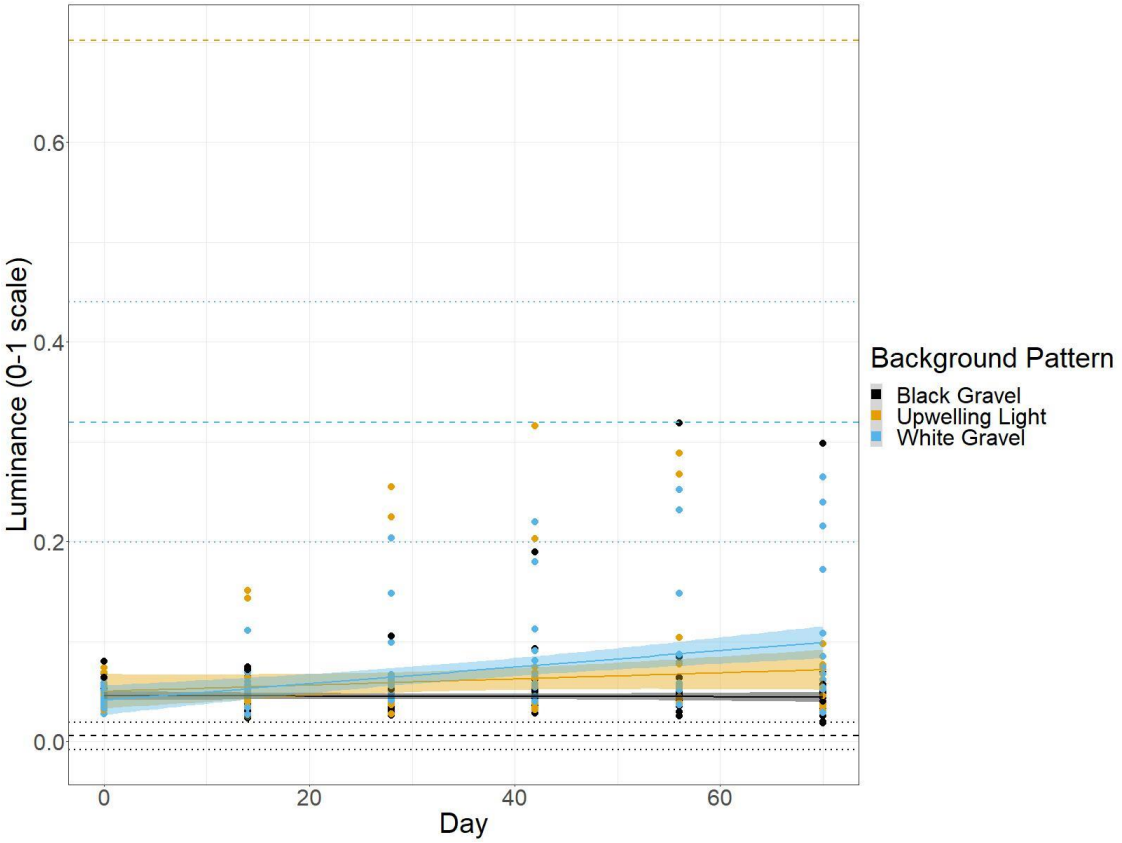

Figure 39: Dichromat luminance values for crabs over time for substrate perception experiments.

Colours correspond to lighting treatment. Straight lines produced via luminance ~ time linear models, with 95% confidence intervals. Lines and confidence intervals do not include one sample - B2, isolated as an outlier. B2 was included on the plot and can be seen as the highest black point on days 28 and 42, and the highest point on days 56 and 70. Horizontal lines indicate luminance values for white (blue lines) and black gravel (black lines) respectively. Dashed lines equal the mean treatment luminance, dotted lines ± std. deviation. NB Black gravel deviation cannot be below 0, as 0 indicative of complete absorbance (no reflectance). The upwelling light treatment only had the mean plotted as the std. deviation spanned beyond the limits of luminance scores. It is included simply to demonstrate that even when the white is well lit (via arc lamp, significantly brighter than over than lights even without restriction), the light treatment will be

perceived significantly brighter, given radiance will be constant regardless of incident light. Graphs plotted with ggplot2 (Wickham 2016).

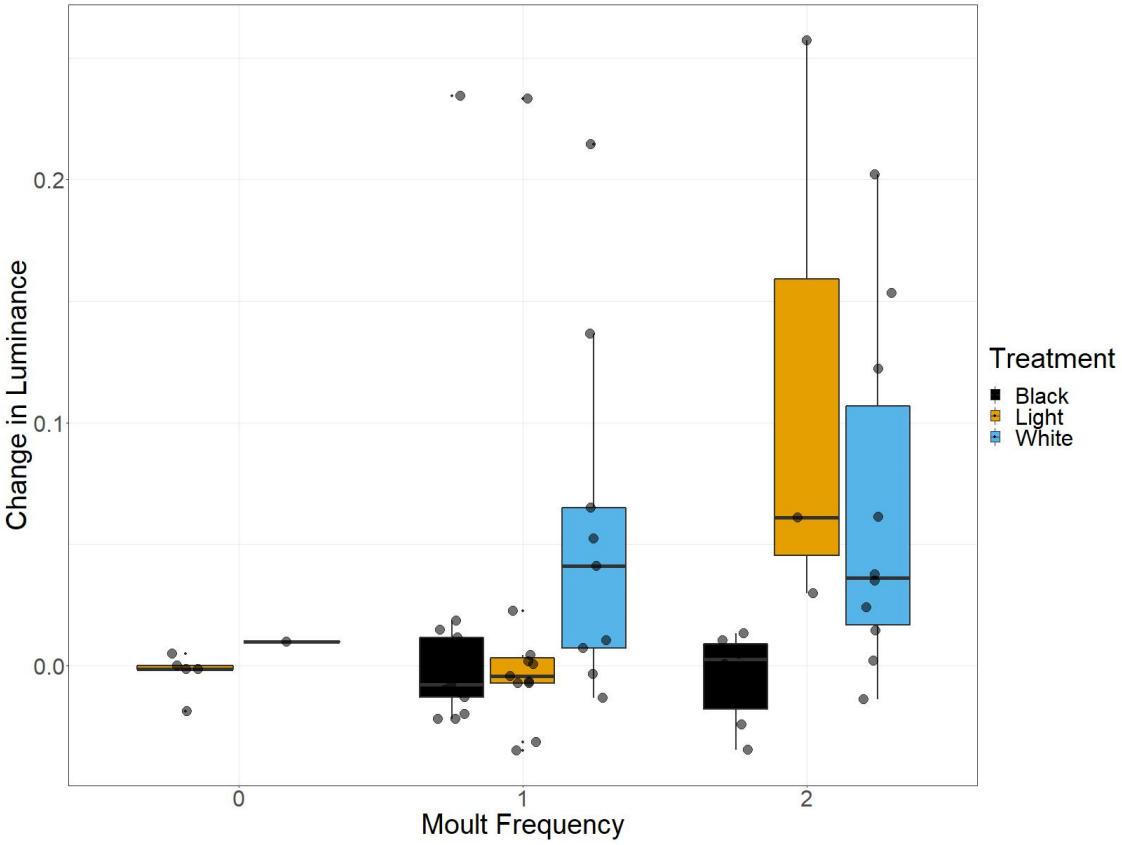

Figure 40: Change in dichromat luminance of crabs for substrate perception experiments on each of the three treatments, split by moult frequency. Fill colour corresponds to experimental treatment. All crabs are included, regardless of duration of time. All crabs were included that survived at least 14 days, as this allowed for a minimum of two digital image collections, and potential colour change to be recorded. All 19 black crabs that survived at least 14 days moulted once, while multiple crabs on light and white did not. Graphs plotted with ggplot2 (Wickham 2016).

Trichromat results

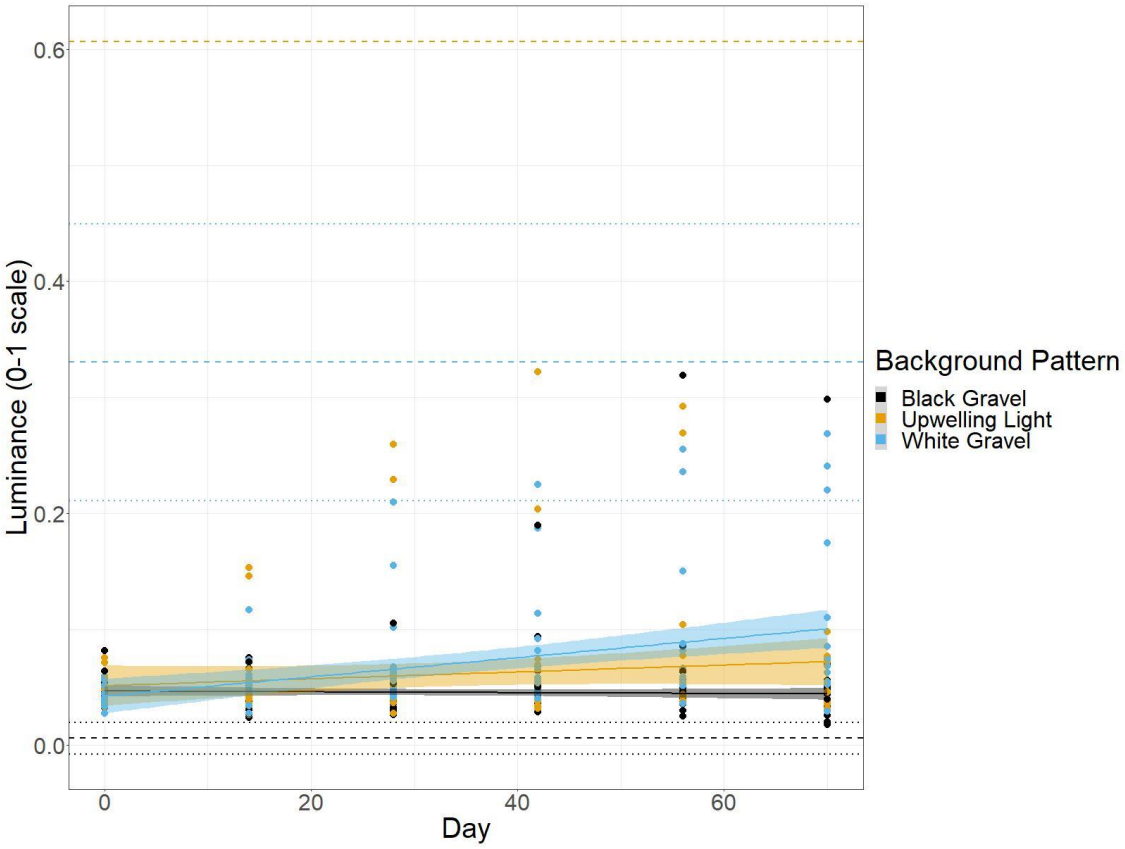

Figure 41: Trichromat luminance values for crabs over time for substrate perception experiments.

Colours correspond to lighting treatment. Straight lines produced via luminance ~ time linear models, with 95% confidence intervals. Lines and confidence intervals do not include one sample - B2, isolated as an outlier. B2 was included on the plot and can be seen as the highest black point on days 28 and 42, and the highest point on days 56 and 70. Horizontal lines indicate luminance values for white (blue lines) and black gravel (black lines) respectively. Dashed lines equal the mean treatment luminance, dotted lines ± std. deviation. NB Black gravel deviation cannot be below 0, as 0 indicative of complete absorbance (no reflectance). The upwelling light treatment only had the mean plotted as the std. deviation spanned beyond the limits of luminance scores. It is included simply to demonstrate that even when the white is well lit (via arc lamp, significantly brighter than over than lights even without restriction), the light treatment will be perceived significantly brighter, given radiance will be constant regardless of incident light. Graphs plotted with ggplot2 (Wickham 2016).

