Phenotype-environment matching and colour change for camouflage in the shore crab *Carcinus maenas*

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I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

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Contents

Abstract

Camouflage is one of the most common antipredator defences found in nature (Ruxton et al., 2004). The shore crab *Carcinus maenas* is vulnerable to attack by shore birds, fish species and even other crabs.. *C. maenas* exhibits a wide range of colours and patterns that appear to be associated with the habitat in which a crab lives. This is commonly referred to as phenotype-environment matching. This thesis investigates whether the colours and patterns provide a camouflage benefit to encompass the wavelengths that can be seen by its putative avian predators. I found that crabs from homogenous, plain mudflat environments have a significantly better camouflage to areas of their own habitat than to backgrounds from other habitat types, in terms of luminance (perceived lightness), colour, and pattern, indicating phenotype specialisation across all appearance metrics. Individual crabs from heterogeneous rockpool and mussel bed environments show mixed levels of phenotype-environment matching. One possible explanation for how shore crabs tune their phenotype to that of the environment is through morphological colour change. I reared crabs on black, white, red, or green backgrounds over short (48 h) and long term (5 weeks) time periods. I found no significant changes in appearance metrics in the short term. However, over a longer time period individuals reared on white backgrounds significantly increased their luminance. Crabs raised on red backgrounds significantly increased their red hue; the changes in luminance and hue significantly improved camouflage as birds were increasingly unable to distinguish them from the background. Phenotype environment matches in *C. maenas* improves camouflage for crabs in homogenous environments, and this appears to be driven by morphological colour change in juveniles. This study provides the first evidence of how species in complex homogenous environments may stay camouflaged to avoid avian predators.

Introduction

The need to avoid detection and attack by predators has resulted in the evolution of prey survival strategies that include crypsis, warning colourations and mimicry (Ruxton et al 2004). Many predators are predominantly visual hunters, which selects for prey that avoid being detected, and ultimately attacked or eaten (Merilaita and Stevens 2011). Though many have evolved bright, high contrast warning colours that signal chemical defences to deter predatory attack (Arenas, Troscianko, & Stevens, 2014; Mappes, Marples, & Endler, 2005; Sami Merilaita & Ruxton, 2007), the most frequent and widely adopted strategy for predator avoidance is that of concealment that can be achieved through background matching, disruptive colouration, etc. (Merilaita and Stevens 2011). Visual camouflage has interested scientists for centuries, and attracted the curiosity of artists such as Abbott Thayer (Thayer, 1896), and has even influenced the military (Behrens 1987).

Visual camouflage includes masquerade, where animals resemble inedible objects such as fish that resemble fallen leaves in the Amazon river (Sazima et al., 2006), and spiders that look like bird droppings (Liu et al., 2014).. Masquerade causes predators to misidentify prey as the inanimate object , and the prey avoid being attacked (Skelhorn et al., 2010). Prey can also avoid being detected by concealing their three-dimensional form through countershading (Rowland et al., 2007; Rowland et al., 2008). Prey can also avoid detection by breaking up the outline of their body through disruptive colouration (Cuthill et al., 2005; Webster et al., 2013). However, many prey simply match the coloration, brightness, or patterns of its surrounding environment (Sami Merilaita & Lind, 2005), known as background matching or phenotype-environment matches (Todd et al., 2006). This is the focus of my thesis, and I will now discuss it in more detail.

Phenotype-environment matching and colour polymorphism

There are many examples of animal's body colours matching the background they are associated, for example pocket mice *Chaetodipus intermedius* have darker coat colourations on dark larval rock and light coats on lighter rock environments of their habitat (Nachman et al., 2003). Oldfield mice *Peromyscus polionotus* in inland habitats have evolved darker coat colours than beach-dwelling subspecies (Steiner et al., 2007). These coat colour differences are the result of mutations in the melanocortin 1 receptor gene (*Mc1r*). This gene has also been implicated in the colour phenotypes in lizards (latin name) of New Mexico. Lizards that live on sand dunes develop lighter body colours than the ancestral brown colour type that live on dark soil (Rosenblum et al., 2010). These polymorphisms in phenotype are also widely observed in crab species. For example, *Charybus annulats* has two colour morphs; a typical brown morph that acts as a generalist and is found across

a range of habitat types, and a more conspicuous orange morph only found in places of high cover (Trivedi & Vachhrajani, 2012). In addition, similar adaptations may be seen in fiddler crabs, which have seen shown to have colours ranging from cryptic brown to obvious blue and white (Hemmi et al., 2006).

Phenotype-environment matching for camouflage

What we do not know from these studies is if these associations enhance camouflage and improve survival. There is a lot of empirical evidence to support background matching's adaptive function (Stevens and Merilaita 2009). For example the differential survival of the peppered moth showed that melanic morphs survived better on dark backgrounds, and typical (white) morphs survived better on lighter background (Cook et al 2015). The peppered moth is often used as a classic example of evolution and adaptation (Kettlewell, 1955, 1956). Using the Galapagos penguin as a predator, (Sumner, 1934) recorded the rate at which penguins consumed fish that were either black or white, on black and white backgrounds. In a classic study by Pietrewicz & Kamil, 1977 blue jays found moths difficult to detect when presented on their appropriate background; i.e. a background that they were more camouflaged against. However, these studies do not explore the visual properties of the animal's body colour as perceived by their predator. This is a problem as the colourations of an animal evolve in response to predator visual systems, which are different from our own. What has been lacking until recently is rigorous analysis of body colours to smaller scale substrate appearance, as opposed to broad subjective associations between animals and their background.

Stevens et al. (2015) studied phenotype- environment matches quantitatively, in a study on the sand flea. Stevens et al. (2015) used specialised photography techniques to assess camouflage through the eyes of an avian predator. Stevens et al. (2015) found that sand fleas match the beaches in which they live better than surrounding beaches. I build on these findings in Chapter 2 of this thesis to assess the phenotype-environment matches in the shore crab, though across a much wider range of visually distinct backgrounds. Most recently Troscianko et al (2015) have shown, for the first time in a natural system, that survival probability of wild birds is directly related to their level of camouflage as perceived by the visual systems of their main predators.

A variety of mechanisms may contribute to the improvement of phenotype–environment matches. For example, some animals actively select backgrounds that more closely match their coloration, known as behavioural crypsis (Kang et al 2012). When given the choice between different background types in laboratory conditions, adult whiting *Merlangius merlangus* show a preference

for backgrounds that match their colour (Atkinson et al,. 2004). Day flying moths select backgrounds that better match their of their forewings (Sargent 1966). Kang *et al.* (2012) and Webster *et al.* (2009) used human subjects to search for moths, and found that participants took significantly longer to detect moths if they had been allowed to position themselves appropriately. Kang *et al* (2014) later analysed the moths using avian visual models to demonstrate both correct substrate choice and body positioning were highly influential in the effectiveness of camouflage not unique to prey species: 'sit and wait' predators such as the crab spiders choose white flowers if they are white, and yellow flowers if they are yellow.

Camouflage by colour change

To achieve effective camouflage in a background environment that is often heterogeneous over space and time, some species have evolved the ability to plastically change colour and sometimes pattern (Stevens et al 2014c). A strong advantage of having the ability to manipulate a phenotype so readily is that an organism may alter the type of camouflage in accordance with different factors in its environment (Stuart-Fox et al 2008).

Visual phenotypes may be adjusted between life stages, as seen in the Caribbean spiny lobster *Panulirus argus* which exhibits disruptive patterning in vulnerable juvenile stages, but red colouration in adulthood when camouflage becomes less important and effective (Anderson et al., 2013). In many motile benthic invertebrates that occupy more exposed areas in adult stages the opposite trend is seen to the Caribbean spiny lobster, and adults develop darker shells as they migrate to more exposed areas (de Bruyn & Gosselin, 2014).

Physiological colour change, where pigment moves around specialised cells called chromatophores (Messenger, 2001), is most extensively studied in benthic level cephalopods, comprising octopus, cuttlefish, and squid. These species have evolved the ability to change extremely rapidly in order to forage freely in multiple habitat types (Barbosa et al., 2008; Hanlon, 2007). These are highly specialised animals, having neural control of colour changing organs, very different from other colour changing animals whose chromatophores are often controlled hormonally (Hanlon, 2007; Messenger, 2001; Zylinski etal., 2009). The abilities of the cuttlefish are so well recognised and so effective they have even inspired synthetic mimics (Yu et al., 2014). Rapid adaptation may also be seen in species such as the file fish which may adapt their appearance in 1-3 seconds (Allen et al., 2015).

Some colour changing animals even have the ability to alter their displays in accordance with the type of predator they are faced with. Dwarf Chameleons exhibit different colour responses in the

presence of birds, which have tetrachromatic vision, than in the presence of trichromatic snakes (Stuart-Fox, Moussalli, & Whiting, 2008). Likewise, the cuttlefish will only display the eye spot signal, also known as the diematic display, when it is needed in the presence of visual predators (Langridge et al., 2007). Rapid colour change is also present in chameleons and salmon, both of which darken when loosing fights to signal they are subordinate (Eaton & Sloman, 2011; Ligon, 2014).

Colour change is also involved in thermoregulation in reptiles and amphibians (Norris & Lowe, 1967). For the purpose of my thesis, I will focus on colour changes that enable more effective camouflage (Stuart-Fox & Moussalli, 2009).

However, the studies on colour change to date have mainly described faster physiological colour change and what is less studied is slower morphological colour changes. These occur due to the changes in the number and density of chromatophores in the dermis layer and usually occur over periods of days, weeks, or months (Thurman, 1988a). These adaptations are frequently seen in crustaceans (Umbers, 2014), for example many shrimp change colour over periods of days or weeks in accordance with their surroundings (Bauer, 1981). In addition, colour changes have been investigated in crab species: Fiddler crab *Uca vomeris* can alter their phenotypes to become more cryptic in the presence of increased predation (Hemmi, Marshall, Pix, Vorobyev, & Zeil, 2006), and the ghost crab *Ocypode ceratophthalmus* has been shown to change its luminance in response to a light background over 4 hours. Colour changes have been documented in the shore crab with evidence indicating they may change their luminance values significantly over a period of 2 hours (Stevens, 2014). What is currently unknown is how these crabs achieve physiological colour change.

I aimed to assess the phenotypic-environment matching of the shore crab (*Carcinus maenas*) and the mechanisms that drive it.

Study species

The shore crab *Carcinus maenas* is a widely abundant species, common to intertidal areas worldwide, a habitat that poses many challenges for species trying to remain hidden. These environments are highly heterogeneous and visually complex, comprising many different colours and patterns. These habitats also frequently change in time as tides go in and out which in turn alters the type of predator that prey are exposed to (Crothers, 1968). These factors pose many challenges for crabs that need to remain hidden to survive.

Native to European shores, *Carcinus maenas* is now one of the world's most invasive species, with populations reaching as far as Australia (Vinuesa, 2007; Walton et al., 2002). Its success as an invasive species is likely due to its hardiness and resilience, which also make it an excellent study

species. Shore crabs are readily located in their habitat and once found they are easy to identify and sex. Life begins for the shore crab when they hatch from eggs into free-swimming planktonic larvae. The larvae of Carcinus have three stages: protozoea, zoea, megalopoa. These planktonic stages mean that *Carcinus* has relatively low levels of genetic variability. Individuals become 'crab like' after 4-5 moults at the megalopoa stage, walking on thoracic legs and swimming with pleopods. After a few days at this stage the crab settles to the seabed and they become the shore crab juvenile form. Crabs then enter frequent moult stages until they reach maturity when they reach approximately 25mm. Individuals have a very varied diet of living and dead matter consisting mainly of worms, molluscs and fish which are detected using antennae. They are subject to attack by an array of predators and are mainly attacked and eaten by shore birds such as herring gulls, but also by fish such as gobies (Crothers 1968). This means *Carcinus* must contend with predators with a range of visual and sensory systems. Individuals encounter such a variety of predators because they live across a range of habitat types such as rockpools, mudflats and mussel beds (Crothers 1968).

C.maenas is notorious for displaying a wide variety of visual phenotypes. This is especially apparent in the juvenile stages where individuals differ greatly from each other in colour and pattern (Stevens et al 2014b). There is some evidence that crab appearance differs across habitats to enable individuals to be camouflaged in their habitat (Stevens et al 2014b). Previous work also indicates shore crabs can alter their appearance over time. Individuals may change their brightness through the use of chromatophores (Powell 1962a, 1962b) and this change can significantly change camouflage over a period of 2 hours (Stevens et al 2014c) *C.maenas* is notorious for displaying a wide variety of visual phenotypes. This is especially apparent in the juvenile stages where individuals differ greatly from each other in colour and pattern (Stevens et al 2014b). There is some evidence that crab appearance differs across habitats to enable individuals to be camouflaged in their habitat (Stevens et al 2014b, Hogarth 1978, Todd at al., 2006,2012). Previous work also indicates shore crabs can alter their appearance over time. Individuals may change their brightness through the use of chromatophores (Powell 1962a, 1962c) and this change can significantly change camouflage over a period of 2 hours (Stevens et al 2014c). Previous work also indicates shore crabs can alter their appearance over time. Individuals may change their brightness through the use of chromatophores (Powell 1962a, 1962b) and this change can significantly change camouflage over a period of 2 hours (Stevens et al 2014c)

Colour change in *Carcinus maenas*

The visual phenotype of the shore crab has been of interest to past research. Powell (1962) noted that the appearance of crabs seem to change with size. Juveniles tended to be highly patterned with

pattern level decreasing as crabs became larger. He also noted that crabs tended to differ in appearances across different sites. *Carcinus* pattern has also been shown to vary between areas with different levels of fucoid algae and mud (Hogarth, 1978). More recently Todd (2006) studied the appearances of crabs across several habitat types in Scotland, finding plainer crabs are associated with macro algal areas, with patterned crabs being found on mussel bed areas. Most comprehensively, combined evidence from several of these papers, across several spatial scales, indicated that crabs have a better match on a micro scale ($<1m^2$) (Todd et al., 2012).These studies are valuable in highlighting the importance of phenotype environment associations in the shore crab. However, they are still partially limited because crabs were largely categorised and analysed using human assessment. Recent studies (Stevens et al., 2014) have used specialised photography and image analysis techniques (Troscianko & Stevens, 2015) to directly quantify the phenotype of the shore crab across several sites in Cornwall. Rather than categorising into appearance types, individuals were viewed as more plastic and several aspects of their phenotype were analysed including hue, saturation, and luminance. Results indicated crabs from the different habitats were indeed different in several appearance metrics. However, despite these variances being apparent, their function is not. It is likely and assumed that they infer a camouflage benefit, though this has never been tested.

Summary of chapters

In C**hapter 2** of my thesis I tested if differences in phenotype across habitats are for camouflage and assess this *in situ* through the eyes of an avian predator. This was done using photographs of both crab and the background in which it was found. These were then compared to assess how well a predator would be able to discriminate between crab and background and therefore indicate its level of camouflage. Crabs were sampled across differing habitat types, two sites comprise a mudflat habitat, two more were selected for rockpool sites, and the third were areas of mussel bed. Analysis was conducted over several spatial scales. Firstly, the camouflage level between crab and background were tested on a micro scale (<1m2) against their own immediate habitat. This was then compared to crabs against other areas within the same habitat type. A third spatial category tested crabs against backgrounds that were from a separate habitat category. This meant phenotype-environment associations could be assessed across different habitats at different spatial scales with the prediction that associations will be highest within their own habitat type. Results showed crabs have a higher level of matching in homogenous mudflat habitats, becoming mismatched and less camouflaged in foreign, heterogeneous habitat types. Crabs from heterogeneous habitats showed

less conclusive results, though juveniles in mussel beds and rockpools did show a greater match to their immediate background, in some aspects.

However, what remains unclear are the drivers and causes of these phenotype-background matches. One possible explanation for such is the alteration and tuning of an individual's phenotype through colour changes. Juvenile *Carcinus* are known to possess chromatophores visible through their cuticle that have the potential to allow them to adapt to the conditions of their environment. These were most extensively studied by (Powell, 1962) who showed on a light background they may condense pigment in chromatophores to appear lighter, and disperse this pigment to become darker on a dark background. Much more recent work (Stevens et al., 2014) has assessed changes in juveniles by leaving them on differently coloured backgrounds and tracking their changes through specialised photography. Again, this means individuals may be mapped to a particular vision, making it relative to the receiver. Stevens et al. found juvenile individuals significantly changed their luminance (perceived brightness) in relation to their background, increasing on white and decreasing on black, though no changes were seen on green or red backgrounds. What remains unclear is what would happen if crabs were left for a longer time period.

Chapter 3 of my thesis built on chapter 2 to assess phenotype changes in two experiments. The first of these focuses on shorter term changes, and images were taken after 0, 2, 4 and 48 hours of crabs being left on white, black, red, and green backgrounds. The second kept crabs on the same colours, but for 5 weeks, documenting changes once a week. Phenotypes were analysed in terms of luminance and colour (hue) to analyse any changes. Results were then also compared in terms of camouflage, to test whether any changes in these metrics afforded them improved camouflage to an avian predator. If significant, this could provide a potential explanation for any phenotype environment associations seen and provide insights as to how this highly adaptive species survives in a highly dynamic and challenging environment. Results indicated that shore crabs have the ability to alter both their luminance and colour, over the long-term 5-week experiment, especially with moulting, though no significance was seen in the short term. All changes significantly improved camouflage over time.

