Camouflage and Pattern Change in the Common Shore Crab *Carcinus maenas*

Volume 1 of 1

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Abstract

In nature, one of the most common and effective adaptions for reducing the threat of predation is to decrease the likelihood of detection through camouflage. The shore crab *(Carcinus maenas)* faces several predators in the wild and is also widely distributed across several habitats differing in substrate. Previous literature has recorded the diversity in shore crab pattern particularly amongst juveniles, linking the variation in pattern to differences in habitat substrate. A possible explanation for these phenotype - environment associations, is morphological pattern change. However, the plasticity of pattern variation in shore crabs has received little attention. Building on previous studies' findings, that the shore crab is capable of changing brightness, for the first time, this study assesses whether shore crabs are also capable of changing carapace pattern under experimental conditions, to improve camouflage over a period of 12 weeks on two artificial backgrounds. My findings show that indeed, shore crabs show plasticity in carapace pattern and brightness, and that, through the eyes of avian and fish vision, this resulted in improved camouflage for individuals on the uniform artificial background. Results for individuals on the patterned artificial background were less conclusive. The second part of this thesis focused on explaining the significance of this plasticity and phenotypic variation in the shore crabs natural habitats. The study quantified two strategies of camouflage; background matching and disruptive colouration. This was to establish differences in strategy between shore crabs from rockpool habitats and shore crabs from mudflat habitats. I further tested for differences between juveniles and adults amongst these habitats. My findings found clear differences between habitats. Using an avian predator model for vision, results for individuals from rockpool habitats highlighted significantly higher edge disruption than shore crabs from mudflats and conversely, shore crabs from mudflat habitats were found to have a significantly better match between carapace pattern and background pattern than rockpool crabs. In addition to this, our findings indicated differences between adults and juveniles. wwThese findings provide support for differences in camouflage strategy between habitats and suggest that the effectiveness of the strategy may change as crabs mature. Overall, the findings pro-

vide evidence for improved camouflage through pattern and luminance change in shore crabs under experimental conditions, and differences in camouflage strategy between crabs collected from homogeneous habitats and those from heterogeneous habitats.

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Chapter 1: Overall Introduction

Camouflage

Animal colouration is a prevailing aspect of camouflage whereby animals blend in with their surroundings, reducing visibility to predators (Carvalho et al., 2006). The strategies employed are varied but all have the same objective; to reduce the chances of detection or recognition from predators. These strategies can include animal body colours or patterns that resemble the general colour of the habitat, termed background matching and this is evident across a variety of species, such as moths (e.g. Kettlewell, 1955; Michalis et al., 2017), carnivores, artiodactyls, lagomorphs (Caro, 2005), African jerboas (Boratynski et al., 2014) and crabs (Todd et al., 2006; Krause - Nehring et al., 2010; Stevens et al., 2014). Examples include the arctic fox, the polar bear, and desert rodents, where it was found that individual rodents with paler coats are found on pale soils whilst those with darker coats are found on blackened beds (Caro, 2005; Belk & Smith, 1996).

One of the most researched forms of camouflage is crypsis, this term incorporates all traits that reduce an animal's risk of being detected, this includes features of physical appearance (e.g. colouration) but also behavioural traits, or both, to prevent detection. Crypsis is a highly effective anti-predator strategy, indeed some of the mechanisms involved in cryptic colouration (disruptive markings, background matching) have been adopted by the military and hunters (Stevens & Merilaita, 2009) as well as thousands of species in nature (countershading).

Disruptive Colouration and Countershading

Animals with a colour gradation of dark to light from the dorsum to the ventrum, likely benefit from a form of crypsis that counterbalances conspicuous shadows on an animal's body, causing the animal to be perceived as optically flat, making recognition by predators more difficult (Ruxton et al., 2004, Kiltie, 1988, Rowland et al., 2007). This mechanism is termed countershading (Thayer, 1896). An example of countershading providing a significant survival advan-

tage against avian predators can be found in caterpillars (Rowland et al., 2007) and isopods, where countershading is achieved by physiological colour change (Körner, 1982).

Reducing visibility to predators can also be achieved through disruptive colouration, typically, this involves bold contrasting adjacent colours, which next to each other, create a false edge on the animal's periphery (Cott 1940; Cuthill et al., 2005). If effective, this false edge across the body will appear more conspicuous than the real body edge (Espinosa & Cuthill, 2014; Cott, 1940; Cuthill et al., 2005; Merilaita, 1998; Stevens & Cuthill, 2006). False edges make it very difficult for predators to detect the prey animal, as shown by a study involving wild avian predators and artificial moth targets, whereby targets with disruptive markings had significantly higher survival rates than targets with background matching patterns (Cuthill et al., 2005).

Cuttlefish are masters of camouflage and research suggests that the diversity of patterns expressed by cuttlefish, enable them to achieve camouflage through disruption (Barbosa et al.,2008; Chiao et al., 2005). Some studies have focused on these components, particularly the white square (a neurophysiologically controlled component of the skin), to assess which features of the background are required to cause the body pattern to change (Barbosa et al., 2008; Kelman et al., 2007; Mathger et al., 2006, 2007). One of which found that disruptive patterns and colouration were stimulated when the experimental gravel background was equivalent in size to the 'white square' component of cuttlefish dorsal patterns (Chiao et al., 2005).

It has been suggested that disruptive colouration can hinder detection by predators, but it is currently still under debate as to whether background matching and disruptive colouration are separate mechanisms of camouflage or whether they are interdependent (Schaefer & Stobbe, 2006).In chapter three, I focus on how these two camouflage strategies differ in the mechanism of predatory avoidance, and how they are linked to the habitat type.

Colour Change

In a heterogeneous environment, the substrate background can vary temporally and seasonally. In these circumstances, cryptic colouration can prove invaluable and animals with an ability to change their phenotype (colour and pattern) to adapt to changes in their habitat, are at a particular advantage (Stevens, 2016; Stuart-fox & Moussalli, 2009; 2008; Caro et al., 2016; Duarte et al., 2017).

Reducing conspicuousness is one of the three main concepts, alongside communication and thermoregulation, proposed to explain changes in colour and pattern in animals (Stuart-fox & Moussalli, 2009). The ability to rapidly change colour has evolved across several animal lineages, vertebrates and invertebrates, terrestrial and marine (Stuart-fox & Moussalli, 2009; Duarte et al., 2017). Members of the Cephalopoda family are renowned for their ability to rapidly change colour, pattern, and texture and this has remarkable significance on their ability to conceal against structures within their habitats (Hanlon, 2007). Rapid colour change, over a period of seconds, minutes, or hours has also been recorded in crustaceans (Thurman, 1988; Stevens et al., 2014; Easley et al., 2015), reptiles - for example the chameleon, (Cooper & Greenberg, 1992; Stuart-Fox et al., 2006, 2008; Teyssier et al., 2015) and amphibians (Kindermann et al., 2014; Kindermann & Hero, 2016). Colour change can result from either morphological or physiological mechanisms (Umbers et al., 2014; Fingerman, 1970; Chapman, 1998). The mechanisms depend on the changes that occur regarding the chromatophore cells and result in a different speed of change. Morphological colour change results from changes in the number and proportion of chromatophore types and pigment content (Bagnara & Hadley, 1973; Bagnara & Matsumoto, 2006) and usually occurs over a course of days to months (Thurman, 1988). Morphological colour change can result in dramatic changes in appearance and moulting which occurs in many species - crabs and caterpillars are just a few examples (Stevens, 2016; Noor et al., 2008). Conversely, physiological colour change occurs as a consequence of dispersion or aggregation of pigments within the chromatophore cells and the outcome is much more rapid, with changes developing over a matter of seconds to hours (Thurman, 1988; Umbers et al., 2014; Bagnara & Hadley, 1973; Bagnara & Matsumoto, 2006).

Most colour change studies involve animals capable of rapid physiological colour change, the cuttlefish *Sepia officinalis* and the tropical flounder *Bothus ocellatus* (Ramachandran, 1996; Kelman et al., 2007; Briffa et al., 2008) are prime examples of this. The speed at which cuttlefish can change colour and pattern in their natural habitat, has enabled them to become model taxa in the study of camouflage (Hanlon et al., 2009). Conversely, much less attention has been given to animals responding to changes in their environment through morphological mechanisms and with slower changes (Stevens, 2016; Duarte et al., 2017). It has been suggested that spatial or temporal changes in an animal's background and therefore changes in the risk of predation, affects the speed of colour change. Indeed, temporal changes in an animal's background may select for gradual colour changes, whereas spatial heterogeneity may select for more rapid colour changes. Examples of slower, more gradual colour change, include the mountain hare, which changes its coat from brown to white in accordance with seasonal variability in its natural habitat (Flux, 1970).

Colour Change in Crabs

The study of predatory avoidance mechanisms in crabs is both varied and longstanding, including cryptic behaviours. For example, studies based on hermit crabs reveal that individuals are capable of making informed decisions over shell choice based on a range of environmental cues, including predation risk (Briffa et al., 2008). Decorator crabs and other species of crab have been recorded using masking material to camouflage themselves - in particular algae has been recorded on the carapaces of crabs from the Majoidea family. This family of crabs has over 900 species, 75% of which have specialised hooked setae to attach material from the environment to a part or all of their body (Ruxton & Stevens, 2015). Algae placed on the carapaces of crabs, not only reduces conspicuousness but also contains unpleasant substances that deter predators from attacking (Stachowicz & Hay, 1999; Hultgren & Stachowicz, 2011; Cruz-Riviera, 2001).

The majority of colour change studies in crabs have focused on the direct changes that occur within chromatophores and pigment dispersion when crabs are under differing lighting conditions and habitats. In 1937, Abramowitz

described the 24 hour circadian colour change in the fiddler crab, showing a pattern of pigment dispersion, resulting in darker colouration during the daytime and lighter pigmentation at night. In 1966, a similar rhythm was recorded in shore crabs *(Carcinus maenas)* - this 24 hour rhythm is prevented when constant exposure to illumination is provided and reversal of the normal 24 hour light dark cycle (Powell, 1966) results in reversal of the pigment dispersion, suggesting that the pigment dispersal is affected by the change in the brightness of the crab's surroundings.

More recently, studies have assessed the causality behind these colour changes as opposed to the mechanisms underpinning them, demonstrating the use of colour change as a response to the need to reduce conspicuousness. Stevens et al., (2013) used digital image analysis to quantify the colour changing abilities of juvenile horned ghost crabs *(Ocypode ceratophthalmus)*. Their results highlighted a circadian 24 hour rhythm, causing crabs to become darker at night and lighter during the day, a closer match to their beach habitat. Fiddler crabs also respond to increases in avian predatory threats, by changing carapace colour to reduce conspicuousness over a period of days (Hemmi et al., 2006). In addition, a study by Bedini in 2002 contradicted previous research that pointed towards patterns remaining stable at ecdysis in crustacea, by highlighting the extent of change seen in colour and pattern in juvenile *Carcinus meanas*. In particular, the change seen between the final moult and adulthood where he referred to habitat as the main factor for this change seen.