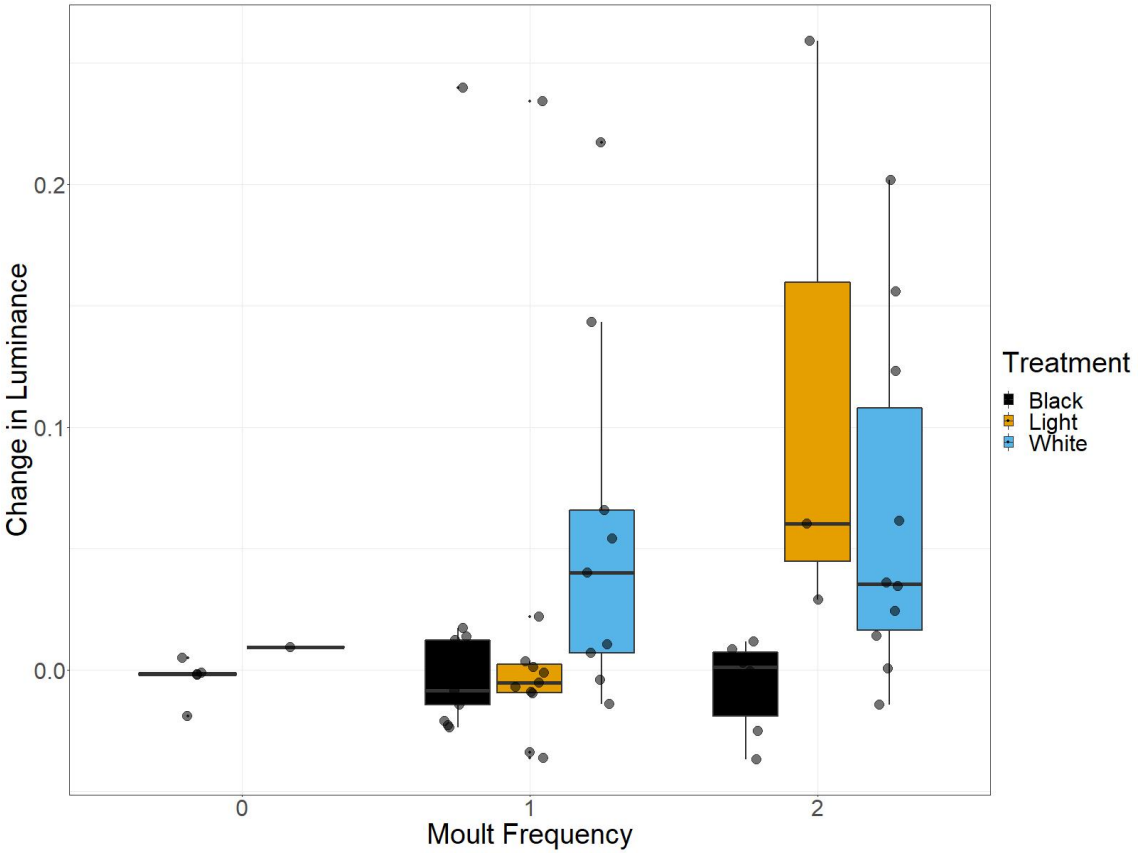

Figure 42: Change in trichromat luminance of crabs for substrate perception experiments on each of the three treatments, split by moult frequency. Fill colour corresponds to experimental treatment. All crabs are included, regardless of duration of time. All crabs were included that survived at least 14 days, as this allowed for a minimum of two digital image collections, and potential colour change to be recorded. All 19 black crabs that survived at least 14 days moulted once, while multiple crabs on light and white did not. Graphs plotted with ggplot2 (Wickham 2016).

Appendix 3B – Examples of crab colour change for substrate perception experiments

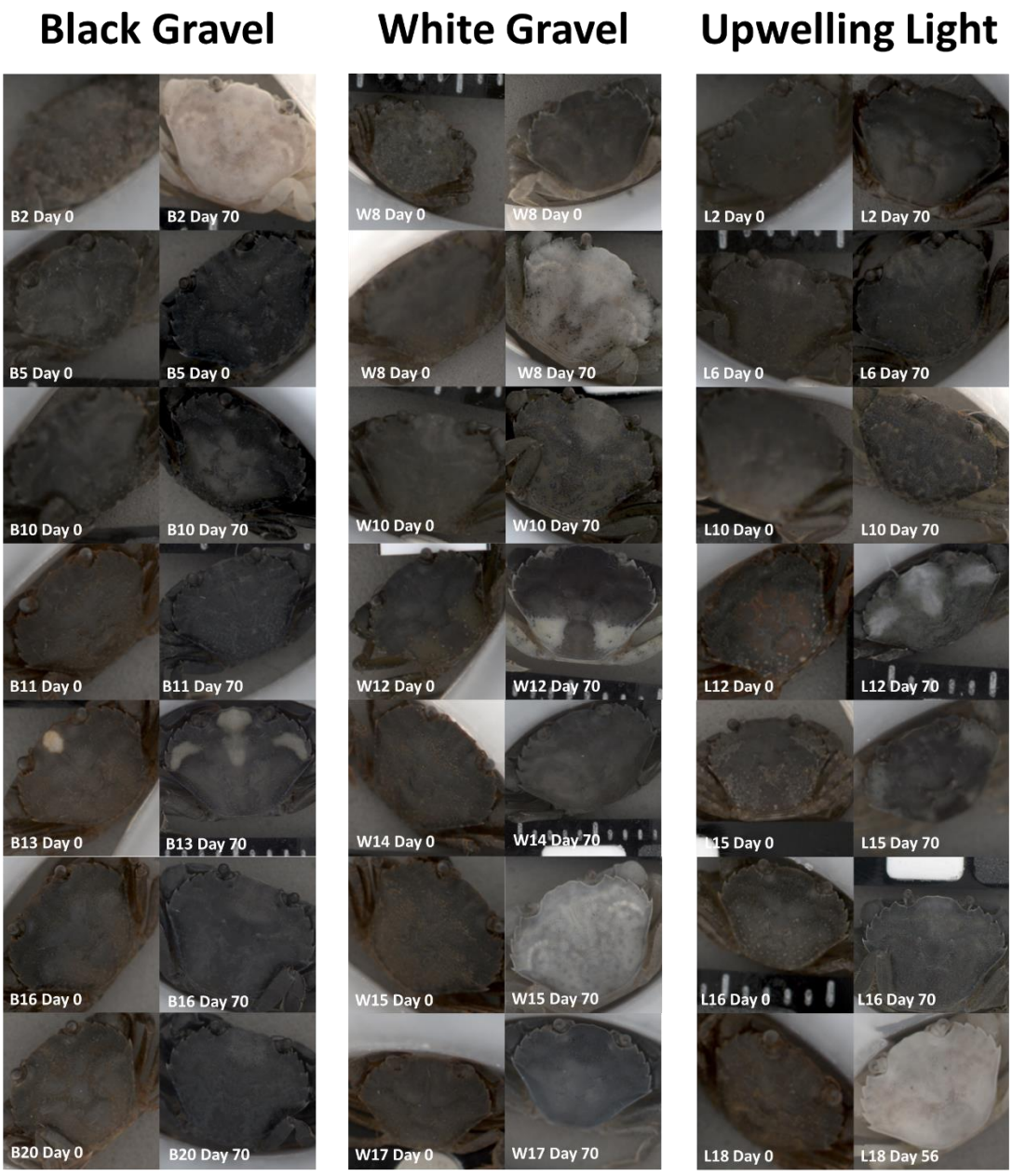

Figure 43: Presentation images of a subsample of crabs from Chapter 3's substrate perception and brightness change experiments.
Photos are from the start (Day 0) and end (Day 70) of experiments. The exception is sample L18, which died prior to final photo graphs, but is included as an example of the brightness achieved in the experiment. Also included is sample B2, to demonstrate difference from other samples on black gravel. Images are false colour RGB images produced from multispectral images generated by the MICA Toolbox in imageJ. Photographs are not scaled.

Appendix 3C – Un-simplified model output from substrate perception experiments in Chapter 4

Table 48: Un-simplified model ANOVA output of longitudinal luminance data for substrate perception experiments in Chapter 4.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Day	0.00142	0.00142	1	265.1117	1.651993	0.19981
Treatment	0.000523	0.000262	2	80.91005	0.304358	0.738437
Moult Count	1.56E-05	1.56E-05	1	80.07415	0.018111	0.893283
Day: Treatment	0.003325	0.001663	2	265.4248	1.934451	0.146535
Day: Moult Count	0.006569	0.006569	1	263.3457	7.643321	0.006101
Treatment: Moult Count	0.000805	0.000402	2	80.36014	0.468309	0.627758
Day: Treatment: Moult Count	0.004175	0.002087	2	263.7132	2.428735	0.090118

Table 49: Un-simplified model ANOVA output of overall change in luminance data for substrate perception experiments in Chapter 4.

Models produced using the lmer() function in the LME4 package

	Sum Sq	Mean Sq	NumDF	DenDF	F value	Pr(>F)
Treatment	0.05433	0.027165	2	51.05495	7.113345	0.001883
Moult Count	0.021271	0.010636	2	51.9528	2.785046	0.070961
Treatment: Moult Count	0.022131	0.007377	3	51.32994	1.931715	0.136072

Bibliography

Akkaynak, Derya. 2014. 'Use of spectroscopy for assessment of color discrimination in animal vision', *Journal of the Optical Society of America A*, 31: A27-A33.

Akkaynak, Derya, Liese A. Siemann, Alexandra Barbosa, and Lydia M. Mäthger. 2017. 'Changeable camouflage: How well can flounder resemble the colour and spatial scale of substrates in their natural habitats?', *Royal Society Open Science*, 4.

Alexander, Jodi L., Andrew Oliphant, David C. Wilcockson, Timothy Brendler-Spaeth, Heinrich Dircksen, and Simon G. Webster. 2020. 'Pigment dispersing factors and their cognate receptors in a crustacean model, with new insights into distinct neurons and their functions', *Frontiers in neuroscience*, 14: 595648-48.

Allen, Justine J, Derya Akkaynak, Arthur U Sugden, and Roger T Hanlon. 2015. 'Adaptive body patterning, three-dimensional skin morphology and camouflage measures of the slender filefish *Monacanthus tuckeri* on a Caribbean coral reef', *Biological Journal of the Linnean Society*, 116: 377-96.

Allen, Justine J, Lydia M Mäthger, Kendra C Buresch, Thomas Fetchko, Meg Gardner, and Roger T Hanlon. 2010. 'Night vision by cuttlefish enables changeable camouflage', *Journal of Experimental Biology*, 213: 3953-60.

Allen, Justine J., Lydia M. Mäthger, Alexandra Barbosa, Kendra C. Buresch, Emilia Sogin, Jillian Schwartz, Charles Chubb, and Roger T. Hanion. 2010. 'Cuttlefish dynamic camouflage: responses to substrate choice and integration of multiple visual cues', *Proceedings of the Royal Society B: Biological Sciences*, 277: 1031-39.

Almeida, Maria João, Augusto A. V. Flores, and Henrique Queiroga. 2008. 'Effect of crab size and habitat type on the locomotory activity of juvenile shore crabs, *Carcinus maenas*', *Estuarine, Coastal and Shelf Science*, 80: 509-16.

Amaral, Valter, Henrique N. Cabral, Stuart Jenkins, Stephen Hawkins, and José Paula. 2009. 'Comparing quality of estuarine and nearshore intertidal habitats for *Carcinus maenas*', *Estuarine, Coastal and Shelf Science*, 83: 219-26.