Chapter 2: Habitat specific camouflage in the shore crab (*Carcinus maenas)*

Abstract

Camouflage is an important adaptation for many species that would otherwise be vulnerable to predation. As a consequence, many have phenotypic associations with their surroundings, matching the colourations of those in their habitat. Given the presence of potential threats within its environment, it is imperative that the shore crab *Carcinus maenas* is well camouflaged. *C. maenas* survives in a diverse range of habitat types, all of which differ visually. Previous work has shown that crab phenotypes differ in several visual aspects between mudflat, rockpool and mussel bed habitats. This study aimed to assess if these changes were due to phenotype environment matching in each of the areas. I tested phenotype environment matching on a microhabitat scale, assessing whether individuals matched their immediate background more than other areas in the same habitat. Crabs were compared to backgrounds on three spatial categories; their own immediate background (microhabitat), backgrounds from the same habitat type, and backgrounds from differing habitat types. I used measures of luminance, colour, and pattern to quantify phenotype in terms of avian predator vision. I found that individuals from homogeneous mudflat habitats had phenotypes that were well matched to their immediate background, and equally well matched to random locations in their own environment. In contrast, crabs from rockpool and mussel bed environments had generalised camouflage across all habitat types. Crabs in heterogeneous rockpool and mussel bed environments may use disruptive camouflage in these complex environments, or have compromise crypsis. This study highlights how animal phenotypes can be specialised to the conditions of their local environment in order to improve camouflage.

Introduction

One of the most widespread ways an organism may conceal itself from predators is to match the visual properties of its environment (Thayer 1909; Cott, 1940; Stevens & Merilaita, 2009) . Known as background matching, this occurs when an animal's phenotype matches the general colour, brightness, and pattern of one or several background types (Stevens and Merilaita, 2009). This phenomenon is also referred to as phenotype environment matching (Stevens et al., 2015; Stevens et al., 2014; Todd et al., 2006).

A variety of desert dwelling rodents, such as oldfield mice (*Peromyscus polionotus*) that live inland are dark in colouration, while mice that inhabit coastal environments are much lighter (Steiner et al 2007). This phenotype environment matching is proposed to increase the match between the mice and the background sand (Steiner et al., 2007). Phenotype environment matching has also been studied on a much larger spatial scales in African Jerboas (*Jaculus jaculus*), which have body colours that reflect the colouration of their habitat (Boratyński et al 2024). Jerboas that live in darker rocky areas having a darker phenotype than those living on sand (Boratyński et al., 2014). In a similar species, the rock pocket mouse (*Chaetodipus intermedius*), are light coloured in areas of light rock, whereas individuals found on dark larval rock have a darker phenotype (Hoekstra et al., 2014; Nachman et al., 2003). Phenotype-environment matches for camouflage are also seen in several lizard species in New Mexico (Rosenblum et al., 2004, 2010). Lizards living in white sand dune environments have light coloration, and this is suggested to match these areas (Rosenblum et al., 2010).

Although phenotype-environment matches appear to be a common adaptation in desert habitats, this is not a phenomenon unique to deserts, and is also seen in forest-living tree squirrels in North America (Chavez & Kenagy, 2014). Squirrels possess ventral coat coloration that changes on a gradient in accordance with tree canopy cover; fur coloration in areas of low canopy cover are significantly lighter, and this is thought to improve camouflage (Chavez & Kenagy, 2014).

While the above studies demonstrate how phenotypes can diverge between habitats, there is only indirect evidence that this improves camouflage matching to visual backgrounds. Assessments of background matching have also generally relied on human vision (Hogarth 1975, Todd et al 2006, 2012). What is lacking in this area of research is formal quantification of camouflage matching by rigorous phenotype assessment from the point of view of the animal's putative predator. The only exception to this observation is a recent study concerning background matching in the sand flea *Hippa testudinaria* (Stevens et al., 2015). The sand flea is a species that possesses phenotypes in a range of colours and brightness. Stevens et al used specialist photography and image analysis

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techniques to assess sand flea camouflage from the perspective of an avian predator. Fleas were tested against backgrounds from their own beaches and to a random sample from other beaches. Fleas had a greater level of camouflage on their own beaches, showing phenotype-environment matching. However, this study did not analyse phenotypes in terms of pattern, and did not analyse matching abilities within habitats that would have provided a greater overview of the phenotype-environment match. My study will address similar questions using the shore crab *Carcinus maenas*, comparing camouflaging abilities of crabs against their own backgrounds to compare to foreign areas of the same habitat, and then across entirely different habitat types.

The shore crab (*Carcinus maenas*) has extremely high phenotypic variation, with individuals displaying a large array of colours and patterns especially at juvenile stages (Bedini, 2002; Hogarth, 1975, 1978; Stevens et al., 2014). Subjectively, when individuals are found they tend to be on colours that reflect that of their own phenotype, and it is therefore widely presumed that their colour patterns are a form of background matching (Bedini, 2002; Todd et al., 2005).

Previous work with *C. maenas* has demonstrated assosciations between the visual properties of the environment and the phenotype of the crab that suggests that their camouflage is tuned to the local environment. Bedini (2002) studied *C. maenas* in sea grass meadows in Mediterranean waters noting that juveniles that hide in these areas contain colour bands, stripes, and blotches resembling elements of their environment, and that these change to a completely plain phenotype in adulthood. Hogarth (1978) tested crab and environment associations analysed the patterns of individuals from around the UK, and found that individuals with less cover had more patterning. However this work used human assessment of pattern, and did not consider predator vision that is a problem because humans are not the main predator of crabs.

More recently, Todd et al. (2006) investigated shore crab phenotypes across Scotland, amongst several visually different environment types encompassing mussel bed, seaweed, sandy beach, and rock. Crabs were placed into categories based on visual appearance of colours and patterns on their carapace, and they found that patterned crabs associated with mussel bed substrates, whereas plain crabs were found on seaweed backgrounds. Continuing this work, Todd *et al* (2012) collated data from several sources and sites around the UK to understand phenotype substrate relationships over three spatial scales: macro (>10,000sm^2), meso (>100 m^2) and micro (<1m^2). Similar to previous results, a greater proportion of patterned crabs were found on mussel beds than on algae and rocks. Todd et al's results clearly show a link between patterned crabs and visually diverse backgrounds, and plainer crabs and more uniformly coloured backgrounds, especially at the micro scale, which

suggests possible behavioural choice by individuals of substrate to improve their own specific camouflage.

The newest research by Stevens et al. (2014), using image analyses and colour modelling of nearly 700 crabs collected from four sites in Cornwall, found that crabs vary significantly in several aspects of appearance, in relation to colour and pattern depending on the location. Individuals were caught across a range of typical crab habitats including mudflat, mussel bed, and rockpool. Crabs from mudflats were dark, highly saturated, and had low pattern. Crabs from mussel beds were plain, with differences in brightness between adults and juveniles. In contrast, crabs from rockpools were highly patterned, with high individual variation.

However, what was lacking from Stevens et al's study was a direct analysis of camouflage and the degree of phenotype-environment matching. In this study I builds upon Stevens et al's work, and directly assesses the camouflage of crabs across mudflats, rockpools, and mussel bed habitats in terms of luminance, colour, and pattern. I measured these phenotype metrcis in terms of the visual system of birds because they are an important predator group (Crothers 1968).

Crabs were compared against their own immediate background, alternative locations from the same habitat and entirely foreign habitats. This meant crabs could be assessed to determine if they had a greater level of camouflage in their own habitat compared to foreign, visually differing environments. In addition crabs were assessed on a much smaller (microhabitat) scale to test if their camouflage was specific to their exact location, and whether a mismatch would occur within a few metres from their exact location.

Methods

Data collection in the field

Three different habitat types were chosen to sample crabs from: rockpool, mudflat, and mussel bed. Two sites were chosen to represent each of the three habitat types, totalling six fieldwork sites, all in the county of Cornwall in the southwest UK (Figure 1). Gyllyngvase beach in Falmouth and Tavern beach in St Mawes were chosen for rockpool sites. The former consisted of a large area of rock with shallow rockpools filled with gravel to the top of the shore, and increasing brown or red macro algal growth over larger rockpools toward the low tide zone. St Mawes offered a much smaller site, with shallower rockpools. Substrates followed a similar patterning with macro algae only in the low tide zone. In contrast, mudflat habitats were very different from these predominantly rocky areas. Penryn estuary is a large area of mudflat at low tide with a covering of brown macroalgae. Helford

Passage is similar, although it consists mainly of more sandy mud, with intermittent covering of algae. Areas of mussel bed were used for the final category; Godrevy and Polzeath.. These areas are similar to rockpool sites, yet substrate coloration is often very different as many of the rocks are covered in blue mussels *Mytilus edulis,* which juvenile shore crabs can often be found hiding amongst. Both Godrevy and New Polzeath consisted of large areas of mussel covered rock, interspersed by rockpools with some algae cover. Sampling was conducted between April and October 2014.
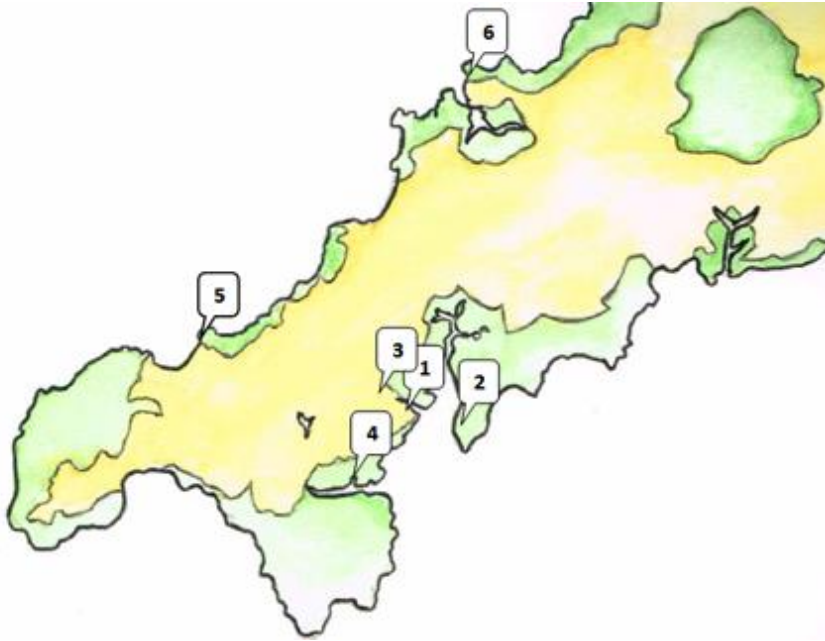
Figure 1- Map showing the location of all 6 field sites. 1) Gyllyngvase beach in Falmouth 2) Tavern beach St Mawes 3) Penryn mud flats 4) Helford Passage 5) Godrevy point 6) New Polzeath.

At each site the shore was measured from the low tide mark to the top of the beach and this measurement was then divided into five sections. Areas closest to the low and high tide marks were disregarded. The middle three sections were used to lay three 30 metre transects across the beach parallel to the shoreline. These represented high, middle, and low zones of the shore. This meant an appropriate sample of the beach was achieved, but areas of extreme high and low tide were avoided. In areas where crabs were scarcer, where necessary, transects were extended up to 40m. A 0.5m^2 quadrat was placed either side of each of the transects, and every 2.5 metres, and each area was searched for shore crabs for up to five minutes (some areas were searched for a shorter time period if it was obvious no crabs would be found; e.g. areas of barren rock). Only one crab was used for photography per quadrat, this being the first to be discovered. Any additional crabs found within the quadrat were disregarded, as these would have shared the same or a very similar background

image as the first crab. It was important to assess a wide range of backgrounds within a habitat, to gain an understanding of the phenotype range within the area. This was especially important in heterogeneous places such as rock pools and mussel beds. It could be argued that this method creates bias as only the most easily seen, and therefore least camouflaged, individuals are located. To overcome this, quadrats were searched thoroughly, using tactile cues in addition to vision. Furthermore, if the most visible individuals were being located, but still shown to have the best camouflage, other crabs must have even better camouflage and my results are conservative. Searching through the areas also disturbed the background, which could potentially alter their visual aspects. Trying to find all the crabs within a quadrat would have required significant alteration of the habitat, meaning background photographs would have been inaccurate.

10 juveniles and 5 adults were photographed per transect totalling 30 juveniles and 15 adults per site. Fewer adults were used due to their scarcity. All sampling was conducted between the months of April and October 2014.

Photography in the field

Once a crab had been located it was taken, gently dried and placed into a grey tray for photography. A series of images were taken in human visible spectrum, and then immediately afterward in ultraviolet (UV) light. A digital Nikon D700 camera, which had undergone a quartz conversion to allow for UV sensitivity (Advanced Camera Services, Norfolk, UK) was used for all photographs. To capture human visible images, a filter (Baader UV/IR Cut filter) was placed in front a Nikon 105 mm Nikkor lens that blocked UV and infrared light and only transmitted waves between 400-700 nm. For the UV images a second filter (Baader Venus U filter) was placed in front of the lens, allowing for UV transmission between 300-400 nm but blocking infrared and human visible light. Photographs were taken in RAW format with fixed aperture settings. Several photographs were taken of the same subject at a range of exposures to avoid capturing any overexposed images, as these cannot be used for analysis. A Spectralon grey standard (Labsphere, Congleton, UK) was placed into each photo which reflects a known amount of light equally at 40% between 300 and 750 nm (Stevens et al., 2007). This allows images to be controlled for changes in lighting conditions, which is especially important in the field as light fluctuates as the sun goes behind clouds. To keep lighting as even as possible across the image, a photographic umbrella was also used.A ruler was also placed into each shot for scale.

Image analysis

Once uploaded, the most appropriately exposed photographs were selected using RGB histograms in the program RAWTherapee (open source from rawtherapee.com). It is important to use photographs with the optimum exposure as any overexposure causes pixel saturation meaning data is lost and images cannot be correctly measured (Troscianko & Stevens, 2015). Once the correct images were selected, custom codes from the 'multispectral image calibration and analysis toolbox' (Troscianko & Stevens, 2015) were used in Image J to create a multispectral image(mspec). This involves the aligning of the human visible and UV images into one entity, which is then split down into differing wavelengths. The result is a stack of images at the relative wavelengths of red shortwave (SW, blue) mediumwave (MW, green), longwave (LW, red) and UV. Because the UV was captured in a separate image, and the images must be refocused, it is also important at this stage to ensure both images are perfectly aligned to avoid false colours being created. During this process the grey standard was selected which had a known reflectance of 40% meaning the rest of the photo could be calibrated to this. Following the calibration of these images, regions of interests (ROIs) could be selected; these were the sections of the photographs that would be measured - in this case the crab or background. If crabs were multi-coloured only the predominant colour of the crab was selected as an ROI. The predominant colour was taken as one that accounted for at least >50% of the overall colour on the carapace of the crab. In most cases, this was not difficult to judge as most multi-coloured individuals had obvious markings that were different to their 'base' colour; i.e. in a brown crab with three white dots on the front of the carapace (a common phenotype) the brown would be selected and the white avoided. This was important for colour results as the code works by taking an average of the information in the ROI, therefore a bright white dot would skew any colour and data, creating a colour that does not actually exist on the crab or background. For pattern analysis, the ROI was selected as the whole of the carapace of the crab. This was drawn as close to the edge as was sensible, so as to get as much information as possible, without the possibility of incidentally measuring unwanted areas; i.e. the tray in the background or the leg/eye of a crab.

Central to this study's uniqueness is viewing camouflage through the eyes of the appropriate receiver (a predator). One of the largest predation threats for *C. maenas* comes from shore birds such as oyster catchers and turnstones (Crothers, 1968), which have a different visual system to that of humans and fall into the avian violet group (VS) system. These birds are still sensitive to UV light, but the ultrashortwave cone type is more sensitive to longer violet wavelengths than in UV bird like blue-tits (Odeen et al., 2010). Therefore, generated mspecs were analysed in terms of an avian vs visual system using the 'batch multispectral analysis' tool provided by Troscianko and Stevens (2015). The vision of the peafowl *Pavo cristatus* falls into this category (Hart, 2002) and is a widely used model species of avian VS vision, and therefore its specific spectral sensitivity data was used

under a D65 standard irradiance spectrum to convert from camera to avian colour space using a polynomial mapping technique (Stevens et al., 2007; Troscianko & Stevens, 2015).

To analyse images in terms of specific camouflage, just noticeable differences or JNDs were used. This data is derived from a model that calculates predicted units of discrimination between two objects, thus calculating how likely it would be for an observer to be able to distinguish between two objects. It can therefore be used to calculate how well camouflaged an object is against a background. A log form of the Vorobyev-Osorio model was used, which is based on single cone avian photon catch values (Vorobyev & Osorio, 1998). A Weber fraction of 0.05 was used as in previous work (Stevens et al., 2014), as well as the relative proportions of the different cone types in the retina of the peafowl *Pavo cristatus* (LW = 0.95, MW = 1, SW = 0.86, UV = 0.45) (Hart, 2002). Values below one indicate that the objects are indistinguishable, with values above three suggesting that they are distinguishable to an increasing extent. This method is used for chromatic differences. For achromatic analysis (luminance) a modified version of this model, based on that used by Siddiqi et al (2004) where comparisons are based on luminance differences obtained from the double cones. Double cones are used because these are widely believed to be involved in achromatic perception in birds (Osorio & Vorobyev 2005).