Several experimental studies have investigated this colour changing ability of shore crabs in an attempt to understand how this may reduce conspicuousness in the wild by enabling crabs to match their backgrounds. Early work by Powell (1962), tested the response of chromatophores to changes in light and background in immature adult crabs. His findings reported three broad types of chromatophore with red, white, and black pigments and he quantified the degree of concentration of each pigments for crabs on white and black backgrounds. He discovered that on a black background, black pigment disperses and white pigment becomes concentrated, with the opposite occurring on a white background. These findings revealed that chromatophores can directly respond to either light or the nature of the background (e.g. brightness). Stevens et al., (2014) and Easley et al., (2015) carried out similar investigations

using more advanced methods of avian vision to establish the change through the vision of one of the shore crabs main predators. Their findings confirmed the ability of shore crabs to significantly change brightness in relation to a black or white background. Furthermore, the authors suggested that this would lead to significant improvements in camouflage. However, to my knowledge, the ability to change carapace pattern to match experimental backgrounds, has not been investigated in shore crabs previously. In chapter two, I use both fish and avian predator visual systems to assess the ability of shore crabs to change carapace pattern over 12 weeks, to better match experimental backgrounds differing in pattern. We us image analysis techniques to quantify the match between carapace and background, as a measure of camouflage.

The experimental studies in the literature, have provided a fascinating insight into one of the possible mechanisms behind shore crabs achieving camouflage in the wild; phenotypic plasticity. However, for many years, field studies have also attempted to directly quantify the diversity observed in shore crab pattern and colour in their natural environments. Indeed, earlier studies analysed the link between this variation and differences between habitat sites, finding direct correlations between maturity, habitat, and carapace colour and pattern (Hogarth, 1978; Powell, 1962; Crothers, 1967). Todd et al., (2006) built on these findings, using distinct categories for crab phenotypes, he found a negative association between carapace pattern cover and the the algal cover of the habitat. His work later found evidence for an association at both the micro (<1m2), meso (100 s m2) and macro (10,000 s m2) scale. A more recent analysis using predator vision models, found support for these findings. The results suggested that there is less diversity in shore crab appearance in homogeneous habitats in comparison to the large amount of variation from heterogeneous habitats (Stevens et al., 2014). However, although these studies suggested a camouflage purpose to this variation, until recently, very few attempts were made to actively quantify the difference in the camouflage of shore crabs differing in pattern and colour between habitats. Using a model of an avian predator's vision, Easley et al., (2015) used image analysis software to quantify how well crabs from different habitats matched their phenotype to their own background and different habitat backgrounds, in terms of luminance and pattern, through a specific form of camouflage; phenotype - environment matching. Our third chapter builds upon this using the most advanced image analysis technology and avian pred-

atory vision, to investigate the possibility that this variation in shore crab pattern is linked to differences between two camouflage strategies; disruptive colouration and phenotype - environment matching.

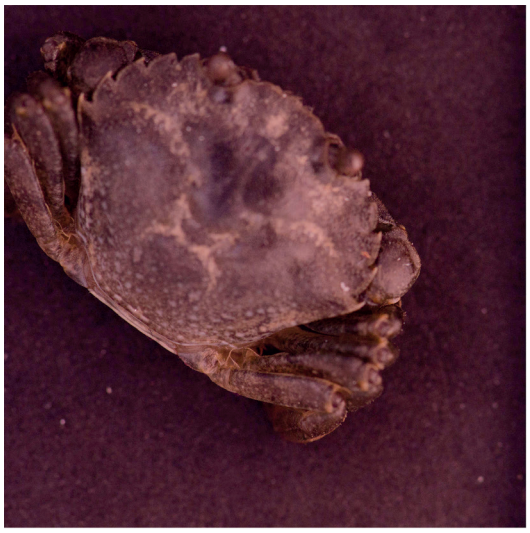
Purpose of this Thesis

Research on pattern change for camouflage is very limited, with the majority of studies focusing on animals that are capable of rapid colour and pattern change, over a period of seconds to hours, such as cephalopods and chameleons (Allen et al., 2015; Stuart-Fox et al., 2006, 2008). Very few studies have focused on slower pattern change for camouflage, over a period of weeks. In addition, despite the emergence of evidence for disruptive colouration as an effective strategy for camouflage (Schaefer & Stobbe, 2006; Stevens & Merilaita, 2008; Kang et al., 2015), there has been no research to date, quantifying both phenotype - environment matching and disruptive colouration camouflage strategies within a species living amongst different habitat types.

This project will focus on answering two very important and unresolved questions within the broader field of camouflage and the more specific, phenotypic plasticity. Our model species, the shore crab, is a very common intertidal species, found amongst a wide range of habitats in the UK, Europe, and other parts of the world (Crothers, 1966, 1968). Subjectively, individuals have camouflaged carapace patterns and are faced with many predators (Todd et al., 2006) and have also been reported changing colour (Powell, 1962; Stevens et al., 2014), this makes them an ideal species to study camouflage and phenotypic plasticity.

In chapter two of this thesis, I investigate whether or not shore crabs are capable of changing carapace pattern over time, in relation to matching artificial backgrounds. In addition, in chapter three, I use field techniques to assess whether or not differences in camouflage strategy exist between shore crab phenotypes and the dependence of this on the habitat substrate.

Chapter 2: Pattern and Brightness Change of Shore Crabs in Response to Artificial Backgrounds



Pre-Tank Entry

First Moult

Six weeks

Second Moult

Abstract

Camouflage is a form of anti-predator defence, which can be achieved through many mechanisms and has been studied across several taxa. One route to successful camouflage is phenotypic plasticity, the ability of an animal to change its appearance in response to its environment. Changing colour and pattern to reduce conspicuousness and avoid predation may be particularly beneficial to species that live in a heterogeneous habitat with temporal and spatial changes in substrate, or animals that can thrive in more than one type of habitat. The common shore crab *(Carcinus maenas)* is found in temperate intertidal habitats ranging from mudflats to rockpools and mussel beds, and is known for its variability in phenotypic appearance, particularly amongst juveniles. Previous work has shown that individuals can change their brightness over a short period on black and white artificial backgrounds. However, this work only studied the change in brightness up to five weeks, did not quantify changes in carapace pattern, and only used the visual system of one of the shore crab's many predators.

Here, for the first time, I extend this period to 12 weeks, allowing crabs to moult up to three times within this period. I quantify changes in carapace pattern in addition to brightness, in response to two artificial backgrounds (patterned and uniform), using individuals collected from two different natural habitats; mudflats and rockpools. I used digital image analysis and models of bird and fish predator vision to investigate whether shore crabs can change their pattern as well as their luminance (perceived lightness), and whether this change is affected by their surroundings. I found that, shore crabs subjected to the uniform treatment did significantly change their carapace pattern and luminance over time, and that these changes resulted in a closer match to the pattern and luminance of the treatment background. No significant change was found for shore crabs subjected to the patterned treatment. Shore crabs collected from mudflat habitats significantly changed pattern on the patterned treatment, however shore crabs from rockpools and subjected to the same treatment, did not significantly change pattern. I also found that the more moults an individual underwent over the experimental period, resulted in a larger change in pattern and luminance recording. These pattern and luminance changes specific to the

uniform background may suggest that changes in pattern over time are also influenced by ontogenetic changes in juveniles, that are not necessarily affected by the environment.

Introduction

Predation constitutes one of the main threats to many wild animals. This is evidenced by the effort invested by animals to reduce the chances of attack, which can be seen across a variety of mechanisms. Camouflage, a classic example of natural selection (Stevens & Merilaita, 2011), reduces the threat of attack by reducing conspicuousness. The majority of cases report animals becoming camouflaged through associations between the phenotype and the surrounding environment (referred to as phenotype - environment associations), which involves animals matching their surroundings by resembling the colour and pattern of markings found in their environment (Stevens & Merilaita, 2011). Various moths have evolved permanent colours and patterns that resemble the bark in their surroundings (Kettlewell, 1955; Kang et al., 2012, 2014). Pelagic and littoral habitat types also lead to phenotypic colour differences in populations of Eurasian perch (Kaekalainen et al., 2009). Further examples of phenotype - environment associations for camouflage include African desert jerboas (Boratynski et al., 2014), invertebrates such as the isopod *Idotea baltica* (Merilaita, 2001), and crustaceans including the sand flea (Stevens et al., 2015) and several species of crab (Todd et al., 2006; Stevens et al., 2013; Stevens et al., 2014; Detto et al., 2008; Easley et al., 2015).

Phenotypic diversification can result from either genetic differentiation or phenotypic plasticity (Levins, 1968; West-Eberhard, 1989; Orr & Smith, 1998; Langerhans et al., 2003). Phenotypic plasticity is defined as the ability of an organism to change its phenotype in response to changes in its environment (Price et al., 2003). These changes may or may not be permanent and can occur over different timescales, from seconds and minutes to hours or weeks (Stevens, 2016). Examples of phenotypic plasticity exist across both plants and animals, and rapid colour change has been studied across a variety of taxa including cephalopods (Hanlon, 2007; Barbosa et al., 2007, 2008), amphibians (Garcia & Sih, 2003; Kinderman et al., 2014), reptiles (Cooper & Greenberg,

1992; Stuart-Fox & Moussalli, 2008), and crustaceans (Thurman, 1988; Stuart-Fox, 2009; Detto et al., 2008; Stevens et al., 2014). However, although many animals have demonstrated colour changing tendencies, several are unable to adapt their phenotypic colouration and pattern to environmental changes.

The ability to change colour over comparatively short time scales (seconds, minutes, days, or weeks) in response to environmental changes may provide a significant advantage in spatially or temporally heterogeneous environments (Caro et al., 2016; Duarte et al., 2017). For example, Idotea crustaceans show transitory colour change and pattern polymorphism as a result of the threat from predatory fish (Wallerstein & Brusca 1982). The different colour phenotypes display an extraordinary adaptation to heterogeneous littoral environments (Salemaa & Ranta, 1991).Although some recent studies have investigated the influence of the animal's environment and substrate surroundings on colour and pattern variation across species (Gamble & Keeble, 1900; Rosenblum, 2006; Todd et al., 2006; Stevens et al., 2014, 2015; Hultgren & Mittelstaedt, 2015; Jensen & Egnotovich, 2015), few studies have experimentally tested the flexibility of this phenotypic plasticity by quantifying this change in response to matching artificial backgrounds. Indeed, even fewer have quantified the camouflage benefit of colour and pattern change, especially over longer time periods (Duarte et al., 2017).

Crabs have become one of the most widely used species for studying colour change and camouflage. In particular, studies have focused on fiddler crabs (*Uca)* (Thurman, 1990), highlighting the 24 hour circadian rhythm mechanism shown across several species and the changes in dispersion or concentration of chromatophore cells (Thurman, 1990; Ranga Rao et al., 1967). More recently, studies have established that ontogenetic changes alongside stress, courtship displays, and background matching, are mainly responsible for the changes in the colour seen (Detto et al., 2008; Brown & Sandeen, 1948). For example, a study of juvenile horned ghost crabs *(Ocypode ceratophthalmus)* used digital image analysis to quantify colour and brightness changes (Stevens et al., 2013) for background matching purposes, revealing that ghost crabs become lighter when placed on white backgrounds and darker on black backgrounds. Similar results have been found in fiddler crabs (*Uca)* (Rao et al., 1967).