Ameyaw-Akumfi, C, and E Naylor. 1987. 'Spontaneous and induced components of salinity preference behaviour in *Carcinus maenas*', *Marine Ecology Progress Series*, 37: 153-58.

Anderson, A. G., and G. N. Dodson. 2015. 'Colour change ability and its effect on prey capture success in female *Misumenoides formosipes* crab spiders', *Ecological Entomology*, 40: 106-13.

Anderson, Joshua R, Angelo J Spadaro, J Antonio Baeza, and Donald C Behringer. 2013. 'Ontogenetic shifts in resource allocation: colour change and allometric growth of defensive and reproductive structures in the Caribbean spiny lobster *Panulirus argus*', *Biological Journal of the Linnean Society*, 108: 87-98.

Aoto, Tomoji. 1963. 'The primary response of white chromatophores and the nauplius-eye in the prawn, *Palaemon paucidens* (With 9 Text-figures)', *北海道大學理學部紀要*, 15: 177-89.

Atkins, D. 1926. 'On nocturnal colour change in the pea-crab *(Pinnotheres veterum*) [4]', *Nature*, 117: 415-16.

Auerswald, Lutz, Ulrich Freier, Andreas Lopata, and Bettina Meyer. 2008. 'Physiological and morphological colour change in Antarctic krill, *Euphausia superba*: a field study in the Lazarev Sea', *Journal of Experimental Biology*, 211: 3850-58.

Bagnara, Joseph T, and J. Matsumoto. 2006. 'Comparative anatomy and physiology of pigment cells in nonmammalian tissues.' in James J Nordlund, Raymond E Boissy, Vincent J Hearing, Richard A King, William S Oetting and Jean-Paul Ortonne (eds.), *The Pigmentary System: Physiology and Pathophysiology* (Blackwell Publishing Ltd: Oxford, UK).

Baldwin, Jamie, and Sönke Johnsen. 2011. 'Effects of molting on the visual acuity of the blue crab, Callinectes sapidus', *Journal of Experimental Biology*, 214: 3055-61.

——. 2012. 'The male blue crab, *Callinectes sapidus*, uses both chromatic and achromatic cues during mate choice', *Journal of Experimental Biology*, 215: 1184-91.

Barbosa, Alexandra, Lydia M. Mäthger, Kendra C. Buresch, Jennifer Kelly, Charles Chubb, Chuan Chin Chiao, and Roger T. Hanlon. 2008. 'Cuttlefish camouflage: The effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns', *Vision Research*, 48: 1242-53.

Barbosa, Alexandra, Lydia M. Mäthger, Charles Chubb, Christopher Florio, Chuan Chin Chiao, and Roger T. Hanlon. 2007. 'Disruptive coloration in cuttlefish: A visual perception mechanism that regulates ontogenetic adjustment of skin patterning', *Journal of Experimental Biology*, 210: 1139-47.

Barnatan, Yair, Daniel Tomsic, and Julieta Sztarker. 2019. 'Unidirectional optomotor responses and eye dominance in two species of crabs', *Frontiers in Physiology*, 10.

Bates, Douglas, Martin Mächler, Ben Bolker, and Steve Walker. 2015. 'Fitting linear mixed-effects models using lme4', *Journal of Statistical Software*, 67: 1 - 48.

Bedini, Roberto. 2002. 'Colour change and mimicry from juvenile to adult: *Xantho poressa* (Olivi, 1792)(Brachyura, Xanthidae) and *Carcinus maenas* (Linnaeus, 1758)(Brachyura, Portunidae)', *Crustaceana*: 703-10.

Bellwood, Orpha. 2002. 'The occurrence, mechanics and significance of burying behaviour in crabs (Crustacea: Brachyura)', *Journal of Natural History*, 36: 1223-38.

Bian, Xue, Mark A Elgar, and Richard A Peters. 2016. 'The swaying behavior of *Extatosoma tiaratum*: motion camouflage in a stick insect?', *Behavioral Ecology*, 27: 83-92.

Boletzky, Sigurd von. 1996. 'Cephalopods burying in soft substrata: agents of bioturbation?', *Marine Ecology*, 17: 77-86.

Bond, Alan B. 2007. "The evolution of color polymorphism: Crypticity, searching images, and apostatic selection." In *Annual Review of Ecology, Evolution, and Systematics*, 489-514.

Bond, Alan B., and Alan C. Kamil. 2002. 'Visual predators select for crypticity and polymorphism in virtual prey', *Nature*, 415: 609-13.

—. 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: 3214-19.

Booth, Carey L. 1990. 'Evolutionary significance of ontogenetic colour change in animals', *Biological Journal of the Linnean Society*, 40: 125-63.

Brian, Jayne V., Teresa Fernandes, Richard J. Ladle, and Peter A. Todd. 2006. 'Patterns of morphological and genetic variability in UK populations of the shore crab, *Carcinus maenas* Linnaeus, 1758 (Crustacea: Decapoda: Brachyura)', *Journal of Experimental Marine Biology and Ecology*, 329: 47-54.

Briolat, Emmanuelle Sophie, Lina María Arenas, Anna E. Hughes, Eric Liggins, and Martin Stevens. 2021. 'Generalist camouflage can be more successful than microhabitat specialisation in natural environments', *BMC Ecology and Evolution*, 21: 151.

Brown Jr, Frank A. 1950. 'Studies on the physiology of *Uca* red chromatophores', *The Biological Bulletin*, 98: 218-26.

Brown Jr, Frank A, and Muriel I Sandeen. 1948. 'Responses of the chromatophores of the fiddler crab, *Uca,* to light and temperature', *Physiological Zoology*, 21: 361-71.

Bruno, Merle S., and Timothy H. Goldsmith. 1974. 'Rhodopsin of the blue crab *Callinectes*: Evidence for absorption differences in vitro and in vivo', *Vision Research*, 14: 653-58.

Bruno, Merle S., Michael I. Mote, and Timothy H. Goldsmith. 1973. 'Spectral absorption and sensitivity measurements in single ommatidia of the green crab, *Carcinus*', *Journal of Comparative Physiology*, 82: 151-63.

Bückmann, Detlef. 1977. 'Morphological colour change: Stage independent, optically induced ommochrome synthesis in larvae of the stick insect, *Carausius morosus* Br', *Journal of Comparative Physiology*, 115: 185-93.

—. 1979. 'Morphological colour change (2): The effects of total and partial blinding on epidermal ommochrome content in the stick insect,*Carausius morosus* Br', *Journal of Comparative Physiology*, 130: 331-36.

Bueno-Villafañe, Diego, Andrea Caballlero-Gini, Marcela Ferreira, Flavia Netto, Danilo Fernández Ríos, and Francisco Brusquetti. 2020. 'Ontogenetic changes in the ventral colouration of post metamorphic *Elachistocleis haroi* Pereyra, Akmentins, Laufer, Vaira, 2013 (Anura: Microhylidae)', *Amphibia-Reptilia*, 41: 191-200.

Bulbert, Matthew W, Thomas E White, Ralph A Saporito, and Fred Kraus. 2017. 'Ontogenetic colour change in *Oreophryne ezra* (Anura: Microhylidae) reflects an unusual shift from conspicuousness to crypsis but not in toxicity', *Biological Journal of the Linnean Society*, 123: 12-20.

Bursey, C. R. 1984. 'Color recognition by the blue crab, *Callinectes sapidus* Rathbun (Decapoda, Brachyura)', *Crustaceana*, 47: 278-84.

Burtt, E. 2009. 'The ability of adult grasshoppers to change colour on burnt ground', *Proceedings of the Royal Entomological Society of London. Series A, General Entomology*, 26: 45-48.

Carter, Emily E., Tom Tregenza, and Martin Stevens. 2020. 'Ship noise inhibits colour change, camouflage, and anti-predator behaviour in shore crabs', *Current Biology*, 30: R211-R12.

Caves, Eleanor M., Nicholas C. Brandley, and Sönke Johnsen. 2018. 'Visual acuity and the evolution of signals', *Trends in Ecology & Evolution*, 33: 358-72.

Chiao, Chuan-Chin, Charles Chubb, and Roger T Hanlon. 2007. 'Interactive effects of size, contrast, intensity and configuration of background objects in evoking disruptive camouflage in cuttlefish', *Vision Research*, 47: 2223-35.

Chiao, Chuan-Chin, Charles Chubb, and Roger T. Hanlon. 2015. 'A review of visual perception mechanisms that regulate rapid adaptive camouflage in cuttlefish', *Journal of Comparative Physiology A*, 201: 933-45.

Chiao, Chuan-Chin, and Roger T Hanlon. 2001a. 'Cuttlefish camouflage: Visual perception of size, contrast and number of white squares on artificial checkerboard substrata initiates disruptive coloration', *Journal of Experimental Biology*, 204: 2119-25.

—. 2001b. 'Cuttlefish cue visually on area—not shape or aspect ratio—of light objects in the substrate to produce disruptive body patterns for camouflage', *The Biological Bulletin*, 201: 269-70.

Chiao, Chuan-Chin, Emma J Kelman, and Roger T Hanlon. 2005. 'Disruptive body patterning of cuttlefish (*Sepia officinalis*) requires visual information regarding edges and contrast of objects in natural substrate backgrounds', *The Biological Bulletin*, 208: 7-11.

Chiao, Chuan-Chin, J. Kenneth Wickiser, Justine J. Allen, Brock Genter, and Roger T. Hanlon. 2011. 'Hyperspectral imaging of cuttlefish camouflage

indicates good color match in the eyes of fish predators', *Proceedings of the National Academy of Sciences*, 108: 9148-53.

Clarke, Cyril A, GS Mani, and Goronwy Wynne. 1985. 'Evolution in reverse: clean air and the peppered moth', *Biological Journal of the Linnean Society*, 26: 189-99.

Clarke, George L., and Harry R. James. 1939. 'Laboratory analysis of the selective absorption of light by sea water*', *Journal of the Optical Society of America*, 29: 43-55.

Cook, L. M., and I. J. Saccheri. 2013. 'The peppered moth and industrial melanism: evolution of a natural selection case study', *Heredity*, 110: 207-12.

Cook, LM, GS Mani, and ME Varley. 1986. 'Postindustrial melanism in the peppered moth', *Science*, 231: 611-13.

Corl, Ammon, Ke Bi, Claudia Luke, Akshara Sree Challa, Aaron James Stern, Barry Sinervo, and Rasmus Nielsen. 2018. 'The genetic basis of adaptation following plastic changes in coloration in a novel environment', *Current Biology*, 28: 2970-77.e7.

Cortesi, Fabio, Zuzana Musilová, Sara M Stieb, Nathan S Hart, Ulrike E Siebeck, Karen L Cheney, Walter Salzburger, and N Justin Marshall. 2016. 'From crypsis to mimicry: changes in colour and the configuration of the visual system during ontogenetic habitat transitions in a coral reef fish', *Journal of Experimental Biology*, 219: 2545-58.

Cott, H. B. 1940. *Adaptive coloration in animals.* (Methuen and Co., Ltd.: London).

Coverdale, Tyler C., Eric E. Axelman, Caitlin P. Brisson, Eric W. Young, Andrew H. Altieri, and Mark D. Bertness. 2013. 'New England salt marsh recovery: Opportunistic colonization of an invasive species and its non-consumptive effects', *PLOS ONE*, 8: e73823.

Cresswell, R. 1862. *Aristotle's History of animals: In ten books: Translated by Richard Cresswell* (H. G. Bohn: London).

Cronin, Thomas W, Sönke Johnsen, N Justin Marshall, and Eric J Warrant. 2014. *Visual ecology* (Princeton University Press).