Luminance and colour JNDs were calculated between each crab on its own immediate background, each crab and a random area from the same habitat type (i.e. a crab from mudflats compared to a random other area of mudflat), and finally each crab and a random area from a completely different habitat type (i.e. a crab from mudflats compared to a background from a rockpool). Further to this, the camouflage of crabs on their own backgrounds was compared: i.e. are crabs from some habitats just better concealed overall?

Pattern is also important in camouflage effectiveness, and so crabs were also assessed in terms of how closely the size and contrast of the crab markings match those of the background. This was quantified using a granularity analysis similar to that previously used to analyse cuttlefish patterns (Barbosa et al., 2008; Chiao et al., 2009) and egg patterns (Stoddard & Stevens, 2010). This method involves filtering each image using a fast Fourier transform and applying ten octave–wide isotropic bandpass filters to each image. Each of these filters acts like a sieve, catching information at different spatial scales; i.e. smaller filter sizes capture large markings of low spatial frequency and vice versa. This information is used to create a granularity spectrum, representing pattern energy against marking size. Therefore, the energy output at each of these levels is used to assess the relative contribution of different marking sizes to the overall body pattern. This procedure was carried out using the 'batch multispectral image analysis' tool (Troscianko & Stevens, 2015) in Image

J. All images were scaled to 13 px/mm, with patterns quantified between 2 and 45.25 px with scale incrementing from 2 to the square root of 2.

To compare crabs to particular backgrounds, pattern energy differences were calculated using a custom made difference calculator in ImageJ (Troscianko & Stevens, 2015). This measures the granularity spectrum of both the crab and background and calculates the absolute difference between the two spectra. Granularity spectra that diverge more in shape and energy (amplitude) will have larger differences, meaning that the crab and background are less well matched for pattern size and contrast. Again, crab patterns were compared to that of their own background, a randomly chosen background from their own habitat, and to a randomly chosen patch from a different habitat. Following this, crab's pattern matches within their own habitat were compared.

Statistics

The colour metrics data were log transformed to meet the requirement of normal distribution. I used a repeated measures ANOVA to compare differences between the crab and backgrounds in terms of luminance JNDs, colour JNDs, and pattern JNDs, when viewed against their own background, a random sample from the same habitat, and a random sample from a different habitat (within subject factor). The between subjects factor was the habitat in which the crabs were collected from, and the age of the crabs (juvenile and adult crabs). I also included size as a covariate. Because of significant sphericity, I applied a Greenhouse-Geisser correction to the F statistic.

I used a one-way ANOVA to compare differences in luminance and colour JNDs, and pattern for juvenile and adult crabs on their own backgrounds, and how this varied depending on the habitat from which they were collected (i.e. to compare the level of camouflage achievable on each habitat). I used planned simple contrasts to compare mudflat crabs which subjectively represents the most simple background to rockpools and mussel beds. All analysis was conducted in IBM SPSS statistics v23.

Results

Luminance analysis

<u>Are crabs more camouflaged in their own habitat than a random sample of the same and a different habitat?</u>

There was no interaction between the size of the crab and the luminance JND for each background it was tested against ($F_{2,524}$= 1.720, p= 0.180). But there was a significant interaction between how well a crab matched its background in terms of luminance and the habitat it came from ($F_{4,524}$= 7.696, p<0.001). There was no significant interaction between how well the crabs matched their background and their age ($F_{2,526}$ =0.618 p=0.539), or between the background type, habitat, and age of the crab ($F_{4,526}$ =1.612 p=0.170). There was a main effect of size ($F_{1,\ 263}$= 4.930, p = 0.027), with larger crabs having smaller JNDs regardless of their habitat or the background on which they were measured. There was also a main effect of habitat ($F_{2,\ 263}$ = 7.044, p = 0.001) with crabs from mudflats having lower JNDs regardless of the background to which they are compared. Differences between habitat types may be seen in Figure 2.
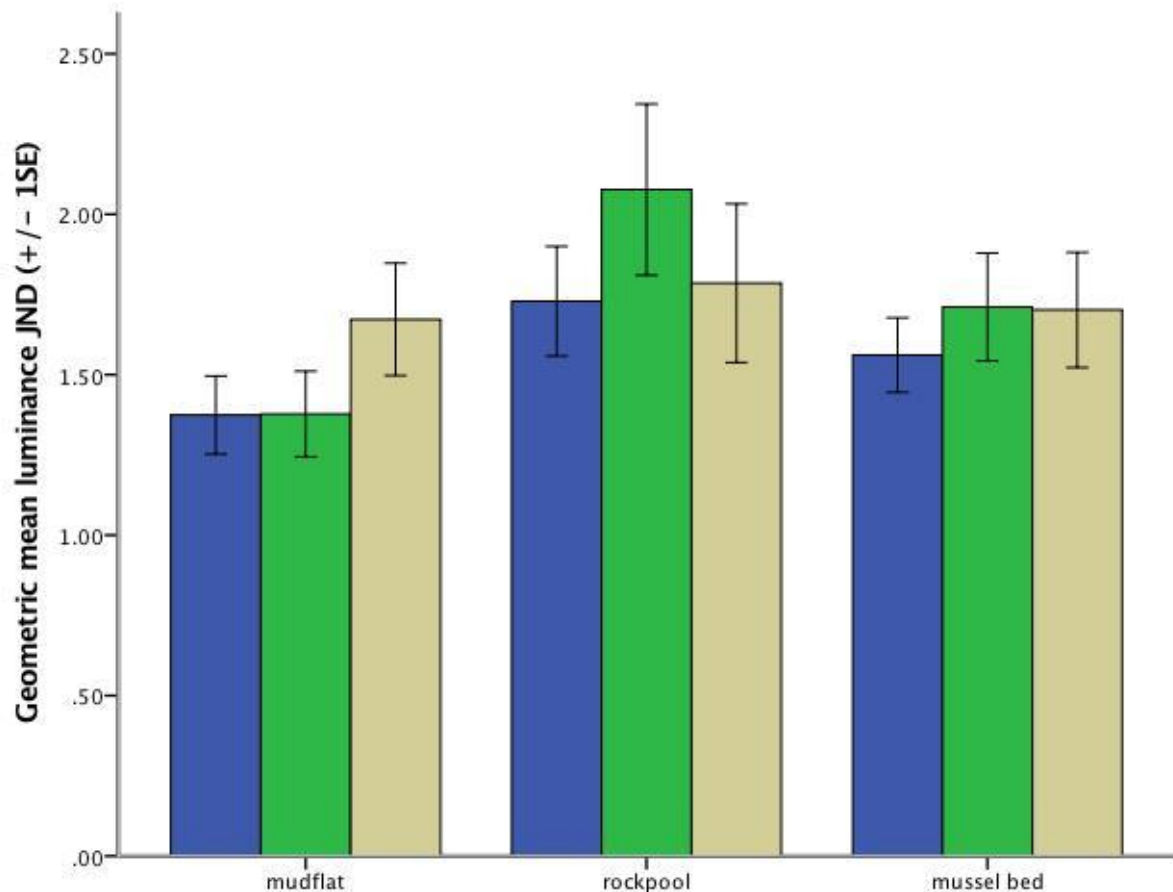
Figure 2 Geometric mean luminance JND (+/- 2 SE) for crabs found in mudflats, rockpools, and mussel beds, measured against their own background (blue bars), random samples of their background (green bars) or a different background (beige bars).

Does camouflage differ between habitats?

Crabs from different habitats showed significantly different levels of luminance matching (blue bars, Figure 2; $F_{2, 263}$, = 7.558, p =0.001). Crabs from mudflats matched their backgrounds significantly better than crabs from rock pools (p<0.001) and marginally better than mussel beds (p=0.055). There was no effect of age ($F_{1, 263}$ = 0.446, p=0.505) and no interaction between age and habitat type ($F_{2, 263}$ =1.616 p=0.201).

Colour analysis

Are crabs more camouflaged in their own habitat?

There was no interaction between the size of the crab and the colour JND for each background it was tested against ($F_{2, 526}$ =1.448, p= 0.236). There was also no significant interaction between how well a crab matched its background in terms of colour and the habitat it came from ($F_{4, 526}$ =1.122,

p=0.345). However, there was a significant interaction between how well the crabs matched their background and their age ($F_{2, 526}$ =6.941 p=0.001). There was no interaction between the background type, habitat, and age of the crab ($F_{4, 526}$ =1.478 p=0.207). There was a main effect of size ($F_{1, 263}$ =4.443, p = 0.036), with larger crabs having smaller JNDs regardless of their habitat or the background on which they were measured. There was also a main effect of age ($F_{1, 263}$ =16.817 p< 0.001). Therefore, analysis was split by age single repeated measures ANOVAs. In adult crabs (Figure 3) there was no significant interaction between the background and the size of the crab ($F_{2,352}$ =2 1.343 p=0.264), habitat (F= $_{4, 352}$ =0.928 0.449). However, in juveniles (Figure 4) there was a weak significant interaction with background ($F_{2, 352}$ =0.632 p=0.050 but no interaction between habitat and size ($F_{2, 352}$ =3.40 p=0.712). There was an interaction between the background they were found on and what habitat they are found in ($F_{4, 352}$ =0.520 p=0.043).
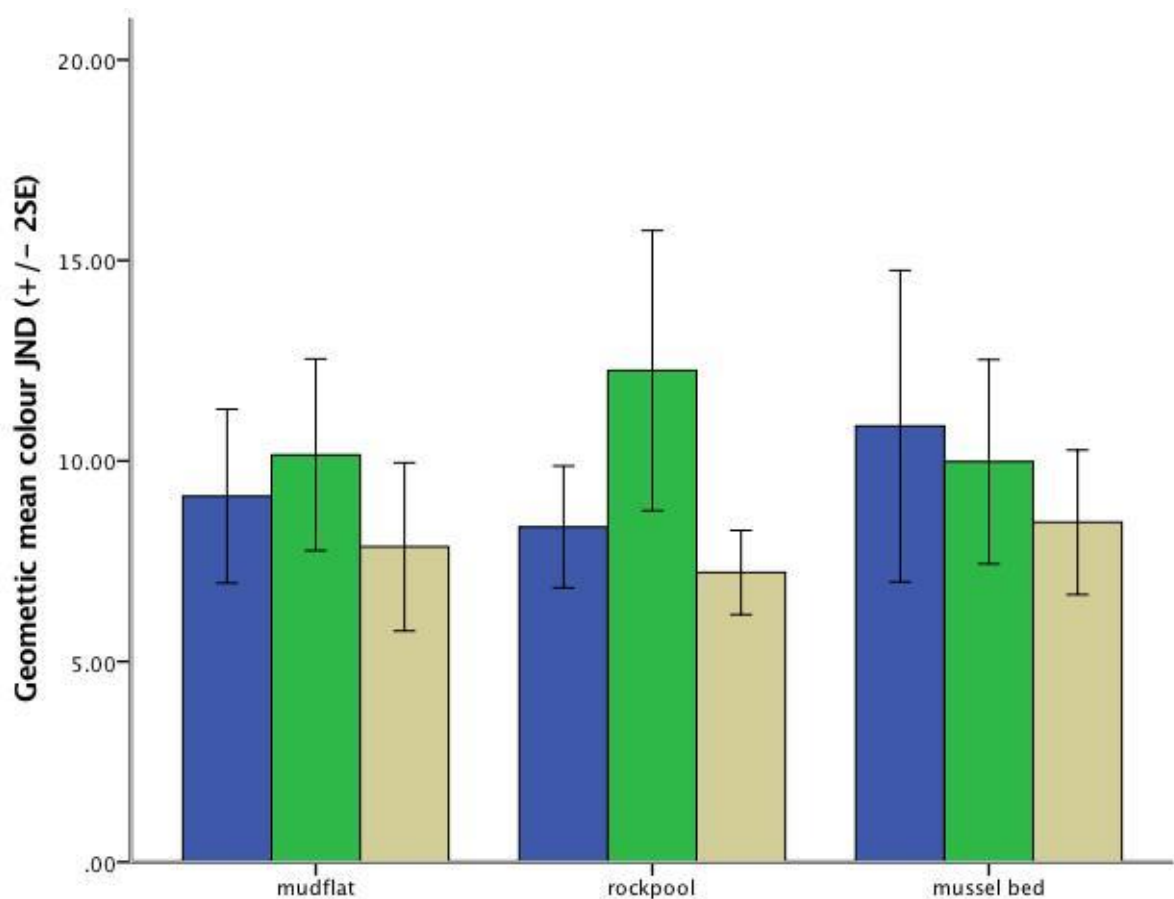

Figure 3 Geometric mean colourJND (+/- 2 SE) for adult crabs on mudflats, rockpool and mussel bed habitats on their own backgrounds (blue bars), a random sample from the same habitat (green bars) and a background from another habitat category (beige bars).
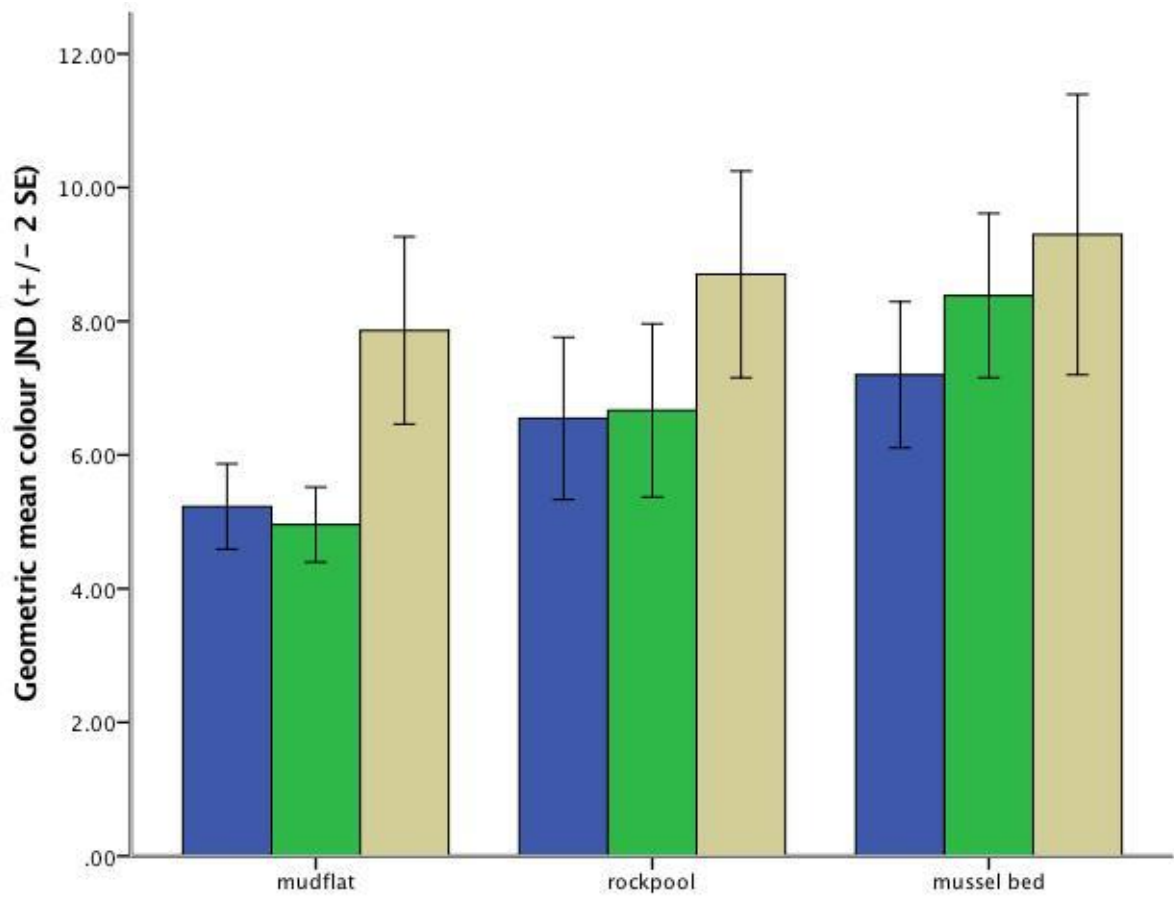
Figure 4– Geometric mean colour JND for juvenile crabs on mudflat, rockpool and mussel bed habitats on their own background (blue bars), other areas of the same habitat (green bars), and a background from another habitat type (beige bars).
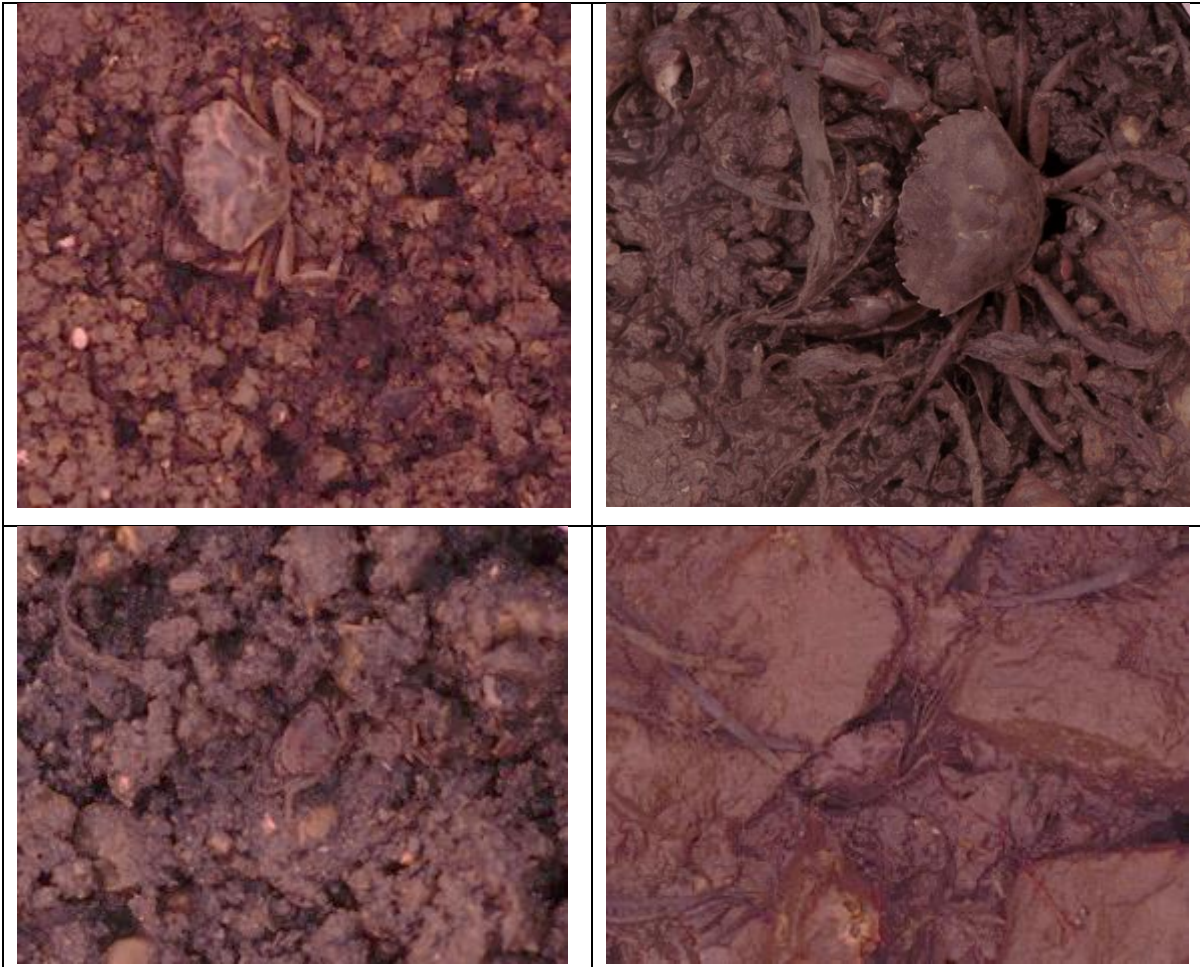
Figure 5- Examples of crabs from mudflat habitats on mudflat backgrounds. Adults are shown at the top, juveniles below.