Another recent study compared appearances of crabs found on sargassum mats, with findings showing that crab appearance depended on the substrate (Russel & Dierssen, 2015). Building on the foundation of Powell's (1962) work, a study recently assessed the colour changing ability of the shore crab, modelling and quantifying these changes using the visual system of an avian predator. Results indicated that shore crabs are capable of significantly changing their brightness to better match their backgrounds, becoming lighter or darker over a period of hours on a black or white background (Stevens et al., 2014). Further work has shown that crabs substantially change brightness over a period of weeks (unpublished experiments) in response to background brightness. Shore crabs are likely capable of changing colour within the same moult and between moults (Stevens, 2016; Todd et al., 2006), making them an ideal study species for phenotypic colour and pattern changes and the associated role in concealment from predators.

To blend in, an animal must resemble the pattern, not just the colour and brightness of its surroundings. Indeed, studies have shown that pattern plays a vital role in the ability of an animal to completely match its surroundings and reduce conspicuousness to predators. Marine invertebrates such as the cuttlefish are capable of camouflaging themselves against almost any background, altering their body pattern to match the pattern, colour intensity, and even texture of their surroundings, to achieve camouflage in seconds (Hanlon, 2007). This ability has become well established amongst cephalopods, but likely also occurs more widely; for example, a recent study assessed pattern change in rock gobies *(Gobius paganellus)* and found that individuals changed their pattern in response to changes in artificial backgrounds (Smithers, 2015). These studies focussed on rapid pattern change with responses to background changes occurring over seconds or minutes. However, very few if any studies have focused on progressive phenotypic changes, occurring over weeks in response to the pattern of the background. Juvenile shore crabs provide an ideal species for studying long term pattern changes in response to artificial backgrounds due to the variation in pattern displayed amongst them, the high frequency of moults they undergo before adulthood, and their resilience as one of the most invasive and widespread animals (Darling et al., 2008). *C.maenas* is highly invasive due to it's ability to tolerate a wide range of salinities and temperatures and to live in

all types of marine and estuarine habitats, including habitats with mud, sand, or rock substrates, submerged aquatic vegetation, and emergent marsh (Cohen et al., 1995). In comparison, other species of crab are not as widely distributed, for example furrowed crabs (Xantho hydrophilus) tend to be restricted to low tide line, and velvet swimmers (Necora puber) tend to be middle to lower tide. Most intertidal crab species occur mainly in rocky shore and are much less common in mudflat habitats. Conversely, shore crabs are common in every habitat. In addition, subjectively, crabs differ in appearance with age and it has been suggested that crab patterns partially or fully disappear as juveniles become adults (Hogarth, 1978, Todd et al., 2006, Crothers, 1968, Bedini, 2002). A more recent study (Stevens et al., 2014) quantified these differences, finding that larger adult crabs tend to be darker, have less saturated colours and less contrasting carapace patterns than smaller juveniles. They also found that as crabs move towards adulthood (growing larger) they become more grey, moving towards an achromatic points and interestingly the spread of variation in appearance decreases as crabs get larger. A very recent paper found further support for pattern differences between adults and juveniles, showing that juvenile crabs had significantly more diverse markings but adults had larger markings (Nokelainen et al., 2017).This provides strong evidence to show that juvenile shore crabs exhibit more variation and more contrasting patterns than larger adult crabs. This may be linked to changes in the visual diversity of the background in which individuals live (Stevens et al., 2014), but also ontogenetic changes without regard to the environment as crabs age/grow (Todd et al., 2012).

In this chapter, I tested the ability of the European green shore crab *(Carcinus maenas)* to change carapace pattern over a period of 12 weeks in response to remaining on either a patterned or uniform achromatic background. Crabs from two habitat types, across seven populations, were used to assess whether the treatment background, habitat type, and experimental time affected pattern change and camouflage between individuals and the background. I selected crabs from rockpool and mudflat habitats. Crabs from these two habitats differ with regards to how variable they are visually, subjectively crabs from homogeneous mudflats appear to have less diversity in carapace pattern than crabs from heterogeneous rockpools . By doing this, we were able to investigate potential effects of originating visual habitat type on pattern change.This is important because, due to the close proximity of rockpools and mudflat, and

the ability of shore crabs to move between 0.5–2 km in a short space of time (Ameyaw-Akumfi & Naylor, 1987), It is probable that shore crabs move between the two habitats, resulting in the necessity for plasticity and the ability to change colour and pattern to match changes in environmental backgound. Most species are preyed upon by more than one predator species, which are likely to differ in foraging styles, perceptual and learning abilities. To account for this, digital image analysis using both avian and aquatic models of predator vision were used to quantify the changes in body pattern.

Methods

Field Sites

Crabs were sampled from two habitat types with very different substrates: rockpool and mudflat. Three mudflat and four rockpool sites were selected to represent the two habitats. All sites are based in the Southwest of the UK, in the county of Cornwall, and they were chosen due to their varied distribution, covering North and South coasts of the county, see figure 2.1. The closest sites were Gyllyngvase rockpool and Penryn mudflats, roughly 6km apart, however these distances ranged up to 50km between hayle mudflats and Perranuthnoe rockpools. This distribution was important as it covered sites differing in substrate composition as well as sites that were close to each other and far apart. Gyllyngvase beach (50° 8’ 39.42” N, -5° 4’ 5.244” W) and Maenporth beach (50° 7’ 33.876” N, -5° 5’ 39.555” W) were chosen as rockpool sites in Falmouth. Kennack Sands (50° 0’ 23.695” N, -5° 9’ 28.258” W) was chosen as a rockpool site located further down the Southwest coast, and Perranuthnoe rockpools (50° 6’ 43.383” N, -5° 26’ 28.142” W) were selected on the South coast. Fewer mudflat sites were available or accessible on the North and West coasts and so on the South, Penryn (50° 9’ 49.335” N, -5° 5’ 2.124” W) and Helford (50° 5’ 23.1” N, -5° 9’ 58.754” W) mudflat sites were chosen, and on the North coast crabs were collected from Hayle (50° 11’ 36.979” N, -5° 25’ 47.973” W) mudflats. Rockpool sites varied in rock and sand composition, Gyllyngvase and Maenporth rockpools provided very similar habitat types, with large clusters of rocks, forming

deep gullies filled with gravel to the top of the shore, with few expanses of sand, see figure 2.1A. Conversely, Perranuthnoe and Kennack Sands consisted of much smaller stretches with shallower rockpools, and substrate composed of sand rather than gravel, see figure 2.1B. Mudflats provide contrasting habitats to rockpools, at low tide, the area consists of large expanses of dark brown mud with little above surface shelter other than dispersed rocks or objects, see figures 2.1C and 2.1D.

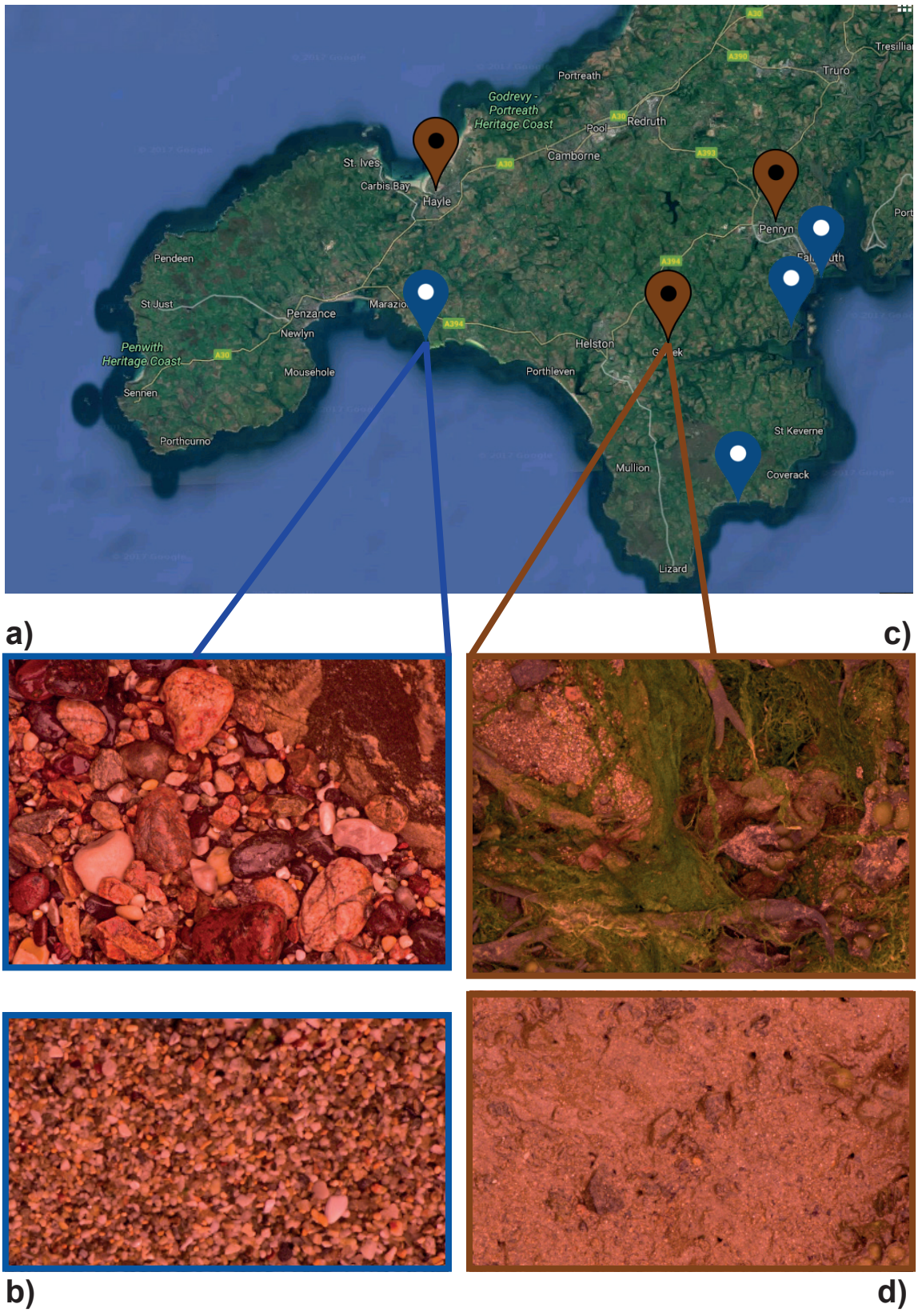


Figure 2.1: A map to show all seven sites visited for crab collection. Blue markers indicate rockpool habitat, and examples of the substrate found there can be seen from figure 2.1 A) and B). Brown markers indicate mudflat habitat, and examples of the substrate found there can be seen from figure 2.1 C) and D).

Method of Crab Collection

Shore crabs were identified by their carapace shape, the distinct five spines either side of the eyes with three spines in between the eyes and the lack of swimming paddles (Crothers, 1968). At each site, collection of crabs was indiscriminate, the area was scanned thoroughly, with all zones included at low tide and any crab found was measured using a 15 cm ruler regardless of carapace colour or pattern. Measurements were taken from the fifth pointed spine at the widest part of the carapace to the same point on the other side. Individuals measuring less than 15 mm were classified as juveniles and were included in the study. Any individuals measuring more than 15mm were put back as they were classified as adults and only juveniles were to be used in the study. This classification was based on the suggestion that crabs larger than 15mm are less likely to change carapace colouration and pattern due to a thickening of the cuticle with age (Crothers, 1968; Powell, 1962). Twenty crabs from each of the seven sites (140 crabs in total) were collected. These individuals were then transported back to the laboratory in clear tanks containing salt water from the sea and enough background substrate from the site to cover the bottom of the tank and provide refuge, to reduce stress during transportation.