Cronin, Thomas W. 2016. 'Camouflage: Being Invisible in the Open Ocean', *Current Biology*, 26: R1179-R81.

Crothers, J. H. 1968. "The biology of the shore crab *Carcinus maenas* (L.) 2. The life of the adult crab." In.

Cummings, M., and S. Johnsen. 2007. '"Light, Effects of" in L.' in Mark W. Denny and Steven D. Gaines (eds.), *Encyclopedia of Tidepools and Rocky Shores* (University of California Press).

Cuthill, I. C. 2019. 'Camouflage', *Journal of Zoology*, 308: 75-92.

Cuthill, Innes C., Samuel R. Matchette, and Nicholas E. Scott-Samuel. 2019. "Camouflage in a dynamic world." In *Current Opinion in Behavioral Sciences*, 109-15. Elsevier Ltd.

Cuthill, Innes C., N. Simon Sanghera, Olivier Penacchio, Paul George Lovell, Graeme D. Ruxton, and Julie M. Harris. 2016. 'Optimizing countershading camouflage', *Proceedings of the National Academy of Sciences of the United States of America*, 113: 13093-97.

Cuthill, Innes C., Martin Stevens, Jenna Sheppard, Tracey Haddocks, C. Atejandro Párraga, and Tom S. Troscianko. 2005. 'Disruptive coloration and background pattern matching', *Nature*, 434: 72-74.

D'Abramo, Louis R., Nancy A. Baum, Clark E. Bordner, and Douglas E. Conklin. 1983. 'Carotenoids as a Source of Pigmentation in Juvenile Lobsters Fed a Purified Diet', *Canadian Journal of Fisheries and Aquatic Sciences*, 40: 699-704.

Daly, Ilse M, Anna E Tetley, Sheryl L Jared, Martin J How, and Nicholas W Roberts. 2017. 'Colour preference in *Odontodactylus scyllarus* (Linnaeus, 1758) (Stomatopoda)', *Journal of Crustacean Biology*, 37: 374-79.

Darnell, M. Zachary. 2012. 'Ecological physiology of the circadian pigmentation rhythm in the fiddler crab *Uca panacea*', *Journal of Experimental Marine Biology and Ecology*, 426-427: 39-47.

Defrize, Jérémy, Claudio R. Lazzari, Eric J. Warrant, and Jérôme Casas. 2011. 'Spectral sensitivity of a colour changing spider', *Journal of Insect Physiology*, 57: 508-13.

Detto, Tanya. 2007. 'The fiddler crab *Uca mjoebergi* uses colour vision in mate choice', *Proceedings of the Royal Society B: Biological Sciences*, 274: 2785-90.

Dimitrova, Marina, and Sami Merilaita. 2014. 'Hide and seek: Properties of prey and background patterns affect prey detection by blue tits', *Behavioral Ecology*, 25: 402-08.

Dimitrova, Marina, Nina Stobbe, H. Martin Schaefer, and Sami Merilaita. 2009. 'Concealed by conspicuousness: distractive prey markings and backgrounds', *Proceedings of the Royal Society B: Biological Sciences*, 276: 1905-10.

Drerup, Christian, and Martin J. How. 2021. 'Polarization contrasts and their effect on the gaze stabilization of crustaceans', *Journal of Experimental Biology*, 224.

Duarte, Rafael C, Gustavo M Dias, Augusto A V Flores, and Martin Stevens. 2020. 'Different ontogenetic trajectories of body colour, pattern and crypsis in two sympatric intertidal crab species', *Biological Journal of the Linnean Society*, 132: 17-31.

Duarte, Rafael C., Augusto A. V. Flores, and Martin Stevens. 2017. 'Camouflage through colour change: Mechanisms, adaptive value and ecological significance', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372.

Duarte, Rafael C., Martin Stevens, and Augusto A. V. Flores. 2018. 'The adaptive value of camouflage and colour change in a polymorphic prawn', *Scientific Reports*, 8: 16028.

Dumas, Jeannette V., and J. D. Witman. 1993. 'Predation by Herring Gulls (*Larus argentatus* Coues) on two rocky intertidal crab species [*Carcinus maenas* (L.) & *Cancer irroratus* Say]', *Journal of Experimental Marine Biology and Ecology*, 169: 89-101.

Eacock, Amy, Hannah M. Rowland, Nicola Edmonds, and Ilik J. Saccheri. 2017. 'Colour change of twig-mimicking peppered moth larvae is a continuous reaction norm that increases camouflage against avian predators', *PeerJ*, 5: e3999-e99.

Eacock, Amy, Hannah M. Rowland, Arjen E. van't Hof, Carl J. Yung, Nicola Edmonds, and Ilik J. Saccheri. 2019. 'Adaptive colour change and background choice behaviour in peppered moth caterpillars is mediated by extraocular photoreception', *Communications Biology*, 2: 286.

Edmunds, M. 1974. *Defence in animals.* (Longman, London: Harlow, Essex).

Edwards, Roy L. 1958. 'Movements of individual members in a population of the shore crab, *Carcinus maenas* L., in the littoral zone', *The Journal of Animal Ecology*, 27: 37-37.

Ellis, T., B. R. Hoowell, and R. N. Hughes. 1997. 'The cryptic responses of hatchery-reared sole to a natural sand substratum', *Journal of Fish Biology*, 51: 389-401.

Endler, John A. 1978. 'A predator's view of animal color patterns.' in Max K. Hecht, William C. Steere and Bruce Wallace (eds.), *Evolutionary Biology* (Springer US: Boston, MA).

—. 2008. 'Progressive background in moths, and a quantitative measure of crypsis', *Biological Journal of the Linnean Society*, 22: 187-231.

Espinosa, Irene, and Innes C. Cuthill. 2014. 'Disruptive colouration and perceptual grouping', *PLOS ONE*, 9: e87153-e53.

Feller, Kathryn D., Camilla R. Sharkey, Alyssa McDuffee-Altekruse, Heather D. Bracken-Grissom, Nathan P. Lord, Megan L. Porter, and Lorian E. Schweikert. 2021. 'Surf and turf vision: Patterns and predictors of visual acuity in compound eye evolution', *Arthropod Structure & Development*, 60: 101002.

Fennell, J. G., L. Talas, R. J. Baddeley, I. C. Cuthill, and N. E. Scott-Samuel. 2019. 'Optimizing colour for camouflage and visibility using deep learning: The effects of the environment and the observer's visual system', *Journal of The Royal Society Interface*, 16.

Figon, Florent, and Jérôme Casas. 2018. 'Morphological and Physiological Colour Changes in the Animal Kingdom.' in, *eLS* (John Wiley & Sons, Ltd).

Filshie, B. K., M. F. Day, and E. H. Mercer. 1975. 'Colour and colour change in the grasshopper, *Kosciuscola tristis*', *Journal of Insect Physiology*, 21: 1763-70.

Fingerman, Milton. 1956. 'Phase difference in the tidal rhythms of color change of two species of fiddler crab', *The Biological Bulletin*, 110: 274-90.

Fingerman, Milton, Mildred E. Lowe, and William C. Mobberly. 1958. 'Environmental factors involved in setting the phases of tidal rhythm of color change in the fiddler crabs *Uca pugilator* and *Uca minax* 1', *Limnology and Oceanography*, 3: 271-82.

Fingerman, Milton, and Yoshihiro Yamamoto. 1967. 'Daily rhythm of melanophoric pigment migration in eyestalkless fiddler crabs, *Uca pugilator* (Bosc)', *Crustaceana*, 12: 303-19.

Forward, Richard B., Thomas W. Cronin, and John K. Douglass. 1988. 'The visual pigments of crabs II. Environmental adaptations', *Journal of Comparative Physiology A*, 162: 479-90.

Foster, David H. 2011. "Color constancy." In *Vision Research*, 674-700. Pergamon.

Fox, Denis L. 1955. 'Astaxanthin in the American flamingo', *Nature*, 175: 942-43.

Fuhrmann, Mona M, Henrik Nygård, Rupert H Krapp, Jørgen Berge, and Iris Werner. 2011. 'The adaptive significance of chromatophores in the Arctic under-ice amphipod *Apherusa glacialis*', *Polar Biology*, 34: 823-32.

Fulgione, D., M. Trapanese, V. Maselli, D. Rippa, F. Itri, B. Avallone, R. Van Damme, D. M. Monti, and P. Raia. 2014. 'Seeing through the skin: dermal light sensitivity provides cryptism in moorish gecko', *Journal of Zoology*, 294: 122-28.

Gačić, Z., I. Damjanović, B. Mićković, A. Hegediš, and M. Nikčević. 2006. 'Spectral sensitivity of the dogfish shark (*Scyliorhinus canicula*)', *Fish Physiology and Biochemistry*, 33: 21.

Gagnon, Yakir Luc, Daniel C. Osorio, Trevor J. Wardill, N. Justin Marshall, Wen-Sung Chung, and Shelby E. Temple. 2016. 'Can chromatic aberration enable color vision in natural environments?', *Proceedings of the National Academy of Sciences*, 113: E6908-E09.

Galloway, James A. M., Samuel D. Green, Martin Stevens, and Laura A. Kelley. 2020. 'Finding a signal hidden among noise: How can predators overcome camouflage strategies?: How predators overcome camouflage', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 375.

Gamble, F. W., and F. W. Keeble. 1900. 'Memoirs: *Hippolyte varians*: A study in colour-change', *Journal of Cell Science*, s2-43: 589-698.

Garate, Alberto Martínez, Eloísa Urrechaga, José Carlos G. Milicua, Ramon Gomez, and George Britton. 1984. 'A blue carotenoprotein from the carapace of the crab, *Carcinus maenas*', *Comparative Biochemistry and Physiology B*, 77: 605-08.

Gillespie, Rosemary G. 1989. 'Diet-induced color change in the Hawaiian happy-face spider *Theridion grallator*, (Araneae, Theridiidae)', *The Journal of Arachnology*, 17: 171-77.

Grayson, J. O. Y., and Malcolm Edmunds. 1989. 'The causes of colour and colour change in caterpillars of the poplar and eyed hawkmoths (*Laothoe populi* and *Smerinthus ocellata*)', *Biological Journal of the Linnean Society*, 37: 263-79.

Greco, G. 2011. 'Natural antifouling in *Carcinus maenas*: lessons for marine antifouling technology.' in, *BEES–ZEPS* (University College Cork: Cork, Ireland).

Greco, G., T. Svaldo Lanero, S. Torrassa, R. Young, M. Vassalli, A. Cavaliere, R. Rolandi, E. Pelucchi, M. Faimali, and J. Davenport. 2013. 'Microtopography of the eye surface of the crab *Carcinus maenas*: an atomic force microscope study suggesting a possible antifouling potential', *Journal of The Royal Society Interface*, 10: 20130122.

Green, Samuel D., Rafael C. Duarte, Emily Kellett, Natasha Alagaratnam, and Martin Stevens. 2019. 'Colour change and behavioural choice facilitate chameleon prawn camouflage against different seaweed backgrounds', *Communications Biology*, 2: 230.

Greene, Erick. 1996. 'Effect of light quality and larval diet on morph induction in the polymorphic caterpillar *Nemoria arizonaria* (Lepidoptera: Geometridae)', *Biological Journal of the Linnean Society*, 58: 277-85.

Groeger, G., P. A. Cotton, and R. Williamson. 2005. 'Ontogenetic changes in the visual acuity of *Sepia officinalis* measured using the optomotor response', *Canadian Journal of Zoology*, 83: 274-79.