Figure 6 Photographs showing typical crabs from rockpool areas, within their habitat, on the backgrounds they were found on. Adults are above and juveniles below.

Does camouflage differ between habitats?

There was no significant difference in colour JNDs (camouflage) between habitat types ($F_{2.263}$=1.503, p=0.224). Adult crabs had significantly larger JNDs than juvenile crabs ($F_{1.263}$=20.267, p<0.001). There was a significant effect of size on JNDs ($F_{1.263}$=5.567, p=0.019) with larger juvenile crabs having smaller JNDs (Pearson correlation = -0.158, p = 0.34) but there was no significant difference in adult size on JNDs (Pearson correlation = -0.170, p = 0.110). There was no significant interaction between habitat and age ($F_{2.263}$=0.804, p=0.449).

Figure 7 –Individuals in typical mussel bed environments, adults are shown at the top, and juveniles at the bottom.
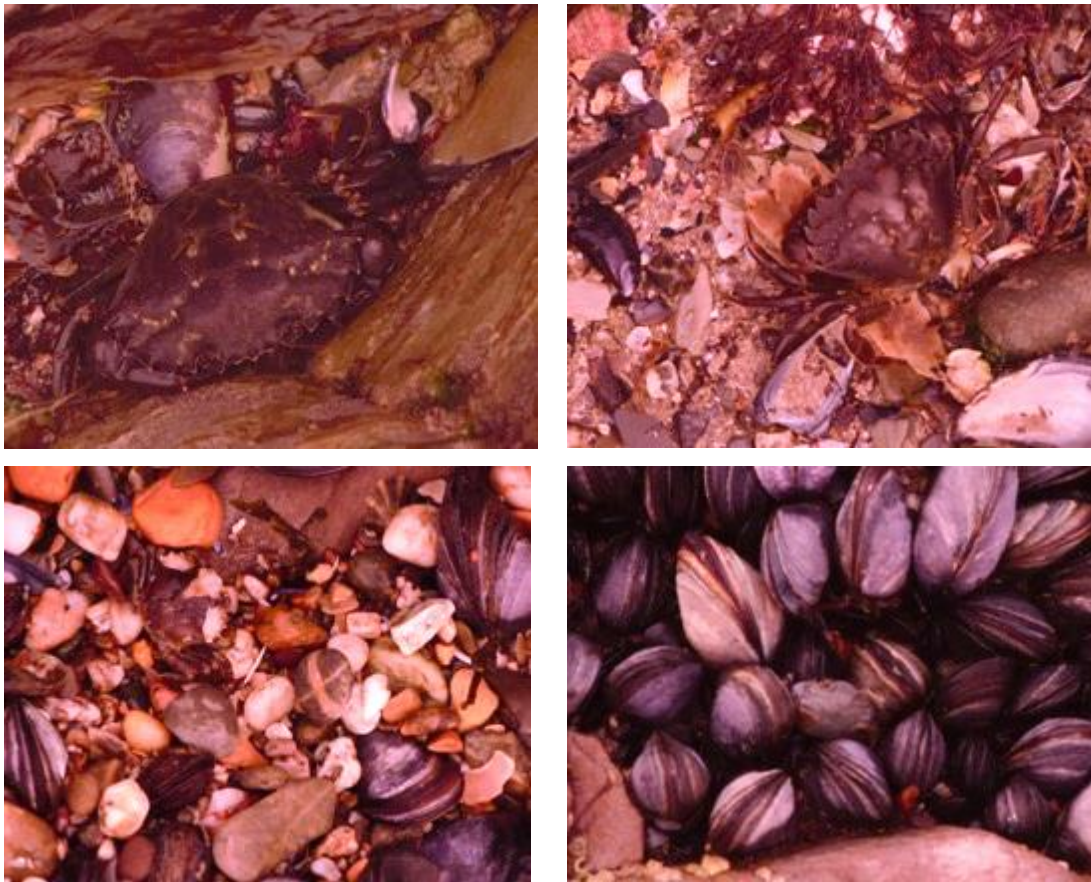
Pattern analysis

Are individuals more camouflaged in their own habitat?

There was no interaction between the size of the crab and the pattern for each background it was tested against ($F_{2, 526} = 1.980$, p= 0.139). There was a significant interaction between how well a crab matched its background in terms of pattern and the habitat it came from ($F_{4, 526} = 11.542$, p<0.001). There was no significant interaction between how well the crabs matched their background and their age ($F_{2, 526} = 2.410$ p=0.091). There was no interaction between the background type, habitat, and age of the crab ($F_{4, 526} = 1.267$ p=0.282). There was a main effect of size ($F_{1, 263} = 4.540$ p=0.034) habitat ($F_{2, 263} = 15.328$, p<0.001) and age ($F_{1, 263} = 7.242$ p=0.008). Differences in pattern across habitats may be seen in Figure 8.
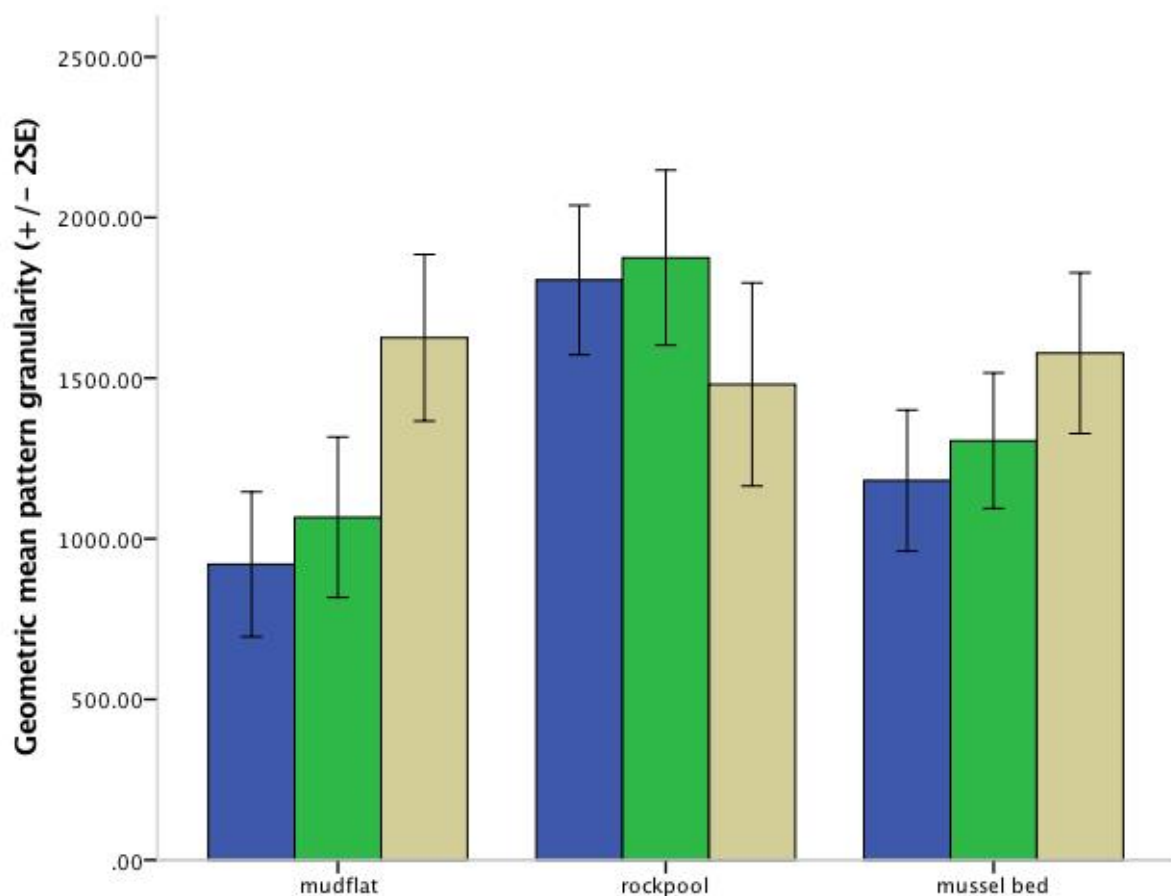

Figure 8 Geometric mean pattern energy difference for crabs on mudflat, rockpool and mussel bed habitats on their own background (blue bars), other areas of the same habitat (green bars), and a background from another habitat type (beige bars).

<u>Does camouflage differ between habitats?</u>

There was a significant effect of size on pattern matching ($F_{1,263}$ =6.659 p=0.10) habitat ($F_{2,263}$ =19.420 p<0.001) and age ($F_{1,263}$ 9.108 p=0.003). There was no interaction between habitat and age ($F_{2,263}$ =1.809, p=0.166). Mudflats were significantly different from rockpools (p<0.001) and mussel beds (p=0.012). Mussel beds were significantly different from rockpools (p=0.003).

Both pattern and colour differences in juveniles throughout these habitats can be seen in Figure 9. To the human eye, stark differences can be seen in these individuals' phenotypes across habitats with mudflat crabs showing very plain brown colorations whereas those from more heterogeneous rockpool and mussel bed areas vary widely.
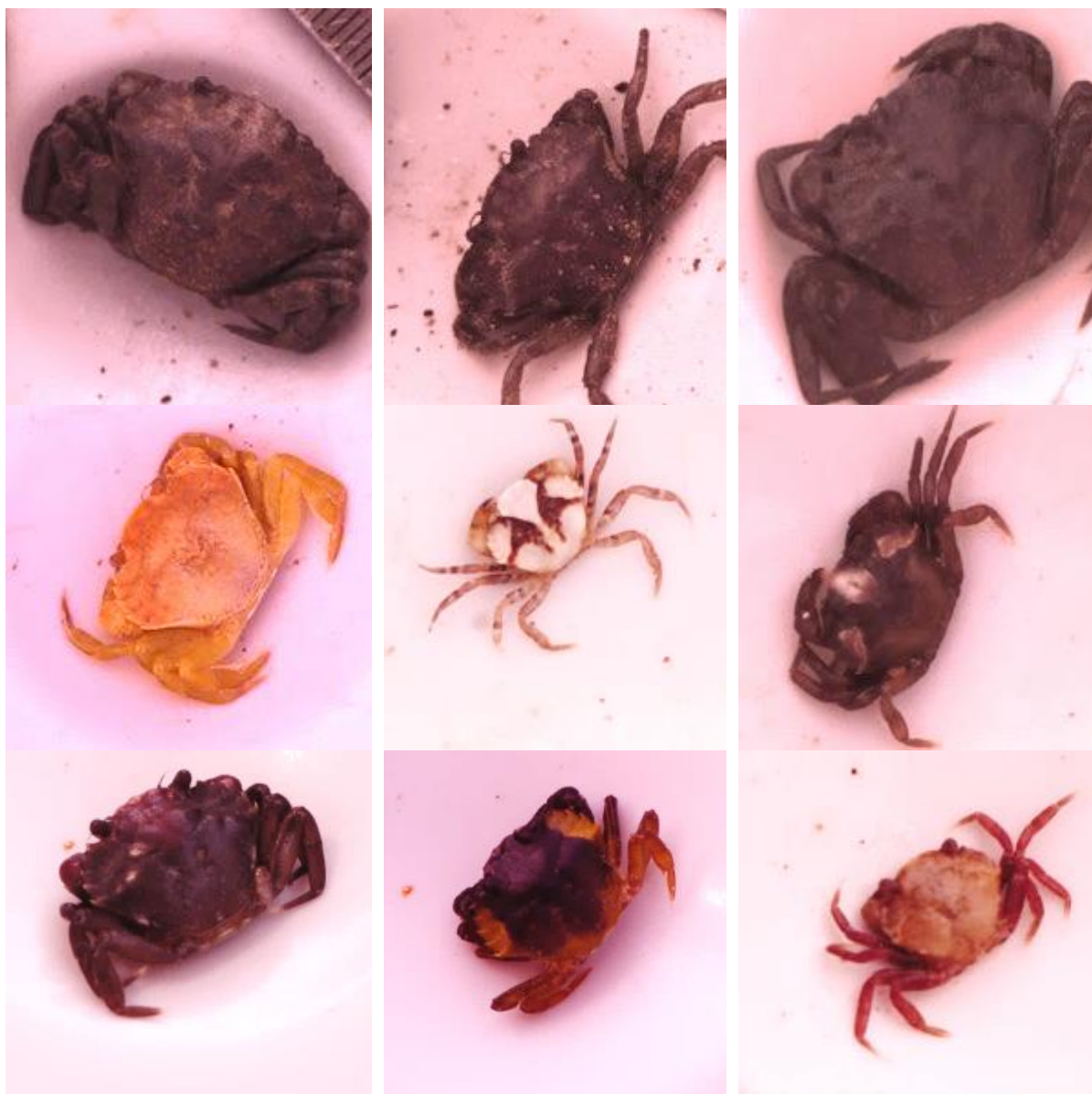

Figure 9 – Random selection of typical juveniles phenotypes from mudflats (top row) rockpools (middle row) and mussel beds (bottom row)

Discussion

The camouflage of crabs from different habitats was assessed using luminance JNDS, colour JNDs, and pattern energy differences between the crabs and backgrounds. All age classes of crabs from mudflats had better luminance camouflage than crabs from mussel beds and rock pools, as measured by JNDs. Mudflat crabs matched their own backgrounds in luminance better than other backgrounds, whereas rockpool and mussel bed crabs had generalised camouflage to all three backgrounds. Juvenile crabs from mudlflats matched their own background in terms of colour better than other backgrounds, whereas rockpool and mussel bed crabs had generalised camouflage to all three backgrounds. This was also true for pattern for crabs from mudflats, which matched their own background better, whereas rockpool and mussel bed crabs had generalised camouflage to all three backgroudns. Larger crabs matched their backgrounds in terms of luminance and colour better than smaller crabs. The findings of this chapter support those of Stevens et al. (2014). This chapter highlights how one species may use different phenotypes in order to become camouflaged across visually different environments. This is especially important for an organism such as the shore crab as it resides in such a wide range of highly differing habitats, all of which contain a wealth of predators.

My results for colour, luminance and pattern matching by crabs from mudflats are relatively unsurprising given the findings from similar uniform environments such as those that oldfield mice (*Peromyscus polionotus*) live in and match (Steiner et al 2007). Mudflats are very uniform, consisting principally of mud and brown coloured macroalgae (personal observation), and this means that crabs from plain uniform backgrounds are very much specialised to these areas

Crabs from more, heterogeneous environments showed more general camouflage against all background, which is expected given that specialism in complex environments is not beneficial (Dimitrova & Merilaita, 2011). It is possible that crabs in heterogeneous environments use microhabitat selection (Kang et al., 2012; Webster et al., 2009). In line with this ,Todd (2012), found the strongest phenotype background matches were at the metre^2 level. it was notable when collecting data that crabs from mudflats differed in behaviour from those from heterogeneous habitats in that they were highly aggressive. This could be due to these individuals being more vulnerable to predation as they have less cover in their environment. Behavioural choice of substrate should be studied in future work.