Photography Pre-Tank Entry

Back at the laboratory, individuals were removed from the tank, gently dried and their carapace width was measured again, to ascertain that they were juveniles. The crab was then placed underneath a tripod set up in a dark photography room. The crab was placed on a spectrally flat sheet of black 2mm thick craft foam (Ethylene-vinyl acetate) with a reflective cylinder surrounding the individual. A black and white reflectance standard was placed by the side of the crab with an identification number. The standard was made from 10 X 10mm sections of zenith diffuse sintered PTFE sheet (Labsphere, Congleton, UK), and was calibrated to reflect 8.2% and 94.8% of all wavelengths respectively, with a scale bar alongside the PTFE to enable pattern measurements to be made. Including a standard in every image allows changes in lighting conditions to be controlled for (Stevens et al., 2007; Troscianko & Stevens., 2015).

A series of images were taken in human visible light and then immediately afterward in ultraviolet light, after being refocused. The images were taken with a digital Nikon D7000 camera, which had undergone a quartz conversion to allow for UV sensitivity (Advanced Camera Services, Norfolk, UK). A filter (Baader UV/IR Cut filer) was placed in front of a Nikorr 105mm Nikon lens that blocked UV and infrared light and only transmitted wavelengths between 400-700nm, this was to capture human visible images. For capturing UV images, a different filter (Baader Venus U filter) was placed in front of the lens, allowing UV transmission between 300-400nm and blocking infrared and human visible light through. Photographs were taken in RAW format with fixed aperture settings. Several photos were taken of the same subject at a range of exposures to avoid over exposure resulting in images, which then cannot be used in analyses.

Tank Preparation

Pattern change experiments were conducted in four glass tanks, each 90 x 45cm with an identical set up. Each tank was divided into 24 equal sized sections using UV transmitting plastic (Penryn plastics UK), each 11 x 15cm and held in place by aquarium safe silicon adhesive. Water circulated through the tank via holes between compartments, the holes were covered with netting to prevent movement of individuals between compartments. Tanks were filled with dechlorinated tap water mixed with instant ocean salt (Aquarium Systems Instant Ocean Salt, Swell UK Ltd., UK) to imitate natural sea water. A refractometer (D&D's Refractometer, Swell UK Ltd., UK) was then used to test the salt water, ensuring salt content was at 30ppt before filling the tank. The tank water was kept clean and at a constant temperature by passing through a filtration systems (Eheim classic 350 EHEIM GmBH & Co. KG, Deizisau, Germany) and cooler (D&D DC300 aquarium cooler 300w cooling power, Swell UK Ltd., UK). Cooler temperature was set to 15 degrees celcius, this matched the temperature of the seawater from the first collection. Two of the compartments in opposite corners were used for the input and output of the filtration system, allowing for water to flow efficiently through the tank. The output filter compartment also housed an air stone, fed by an airpump (Aquarline High Output Air Compres-

sor, 2880 Litre/Hour), to ensure oxygen flow through the chambers was at its optimum level. Above the tanks, three lights were suspended, two were daylight spectrum and one was near UV (Grobeam600 Ultima and AquaBeam 600 Ultima MW, Tropical Marine Centre UK). It was important to create a constant light cycle for the tanks as the laboratory had no source of natural light and studies have shown that chromatophores in *Carcinus maenas* follow a circadian rhythm, becoming darker in the day and blanching at night (Powell, 1962). To enable this circadian rhythm to continue, the lights were controlled by a timer and so they faded in at 08.00 am and faded off at 20.00 pm.

For the experimental treatments, compartments were filled with either patterned or uniformly grey gravel, to represent a patterned and unpatterned treatment. The patterned gravel included black, white, and grey gravel pieces, from a mixed bag (Unipac Grigio silver mix 3-6 mm) and was 3-6 mm in size. Uniform grey gravel was from the same manufacturer and consisted of purely grey gravel (Unipac Lunar silver 3-6 mm) of the same size; see figure 2.2. The same volume of gravel was used for both patterned and uniform treatments, ensuring that all conditions were the same for each compartment, other than the colour of the gravel. Crabs often spend time buried beneath their substrates and so backgrounds were created to cover the clear plastic underneath the gravel. To ensure the paper backgrounds were as similar as possible to the gravel treatments, photographs were taken of the patterned and uniform gravel composition in the dark room, using the same set up as above and this was then printed onto waterproof paper (HP laser jet tough paper), glued and stuck down in each compartment, covering the rectangular base and approximately 2 cm up from the base, on all four compartment walls. Once all images and measurements had been taken, the individual was randomly assigned one of the compartments numbered (1-20) and was then placed in the tank, the starting size of the crab and the treatment of the compartment was recorded. Photographs of each individual were taken every three weeks for 12 consecutive weeks, see figure 2.3. Crabs were fed once a day with TetraCrusta complete food pellets. One pellet was dropped into each tank compartment, housing one crab, twice a day, in the morning and afternoon. High standards of animal welfare were maintained through regular cleaning of the tanks themselves and the filters. Crabs were checked on twice a day and tanks were cleaned once a week, using a suction tube to remove excess food, clean the gravel and the sides of the compartent.

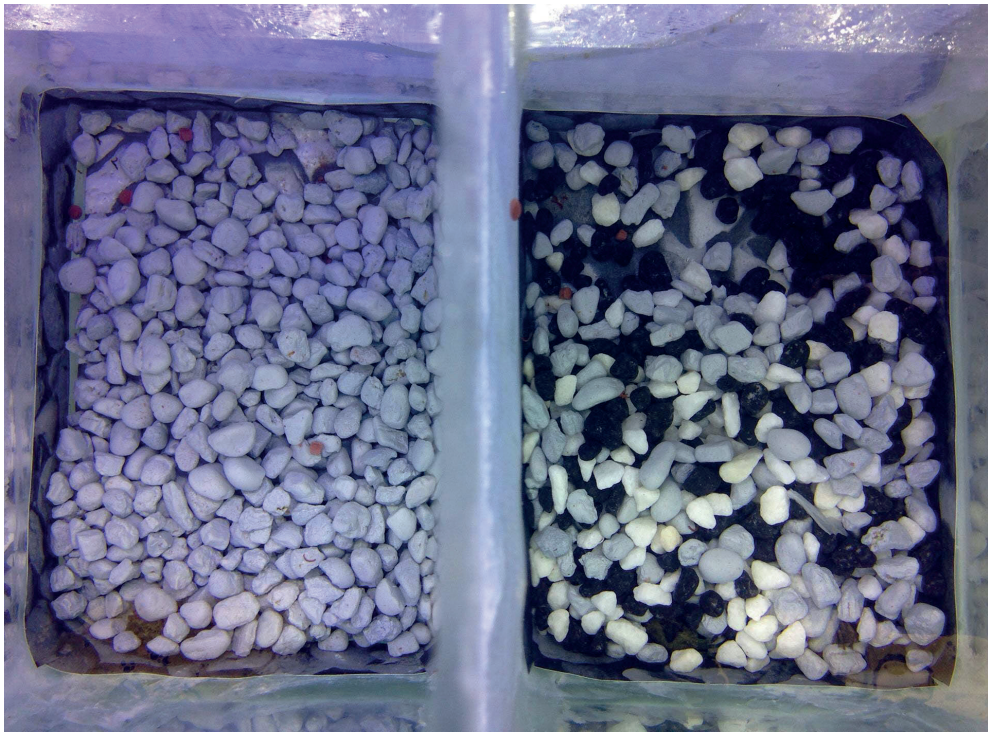


Figure 2.2: Image of the tank set up with uniform and patterned treatment backgrounds. All compartment conditions were identical except for the gravel treatment.

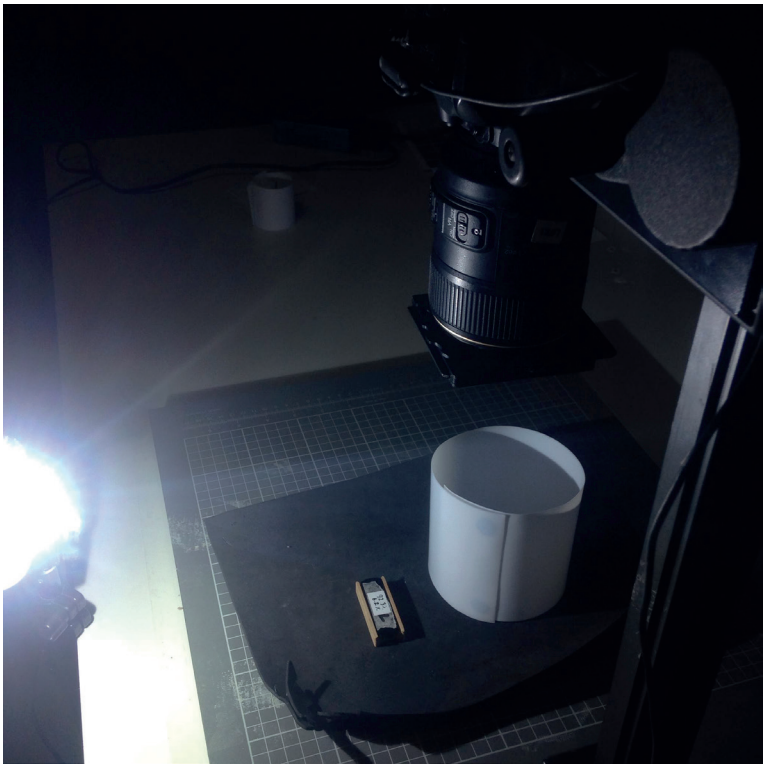


Figure 2.3: Image of the photography stand setup used to photograph crabs over the experimental period. Photographs were taken every three weeks.

Image Analysis

The programme RawTherapee was used to view the RGB histograms of all images taken. One human-visible and one UV image with optimal exposure was then selected for each individual at each timescale. Multispectral images were then created using custom codes from the 'multispectral image calibration and analysis toolbox' in the program Image J (Troscianko & Stevens, 2015). During this process, UV and visible images are manually aligned to form one image, which is then split resulting in a stack of images of relative wavelengths: shortwave (SW), mediumwave (MW), longwave (LW) and UV. Alignment is vital to ensure that any movement captured between filters is rectified without false colour being formed. During production of multispectral images, the white and grey standards were selected to allow images to be linearised with respect to radiance and standardised to control for effects of light conditions (Stevens et al., 2007, Troscianko & Stevens, 2015). Once these images had been normalised, regions of interest (ROIs) were then selected for measurement. For the purpose of this study, the crab's carapace, excluding legs and pincers were selected as ROI's. To allow the measurement and comparison of carapace patterns between images, a 30mm scale bar was attached to the standard and included in every image. Selecting the scale bar size when creating each multi spectral image (mspec), allowed pattern measurements to be accurately scaled and measured.

The main purpose of this study is to analyse changes in pattern in relation to substrate background, with the hypothesis that crabs will change over time to match their background, reducing conspicuousness to predators. It is therefore essential to consider how any changes would be perceived by potential predators. One of the main predators of shore crabs is shore birds. Most birds are potential tetrachromats, as opposed to humans, which are trichromats (Cuthill, 2006). Avian colour vision is constructed from four cone types, which are sensitive to LW, MW, SW, and UV light (Cuthill, 2006). The peafowl is one of many birds that fall into a violet sensitive visual system, with reduced ultraviolet sensitivity (Hart & Hunt, 2007; Odeen et al., 2010). As most shore birds also fall into this category, the peafowl *(Pravo cristatus)* was chosen as a model system of shore crab arial predators. The peafowl model has previously been analysed

and used as a model for shore crab predation (Hart, 2002). Shore crabs are often submerged in rock pools, and whilst this may provide more protection from birds, they are often exposed to marine intertidal fish species. To model the dichromatic visual system of a predatory fish, the SW and LW cones of the pollack *(Pollachius pollachius)* was used (Shand et al., 1988).