Grosholz, Edwin D., and Gregory M. Ruiz. 1996. 'Predicting the impact of introduced marine species: Lessons from the multiple invasions of the European green crab Carcinus maenas', *Biological Conservation*, 78: 59-66.

Guerra-Bobo, M, and TE Brough. 2011. 'Neighbour density, body size and anti-predator hiding time in the New Zealand mud-crab *Austrohelice crassa*', *Journal of the Marine Biological Association of the United Kingdom*, 91: 691-94.

Guilford, Tim, and Marian Stamp Dawkins. 1991. 'Receiver psychology and the evolution of animal signals', *Animal Behaviour*, 42: 1-14.

Hailman, Jack P. 1978. 'Optical signals: animal communication and light', *The Auk*, 95: 213-15.

Hanlon, R. T., C. C. Chiao, L. M. Mäthger, A. Barbosa, K. C. Buresch, and C. Chubb. 2009. "Cephalopod dynamic camouflage: Bridging the continuum between background matching and disruptive coloration." In *Philosophical Transactions of the Royal Society B: Biological Sciences*, 429-37. Royal Society.

Hanlon, R. T., J. W. Forsythe, and D. E. Joneschild. 2008. 'Crypsis, conspicuousness, mimicry and polyphenism as antipredator defences of foraging octopuses on Indo-Pacific coral reefs, with a method of quantifying crypsis from video tapes', *Biological Journal of the Linnean Society*, 66: 1-22.

Hanlon, R. T., J. B. Messenger, and John Zachary Young. 1988. 'Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behaviour', *Philosophical Transactions of the Royal Society of London. B, Biological Sciences*, 320: 437-87.

Hanlon, Roger. 2007. 'Cephalopod dynamic camouflage', *Current Biology*, 17: R400-R04.

Hanlon, Roger T., Chuan-Chin C. Chiao, Lydia M. Mäthger, Kendra C. Buresch, Alexandra Barbosa, Justine J. Allen, Liese Siemann, and Charles Chubb. 2011. 'Rapid adaptive camouflage in cephalopods.' in Martin Stevens and Sami Merilaita (eds.), *Animal camouflage: mechanisms and function* (Cambridge University Press: Cambridge).

Hart, Nathan S. 2002. 'Vision in the peafowl (Aves: *Pavo cristatus*)', *Journal of Experimental Biology*, 205: 3925-35.

Håstad, Olle, Julian C. Partridge, and Anders Ödeen. 2009. 'Ultraviolet photopigment sensitivity and ocular media transmittance in gulls, with an evolutionary perspective', *Journal of Comparative Physiology A*, 195: 585-90.

Healey, EG. 1999. 'The skin pattern of young plaice and its rapid modification in response to graded changes in background tint and pattern', *Journal of Fish Biology*, 55: 937-71.

Hemmi, Jan M., Justin Marshall, Waltraud Pix, Misha Vorobyev, and Jochen Zeil. 2006. 'The variable colours of the fiddler crab *Uca vomeris* and their relation to background and predation', *Journal of Experimental Biology*, 209: 4140-53.

Hewson, Raymond. 1973. 'The moults of captive Scottish ptarmigan (*Lagopus mutus*)', *Journal of Zoology*, 171: 177-87.

Hogarth, P. J. 1975. 'Pattern polymorphism and predation in the shore crab, *Carcinus maenas* (L.)', *Crustaceana*, 28: 316-19.

—. 1978. 'Variation in the carapace pattern of juvenile *Carcinus maenas*', *Marine Biology*, 44: 337-43.

—. 1983. 'Crabs in labs: the shore crab (*Carcinus maena*s) as teaching material', *Journal of Biological Education*, 17: 105-11.

Horch, K., M. Salmon, and R. Forward. 2002. 'Evidence for a two pigment visual system in the fiddler crab, *Uca thayeri*', *Journal of Comparative Physiology A*, 188: 493-99.

Horridge, George Adrian, D. C. Sandeman, and Harold Garnet Callan. 1964. 'Nervous control of optokinetic responses in the crab *Carcinus*', *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 161: 216-46.

Horseman, B. Geoff, Martin W. S. MacAuley, and W. Jon P. Barnes. 2011. 'Neuronal processing of translational optic flow in the visual system of the shore crab Carcinus maenas', *Journal of Experimental Biology*, 214: 1586-98.

Houston, A. I., M. Stevens, and I. C. Cuthill. 2007. 'Animal camouflage: compromise or specialize in a 2 patch-type environment?', *Behavioral Ecology*, 18: 769-75.

How, Martin J, John H Christy, Shelby E Temple, Jan M Hemmi, N. Justin Marshall, and Nicholas W Roberts. 2015. 'Target detection Is enhanced by polarization vision in a fiddler crab', *Current Biology*, 25: 3069-73.

Hughes, Anna, Eric Liggins, and Martin Stevens. 2019. 'Imperfect camouflage: How to hide in a variable world?', *Proceedings of the Royal Society B: Biological Sciences*, 286.

Hultgren, Kristin M., and Hannah Mittelstaedt. 2015. 'Color change in a marine isopod is adaptive in reducing predation', *Current Zoology*, 61: 739-48.

Hultgren, Kristin M., and John J. Stachowicz. 2008. 'Alternative camouflage strategies mediate predation risk among closely related co-occurring kelp crabs', *Oecologia*, 155: 519-28.

Jacobsen Jr, Erling E, Clayton M White, and William B Emison. 1983. 'Molting adaptations of rock ptarmigan on Amchitka island, Alaska', *The Condor*, 85: 420-26.

Jensen, Gregory C., and Michael S. Egnotovich. 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: 729-38.

Johnson, Alan, Frank Cézilly, and Vincent Boy. 1993. 'Plumage development and maturation', *Ardea*, 81: 25-34.

Johnson, Magnus L., Edward Gaten, and Peter M. J. Shelton. 2002. 'Spectral sensitivities of five marine decapod crustaceans and a review of spectral

sensitivity variation in relation to habitat', *Journal of the Marine Biological Association of the United Kingdom*, 82: 835-42.

Kang, C. K., J. Y. Moon, S. I. Lee, and Piotr G. Jablonski. 2012. 'Camouflage through an active choice of a resting spot and body orientation in moths', *Journal of Evolutionary Biology*, 25: 1695-702.

Kang, Changku, Ye Eun Kim, and Yikweon Jang. 2016. 'Colour and pattern change against visually heterogeneous backgrounds in the tree frog *Hyla japonica*', *Scientific Reports*, 6: 22601.

Kang, Changku, Martin Stevens, Jong-yeol Moon, Sang-Im Lee, and Piotr G. Jablonski. 2014. 'Camouflage through behavior in moths: the role of background matching and disruptive coloration', *Behavioral Ecology*, 26: 45-54.

Kassambara, Alboukadel. 2021. "rstatix: Pipe-friendly framework for basic statistical tests." In.

Kawamura, Gunzo, Teodora Uy Bagarinao, Hue Sin Cheah, Hiroaki Saito, Annita Seok Kian Yong, and Leong-Seng Lim. 2020. 'Behavioural evidence for colour vision determined by conditioning in the purple mud crab *Scylla tranquebarica*', *Fisheries Science*, 86: 299-305.

Keeble, Frederick William, Frederick William Gamble, and Sydney John Hickson. 1900. 'The colour-physiology of *Hippolyte varians*', *Proceedings of the Royal Society of London*, 65: 461-68.

Kelber, Almut, Misha Vorobyev, and Daniel Osorio. 2003. 'Animal colour vision – behavioural tests and physiological concepts', *Biological Reviews of the Cambridge Philosophical Society*, 78: S1464793102005985-S85.

Kelber, Almut, Carola Yovanovich, and Peter Olsson. 2017. 'Thresholds and noise limitations of colour vision in dim light', *Philosophical transactions of the Royal Society of London. Series B, Biological sciences*, 372: 20160065.

Kelman, Emma J, Daniel Osorio, and Roland J Baddeley. 2008. 'A review of cuttlefish camouflage and object recognition and evidence for depth perception', *Journal of Experimental Biology*, 211: 1757-63.

Kelman, Emma J., Palap Tiptus, and Daniel Osorio. 2006. 'Juvenile plaice (*Pleuronectes platessa*) produce camouflage by flexibly combining two separate patterns', *Journal of Experimental Biology*, 209: 3288-92.

Kettlewell, H. B. D. 1955. 'Selection experiments on industrial melanism in the Lepidoptera', *Heredity*, 9: 323-42.

Kettlewell, H. B. D., and D. L. T. Conn. 1977. 'Further background-choice experiments on cryptic Lepidoptera', *Journal of Zoology*, 181: 371-76.

Kjernsmo, Karin, and Sami Merilaita. 2012. 'Background choice as an anti-predator strategy: The roles of background matching and visual complexity in the habitat choice of the least killifish', *Proceedings of the Royal Society B: Biological Sciences*, 279: 4192-98.

Krauss, Andrea, and Christa Neumeyer. 2003. 'Wavelength dependence of the optomotor response in zebrafish (*Danio rerio*)', *Vision Research*, 43: 1275-84.

Kuznetsova, Alexandra, Per B. Brockhoff, and Rune H. B. Christensen. 2017. 'lmerTest package: Tests in linear mixed effects models', *Journal of Statistical Software*, 82: 1 - 26.

Lall, Abner B., and Thomas W. Cronin. 1987. 'Spectral sensitivity of the compound eyes in the purple land crab *Gecarcinus lateralis* (Freminville)', *Biological Bulletin*, 173: 398-406.

Land, Michael F, and Dan-Eric Nilsson. 2012. *Animal eyes* (OUP Oxford).

Land, Michael F. 1997. 'Visual acuity in insects', *Annual Review of Entomology*, 42: 147-77.

Le Roux, P. J., G. M. Branch, and M. A. P. Joska. 1990. 'On the distribution, diet and possible impact of the invasive European shore crab *Carcinus maenas* (L.) along the South African coast', *South African Journal of Marine Science*, 9: 85-93.

Lee, Karen T., and Jessica L. Vespoli. 2015. 'Tracking color change in individual green crabs, *Carcinus maenas* (L.)', *Northeastern Naturalist*, 22: 413-23, 11.

Lee, Welton L. 1966. 'Color change and the ecology of the marine isopod *Idothea* (Pentidothea) Montereyensis Maloney, 1933', *Ecology*, 47: 930-41.

Macevoy, Sean P., and Michael A. Paradiso. 2001. 'Lightness constancy in primary visual cortex', *Proceedings of the National Academy of Sciences*, 98: 8827-31.

Magee, Barry, and Robert W. Elwood. 2013. 'Shock avoidance by discrimination learning in the shore crab (*Carcinus maenas*) is consistent with a key criterion for pain', *Journal of Experimental Biology*, 216: 353-58.

Majerus, Michael E. N. 2009. 'Industrial melanism in the peppered moth, *Biston betularia*: An excellent teaching example of Darwinian evolution in action', *Evolution: Education and Outreach*, 2: 63-74.

Manríquez, Patricio H, Nelson A Lagos, María Elisa Jara, and Juan Carlos Castilla. 2009. 'Adaptive shell color plasticity during the early ontogeny of an intertidal keystone snail', *Proceedings of the National Academy of Sciences*, 106: 16298-303.

Marshall, J., J. Kent, and T. Cronin. 1999. 'Visual adaptations in crustaceans: Spectral sensitivity in diverse habitats.' in S. N. Archer, M. B. A. Djamgoz, E. R. Loew, J. C. Partridge and S. Vallerga (eds.), *Adaptive Mechanisms in the Ecology of Vision* (Springer Netherlands: Dordrecht).

Marshall, N. J., J. P. Jones, and T. W. Cronin. 1996. 'Behavioural evidence for colour vision in stomatopod crustaceans', *Journal of Comparative Physiology A*, 179: 473-81.