The generalised match of mussel bed and rockpool crabs may be partly due to the fact that a large percentage of the mussel bed backgrounds also consisted of areas similar to the substrates found in rockpools, and therefore they may have matched these areas equally as well as their own habitats

(Houston, Stevens, & Cuthill, 2007; Merilaita et al., 1999). in heterogeneous environments, pattern may become more important than colour which may be too variable to match. New research (Todd et al., 2015) indicates that pattern is in fact a central part of shore crab camouflage with patterned individuals being much harder to detect to the human eye.

Predation pressure from birds may also be lower in rockpool habitats if crabs are more protected in these places, as they have a lot more cover from visually hunting predators (Palma & Steneck, 2001; Todd et al., 2009). Furthermore, studies have shown that prey detection is more difficult in complex heterogeneous habitats (Bond & Kamil, 2006; Dimitrova & Merilaita, 2011), which may mean pressure to match the colour of complex environments such as rockpools may be lower than selection to match homogenous uniform habitat such as mudflats. More simply, it could be that it is much easier to adopt and develop a green-brown coloration to match areas of mudflat as the background is always the same colour and patterning, and matching rockpool areas is simply too difficult. It is also possible however, that rockpool crabs do not use background matching, but instead adopt coloration and patterns that are instead disruptive (Cuthill et al., 2005). This is supported by the fact that the markings are highly contrasting and touch the edge of their carapace, both of which are characteristic of disruptive coloration. Such markings function by breaking up the visual outline of the animal by creating false edges (Cuthill et al., 2005; Stevens & Cuthill, 2006; Webster et al., 2013). This was also reported by Todd et al., (2005) who observed that a large majority of patterned phenotypes possess white spots on the edge of the carapace which are areas often exposed when crabs peer out from under things in their habitat.

There is much more to learn from *C. maenas* in terms of phenotype adaptation and modification, especially in relation to behavioural adaptations. For example, there is scope to investigate whether crabs are actively choosing areas that match their phenotype to consequently improve their camouflage. This could be the case for individuals that show evidence of microhabitat selection in heterogeneous environments, such as juveniles from rockpools. Individuals from these environments could be given a choice between sediments and tested to see if they actively choose to be on backgrounds that match their phenotype. These types of experiments have been conducted with similar species such as the adult whiting *Merlangius merlangus*, which, when given the choice, choose sandy backgrounds which more closely match their colour (Atkinson et al,. 2004). Additional or alternative mechanisms could also be responsible for phenotype matches, for example colour change. Previous work indicates juvenile shore crabs have the ability to physically change their phenotype over time. This was most extensively studied by Powell (1962a, 1962b) who showed they may alter their brightness appearance through the use of chromatophores with most recent work

indicating they may significantly change luminance to improve camouflage over a period of 2 hours (Stevens et al., 2014). This subject will be investigated in chapter 2 of this study where the colour changing abilities of *C. maenas* are tested on black and white and red a green backgrounds over a period of several weeks.

In the future it would be worthwhile to analyse images in terms of dichromatic fish vision to assess whether this camouflage also translates across species. Furthermore, given that *Carcinus maenas* is also an invasive species (Breen et al., 2011; Rossong et al., 2006; Walton et al., 2002) it would be interesting to conduct work over larger scales, across continents, to compare phenotype-environment matches and the effects on camouflage.

Chapter 3: Colour change to improve background matching in the shore crab *Carcinus maenas*

Abstract

Avoiding being detected by predators is crucial for prey survival, and consequently many prey species have evolved to match visual aspects of their surroundings. How individuals achieve the match between their phenotype and their background is still not fully understood. One mechanism some animals employ is colour change, where the phenotype is modified to match the surroundings of the animal. This chapter investigated the colour change phenomenon in the shore crab *Carcinus maenas* under lab conditions. I conducted two types of experiment that differed in time scale: short vs. long term time periods, and I manipulated the colour of the background on which crabs were maintained (black or white and red or green backgrounds). I measured colour change using specialised digital photography methods after 0, 2, 4, and 48 hrs for short-term experiments, and once a week for 5 weeks for long term experiments. I found that crabs showed very little change in colour metrics over the course of short-term measurements, and this was true on any colour background. In contrast, in the longer term I found that crabs maintained on a white background increased their luminance significantly over time compared to those maintained on black black backgrounds. Similarly, crabs on red backgrounds increased in red hue over time, whereas those on green showed little change. I quantified these changes using visual modelling of predator perception to calculate Just Noticeable Differences (JNDs). Using this method I found that the changes in white and red improved the camouflage of crabs significantly, in terms of avian vision. The largest changes in both luminance and hue were observed between moult stages of the crab. This study demonstrates the abilities of the shore crab to alter its phenotype through colour change in order to improve levels of camouflage. I discuss the potential mechanism by which crabs may successfully match the conditions of their environment, and how this may be especially important for animals in heterogeneous environments.

Introduction

In order to remain hidden from visually hunting predators, many prey species have evolved an array of methods to avoid detection and attack. Many animals have evolved to match the colours and patterns of their habitat, known as background matching (Merilaita & Stevens, 2011; Sami Merilaita & Lind, 2005; Stevens & Merilaita, 2009). While the evidence for the ultimate benefit of background matching is extensive (Hemmi et al 2006), the degree of matching and survival benefit is known to

be dependent on not moving (Kang et al 2012,2014), and detectability increases for moving prey, especially in a habitat that is visually variable (Crook 1997).

Many cryptic species adopt behavioural mechanisms to improve their background matching (Kang et al 2012,2014). Some choose areas within their habitat that resemble their own colours (Atkinson et al., 2004; Sargent, 1966), whereas others reduce detectability by orientating themselves to align their own body patterns with those of the background (Kang et al., 2012; Webster et al., 2009). One of the more advanced behavioural methods of cryptic animals is the ability to manipulate the visual aspects of their phenotype by colour change (Stuart-Fox et al 2009, Stevens et al 2014a,c Umbers et al 2014).

Two types of colour change have been described in animals:physiological and morphological. In physiological colour change, the movement of pigment in chromatophores facilitates colour changes in a matter of seconds (Thurman, 1988b). Rapid colour changes is seen in chameleons (Ligon, 2014) and in cephalopods such as cuttlefish (Barbosa et al., 2008; Zylinski et al., 2011). The mechanisms of rapid physiological colour change are well studied, but less is known about the abilities of species that undergo slower morphological colour change (Stuart-Fox et al 2009)). Slower morphological colour changes are characterised by time periods of hours, days or weeks, and are caused by changes in the density of chromatophores (Powell 1962).

Morphological colour change has been documented in the kelp crab (*Pugettia product*). *Pugettia* sequesters pigment from the algae that it eats (Hultgren and Stachowicz 2010). Kelp crabs that live and feed in red algae change colour to appear red, and crabs that feed on orangekelp turn amber (Huktgren and Stachowicz 2010). This colour change enables individuals to subjectively blend into their surrounding backgrounds The fiddler crab changes colour by pigment changes in its chromatophores in response to light and temperature: becoming lighter in warmer or lighter conditions, and darker in colder ones with less light (Silbiger & Munguia, 2008). Detto et al., (2008) described changes in the colours of fiddler crabs as they go through moult stages: crabs with blue dots become whiter throughout successive moults, and can even turn yellow in larger males. The ghost crab *Ocypode ceratophthalmus* shows a 24 hr cycle of colour change, becoming significantly lighter towards midday and darker at night, which is proposed to help them to become better camouflaged against the light sand substrate where they live (Stevens et al., 2013).

Colour change for camouflage has been documented in the common shore crab *Carcinus maenas,* and this chapter focuses on the time frame this occurs within. It is evident that shore crabs are highly phenotypically variable especially in juvenile stages with individuals possessing a large

diversity in pattern and colour (Hogarth, 1978; Stevens et al., 2014). This is somewhat linked to the habitat in which they reside as reported in chapter 2 (see also Stevens et al., 2014; Todd et al., 2006). Similar to other colour changing species *Carcinus* possesses chromatophores that contain black, white and red pigments, visible through the dermis of juvenile crabs. This area has been most extensively studied by Powell (1962a, 1962b) who describes chromatophores having two kinds of responses: a primary response being the direct effect of light on chromatophores, and secondary response being the direct effects of the background on appearance. Powell tested crabs on black and white backgrounds and recorded that the pigment becomes more dispersed when crabs are on a black background so they may look dark, but concentrated when on a white background enabling them to appear light. This response depended on the level of light intensity that the crabs experienced, yet this was overridden by the secondary response i.e. the background conditions or substrate choice. Additionally, Powell's studies show shore crabs display a 24hr circadian rhythm of colour change, becoming darker within the day. Although these experiments are valuable, crabs were only tested on black and white backgrounds and there colour was not quantitatively measured.

More recent work has measured luminance changes of *Carcinus maenas* over a period of a few hours, with modern methods utilising digital photography that can then be mapped onto the relative predator vision (Stevens et al., 2014). Stevens et al found that shore crabs change their luminance in response to black and white backgrounds over a period of 1-2 hours, with brightness increasing on a white substrate and decreasing on a black. Stevens et al also tested crabs on red and green backgrounds, but only small changes were recorded in colour in this time period. It remains unclear as to whether shore crabs continue to change colour, if they are left for longer on particular backgrounds.

This project aimed to extend the time frame of previous experiments up to five weeks to see if any further colour changes occur in the shore crab. Given that previous work (Powel 1962) provided initial evidence that they could change colour over a period of 18 days, I proposed that there is potential for longer-term changes. Crabs were first tested in short-term experiments, with photographs being taken to record changes at 0, 2, 4 and 48 hours. A second longer-term experiment was then conducted over five weeks with new crabs photographed once every week. This longer time period was designed to enable individuals to go through the natural process of moulting, which has potential to further change their colorations.

Methods

Tank set up

All colour change experiments were conducted in four identical glass tanks, each measuring 90 x 45cm. Tanks were divided into 24 equal sections, approximately 11 x 15cm, using UV transmitting plastic (Penryn plastics UK). Plastic was held in place using aquarium safe silicone adhesive. Each piece of plastic contained a hole covered in fine netting, which allowed the circulation of water around the tank. Tanks were filled with de-chlorinated tap water mixed with instant ocean salt to imitate natural sea water (Aquarium Systems Instant Ocean Salt, Swell UK Ltd., UK). A refractometer was used to ensure the salinity of the water was held constant at 30ppt before being placed into the tanks (D&D's Refractometer, Swell UK Ltd., UK). Water was passed through a filtration system (Eheim classic 350 EHEIM GmBH & Co. KG, Deizisau, Germany) and cooler (D&D DC300 aquarium cooler 300w cooling power, Swell UK Ltd., UK), to keep the water both clean and at a constant temperature. The temperature was held constant at 14°C to match that of the sea at the time of crab collection. Two sections were not used to accommodate crabs, but instead housed the inputs and outputs of the filtration system to allow for maximum water flow through each section of the tank. An air stone fed by an airpump (Aquarline High Output Air Compressor, 2880 Litre/Hour) accompanied the filter output section, to allow as much oxygen to flow through the tank as possible. Three lights were suspended above each tank, two of which were daylight and one near UV (Grobeam600 Ultima and AquaBeam 600 Ultima MW, Tropical Marine Centre UK). To establish a constant light cycle, a timer controlled lighting so that they faded in at 0800 hrs and off at 2000hrs. A constant light cycle was important to establish because it has been shown that chromatophores in *Carcinus maenas* follow a circadian rhythm, becoming darker in the day time and blanching at night (Powell, 1962b). Backgrounds were printed onto waterproof paper (HP laser jet tough paper). To create a grey coloured background a grid of grey squares with differing reflectance was generated on the computer and printed onto the same waterproof paper as used in the experiment. This paper was then photographed in the darkroom alongside a grey standard meaning that the exact reflectance could be measured from the photograph. A grey was chosen for the experiments from this grid that had approximately 50% reflectance. A similar method was followed to create the green and red colours. A grid of reds and greens was printed and photographed. The reflectance of each was measured using the same photography method as previously stated. The red and the green with the most similar measures of reflectance were used. Ensuring both colours had similar reflectance was important as it meant any changes seen in crabs were caused by colour, not luminance. Background colours were divided so that each tank contained both colour types, ensuring that conditions in the tanks did not affect results.

Crab collection

All crabs were collected from Gyllyngvase beach in Falmouth, Cornwall UK (5°4'8"W, 50°8'31"N) at low tide. Crabs were immediately transported back to the lab in a grey bucket in order to minimise any potential colour change. Only juveniles with a carapace width of < 15mm were used because colour change is less likely to happen in larger crabs owing to the thickening of the cuticle with age (Crothers, 1968; Powell, 1962b). Short-term experiments were repeated twice. N=16 crabs were placed on black backgrounds, and n=19 on white. N= 18 crabs were tested on red backgrounds, and N=19 on green. In short term experiments, crabs were placed into prepared tanks with a grey background to acclimatise, and after being left on this colour for 24 hours the first set of photos were taken. After two initial hours on their respective colours, a second set of photos were taken, and then again after a further two hours. After this stage they were left in the tank for a further 24 hours and final set of images were taken before all crabs were returned to the sea. For long-term experiments new crabs were caught from Gyllyngvase beach. 22 crabs were collected for each of the four treatments (black, white, red, and green) totalling 88 crabs. All crabs were photographed immediately on entry to the lab and then placed onto a white, black, red or green background in a tank.

Photography methods

Images were captured using a Nikon D700 digital camera that had been adapted to enable UV photography (Advanced Camera Services, Norfolk, UK) and fitted with a Nikor 105mm lens. For each individual a series of photographs were taken, firstly in the human visible spectrum and then in UV. For human visible images a filter was placed in front of the lens that blocked UV and infrared (IR) light and only transmitted wavelengths between 400-700nm (Baader UV/IR Cut Filter). For UV images the filter was switched for one allowing UV but blocking IR and transmitting wavelengths between 300-400nm (Baader U Filter). Images were taken in RAW format with fixed aperture settings and manual white balance. Each crab was gently dried with paper towel before photography in order to minimise any areas of high reflectance that may affect results. A full spectrum arc Lamp provided the light source for all photographs (70W 1.0A power source; EYE Color Arc Lamp with Ventronic, Venture Lighting Europe Ltd. Hertfordshire, UK). A photographic umbrella was used to ensure that the light was even across the whole image (Neewer, Guangdong, China). For each photograph, the crab was placed next to a scale bar, as well as a colour standard made from two 10 x 10mm sections of Zenith diffuse sintered PTFE sheet, calibrated to 8.6% and 95.8% reflectance. This controlled for any changes in lighting conditions between images to allow one image to be compared to another.

Image analysis

Using the RGB histograms in the programme RawTherapee, I chose the optimally exposed photographs for human visible and UV visible images. Custom codes in Image J (Troscianko & Stevens, 2015) were used to create a multispectral image (mspec). This is a stack of images broken down to relative wavelengths: shortwave (SW, blue) mediumwave (MW, green), longwave (LW, red) and UV. Black (8.5% reflectance) and white (96% reflectance) standards were selected so that the images could be linearized with respect to radiance, and standardised to control for effects of light conditions (Stevens et al. 2007, Troscianko & Stevens 2015). Images were then manually checked to ensure they were aligned. This is important because any camera movement or refocusing between visible and UV shots may cause misalignment, and this causes false colours to be formed. Once the images had been converted and normalised, regions of interests (ROIs) were selected for measurement. Only the crab's carapace was selected. Areas of specular reflectance (where light simply bounces back off the carapace) were avoided so as to prevent any colour bias. Mspecs were saved alongside the ROIs

Key to this study is the need to analyse the colour change of the crab, as potential predators would perceive it. Shore crabs face many predators in a rockpool habitat, but one of their main threats come from shore birds (Crothers, 1966). Birds are tetrachromats and so have a vastly different visual system to that of trichromatic humans, with four cones types used in colour vision for LW, MW, SW and UV light (Cuthill 2006). Most shore birds have vision that falls into the violet sensitive (VS) visual system (Hart & Hunt, 2007; Odeen et al., 2010). Therefore multispectral images were mapped to peafowl vision (*Pravo cristatus*) as a model for VS birds (Hart, 2002a).

Once mapped to the particular visual capabilities of the predator, images were analysed and appropriate metrics of colour were calculated. Luminance was calculated using the double cones values, which is a measure of perceived lightness of an object as perceived by a certain visual system (Osorio & Vorobyev 2005). Luminance can inform us of how light or dark an object is, and was therefore used to explore changes in individuals on black and white backgrounds. For the red and green experiments, measures of colour, as opposed to luminance, were used. Saturation, described as the richness of a colour compared to white light (e.g. red versus pink) was calculated using LW, MW, SW and UV values to plot a point in tetrahedral colour space, with points further towards the edge of the space giving higher values of saturation (Endler & Mielke, 2005; Stevens, 2011). Hue, the colour type (e.g. red versus blue), was also calculated based on opponent colour channels. A principal component analysis (PCA) was performed to determine which colour channels to use. The resulting principal components indicated that PC1 explained 94% of the variance, and therefore this

was used to analyse hue. The resulting equation read LW+MW/SW+UV with higher numbers representing red colours and low numbers indicating green colours (Spottiswoode & Stevens, 2011; Stevens et al., 2014). To analyse how any potential colour change would affect the camouflage of the crab against the background just noticeable differences or JNDs were calculated. This was done using a log form of the Vorobyev-Osorio model, which is based on single cone avian photon catch values (Vorobyev & Osorio, 1998). A JND gives a value that indicates how discriminable two objects are from one another. In this case these two objects are the crab and background. Values between 1 and 3 mean the two are unlikely to be discriminated by a predator with increasing number indicating increasing camouflage (Siddiqi et al., 2004).A Weber fraction of 0.05 was used (Stevens et al., 2014), as well as the relative proportions of the different cone types in the retina of the peafowl *Pavo cristatus* (LW = 0.95, MW = 1, SW = 0.86, UV = 0.45) (Hart, 2002).