Image Processing

Images were analysed in terms of luminance (perceived brightness) and pattern changes, and the effects that these changes have in terms of camouflage with the treatment background. Both bird (peafowl) and fish (pollack) visual systems were used to allow for detection of changes in carapace pattern and luminance through the vision of two very different shore crab predators. The images were mapped to these visual systems using the 'Batch Multispectral Analysis Tool' (Troscianko & Stevens, 2015). The tool uses the spectral sensitivity data of the respective predators (Hart, 2002) under the D65 standard irradiance spectrum. D65 is a standard illuminant part of the CIE (international commission on illumination) D series of illuminants, which portrays standard illumination conditions at open air, in different parts of the world (Janos, 2007). The tool then converts from camera sensitivities to the predator's (bird or fish) colour space using a polynomial mapping technique, which generates animal photoreceptor cone catch values from the camera's photoreceptor values (Stevens et al., 2007; Troscianko & Stevens, 2015).

To quantify the change in carapace pattern of shore crabs over the experiment, the size and 'energy' of pattern markings were measured. This was carried out using a granularity analysis, a method which has previously been used to analyse patterns in other animals, including cuttlefish (Barbosa et al., 2008; Chiao et al., 2009), bird eggs (Stoddard & Stevens, 2010), and shore crab markings (Stevens et al., 2014). In a granularity analysis, each image is filtered using Fast Fourier bandpass filtering, at multiple spatial frequency scales, followed by quantifying 'energy' at different spatial scales. The energy at each of these scales is measured as the sum of the squared pixel values (Chiao et al., 2009; Stoddard & Stevens, 2010), with larger markings of low spatial frequency captured by smaller filter sizes, and larger filter sizes capturing information

concerning smaller markings of higher spatial frequency. The resulting granularity spectrum and the descriptive summary statistics outputted from this can be used to assess the relative change in pattern and the contribution of different marking sizes to the overall body. Specifically, we used the total pattern energy and the maximum frequency (the spatial frequency with the highest energy, corresponding to dominant marking size) outputted from the granularity analysis, to assess the overall carapace pattern of individuals and the dominant pattern markings. The total pattern energy is calculated as the sum of the squared pixel values in each image divided by the number of pixels in the image, with the actual scale being arbitrary (Chiao et al., 2009). To assess whether there was a significant change in the carapace pattern of individuals over the experiment, we used the carapace pattern energy of individuals at the start and end of the experiment. Using this data, we carried out further analyses, using the 'pairwise pattern difference calculator ' in imageJ to test for a significant difference in pattern energy over the experiment.

For luminance analyses, double cone values were used for avian predators, since these are widely thought to underlie achromatic vision in birds (Osorio & Vorobyev, 2005). The average carapace luminance value for individuals was used for comparison of change over time and in relation to the treatment background.

To analyse images in terms of specific camouflage, just noticeable differences (JNDs) were used for luminance analyses. These values are generated by a model that calculates predicted units of discrimination between two objects, and can therefore be used as a measure of how well camouflaged an object is against a background. A modified version of the Vorobyev-Osorio model, based on that used by Siddiqi et al., (2004) which makes comparisons based on luminance differences obtained from double cones (Osorio & Vorobyev, 2005), was used. For pattern analyses, pattern energy difference (PED) values were used, these were generated by a custom made difference calculator in imageJ (Troscianko & Stevens, 2015), which works out the absolute difference between the spectra of two images/objects, across the spatial scales measured (Troscianko & Stevens, 2015; Troscianko et al., 2016). Any two patterns with similar amounts of energy across the spatial scales will produce low pattern difference values.

Predictions

Shore crabs are capable of changing carapace luminance to better match their experimental background (Stevens et al., 2014; Stevens, 2016). Based on those findings, I predict that shore crabs will also show plasticity in carapace pattern, showing a larger increase in total pattern energy and size when on a patterned treatment background than when kept on a uniform treatment background. I also predict that these changes will result in a reduction in conspicuousness, due to an increase in similarity between the treatment background and carapace pattern and luminance.

Statistical Analyses

Analyses of luminance and pattern change against either patterned or uniform treatment backgrounds, were conducted using a generalized linear mixed model (GLMM) . Treatment background, habitat type, time, and their 2- and 3-way interactions were all included in the model as fixed effects. Collection site was included as a random effect. Model simplification was used to identify the final model with only significant main effects and interactions. Where normality was not met, log transformations were used on variables or alternatively non-parametric tests were used to avoid violation of test assumptions. All analyses were conducted in the statistical program R.

Results

Pattern Analyses

Change in carapace pattern over time

To assess whether, on average, the total carapace pattern energy of individuals changed over the experiment and whether this change was affected by the treatment background and the collection habitat of individuals, a generalized linear mixed model (GLMM) analysis was performed. Due to data being non normal, crossed random effects and a slightly unbalanced design, a penalized Quasi-Likelihood (PQL) method was used for the GLMM model.

The results found that the time point (start or end) at which the measurement of carapace total pattern energy was taken, had a significant effect on the value (GLMM, $F_{1,208}$= 19.464, p = <0.05), indicating that there was a significant increase in carapace pattern of individuals from the start to the end of the experiment . Using model simplification, the significance of the main effects (treatment background, habitat type, and time) and the interaction terms were established. Non significant interactions were removed to select the model with the best fit. The results from this final model (see table 2.1) indicated that the affect of the treatment background, on the change in carapace pattern, was also dependent on the habitat that individuals originated from (collected from - rockpools or mudflats). Further to the GLMM models, two separate Wilcoxon ranked sum tests were performed for each treatment, finding that individuals on the uniform treatment significantly increased their carapace pattern on average (Wilcoxon: V = 251, p< 0.001; figure 2.4a) and individuals from the patterned treatment did not (Wilcoxon: V = 572 , p = 0.14; figure 2.4b).

The model also found a significant interaction with the habitat crabs originated from (GLMM, $F_{1,207}$= 5.14, p = <0.05), indicating that shore crabs collected from mudflat habitats, subjected to the uniform treatment, had significantly different carapace pattern energy to crabs collected from rock pools and subjected to the same treatment. Indeed, the largest increase in pattern came from mud-

flat individuals subjected to a uniform background. This may be due to substantial variation in the starting total energy of rock pool individuals on the patterned treatment, as seen in figure 2.5a., which is much larger than the variation for mudflat individuals and whereby rock pool crabs are often highly patterned to begin with (see figure 2.5B.). When I ran this GLMM model using pollack vision, significant results were found for the same terms - habitat and time, and the interaction term - treatment : habitat.

	D.F	F	p
Treatment	210	0.01	0.19
Habitat	209	11.06	< 0.05
Time	208	19.46	< 0.05
Treatment: Habitat	207	5.14	0.038

Table 2.1: Results from the final GLMM model analysis for peafowl vision. Showing the significant effect of time and habitat type on the total pattern energy of crab carapaces and the significant interaction effect of treatment background and habitat on the carapace total pattern energy.

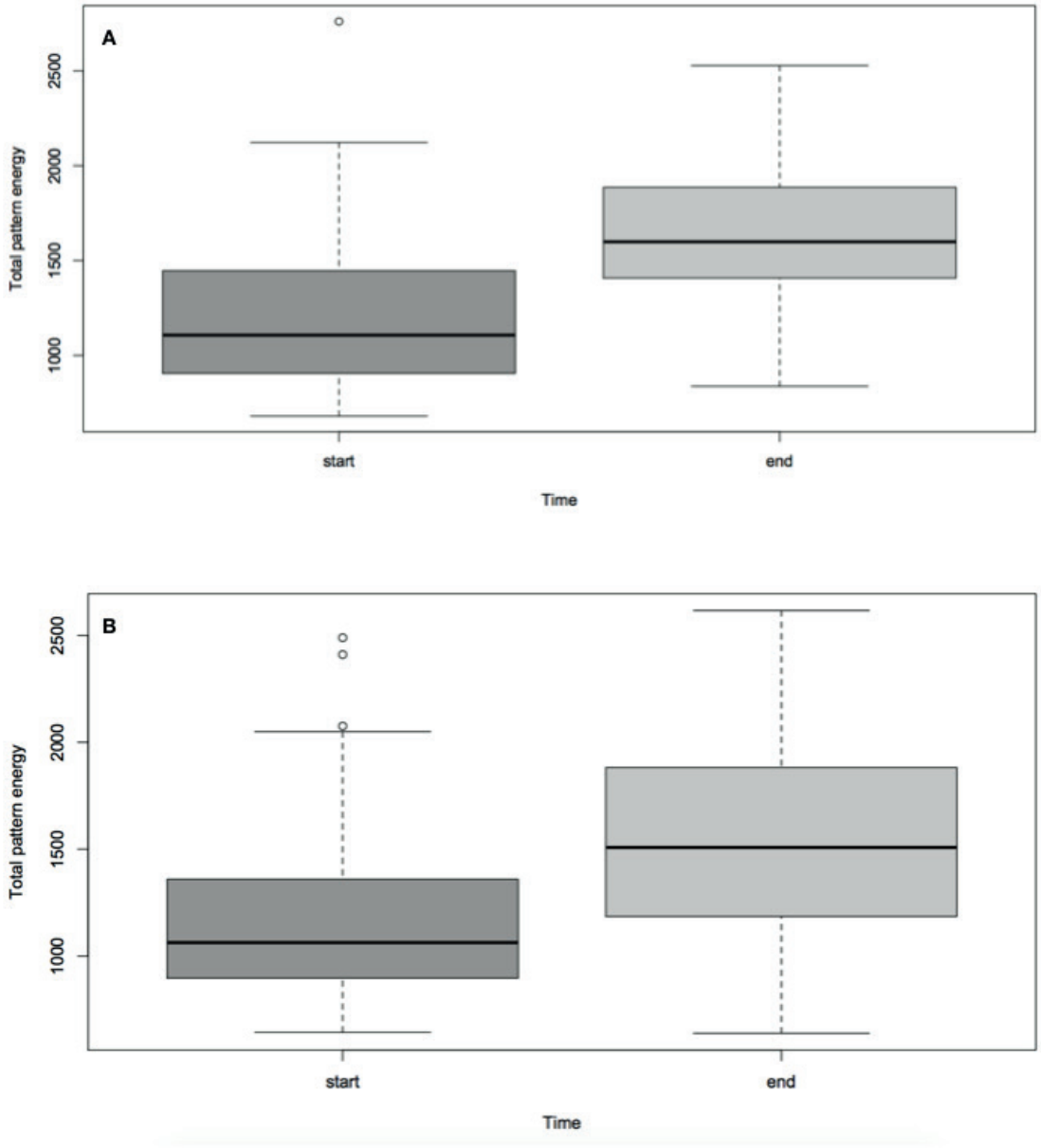
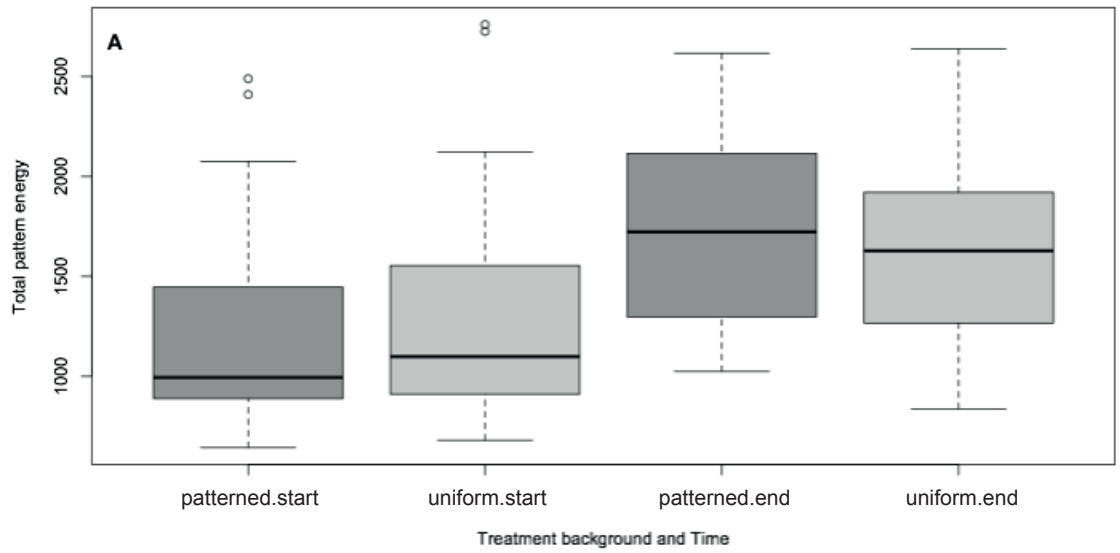