Marshall, N. J., and J. B. Messenger. 1996. 'Colour-blind camouflage', *Nature*, 382: 408-09.

Marshall, N. Justin, Thomas W. Cronin, and Tamara M. Frank. 2003. 'Visual adaptations in crustaceans: Chromatic, developmental, and temporal aspects.' in Shaun P. Collin and N. Justin Marshall (eds.), *Sensory Processing in Aquatic Environments* (Springer New York: New York, NY).

Martin, Francis G., and Michael I. Mote. 1982. 'Color receptors in marine crustaceans: A second spectral class of retinular cell in the compound eyes of *Callinectes* and *Carcinus*', *Journal of Comparative Physiology*, 145: 549-54.

Mäthger, Lydia M., Alexandra Barbosa, Simon Miner, and Roger T. Hanlon. 2006. 'Color blindness and contrast perception in cuttlefish (*Sepia officinalis*) determined by a visual sensorimotor assay', *Vision Research*, 46: 1746-53.

Mäthger, Lydia M., Chuan-Chin Chiao, Alexandra Barbosa, and Roger T. Hanlon. 2008. 'Color matching on natural substrates in cuttlefish, *Sepia officinalis*', *Journal of Comparative Physiology A*, 194: 577.

Medina, Iliana, Regina Vega-Trejo, Thomas Wallenius, Matthew R. E. Symonds, and Devi Stuart-Fox. 2020. 'From cryptic to colorful: Evolutionary decoupling of larval and adult color in butterflies', *Evolution Letters*, 4: 34-43.

Melville-Smith, Roy, Yuk Wing Cheng, and Adrian W. Thomson. 2003. 'Factors affecting colour change in ‘white’ western rock lobsters, *Panulirus cygnus*', *Journal of Experimental Marine Biology and Ecology*, 291: 111-29.

Merilaita, S., A. Lyytinen, and J. Mappes. 2001. 'Selection for cryptic coloration in a visually heterogeneous habitat', *Proceedings of the Royal Society B: Biological Sciences*, 268: 1925-29.

Merilaita, Sami. 2003. 'Visual background complexity facilitates the evolution of camouflage', *Evolution*, 57: 1248-54.

Merilaita, Sami, Nicholas E. Scott-Samuel, and Innes C. Cuthill. 2017. 'How camouflage works', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 372.

Merilaita, Sami, and Martin Stevens. 2011. 'Crypsis through background matching', *Animal camouflage: mechanisms and function*: 17-33.

Merilaita, Sami, Juha Tuomi, and Veijo Jormalainen. 1999. 'Optimization of cryptic coloration in heterogeneous habitats', *Biological Journal of the Linnean Society*, 67: 151-61.

Michalis, Constantine, Nicholas E Scott-Samuel, David P Gibson, and Innes C Cuthill. 2017. 'Optimal background matching camouflage', *Proceedings of the Royal Society B: Biological Sciences*, 284: 20170709.

Mills, L. Scott, Marketa Zimova, Jared Oyler, Steven Running, John T. Abatzoglou, and Paul M. Lukacs. 2013. 'Camouflage mismatch in seasonal coat color due to decreased snow duration', *Proceedings of the National Academy of Sciences of the United States of America*, 110: 7360-65.

Moksnes, P. O. , L. Pihl, and J. van Montfrans. 1998. 'Predation on postlarvae and juveniles of the shore crab *Carcinus maenas*: importance of shelter, size and cannibalism', *Marine Ecology Progress Series*, 166: 211-25.

Moksnes, Per-Olav. 2002. 'The relative importance of habitat-specific settlement, predation and juvenile dispersal for distribution and abundance of young juvenile shore crabs *Carcinus maenas* L', *Journal of Experimental Marine Biology and Ecology*, 271: 41-73.

——. 2004. 'Self-regulating mechanisms in cannibalistic populations of juvenile shore crabs *Carcinus maenas*', *Ecology*, 85: 1343-54.

Moles, Adam, and Brenda L. Norcross. 1995. 'Sediment preference in juvenile pacific flatfishes', *Netherlands Journal of Sea Research*, 34: 177-82.

Mynott, Sara. 2019. 'The impact of climate change on intertidal species, camouflage and predation (Unpublished doctoral thesis)', Doctoral Thesis, University of Exeter.

Nafus, Melia G., Jennifer M. Germano, Jeanette A. Perry, Brian D. Todd, Allyson Walsh, and Ronald R. Swaisgood. 2015. 'Hiding in plain sight: a study on camouflage and habitat selection in a slow-moving desert herbivore', *Behavioral Ecology*, 26: 1389-94.

Nagorsen, David W. 1983. 'Winter pelage colour in snowshoe hares (*Lepus americanus*) from the Pacific Northwest', *Canadian Journal of Zoology*, 61: 2313-18.

Nakajima, Ryuta, Zdeněk Lajbner, Michael J. Kuba, Tamar Gutnick, Teresa L. Iglesias, Keishu Asada, Takahiro Nishibayashi, and Jonathan Miller. 2022. 'Squid adjust their body color according to substrate', *Scientific Reports*, 12: 5227.

Naylor, E. 1958. 'Tidal and diurnal rhythms of locomotory activity in *Carcinus maenas* (L.)', *Journal of Experimental Biology*, 35: 602-10.

Neal, K.J., and P.F Pizzolla. 2008. '*Carcinus maenas* Common shore crab.', Plymouth: Marine Biological Association of the United Kingdom., Accessed 04/02/2022.

Nielsen, Hans Ingolf, and Jan Dyck. 1978. 'Adaptation of the tree frog, *Hyla cinerea*, to colored backgrounds, and the rôle of the three chromatophore types', *Journal of Experimental Zoology*, 205: 79-94.

Nilsson, Dan-E., and Jochen Smolka. 2021. 'Quantifying biologically essential aspects of environmental light', *Journal of The Royal Society Interface*, 18: 20210184.

Niu, Yang, Martin Stevens, and Hang Sun. 2021. 'Commercial harvesting has driven the evolution of camouflage in an alpine plant', *Current Biology*, 31: 446-49.e4.

Nokelainen, Ossi, Nik Hubbard, Alice E Lown, Louisa E Wood, and Martin Stevens. 2017. 'Through predators' eyes: phenotype–environment associations in shore crab coloration at different spatial scales', *Biological Journal of the Linnean Society*, 122: 738-51.

Nokelainen, Ossi, Ruth Maynes, Sara Mynott, Natasha Price, and Martin Stevens. 2019. 'Improved camouflage through ontogenetic colour change confers reduced detection risk in shore crabs', *Functional Ecology*, 33: 654-69.

Noor, Mohamed AF, Robin S Parnell, and Bruce S Grant. 2008. 'A reversible color polyphenism in American peppered moth (*Biston betularia cognataria*) caterpillars', *PLOS ONE*, 3: e3142.

Norris, Kenneth S, and Charles H Lowe. 1964. 'An analysis of background color-matching in amphibians and reptiles', *Ecology*, 45: 565-80.

Oguro, Chitaru. 1962. 'On the physiology of melanophores of the marine isopod, *Idotea japonica*. III. The role of the eyes in background response', *Crustaceana*, 4: 85-92.

Olsson, Peter, Olle Lind, and Almut Kelber. 2015. 'Bird colour vision: Behavioural thresholds reveal receptor noise', *Journal of Experimental Biology*, 218: 184-93.

Osorio, D., N. Justin Marshall, and Thomas W. Cronin. 1997. 'Stomatopod photoreceptor spectral tuning as an adaptation for colour constancy in water', *Vision Research*, 37: 3299-309.

Osorio, D., and M. Vorobyev. 2005. "Photoreceptor spectral sensitivities in terrestrial animals: Adaptations for luminance and colour vision." In *Proceedings of the Royal Society B: Biological Sciences*, 1745-52. Royal Society.

Peralta-Rincon, Juan Ramon, Graciela Escudero, and Pim Edelaar. 2017. 'Phenotypic plasticity in color without molt in adult grasshoppers of the genus *Sphingonotus* (Acrididae: Oedipodinae)', *Journal of Orthoptera Research*, 26: 21-27.

Phlippen, M.K., S.G. Webster, J.S. Chung, and H. Dircksen. 2000. 'Ecdysis of decapod crustaceans is associated with a dramatic release of crustacean cardioactive peptide into the haemolymph', *Journal of Experimental Biology*, 203: 521-36.

Polo-Cavia, Nuria, and Ivan Gomez-Mestre. 2017. 'Pigmentation plasticity enhances crypsis in larval newts: associated metabolic cost and background choice behaviour', *Scientific Reports*, 7: 39739.

Poulton, E. B. 1890. *The Colours of Animals* (Kegan Paul, Trench, Trübner & Co.,: London).

Poulton, Edward B. 1903. 'XVIII. Experiments in 1893, 1894, and 1896 upon the colour-relation between lepidopterous larvæ and their surroundings, and especially the effect of lichen-covered bark upon *Odontopera bidentata*, Gastropacha quercifolia, etc', *Transactions of the Royal Entomological Society of London*, 51: 311-74.

Powell, B. L. 1962a. 'Types, distribution and rhythmical behaviour of the chromatophores of juvenile *Carcinus maenas* (L.)', *Journal of Animal Ecology*, 31: 251-61.

Powell, B.L. 1962b. 'The responses of the chromatophores of *Carcinus maenas* (L., 1758) to light and temperature', *Crustaceana*, 4: 93-102.

Price, Natasha, Samuel Green, Jolyon Troscianko, Tom Tregenza, and Martin Stevens. 2019. 'Background matching and disruptive coloration as habitat-specific strategies for camouflage', *Scientific Reports*, 9: 1-10.

R Development Core Team. 2020. "R: A language and environment for statistical computing." In. Vienna, Austria: R Foundation for Statistical Computing.

Rajkumar, Premraj, Stephanie M. Rollmann, Tiffany A. Cook, and John E. Layne. 2010. 'Molecular evidence for color discrimination in the Atlantic sand fiddler crab, *Uca pugilator*', *The Journal of experimental biology*, 213: 4240-48.

Ramachandran, VS, CW Tyler, RL Gregory, D Rogers-Ramachandran, S Duensing, C Pillsbury, and C Ramachandran. 1996. 'Rapid adaptive camouflage in tropical flounders', *Nature*, 379: 815-18.

Ramirez, M. Desmond, and Todd H. Oakley. 2015. 'Eye-independent, light-activated chromatophore expansion (LACE) and expression of phototransduction genes in the skin of *Octopus bimaculoides*', *Journal of Experimental Biology*, 218: 1513-20.

Rangeley, R. W., and M. L. H. Thomas. 1987. 'Predatory behaviour of juvenile shore crab *Carcinus maenas* (L.)', *Journal of Experimental Marine Biology and Ecology*, 108: 191-97.

Rao, K Ranga, Milton Fingerman, and Clelmer K Bartell. 1967. 'Physiology of the white chromatophores in the fiddler crab, *Uca pugilator*', *The Biological Bulletin*, 133: 606-17.

Ratcliffe, Herbert L. 1936. 'Diet and colour of birds', *Nature*, 137: 402-02.

Reid, D. G., P. Abelló, M. J. Kaiser, and C. G. Warman. 1997. 'Carapace Colour, Inter-moult Duration and the Behavioural and Physiological Ecology of the Shore Crab *Carcinus maenas*', *Estuarine, Coastal and Shelf Science*, 44: 203-11.

Robinson, Elizabeth M., Delbert L. Smee, and Geoffrey C. Trussell. 2011. 'Green crab (*Carcinus maenas*) foraging efficiency reduced by fast flows', *PLOS ONE*, 6: e21025.