Statistical methods

I analysed the data for saturation and luminance JNDs (black and white experiment), and hue and colour JNDs (red and green experiment) using generalized linear mixed effects models. For this I included time (hours) and test background as fixed factors and crab identification (ID) and trial as random factors. I included all two way interactions in the initial model and used model simplification to test for significant interactions and fixed factors using a likelihood ratio test.

Results

Short term colour change

Black and white

To analyse changes in crabs on black and white backgrounds, luminance (defined as the amount of light as perceived by the predator) was calculated, with higher values indicating a higher luminance. Results (Figure 10) from the first two hours show an expected increase in luminance in those crabs on a white background and a decrease in those on black. This trend did not continue for the remainder of the experiment and a linear mixed model indicated that there were no significant changes in luminance over 48hours (χ^2_3= 5.53 p=0.14).

Luminance JND values were then calculated (Figure 11) and analysed to test how any changes affected the camouflaging abilities of crabs against their assigned backgrounds. No significant changes were seen here, (χ^2_3=3.32 p=0.35) yet it is clear that crabs on a black background have consistently much lower values indicating their phenotypes were originally and continually far better camouflaged on a dark background.
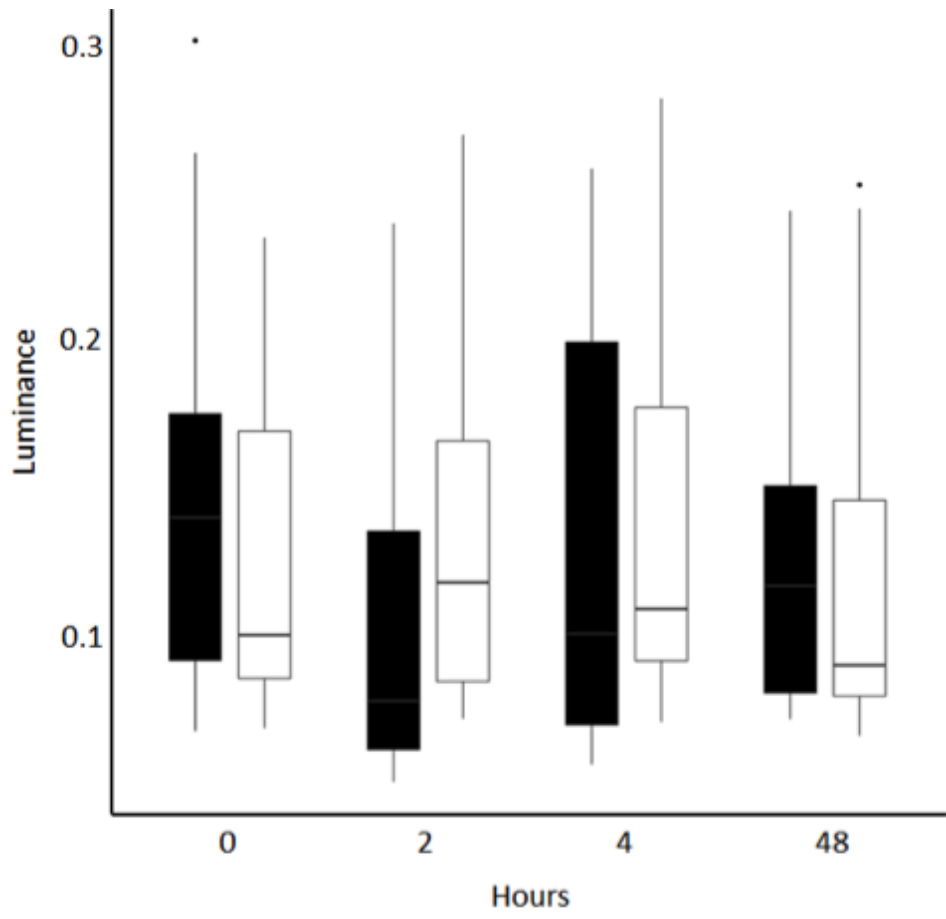
Figure 10 - Luminance (perceived brightness) values of crabs on black and white backgrounds after 0, 2, 4, and 48 hours. Plots show median JND at each time point with quartiles and ranges.
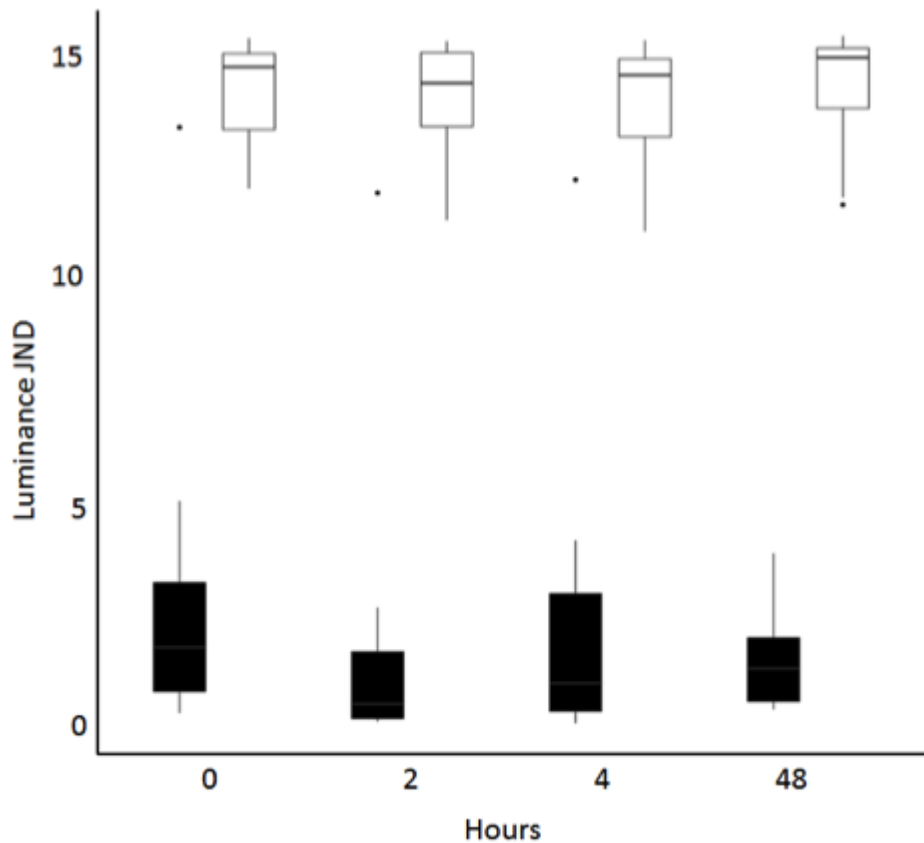
Figure 11 – JND values for crabs on black and white backgrounds show no changes over 0, 2, 4, and 48 hours. However, crabs on black backgrounds are much more camouflaged as a whole than those on white. Plots show median luminance at each time point with quartiles and ranges.

Red and green

The hue of crabs on red and green backgrounds (Figure 12) show no trend across the time points.. A linear mixed model indicated no significant difference in change in hue over the short term between backgrounds. Colour JNDS (Figure 13) were then analysed and unsurprisingly showed no significant difference over time (χ^2_3=4.88, p=0.18). However, it should be noted again that values were much lower on green backgrounds, indicating crabs naturally matched one of their backgrounds compared to the other.
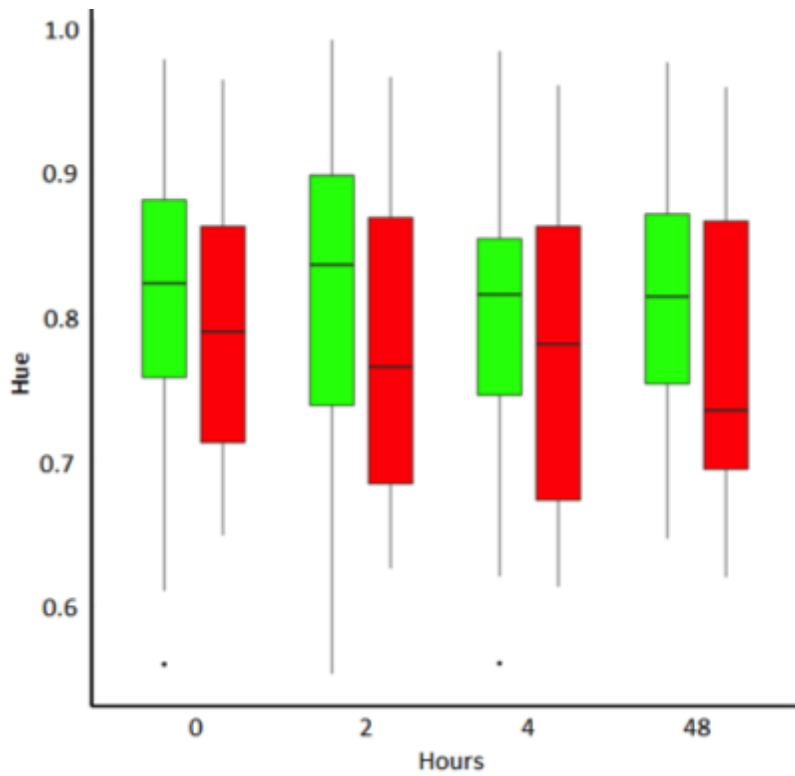
Figure 12- Hue values for crabs on red and green backgrounds at 0, 2, 4, and 48 hours show no changes over this period. Plots show median hue at each time point with quartiles and ranges.
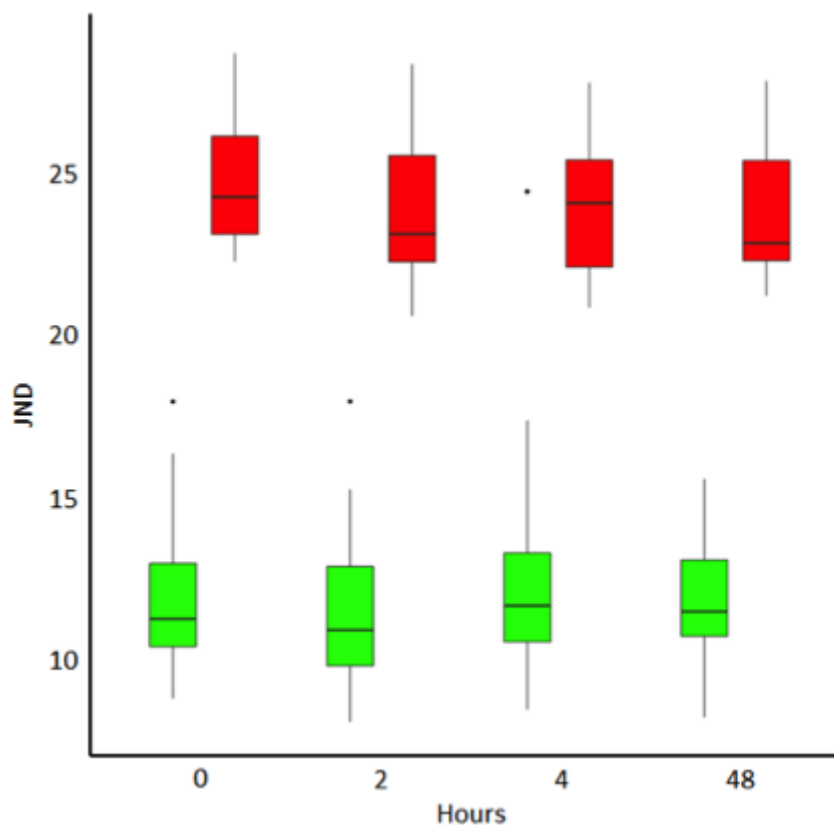

Figure 13- Colour JNDs show no changes over time at 0, 2, 4, or 48 hours, though it can be seen that crabs on green have better camouflage than those on red. Plots show median JND at each time point with quartiles and ranges.

Long term results

Experiment 3: Black and white

Luminance (brightness) was calculated for all crabs on black and white backgrounds.For crabs on white, luminance values showed an increase as those on black remained low (Figure 14) Linear models show the influence of background on crab luminance is dependent on whether or not the crab has moulted, and how long the crab is on the background. Crabs significantly increased their luminance when on a white background for longer (χ^2_8=96.0, p<0.00). It was evident through data collection that crabs went through the biggest changes after a a moult stage. Statistic indicatecrabs on white backgrounds which have moulted at that point in time have significantly greater luminance (χ^2_8=25.37 p<0.00). Figure 15 shows the difference in luminance between moulted and non-moulted crabs in the experiment with those that have moulted showing a much higher luminance value on white. Finally, luminance JNDs (Figure 16) were calculated and plotted again with those on black starting and remaining low and those on white decreasing over time to become less detectable on their background. Statistics show a significant difference in JND values between crabs on different backgrounds over time (χ^2_{16}=14.23, p<0.01) with crabs that have moulted having a significantly different JND value to those which haven't (χ^2_{16}=10.34, p=0.00). Changes over this time period are very much detectable to human vision, as demonstrated in Figure 16.
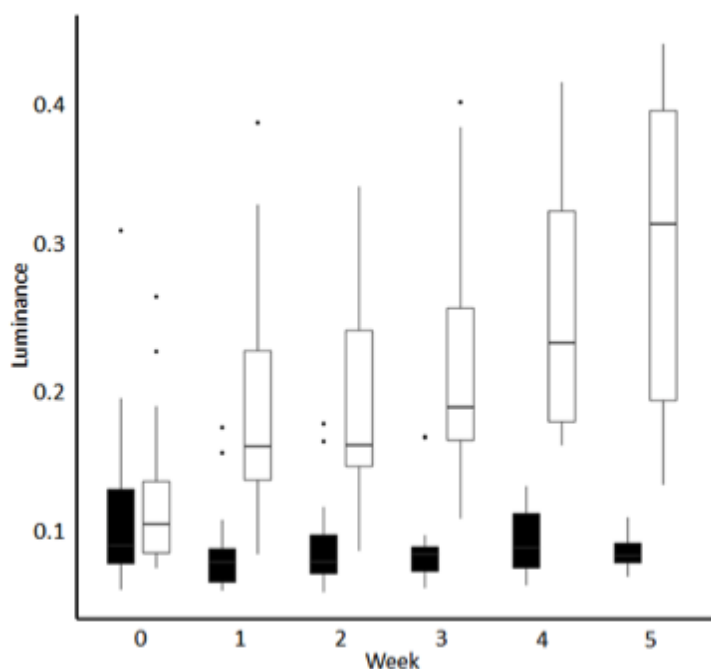