Figure 2.4: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. Graphs show the average total pattern energy at the start and end of the experiment for individuals on either the patterned or uniform treatment background. A) Total Pattern Energy at the start and end of the experiment for Uniform treatment individuals. B) Total Pattern Energy at the start and end of the experiment for Patterned treatment individuals.

Rockpool



Mudflat

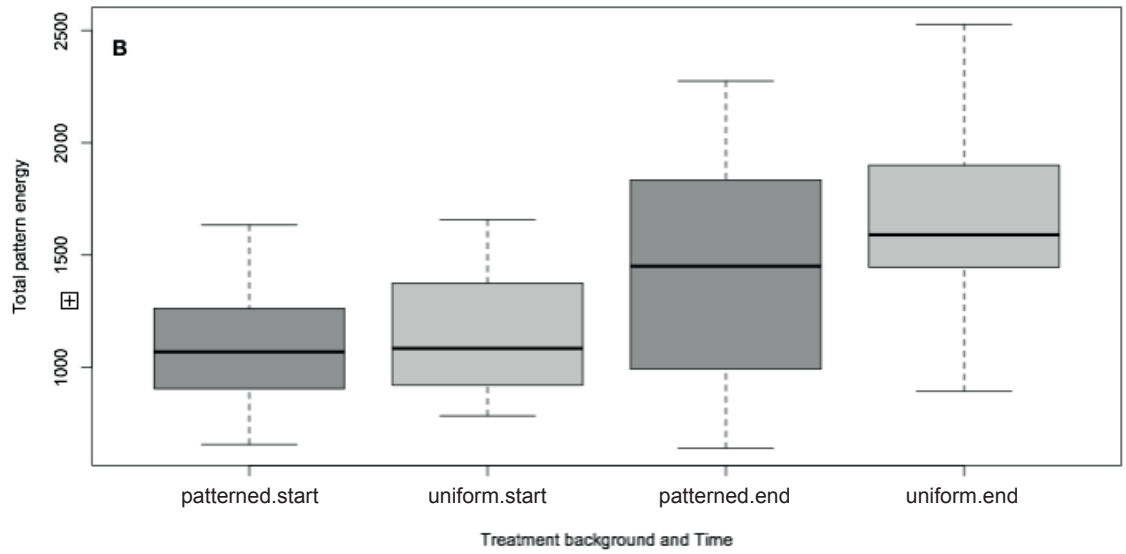


Figure 2.5: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. Graphs show the average total pattern energy for individuals on patterned and uniform treatments, at the start and end of the experiment, categorised by habitat type. A) Total Pattern Energy of crabs collected from rock pool habitats at the start and end of the experiment after being subjected to patterned or uniform treatments B) Total Pattern Energy of crabs collected from mudflat habitat at the start and end of the experiment after being subjected to patterned or uniform treatments.

Carapace pattern - predominant marking size

Wilcoxon ranked sum tests were used to assess the change in predominant marking size of carapaces at the start and end of the experiment. When modelled through avian vision, shore crabs showed a significant increase in dominant marking size from the start of the experiment to the end of the experiment on both the patterned (Wilcoxon: $V = 364$, $p = <0.05$; figure 2.6A) and uniform treatments (Wilcoxon: $V = 250$, $p = <0.005$; figure 2.6B). This result was also significant for fish vision - uniform treatment (Wilcoxon: $V = 239$, $p = <0.05$), and patterned treatment (Wilcoxon: $V = 306$, $p = <0.05$).

This difference over time was confirmed when further GLMM analyses indicated a significant effect of time on the carapace dominant marking size, however the analysis did not indicate that the treatment background or habitat type had any significant effect on this change over time.

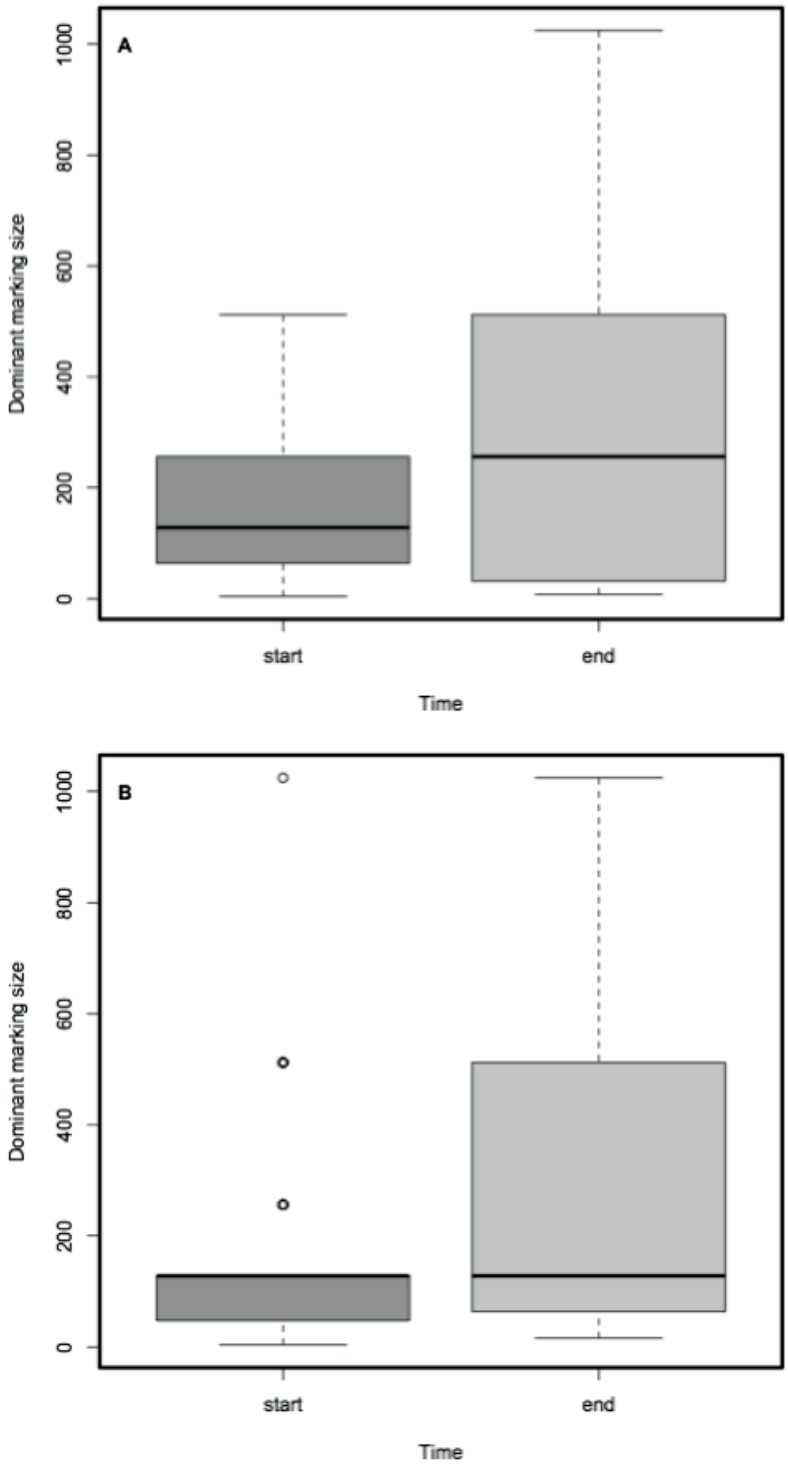


Figure 2.6: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. Graphs show the dominant marking size values (the spatial frequency with the highest energy) at the start and end of the experiment for individuals on either the patterned or uniform treatment background. A) Dominant marking size of shore crabs on the patterned treatment at the start and end of the experiment. B) Dominant marking size of shore crabs on the uniform treatment at the start and end of the experiment. Both graphs are from the data modelled through avian vision.

Change in carapace - background camouflage over time

Pattern energy difference (PED) values were used to determine the difference in pattern energy between two images, in this instance, the carapace of an individual and the background treatment. Larger values indicate less similarity in pattern between the individual and its background, a decrease in this value over time would indicate that the individual has changed pattern to become more similar to its background. To analyse changes in match between the carapace of individuals and the background, a GLMM was used. The results indicated that the difference in pattern between the carapace and the treatment background, was determined by the treatment background the individual was subjected to (GLMM, $F_{1,197}= 14.34$, $P = < 0.01$), and that this was dependent on the time at which the measurement was taken; at the start or end of the experiment (GLMM, $F_{1,197}= 12.90$, $P = < 0.01$). See table 2.2 for the final, simplified model. Specifically, shore crabs subjected to the uniform treatment background showed a significant reduction in PED value, resulting in a significant increase in carapace similarity to the background, whereas crabs subjected to the patterned treatment showed no significant change in match to the background. Further Wilcoxon ranked sum tests confirmed this finding, showing that the PED between the uniform treatment background and the carapace of shore crabs subjected to this treatment, was significantly lower at the end of the experiment in comparison to at the start (Wilcoxon: $V = 1116$, $p = < 0.001$; figure 2.7B). However, shore crabs subjected to the patterned treatment background, did not significantly differ in carapace to background PED values at the start and end of the experiment (Wilcoxon: $V = 889$, $p = 0.069$; see figure 2.7A). When the data was modelled through fish vision, the same results as those for bird vision were found for both patterned and uniform treatments (uniform treatment - Wilcoxon: $V = 944$, $p = < 0.001$; patterned treatment - Wilcoxon: $V = 779$, $p = 0.173$).