Rodgers, Gwendolen M, Nicholas W Gladman, Hannah F Corless, and Lesley J Morrell. 2013. 'Costs of colour change in fish: food intake and behavioural decisions', *Journal of Experimental Biology*, 216: 2760-67.

Rossong, MA, PA Quijon, PJ Williams, and PVR Snelgrove. 2011. 'Foraging and shelter behavior of juvenile American lobster (*Homarus americanus*): the influence of a non-indigenous crab', *Journal of Experimental Marine Biology and Ecology*, 403: 75-80.

Roth, Lina S. V., and Almut Kelber. 2004. 'Nocturnal colour vision in geckos', *Proceedings of the Royal Society B: Biological Sciences*, 271 Suppl 6: S485-S87.

Rothschild, Miriam. 1942. 'Change of pelage in the stoat *Mustela erminea* L', *Nature*, 149: 78-78.

Rowe, Zeke W., Daniel J. D. Austin, Nicol Chippington, William Flynn, Finn Starkey, Edward J. Wightman, Nicholas E. Scott-Samuel, and Innes C. Cuthill. 2021. 'Background complexity can mitigate poor camouflage', *Proceedings of the Royal Society B: Biological Sciences*, 288: 20212029.

Rowland, Hannah M, Innes C Cuthill, Ian F Harvey, Michael P Speed, and Graeme D Ruxton. 2008. 'Can't tell the caterpillars from the trees: countershading enhances survival in a woodland', *Proceedings of the Royal Society B: Biological Sciences*, 275: 2539-45.

Rowland, Hannah M., Robert P. Burriss, and John Skelhorn. 2020. 'The antipredator benefits of postural camouflage in peppered moth caterpillars', *Scientific Reports*, 10: 21654.

RStudio Team. 2020. "RStudio: Integrated development for R." In. Boston, MA: RStudio, PBC.

Rushton, Simon K., Mark F. Bradshaw, and Paul A. Warren. 2007. 'The pop out of scene-relative object movement against retinal motion due to self-movement', *Cognition*, 105: 237-45.

Ruxton, Graeme D, William L Allen, Thomas N Sherratt, and Michael P Speed. 2019. *Avoiding attack: the evolutionary ecology of crypsis, aposematism, and mimicry* (Oxford University Press: Oxford).

Ruxton, Graeme D. 2011. 'Evidence for camouflage involving senses other than vision.' in Martin Stevens and Sami Merilaita (eds.), *Animal camouflage: mechanisms and function* (Cambridge University Press: Cambridge).

Ryer, Clifford H., Jena L. Lemke, Kate Boersma, and Stephen Levas. 2008. 'Adaptive coloration, behavior and predation vulnerability in three juvenile north Pacific flatfishes', *Journal of Experimental Marine Biology and Ecology*, 359: 62-66.

Schaefer, H. Martin, and Nina Stobbe. 2006. 'Disruptive coloration provides camouflage independent of background matching', *Proceedings of the Royal Society B: Biological Sciences*, 273: 2427-32.

Schaerer, Stephanie, and Christa Neumeyer. 1996. 'Motion detection in goldfish investigated with the optomotor response is “color blind”', *Vision Research*, 36: 4025-34.

Schneider, Caroline A., Wayne S. Rasband, and Kevin W. Eliceiri. 2012. 'NIH Image to ImageJ: 25 years of image analysis', *Nature Methods*, 9: 671-75.

Shand, Julia, J. C. Partridge, S. N. Archer, G. W. Potts, and J. N. Lythgoe. 1988. 'Spectral absorbance changes in the violet/blue sensitive cones of the juvenile pollack, *Pollachius pollachius*', *Journal of Comparative Physiology A*, 163: 699-703.

Sharman, Rebecca J., and P. George Lovell. 2019. 'Edge-enhanced disruptive camouflage impairs shape discrimination', *i-Perception*, 10: 2041669519877435.

Shaw, Stephen R. 1966. 'Polarized light responses from crab retinula cells', *Nature*, 211: 92-93.

Shelton, R. G. J., and A. M. Mackie. 1971. 'Studies on the chemical preferences of the shore crab, *Carcinus maenas* (L.)', *Journal of Experimental Marine Biology and Ecology*, 7: 41-49.

Shibley, Gilbert A. 1968. 'Eyestalk function in chromatophore control in a crab, *Cancer magister*', *Physiological Zoology*, 41: 268-79.

Siegenthaler, Andjin, Alexander Mastin, Clément Dufaut, Debapriya Mondal, and Chiara Benvenuto. 2018. 'Background matching in the brown shrimp *Crangon crangon*: adaptive camouflage and behavioural-plasticity', *Scientific Reports*, 8: 3292.

Silva, Ines, Maria Alves, Jose Paula, and Stephen Hawkins. 2010. 'Population differentiation of the shore crab *Carcinus maenas* (Brachyura: Portunidae) on the southwest English coast based on genetic and morphometric analyses', *Scientia Marina*, 74: 435-44.

Simpson, Elisha E., N. Justin Marshall, and Karen L. Cheney. 2016. 'Coral reef fish perceive lightness illusions', *Scientific Reports*, 6: 35335.

Skelhorn, John. 2015. 'Masquerade', *Current Biology*, 25: R643-R44.

Skelhorn, John, and Candy Rowe. 2015. 'Cognition and the evolution of camouflage', *Proceedings of the Royal Society B: Biological Sciences*, 283.

Smith, Austin Gerard. 1980. 'Environmental factors influencing pupal colour determination in Lepidoptera. II. Experiments with *Pieris rapae, Pieris napi* and *Pieris brassicae*', *Proceedings of the Royal Society of London. Series B. Biological Sciences*, 207: 163-86.

Smithers, Samuel P. , Nicholas W. Roberts, and Martin J. How. 2019. 'Parallel processing of polarization and intensity information in fiddler crab vision', *Science Advances*, 5: eaax3572.

Smithers, Samuel P., Alastair Wilson, and Martin Stevens. 2017. 'Rock pool gobies change their body pattern in response to background features', *Biological Journal of the Linnean Society*, 121: 109-21.

Smolka, Jochen, and Jan M. Hemmi. 2009. 'Topography of vision and behaviour', *Journal of Experimental Biology*, 212: 3522-32.

Snyder, Allan W. 1977. 'Acuity of compound eyes: Physical limitations and design', *Journal of Comparative Physiology*, 116: 161-82.

Spottiswoode, Claire N., and Martin Stevens. 2010. 'Visual modeling shows that avian host parents use multiple visual cues in rejecting parasitic eggs', *Proceedings of the National Academy of Sciences*, 107: 8672-76.

Stavenga, D. G., and J. Schwemer. 1984. 'Visual Pigments of Invertebrates.' in M. A. Ali (ed.), *Photoreception and Vision in Invertebrates* (Springer US: Boston, MA).

Stavenga, D. G., R. P. Smits, and B. J. Hoenders. 1993. 'Simple exponential functions describing the absorbance bands of visual pigment spectra', *Vision Research*, 33: 1011-17.

Stevens, Martin. 2007. 'Predator perception and the interrelation between different forms of protective coloration', *Proceedings of the Royal Society B: Biological Sciences*, 274: 1457-64.

—. 2016. 'Color Change, phenotypic plasticity, and camouflage', *Frontiers in Ecology and Evolution*, 4.

Stevens, Martin, Annette C. Broderick, Brendan J. Godley, Alice E. Lown, Jolyon Troscianko, Nicola Weber, and Sam B. Weber. 2015. 'Phenotype - environment matching in sand fleas', *Biology Letters*, 11.

Stevens, Martin, and Innes C Cuthill. 2006. 'Disruptive coloration, crypsis and edge detection in early visual processing', *Proceedings of the Royal Society B: Biological Sciences*, 273: 2141-47.

Stevens, Martin, Innes C Cuthill, Amy MM Windsor, and Hannah J Walker. 2006. 'Disruptive contrast in animal camouflage', *Proceedings of the Royal Society B: Biological Sciences*, 273: 2433-38.

Stevens, Martin, Alice E. Lown, and Alexander M. Denton. 2014. 'Rockpool gobies change colour for camouflage', *PLOS ONE*, 9: e110325.

Stevens, Martin, Alice E. Lown, and Louisa E. Wood. 2014a. 'Camouflage and individual variation in shore crabs (*Carcinus maenas*) from different habitats', *PLOS ONE*, 9: e115586-e86.

—. 2014b. 'Color change and camouflage in juvenile shore crabs *Carcinus maenas*', *Frontiers in Ecology and Evolution*, 2: 14-14.

Stevens, Martin, and Sami Merilaita. 2009a. 'Animal camouflage: current issues and new perspectives', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364: 423-27.

—. 2009b. 'Defining disruptive coloration and distinguishing its functions', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364: 481-88.

—. 2011. *Animal camouflage: mechanisms and function* (Cambridge University Press).

Stevens, Martin, C. Alejandro Párraga, Innes C. Cuthill, Julian C. Partridge, and Tom S Troscianko. 2007. 'Using digital photography to study animal coloration', *Biological Journal of the Linnean Society*, 90: 211-37.

Stevens, Martin, Cheo Pei Rong, and Peter A. Todd. 2013. 'Colour change and camouflage in the horned ghost crab Ocypode ceratophthalmus', *Biological Journal of the Linnean Society*, 109: 257-70.

Stevens, Martin, and Graeme D. Ruxton. 2019. 'The key role of behaviour in animal camouflage', *Biological Reviews*, 94: 116-34.

Stevens, Martin, Jolyon Troscianko, Jared K. Wilson-Aggarwal, and Claire N. Spottiswoode. 2017. 'Improvement of individual camouflage through background choice in ground-nesting birds', *Nature Ecology and Evolution*, 1: 1325-33.

Stevens, Martin, Isabel S. Winney, Abi Cantor, and Julia Graham. 2009. 'Outline and surface disruption in animal camouflage', *Proceedings of the Royal Society B: Biological Sciences*, 276: 781-86.

Stoddard, Mary Caswell, and Martin Stevens. 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: 1387-93.

Stuart-Fox, Devi, and Adnan Moussalli. 2009. "Camouflage, communication and thermoregulation: Lessons from colour changing organisms." In *Philosophical Transactions of the Royal Society B: Biological Sciences*, 463-70. Royal Society.

Stuart-Fox, Devi, Adnan Moussalli, and Martin J. Whiting. 2008. 'Predator-specific camouflage in chameleons', *Biology Letters*, 4: 326-29.

Stuart-Fox, Devi, Martin J. Whiting, and Adnan Moussalli. 2006. 'Camouflage and colour change: antipredator responses to bird and snake predators across multiple populations in a dwarf chameleon', *Biological Journal of the Linnean Society*, 88: 437-46.

Stubbs, Alexander L., and Christopher W. Stubbs. 2016. 'Spectral discrimination in color blind animals via chromatic aberration and pupil shape', *Proceedings of the National Academy of Sciences*, 113: 8206-11.

Styrishave, Bjarne, Kim Rewitz, and Ole Andersen. 2004. 'Frequency of moulting by shore crabs *Carcinus maenas* (L.) changes their colour and their success in mating and physiological performance', *Journal of Experimental Marine Biology and Ecology*, 313: 317-36.

Sumner, Francis B. 1911. 'The adjustment of flatfishes to various backgrounds: A study of adaptive color change', *Journal of Experimental Zoology*, 10: 409-506.

Sumner, Francis B., and Ancel B. Keys. 1929. 'The effects of differences in the apparent source of illumination upon the shade assumed by a flatfish on a given background', *Physiological Zoology*, 2: 495-504.