Figure 14 – Luminance (perceived brightness) in crabs on black and white backgrounds over a 5 week period. Plots show median luminance value at each time point with quartiles and ranges.
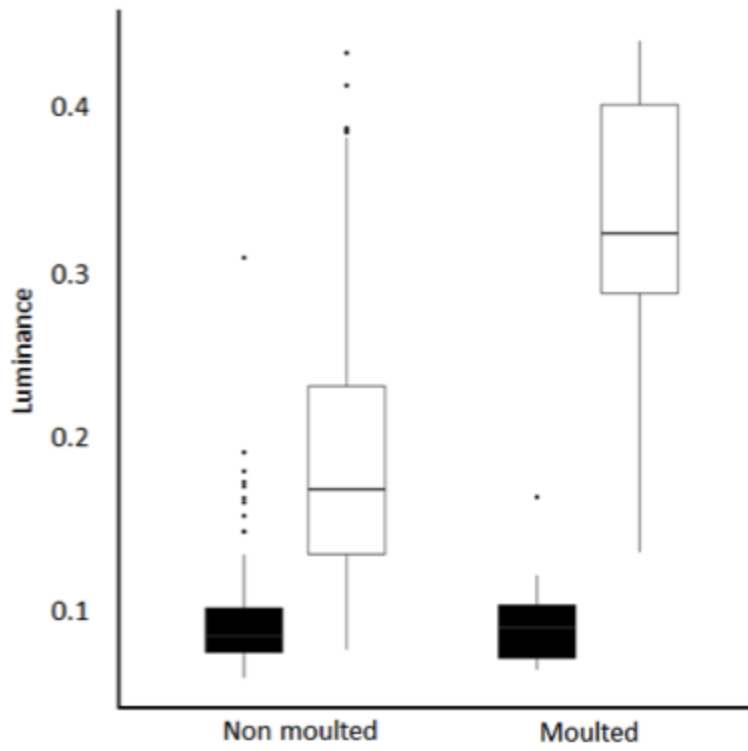
Figure 15 – Luminance values of crabs that did not moult in the experiment (left) compared to those that did (right) at experiment end point. Plots show median luminance value at each time point with quartiles and ranges.
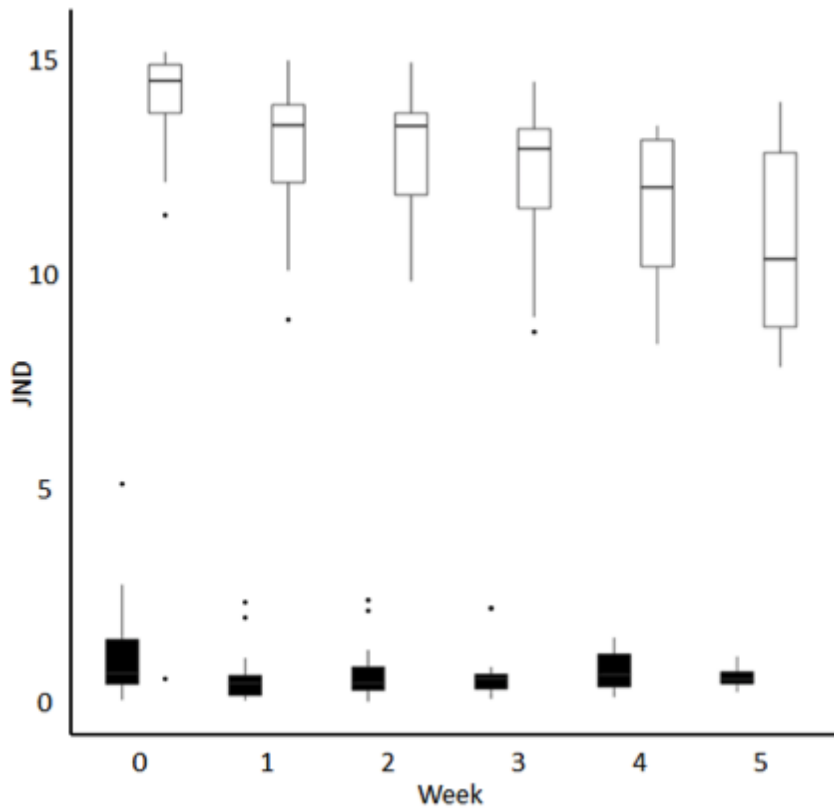
Figure 16 – Luminance JNDs of crabs on black and white backgrounds over a 5 week period. Plots show median JND at each time point with quartiles and ranges.
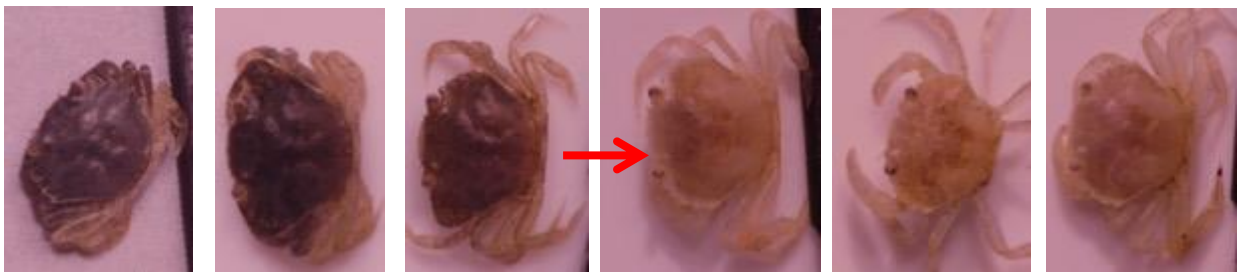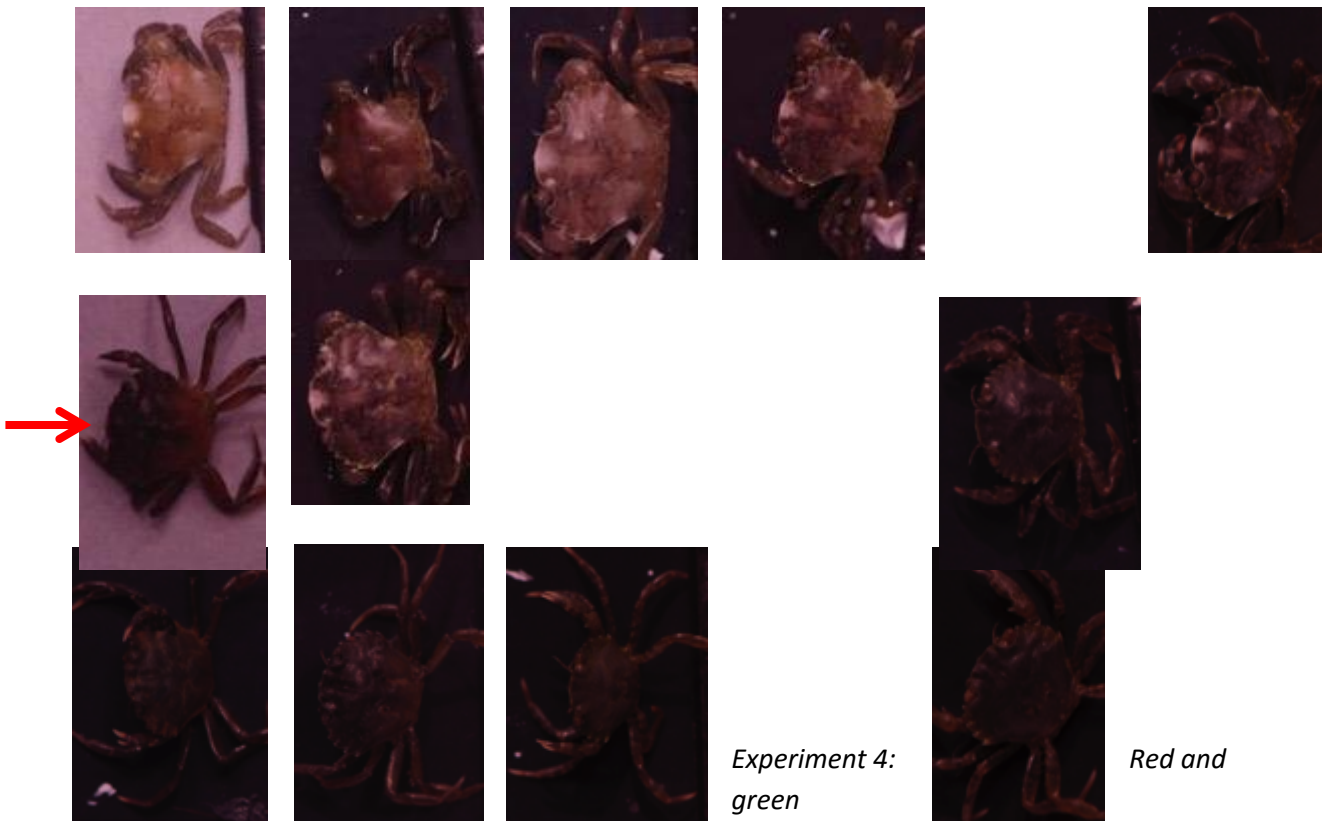

Figure 17 – Two different crabs top and bottom, both left on a white backgrounds for the long term experiment. Images show week 0 on the left, progressing through to week 5 on the right. The red arrow indicates when a crab had moulted.

Experiment 4:   Red and green

on black backgrounds for the long term ...ided evidence of colour change in crabs on red and green. Hue was used ... through to week 5 on the right. The red ...ge, which was calculated, as previously described with PCA analysis. ...e top did not moult.

Over time the hue of crabs on a red backgrounds increased significantly (χ^2_{16}=77.52, p<0.00) indicating an increase in redness, whereas those on a green background remained at a low level (Figure 19). Moulting played a role in colour change with crabs on a red background which moulted showing a significantly greater hue (χ^2_8=10.75, p=0.00). This can be seen further in Figure 20 and 22 with individuals that moulted in the experiment having a much higher hue value on red. Finally, JNDs were calculated and Figure 21 enables us to see red JNDs decreasing as their hue increases Statistics also showing over time crabs on red backgrounds had significantly lower JND values (χ^2_{16}=78.80, p<0.00).
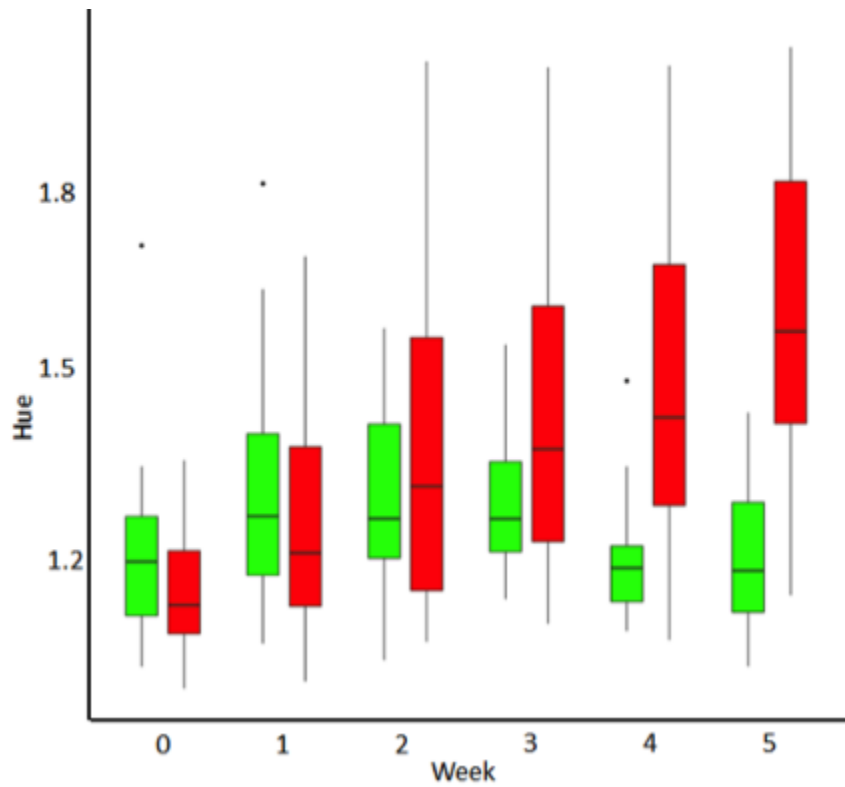
Figure 19 – Hue values for crabs on red and green backgrounds over a 5 week period. An increase in value indicates an increase in red hue, with lower values indicating a blue/green colour. Plots show median hue value at each time point with quartiles and ranges.
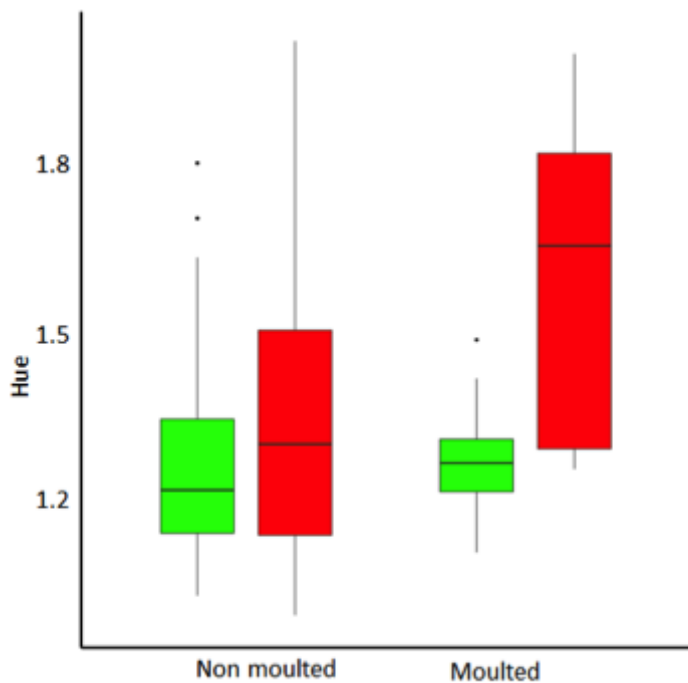

Figure 20 – Hue values of crabs that did (left) and did not moult (right). Increased values indicate a greater red hue. Plots show median hue value at each time point with quartiles and ranges.
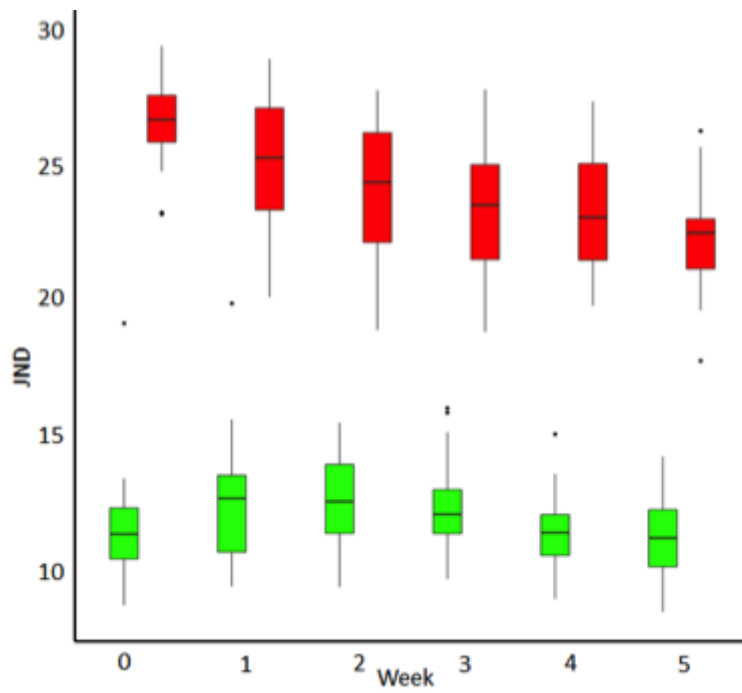
Figure 21 – Colour JNDs for crabs across a 5 week period indicate an increase in red hue improved camouflage, with crabs on green backgrounds remaining unchanged. Plots show median JND at each time point with quartiles and ranges.
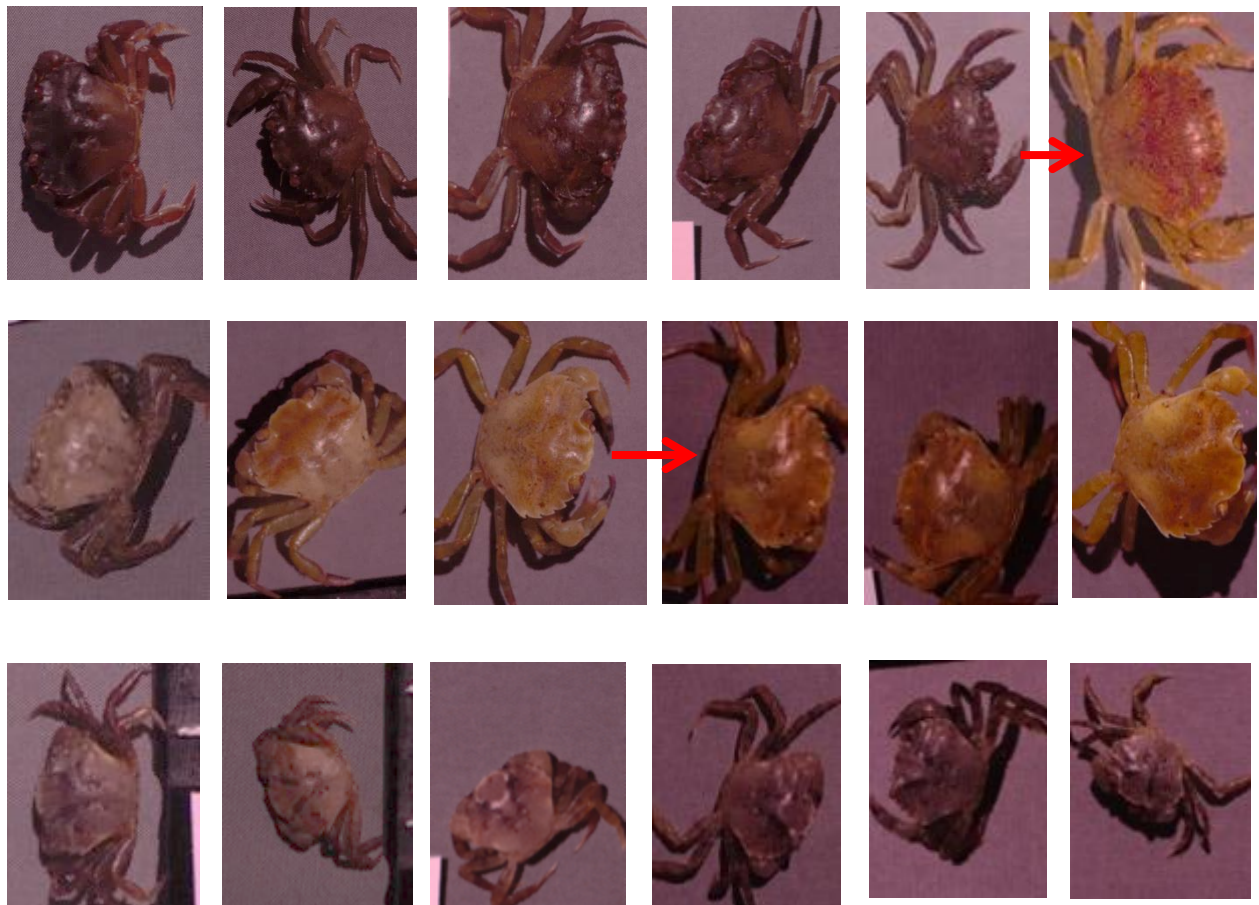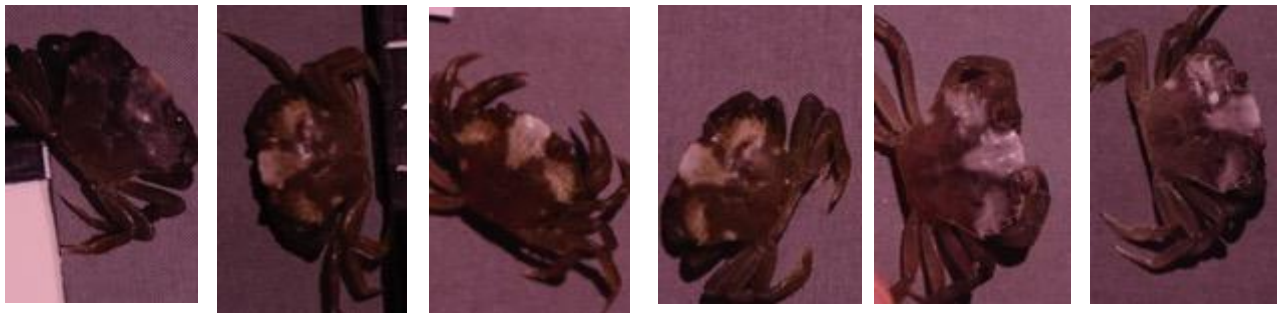
Figure 22 – Four different crabs from long term colour change experiment. Images show week 0 on the left, progressing through to week 5 on the right. The red arrow indicates when a crab had moulted. The crab at the top did not moult. The top two crabs were left on a red background, with the bottom two being left on green.