A further GLMM model analysed differences in the carapace - background match of individuals from different habitat types and subjected to different treatment backgrounds. Habitat type was not found to have a significant affect on the change in PED values of the crab carapace and background treatment. However, when the data was modelled through fish vision rather than the previous model using bird vision, GLMM analyses found a significant interaction between the treatment background and the habitat type (GLMM, $F_{1,193}$

= 14.54, p = < 0.01 ; see table 2.3). This would suggest that the effect of the treatment on carapace to background PED, depends on the habitat type the individual was originally collected from. However, it should be noted that time was not included in this model.

	D.F	F	p
Treatment	197	14.34	< 0.01
Time	197	12.90	< 0.01
Treatment: Time	197	12.69	< 0.01

Table 2.2: Results from the final GLMM model analysis for peafowl vision. Showing the significant effect of the treatment background and time, on the difference in pattern energy between the carapace and the background, as well as the interaction between these two factors.

	D.F	F	p
Habitat	193	22.20	< 0.01
Treatment	193	672.58	< 0.01
Habitat : Treatment	193	14.54	< 0.01

Table 2.3: Results from the final GLMM model analysis for pollack vision. Showing the significant effect of the treatment background and habitat, on the difference in pattern energy between the carapace and the background, as well as the interaction between these two factors.

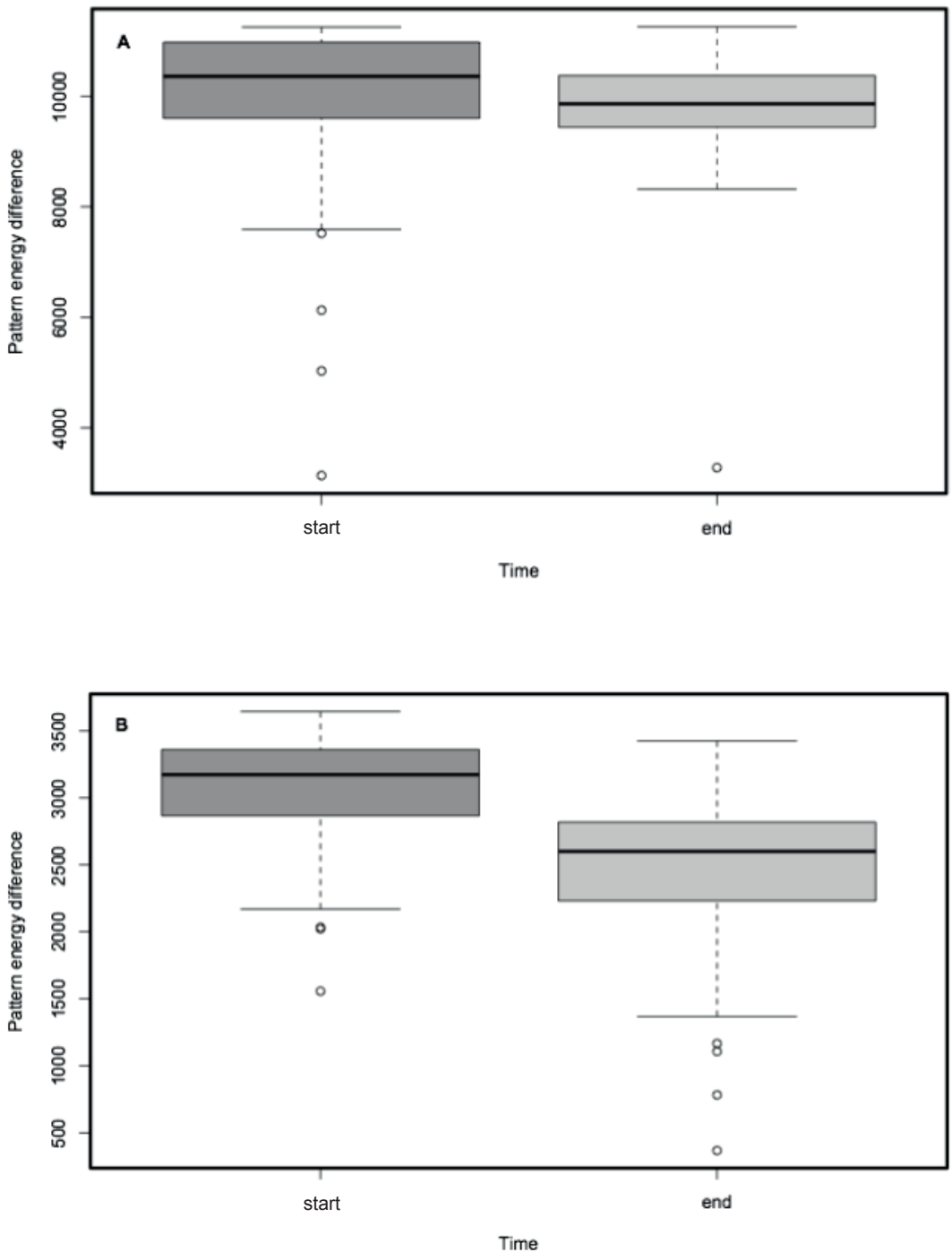


Figure 2.7: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. Graphs show the pattern energy difference values between the carapace and the background at the start and end of the experiment for individuals on either the patterned or uniform treatment background. A) Pattern energy difference values at the start and end of the experiment for individuals from the patterned treatment B) Pattern energy difference values at the start and end of the experiment for individuals from the uniform treatment.

Luminance Analyses

Change in carapace luminance over time

We used Wilcoxon ranked sum tests to assess whether, on average, the carapace luminance of individuals changed over the experiment and whether this change was different across the patterned and uniform treatment backgrouds. Shore crabs assigned to the uniform treatment background, showed a significant increase in carapace luminance over the experimental period (Wilcoxon: $V = 276$, $p<0.001$; figure 2.8A), whilst shore crabs subjected to the patterned treatment background did not significantly change carapace luminance between the start and end of the experiment (Wilcoxon: $V = 756$, $p = 0.91$; figure 2.8B). This result remained constant for patterned and luminance treatments when ran through a bird vision and a fish vision model (Pollack results: uniform treatment - Wilcoxon: $V = 189$, $p = <0.001$; patterned treatment - Wilcoxon: $V = 673$, $p = 0.73$).

Further analyses confirmed this differentiation, using a GLMM we found that there was a significant interaction indicating that the effect of treatment background was dependent on the time (GLMM, $F_{1,206}= 6.19$, $p = 0.013$). The simplified final model with all significant main affects and interactions can be seen in table 2.4.

From figure 2.8A we can see that the carapace luminance significantly increased over the experimental period, for individuals assigned to the uniform treatment. However, this was not a significant increase for individuals on the patterned treatment, as seen in figure 2.8B. It is clear that on average the highest carapace luminance is found from individuals on the uniform treatment, at the end of the experiment, even though uniform individuals started off with the lowest carapace luminance, on average (figure 2.9A). Figure 2.9A shows that there seems to be very little change in carapace luminance for individuals from the patterned treatment, however figure 2.9B plots the interaction between treatment and time, indicating that the individuals on the patterned treatment, on average had a lower carapace luminance at the end of the experiment than their initial starting carapace luminance, however this change was not significant.

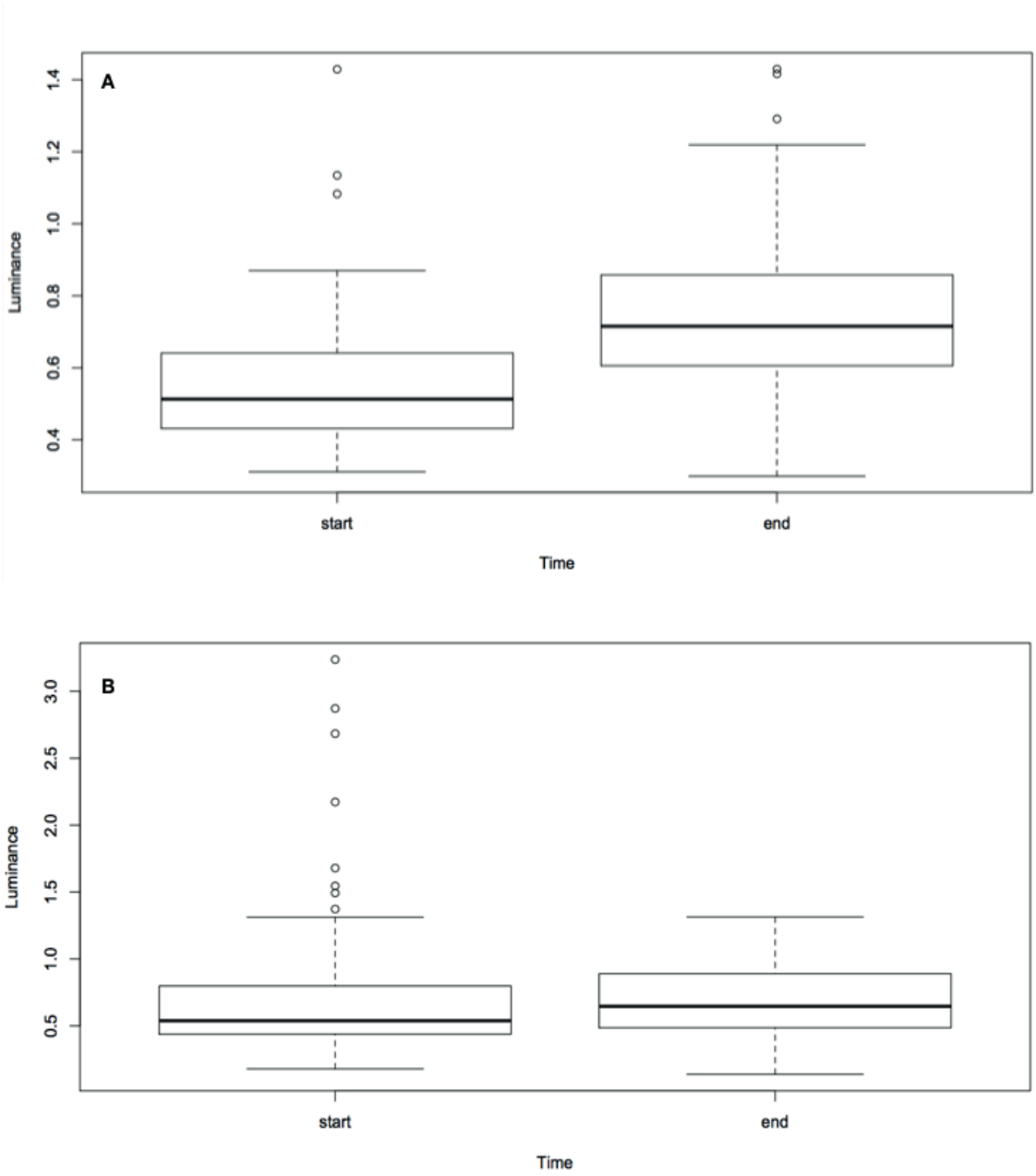
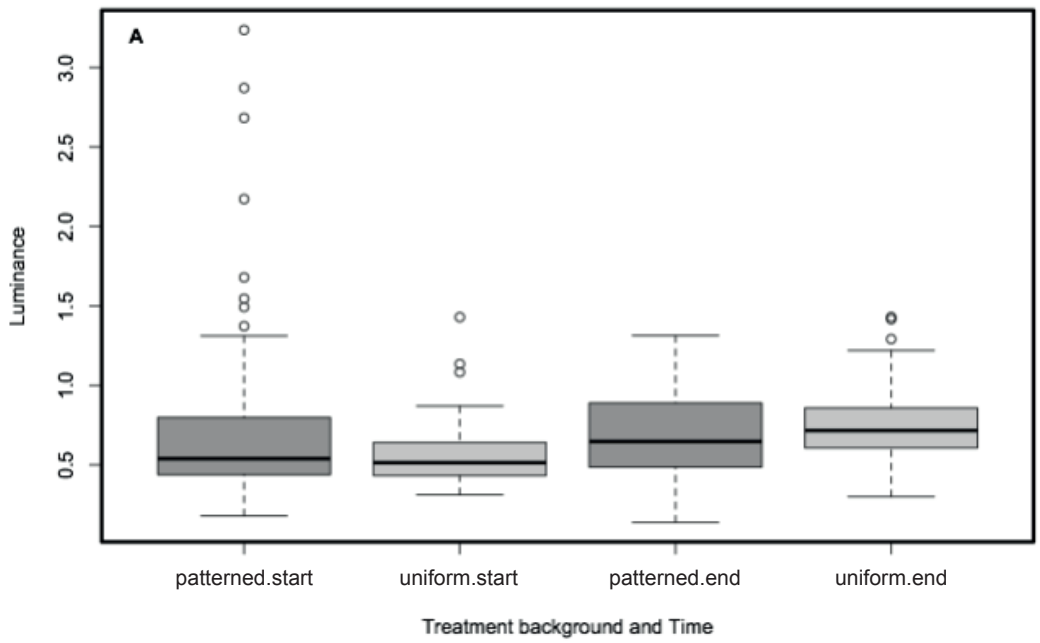