Takeda, Satoshi. 2006. 'Behavioural evidence for body colour signaling in the fiddler crab *Uca perplexa* (Brachyura: Ocypodidae)', *Journal of Experimental Marine Biology and Ecology*, 330: 521-27.

Tanaka, Seiji, Ken-ichi Harano, and Yudai Nishide. 2012. 'Re-examination of the roles of environmental factors in the control of body-color polyphenism in solitarious nymphs of the desert locust *Schistocerca gregaria* with special reference to substrate color and humidity', *Journal of Insect Physiology*, 58: 89-101.

Thayer, Abbott H. 1896. 'The law which underlies protective coloration', *The Auk*, 13: 124-29.

Thayer, Gerald Handerson, and Abbott Handerson Thayer. 1909. *Concealing-coloration in the animal kingdom; an exposition of the laws of disguise through color and pattern: being a summary of Abbott H. Thayer's discoveries* (The Macmillan Co.: New York).

The MathWorks, Inc. 2018. "MATLAB." In. Natick, Massachusetts, United States.

Théry, Marc, and Jérôme Casas. 2009. 'The multiple disguises of spiders: Web colour and decorations, body colour and movement', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364: 471-80.

Todd, P. A., R. A. Briers, R. J. Ladle, and F. Middleton. 2006. 'Phenotype-environment matching in the shore crab (*Carcinus maenas*)', *Marine Biology*, 148: 1357-67.

Todd, Peter A., Joelyn Oh, Lynette H. L. Loke, and Richard J. Ladle. 2012. 'Multi-scale phenotype-substrate matching: Evidence from shore crabs (*Carcinus maenas* L.)', *Ecological Complexity*, 12: 58-62.

Toh, Kok Ben, and Peter Todd. 2017. 'Camouflage that is spot on! Optimization of spot size in prey-background matching', *Evolutionary Ecology*, 31: 447-61.

Troscianko, Jolyon, Alice E. Lown, Anna E. Hughes, and Martin Stevens. 2013. 'Defeating crypsis: Detection and learning of camouflage strategies', *PLOS ONE*, 8: e73733-e33.

Troscianko, Jolyon, Ossi Nokelainen, John Skelhorn, and Martin Stevens. 2021. 'Variable crab camouflage patterns defeat search image formation', *Communications Biology*, 4: 287-87.

Troscianko, Jolyon, John Skelhorn, and Martin Stevens. 2017. 'Quantifying camouflage: how to predict detectability from appearance', *BMC Evolutionary Biology*, 17: 1-13.

—. 2018. 'Camouflage strategies interfere differently with observer search images', *Proceedings. Biological sciences*, 285.

Troscianko, Jolyon, and Martin Stevens. 2015. 'Image calibration and analysis toolbox - a free software suite for objectively measuring reflectance, colour and pattern', *Methods in Ecology and Evolution*, 6: 1320-31.

Troscianko, Jolyon, Jared Wilson-Aggarwal, David Griffiths, Claire N. Spottiswoode, and Martin Stevens. 2017. 'Relative advantages of dichromatic and trichromatic color vision in camouflage breaking', *Behavioral Ecology*, 28: 556-64.

Troscianko, Jolyon, Jared Wilson-Aggarwal, Martin Stevens, and Claire N. Spottiswoode. 2016. 'Camouflage predicts survival in ground-nesting birds', *Scientific Reports*, 6: 19966.

Troscianko, Jolyon, Jared Wilson-Aggarwal, Claire N Spottiswoode, and Martin Stevens. 2016. 'Nest covering in plovers: How modifying the visual environment influences egg camouflage', *Ecology and evolution*, 6: 7536-45.

Troscianko, Tom, Christopher P. Benton, P. George Lovell, David J. Tolhurst, and Zygmunt Pizlo. 2009. 'Camouflage and visual perception', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364: 449-61.

Tyrie, Elizabeth K, Roger T Hanlon, Liese A Siemann, and Maria C Uyarra. 2015. 'Coral reef flounders, *Bothus lunatus,* choose substrates on which they can achieve camouflage with their limited body pattern repertoire', *Biological Journal of the Linnean Society*, 114: 629-38.

Umbers, Kate D. L., Scott A. Fabricant, Felipe M. Gawryszewski, Ainsley E. Seago, and Marie E. Herberstein. 2014. 'Reversible colour change in Arthropoda', *Biological Reviews*, 89: 820-48.

Utne-Palm, Anne C., and James K. Bowmaker. 2006. 'Spectral sensitivity of the two-spotted goby *Gobiusculus flavescens* (Fabricius): a physiological and behavioural study', *Journal of Experimental Biology*, 209: 2034-41.

Uy, Floria M. K., Suvetha Ravichandran, Krisha S. Patel, Jeffrey Aresty, Patricia P. Aresty, Raymond M. Audett, Kelvin Chen, Lauren Epple, Sterling F. Jeffries, Gilbert N. Serein, Phallon Tullis-Joyce, and J. Albert C. Uy. 2017. 'Active background choice facilitates crypsis in a tropical crab', *Biotropica*, 49: 365-71.

Valverde, J. Pablo, and Holger Schielzeth. 2015. 'What triggers colour change? Effects of background colour and temperature on the development of an alpine grasshopper', *BMC Evolutionary Biology*, 15: 168.

Van Hateren, J. H. 1993. 'Spatial, temporal and spectral pre-processing for colour vision', *Proceedings of the Royal Society B: Biological Sciences*, 251: 61-68.

Vignieri, Sacha N., Joanna G. Larson, and Hopi E. Hoekstra. 2010. 'The selective advantage of crypsis in mice', *Evolution*, 64: 2153-58.

Vorobyev, M., and D. Osorio. 1998. 'Receptor noise as a determinant of colour thresholds', *Proceedings of the Royal Society B: Biological Sciences*, 265: 351-58.

Wale, Matthew A., Stephen D. Simpson, and Andrew N. Radford. 2013. 'Noise negatively affects foraging and antipredator behaviour in shore crabs', *Animal Behaviour*, 86: 111-18.

Wallace, Alfred Russel. 1867. *Mimicry, and other protective resemblances among animals* (Read Books Ltd).

—. 1877. 'The Colors of Animals and Plants', *The American Naturalist*, 11: 641-62.

—. 1889. *Darwinism: an exposition of the theory of natural selection with some of its applications* (Cosimo, Inc.).

Walton, Olivia C., and Martin Stevens. 2018. 'Avian vision models and field experiments determine the survival value of peppered moth camouflage', *Communications Biology*, 1: 118.

Warrant, E., and D.E. Nilsson. 2006. *Invertebrate Vision* (Cambridge University Press).

Warrant, Eric, Thomas Porombka, and Wolfgang H. Kirchner. 1996. 'Neural image enhancement allows honeybees to see at night', *Proceedings of the Royal Society B: Biological Sciences*, 263: 1521-26.

Webster, Richard J, Alison Callahan, Jean-Guy J Godin, and Thomas N Sherratt. 2009. 'Behaviourally mediated crypsis in two nocturnal moths with contrasting appearance', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364: 503-10.

Wenner, Adrian M. 1972. 'Incremental color change in an anomuran decapod *Hippa pacifica* Dana', *Pacific Science*, 26: 346-53.

Wickham, Hadley. 2016. *Ggplot2: Elegant graphics for data analysis* (Springer International Publishing: Cham, Switzerland).

Wickham, Hadley, Mara Averick, Jennifer Bryan, Winston Chang, Lucy McGowan, Romain François, Garrett Grolemund, Alex Hayes, Lionel Henry, Jim Hester, Max Kuhn, Thomas Pedersen, Evan Miller, Stephan Bache, Kirill Müller, Jeroen Ooms, David Robinson, Dana Seidel, Vitalie Spinu, Kohske Takahashi, Davis Vaughan, Claus Wilke, Kara Woo, and Hiroaki Yutani. 2019. 'Welcome to the Tidyverse', *Journal of Open Source Software*, 4: 1686.

Wiklund, C. 1972. 'Pupal coloration in *Papilio machaon* in response to the wavelength of light', *Naturwissenschaften*, 59: 219-19.

Williams, Thomas L., Stephen L. Senft, Jingjie Yeo, Francisco J. Martín-Martínez, Alan M. Kuzirian, Camille A. Martin, Christopher W. DiBona, Chun-Teh Chen, Sean R. Dinneen, Hieu T. Nguyen, Conor M. Gomes, Joshua J. C. Rosenthal, Matthew D. MacManes, Feixia Chu, Markus J. Buehler, Roger T. Hanlon, and Leila F. Deravi. 2019. 'Dynamic pigmentary and structural coloration within cephalopod chromatophore organs', *Nature Communications*, 10: 1004.

Wilson, David, Robert Heinsohn, and John A. Endler. 2007. 'The adaptive significance of ontogenetic colour change in a tropical python', *Biology Letters*, 3: 40-43.

Yamada, S., Y. Tanaka, M. Sameshima, and Y. Ito. 1990. 'Pigmentation of prawn (*Penaeus japonicus*) with carotenoids. I. Effect of dietary astaxanthin, β-carotene and canthaxanthin on pigmentation', *Aquaculture*, 87: 323-30.

Yamaguchi, Satoko, Reinhard Wolf, Claude Desplan, and Martin Heisenberg. 2008. 'Motion vision is independent of color in *Drosophila*', *Proceedings of the National Academy of Sciences*, 105: 4910-15.

Youn, Sean, Corey Okinaka, and Lydia M. Mäthger. 2019. 'Elaborate pupils in skates may help camouflage the eye', *Journal of Experimental Biology*, 222.

Young, Alan M., and James A. Elliott. 2020. 'Life history and population dynamics of green crabs (*Carcinus maenas*)', *Fishes*, 5: 4.

Zeil, J, G Nalbach, and HO Nalbach. 1986. 'Eyes, eye stalks and the visual world of semi-terrestrial crabs', *Journal of Comparative Physiology A*, 159: 801-11.

Zimova, Marketa, Sean T. Giery, Scott Newey, J. Joshua Nowak, Michael Spencer, and L. Scott Mills. 2020. 'Lack of phenological shift leads to increased camouflage mismatch in mountain hares', *Proceedings of the Royal Society B: Biological Sciences*, 287: 20201786-86.

Zimova, Marketa, Klaus Hackländer, Jeffrey M Good, José Melo-Ferreira, Paulo Célio Alves, and L Scott Mills. 2018. 'Function and underlying mechanisms of seasonal colour moulting in mammals and birds: what keeps them changing in a warming world?', *Biological Reviews*, 93: 1478-98.

Zimova, Marketa, L. Scott Mills, and J. Joshua Nowak. 2016. 'High fitness costs of climate change-induced camouflage mismatch', *Ecology Letters*, 19: 299-307.

Zylinski, S., M. J. How, D. Osorio, R. T. Hanlon, and J. Marshall. 2011. 'To be seen or to hide: Visual characteristics of body patterns for camouflage and communication in the Australian giant cuttlefish *Sepia apama*', *The American Naturalist*, 177: 681-90.

Zylinski, S., D. Osorio, and A. J. Shohet. 2009a. 'Cuttlefish camouflage: Context-dependent body pattern use during motion', *Proceedings of the Royal Society B: Biological Sciences*, 276: 3963-69.

—. 2009b. 'Perception of edges and visual texture in the camouflage of the common cuttlefish, *Sepia officinalis*', *Philosophical Transactions of the Royal Society B: Biological Sciences*, 364: 439-48.

Zylinski, Sarah, D. Osorio, and Sonke Johnsen. 2016. 'Cuttlefish see shape from shading, fine-tuning coloration in response to pictorial depth cues and

directional illumination', *Proceedings of the Royal Society B: Biological Sciences*, 283: 20160062.