Discussion

We tested the short term and long term colour changing abilities of the shore crab *Carcinus maenas* using digital image analysis and a model of avian predator vision. Short-term experiments examined crabs over a period of 48 hrs on black and white, and red and green backgrounds. Although some initial changes were seen over the first two hours on black and white backgrounds, no significant changes were detected at any time point. Longer-term experiments carried out over a 5-week period showed more substantial changes, with crabs on white increasing their luminance far higher than those on black. Results indicated that this change in luminance improved crab camouflage in terms of avian vision. Long-term changes were also seen in terms of colour, with crabs tested on a red background becoming increasingly red over the course of the experiment that again, significantly improved their camouflage. Furthermore, the event of a crab moulting was a significant factor in these changes with those that moulted changing their appearance dramatically.

Given that changes have been seen in as little as 2 hours in other studies (Stevens et al., 2014b; Stevens et al., 2013) and there is evidence of *Carcinus maenas* having a 24 hr cycle of change as well as brightness in response to black and white backgrounds (Powell, 1962a), it was suprising there were minimal changes in luminance in the short term, This could be due to a number of reasons. Firstly, crabs in this experiment were, on average, bigger than those tested in Stevens et al., (2014b) as the maximum size threshold was increased to 15mm (from 12mm) primarily to improve survivability throughout the experiment, and prevent damage when moving in and out of tanks for photography. It would therefore be worthwhile to repeat the experiment with a range of sizes of equal numbers, so as to see its effects on luminance changes. Results in chapter two do suggest that size has an effect on luminance. Second, Stevens et al., (2014) used a paired design, with each crab being tested on each background, and it is possible that this more powerful design more readily picked up changes. If I were to repeat this experiment could be repeated, I would follow Stevens et al's design. Third, it could also simply be that the diversity in crab appearances masked any changes that happened in some individuals. Fourth, crabs for this experiment were used at different times of the year, with those in the previous paper being used in late spring and early summer, whereas crabs for short term experiments were caught and used in October. It is possible that, just as crabs change with circadian rhythm, they also have an annual cycle, and it would be worthwhile testing crab's colour changing abilities at different times of the year. Fifth, and finally, the crabs in my black background treatment, by chance, happened to have a median brightness that was greater than the crabs allocated to our white treatment, which will have reduced our power to detect change in the opposite direction.

Our long term experiments provided more of an insight into the camouflaging abilities of *Carcinus maenas* consistent with previous studies (Powell, 1962a; Stevens et al., 2014b) Luminance values for those on a white substrate increased dramatically in the first week with a steady increase continuing through each week of the experiment. Those on a black substrate did not show as much of a dramatic change in their phenotype, with the level of luminance initially decreasing but then remaining low without much change. This reflects the fact that those on a black background were already well camouflaged to their assigned substrates, as revealed by their JND values. A JND value below 3 indicates the object is indistinguishable from its background (using only the parameter under consideration) and therefore well camouflaged in terms of avian vision (Siddiqi et al., 2004). For those on a black background all values except one (5.17) were below 3, with the vast majority even being below 1, suggesting a big change may not have been seen in these crabs simply because there was no need for them to spend energy furthering their camouflage. Conversely, those on a white substrate stood out dramatically from their background with JNDs ranging from ~15-10 and so would have been very visible and likely vulnerable to avian predators, making it unsurprising that they altered their luminance so rapidly. It would be interesting to keep crabs for longer to assess whether this trend would continue and whether crab JNDs would drop below the threshold to become indistinguishable.

Past literature has noted changes in the shore crab regarding luminance (Powell, 1962; Stevens et al., 2014b). What is more novel and interesting here, are the changes seen in colour. Previous experimentation (Stevens et al., 2014b) failed to show a significant colour change in crabs in the short term. Long term experiments, allowing crabs to be within a particular coloured environment for a much longer period of time have allowed these changes to happen. Although changes may be seen from time point one, the hue levels of red and green crabs continued to diverge for at least 4 weeks, showing changes in hue are a lot slower than changes in luminance.

By far the biggest changes, both in terms of luminance and colour were seen when a crab goes through a moult. Moulting in shore crabs is asynchronous and is believed to be mediated by food availability and favourable environmental conditions such as light and temperature and controlled by hormones located in the eyestalk (Crothers, 1966). It is likely such large changes occurred associated with moulting as crabs were adapting the colour of the new cuticle below the current one. Studies into the physiology of this process in juveniles would be very interesting. Changes in colour through moults have been previously studied in adult males, which have been reported to be green when first moulted, turning to a red colour as they age through the intermoult stage

(Styrishave, Rewitz, & Andersen, 2004) It may also therefore be interesting for future studies to assess colour change in adults through moults.

Future work should also address the mechanisms by which this colour is detected, for example the eyestalks of crabs may be painted so as to test their responses to backgrounds without a visual aid such as in (Powell, 1962a). Similarly, the visual system of the crab should be further investigated in respect to this to assess what colours they can actually perceive as it is possible for an organism to camouflage itself even when having limited vision, for example it has been shown that the cuttlefish are colour blind yet cephalopods are evidently masters of camouflage (Barry et al., 2014; Mäthger et al., 2006).

This experiment lends itself to many other areas worth exploring. For example, all crabs used were from one section of rockpool habitat and, as mentioned in the previous chapter shore crabs may be found in a range of habitats, from mudflats to mussel beds. It is also known that crabs from these areas have significantly differing phenotypes so as to be camouflaged in their habitat (Stevens, Lown, & Wood, 2014b). It would be worthwhile to test the colour changing abilities of crabs from these different habitats as it may be that those from heterogeneous environments have a greater ability to adapt their phenotype than those from more uniform areas such as mudflats. To expand upon this, it may be that shore crabs from other geographical areas have different responses. For example, crabs from colder areas such as Scotland may respond less readily to changes as interest may lie in thermoregulation as Powel (1962a) also found that crabs kept at 6 degrees had their 24 hour rhythms overridden, and remained constantly black. It would also be worthwhile to assess if they show any behaviours to enhance their camouflage, such as choosing backgrounds appropriate to their phenotype.

This is by no means an adaptation that is unique to the shore crab. Similar morphological colour changes have been seen across crab species, such as in the ghost crab (Stevens et al., 2013) which has been shown to change its luminance in response to backgrounds. Other crab species may also change colour through the sequestering of pigment in its surroundings such as the kelp crab (Hultgren & Stachowicz, 2010). The dramatic changes here seen in moults are also not a unique adaption to the shore crab and the fiddler crab has also been shown to change colour through moults (Detto et al., 2008) with blue patterning turning whiter, and even to yellow and reds in adult males. Research in this area is beginning to reveal that these types of colour changes are quite frequent, especially in intertidal, rockpool habitats. This is likely due to these environments being highly heterogeneous, spatially and temporally, with a range of predators. Examples are seen in the goby which demonstrates background matching in minutes (Stevens et al., 2014) as do prawns such

as *Hepatacarpus pictus* and *H. paladicola* (Bauer, 1981). It is likely that similar adaptations are seen in other species which inhabit these areas, and future work could investigate this.

Overall this study provides valuable insights into the camouflaging abilities of species residing in challenging heterogeneous habitats. It demonstrates the colour change capabilities of the shore crab using the latest colour analysis methodologies in real species. Colour change is not a unique adaptation to *Carcinus maenas* and it is likely it exists in many other species not yet investigated, especially those in intertidal habitats or indeed any that are spatially or temporally heterogeneous.

Chapter 4: General Discussion

The chapters of this thesis explore phenotype-environment matching for camouflage, and the mechanisms by which this may be achieved using the common shore crab *Carcinus maenas*. In Chapter 2 I investigated phenotype-environment matching across crabs from mudflats, rockpools, and mussel beds. The appearances of individual crabs were compared to their own immediate backgrounds, backgrounds from the same habitat type, and then to foreign habitat types. I found that crabs from plain homogeneous mudflat habitats had high phenotypic matches to their own backgrounds, and were equally well matched to other areas of mudflat across all camouflage metrics (colour, luminance, and pattern). However, as predicted, their phenotypes did not afford them good camouflage in either rockpools or mussel beds. Results from heterogeneous rockpool and mussel bed habitats showed that these crabs had generalised or compromised crypsis (Houston, Stevens, & Cuthill, 2007; Merilaita et al., 1999). Previous work (Brian et al., 2006; Stevens et al., 2014; Todd, Briers et al., 2006) has noted differences in crab phenotypes across habitats, with crabs appearances being noticeably different in differing areas. But results from this experiment indicate these phenotype differences can provide a camouflage benefit primarily in homogenous environments.

What chapter two left unanswered, however, was the mechanisms that drive these camouflage matches. One explanation for this is that shore crabs may be changing their appearances to tune their phenotype to the conditions of the background where they live. This was investigated in Chapter 3. Both short (48hrs) and long term (5 weeks) experiments were conducted by leaving crabs on black, white, red, or green backgrounds and photographing them at regular intervals to track changes in luminance or colour. Results showed no changes over the short term. However, long-term experiments showed that crabs on a white background increased their luminance significantly over time, whereas those on black remain low. Red and green experiments show crabs significantly increasing their red hue over time, with those on green remaining unchanged. Most importantly all changes seen also significantly improved a crab's camouflage as a result. Therefore, it can be concluded that in juvenile shore crabs, phenotype-environment matches may be facilitated by colour and luminance changes.

Phenotypic differences across habitats

Phenotypic differences across habitats have been widely and frequently noted in the shore crab (Hogarth, 1978; Stevens et al. 2014; Todd et al., 2006; Todd et al., 2012) and Chapter 2 provides the first evidence that this allows crabs to be more camouflaged, in terms of background matching, amongst homogenous environments. Simple environments such as mudflats are easy to match,

likely due to their consistent uniformity of brown mud and brown macroalgae. These are also areas of little cover and crabs are relatively vulnerable in comparison to those in rockpools. This, in conjunction with the high levels of predation the shore crab endures (Crothers, 1968) means such strong environment matches could be driven by predation pressure.

In heterogeneous environments, background matching was more generalised. It is possible that shore crabs are adopting a different form of camouflage. Disruptive colouration, seen in many species, which describes a method by which contrasting patterning at the edge of the body act to break up the outline of an animal (Cuthill et al., 2005; Schaefer & Stobbe, 2006; Stevens & Cuthill, 2006; Webster et al., 2013). The appearance of many individuals in these areas appeared to fit this criteria, with Todd et al., (2005) observing that many possess patterning towards the front of their carapace. This type of camouflage may make more sense in these environments that are frequently changing and highly diverse as it relies less on colour matching.

Colour change for camouflage

Chapter 3 of this project demonstrated that the juvenile phenotype, in the long term, can be manipulated through phenotypic plasticity (Stevens et al 2014c). All crabs used for laboratory experiments were collected from Gyllyngvase beach, a rockpool habitat. Although no clear changes were seen in the short term (48hrs), long term experiments showed these crabs may alter their phenotype in accordance with their surroundings over weeks, which in turn improves camouflage. It is possible that the colour changes in this species occur to enable individuals to adapt to different habitat types in the long term. Shore crabs have low levels of genetic variability, likely due to their planktonic larval stage. Brian et al., (2006) showed that only 20% of phenotypic variability in shore crabs was associated with patterns of genetic similarity. Therefore, it may be that these adaptations are to allow phenotype-environment matchings to occur wherever a crab resides.

Short term colour change

Regarding the lack of colour change seen in the short term, this result could have arisen for a number of reasons. First, the crabs used for this experiment were caught and used in October, whereas those used in Stevens et al., (2014b) which found luminance changes in as little as 2 hours, were from spring time. It is possible that, as well as a daily circadian rhythm (Powell, 1962a), shore crabs also have an annual cycle, and it would therefore be worthwhile testing this possibility. In addition to this, crabs from these experiments were larger than those used by Stevens et al., (2014b) as the size threshold was moved from 12mm to 15, to improve survivability throughout the experiment and prevent any damage. It would therefore be valuable repeating experiments with

crabs in a range of sizes to see its effects on colour changing abilities in individuals. However, it is possible that changes were simply not detected due to the design of the experiment. Stevens et al., (2014b) used a paired design, by testing crabs on both background treatments, which is a more powerful system to pick up any possible changes. Future work should follow this previous design.

Long term colour change

In contrast the short-term findings, the most drastic changes in both luminance and colour in this study, also clearly visible to the human eye, is seen when a crab went through a moulting stage, replacing its entire cuticle. This has previously been noted in adult males, which are green when first moulted, turning red as they age through the intermoult stage (Styrishave et al., 2004). It is also in accordance with work on fiddler crabs (Detto, 2007) which show colourful spots on individuals change through moults, beginning with blue colours, fading to white and even yellow. Future work should further test this aspect. Moulting in crabs is entirely asynchronous, and mediated by factors such as food availabilies and temperature (Crothers, 1966). Therefore it would be interesting to repeat experiments at a range of temperatures, in order to test moulting causes.

Future directions

One of the biggest unanswered questions in this study is how crabs are sensing the colour of the background that they are on in order to adapt accordingly. To find out if this is done visually, the eyes of crabs could be painted in a manner that would render their visual capabilities redundant as in (Powell, 1962b). Experiments may then be repeated in the same manner and background responses tested. It is possible individuals may adapt to their surroundings, even without specific colour visual cues, which has been seen in the cuttlefish which, despite being colour blind, use responses to changes in brightness to achieve outstanding camouflage capabilities (Barry et al., 2014; Mäthger et al., 2006).

Regarding topic of Chapter 2, future studies should focus on determining the type of camouflage that is used by individuals, as it is possible that crabs in homogenous environments use background matching, whereas those in more complex environments could potentially use disruptive coloration. This thesis also only examines one possible behavioural mechanism by which animals may improve their phenotype-environment matches. Species have other mechanism by which they can improve this, for example they may choose backgrounds which more match their phenotype (Atkinson et al., 2004; Kettlewell, 1955; Sargent, 1966; Treatment, 1966) or orientate themselves in a way that improves the match (Kang et al., 2014; Webster et al., 2009). Experiments could investigate whether this is a behaviour also used by crabs by giving them a choice between substrates. It would be

interesting to also test this with crabs from different habitat types i.e. give crabs from mudflats a choice between mud or rocks and test if they choose the substrate from their habitat.

Considering the enormous range of predators *C. maenas* must avoid, it would also be worthwhile testing how they appear to other species. Fish are another major predator species of the shore crab, and many species have a dichromatic visual system such as the Pollack (Shand, et al., 1988), in comparison to the tetrachromacy seen in birds such as the peafowl (Hart, 2002b) and it would be interesting to test if phenotype-environment matches and colour change translated across other species. This is likely owing to the fact that matches and colour changes are also apparent to the human eye. However, fish also have different hunting strategies to many bird species, which can lift up and turn over rocks, meaning it is possible that it is more important for *C. maenas* to be camouflaged to a dichromatic eye. In addition, shore crabs do also attack and eat one another, and it would be worthwhile in the future to source and map crab vision to see if camouflage may protect them from one another.

<u>Conclusion</u>

This studies in this thesis highlights just some of the many routes species have gone down in order to avoid predation. Phenotype-environment matches are ubiquitous across the animal kingdom; with colour change being only one of many possible way individuals have to manipulate this. Unsurprisingly, it is frequently seen in rockpool animals such as crabs, but also in gobies (Bulletin, 2013; Stevens et al., 2014), and shrimp (Bauer, 1981). Given the frequent changes in this environment, both spatially and temporally, it is likely that adaptions such as colour change are seen in more species that dwell in intertidal areas. This is a habitat that should be explored more in terms of camouflage and phenotype environment relationships.

Overall, studies into phenotype-environment matches and the mechanisms by which they are achieved can help us to further understand the evolution of animals in complex, heterogeneous environments, and across habitats. The methods used in this study provide the most novel approach to date for assessing the camouflage abilities of species, importantly through the eyes of a predator. These methods have allowed for the first direct assessment of whether shore crab coloration functions to improve camouflage. This study also provides valuable insights into the rate of phenotype change in the shore crab. Future work should aim to assess the potential use of disruptive camouflage in the shore crabs in heterogeneous environments as well as investigating other ways in which they could achieve phenotype matching (i.e. background selection). It is likely that long term colour changes are far more frequent in the natural world, especially in complex

environments. Given that much previous literature mainly uses artificial prey, there is a strong need for similar studies to this, assessing camouflage in terms of predator visual systems with real animals in real situations.

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