Figure 2.8: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. Graphs show the average luminance values for individuals on patterned and uniform treatment backgrounds at the start and end of the experiment. A) Carapace luminance of individuals on the uniform treatment at the start and end of the experiment. B) Carapace luminance of individuals on the patterned treatment at the start and end of the experiment. Both graphs are from the data modelled through avian vision.



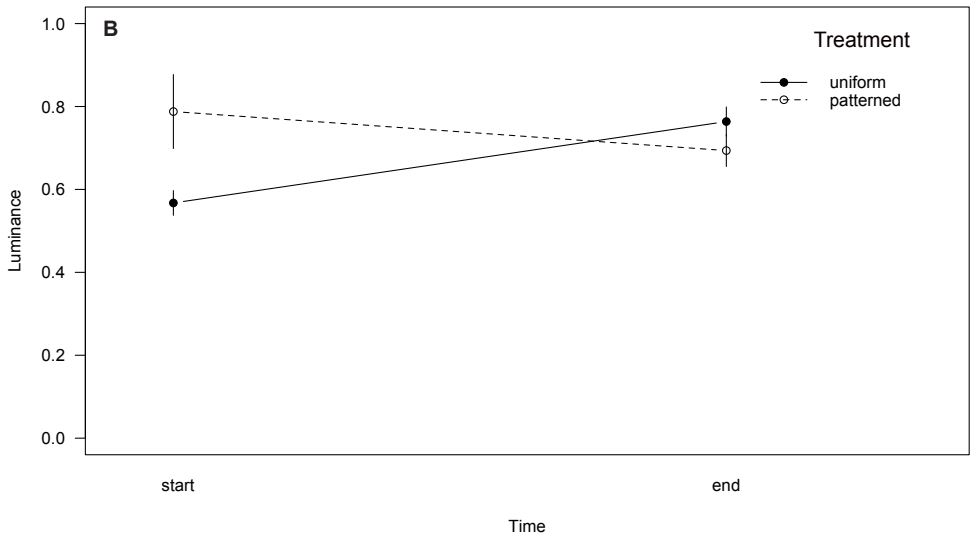


Figure 2.9: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. SD error bars have been included on the average carapace luminance. Graphs show the average luminance values for individuals on patterned and uniform treatment backgrounds at the start and end of the experiment. A) The average carapace luminance of individuals from both treatments at the start and end of the experiment. B) The average carapace luminance of individuals on patterned and uniform treatments, at the beginning and end of the experiment. The data in the graphs are modelled from avian vision.

	D.F	F	p
Habitat	88	1.87	0.17
Treatment	88	0.06	0.79
Time	88	5.91	0.01
Habitat: Treatment	88	3.80	0.05
Treatment: Time	88	6.19	0.01

Table 2.4: Results from the final GLM model analysis for peafowl vision. Showing the significant effect of time, on the change in carapace luminance, as well as the significant interaction between the treatment background and time.

Change in carapace - background camouflage over time : Luminance

Just noticeable difference (JND) values were used to determine the difference in luminance between two objects, in this instance, an individual's carapace and the background treatment it was on. A smaller value indicates more similarity in luminance between the two regions and a decrease in JND values over time would indicate that the change in carapace luminance has resulted in a closer match to the individual's background.

A paired T Test was used to assess changes in this JND from the start to the end of the experiment. This test was chosen for the uniform treatment as data was normal and taken at the start and end of the experiment and therefore paired. Initial results, when mapped through avian vision (peafowl), showed that the luminance JND's between the uniform treatment background and the carapace of shore crabs subjected to this treatment, was significantly lower at the end of the experiment in comparison to at the start (paired $t(35) = 4.5075$, $p = < 0.001$; figure 2.10A.), indicating that the carapace luminance was more similar to the background luminance at the end of the experiment, suggesting an increase in camouflage.

Conversely, data from the patterned treatment was non normal and so a Wilcoxon test was used to assess the change in luminance JND from the start to the end of the experiment. Shore crabs allocated to the patterned treatment did not show a significant change in luminance JND of the carapace to back-

ground between the start and the end of the experiment (Wilcoxon : V = 743, p = 0.45). When the data was modelled through fish vision, this result was the same for both patterned and uniform treatments (Wilcoxon uniform: V = 950, p = < 0.001; wilcoxon patterned: V = 631, p = 0.95).

A GLMM analysis was also used to confirm these differences, indeed the results showed that the difference in carapace to background JND are dependent on the treatment background the individual was subjected to and the time at which the measurement of luminance was taken; at the start or end of the experiment (GLMM, F1,196 = 5.88, p = 0.01; table 2.5 ; figures 2.10A and 2.10B; figures 2.10A and 2.11A). The avian model GLM also found that the effect of the treatment background was dependent on the habitat type that the individual was originally collected from; rock pool or mudflat habitats (GLMM, F1,197 = 4.55, p = 0.03; table 2.5 ;figures 2.10B and 2.11B), showing that individuals from mudflats on uniform backgrounds had the smallest JND and therefore the closest match to the background , however mudflat individuals on patterned backgrounds had the highest JND's. GLMM tests for data from a fish vision model found the same significant interaction effect of the treatment background and time on the JND values (GLMM, F1,188 = 8.07, p = < 0.005 ; table 2.6). However habitat was not found to significantly effect the JND values when modelled through fish vision.

A summary of the results, from all pattern and luminance analyses, and for both bird and fish vision can be seen in tables 2.9 and 2.10.

	D.F	F	p
Treatment	196	49.62	< 0.001
Habitat	196	1.67	0.19
Time	196	11.64	< 0.001
Treatment: Habitat	196	4.55	0.03
Treatment: Time	196	5.88	0.02

Table 2.5: Results from the final GLMM model analysis for peafowl vision. Showing the significant effect of the treatment background and time, on the change in similarity between carapace and treatment background, as well as the significant interaction between the treatment background and time and treatment background and habitat type.

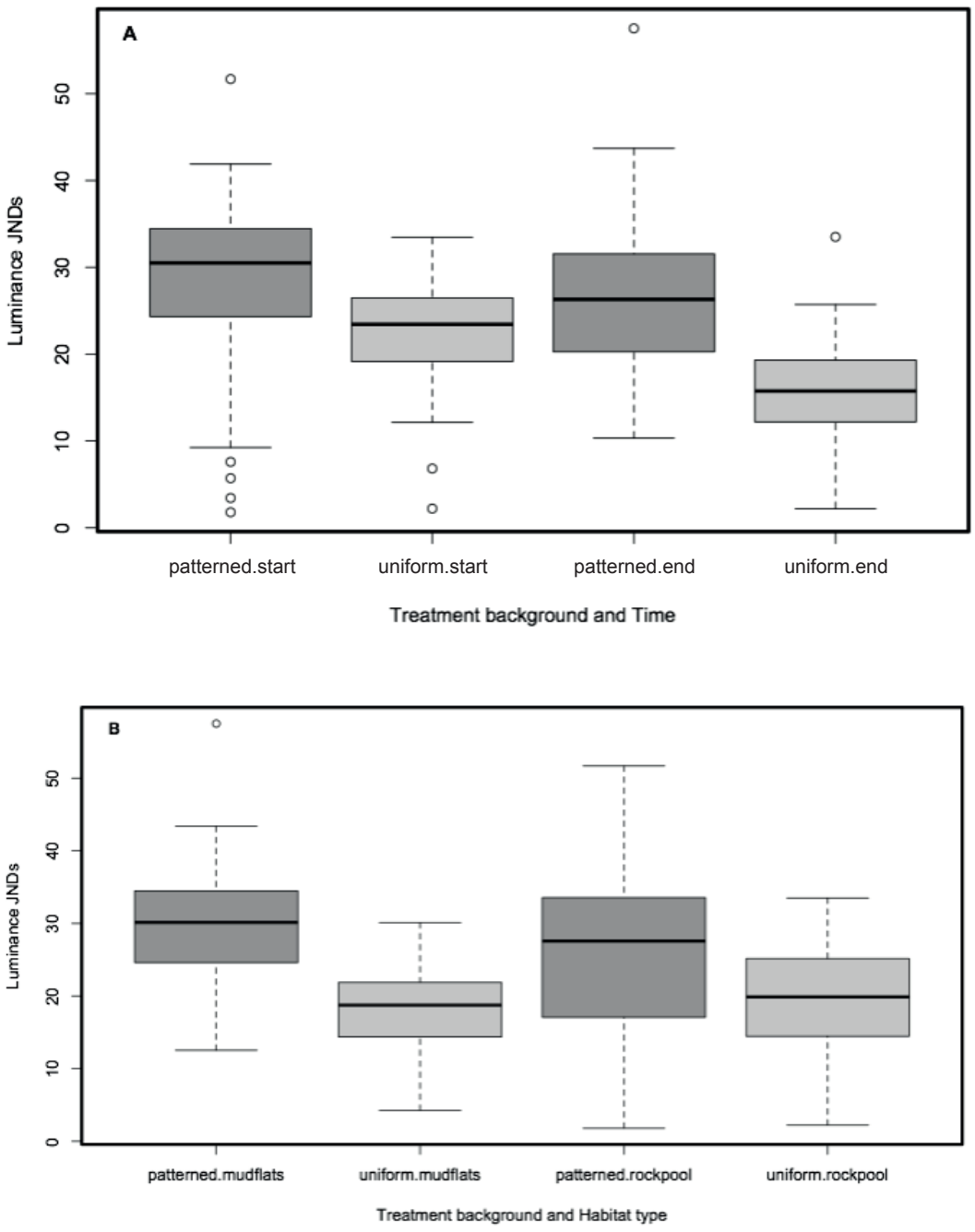


Figure 2.10: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. Graphs show the average carapace to background luminance JND values for individuals, categorised by the treatment background, habitat type and time of measurement. A) The average luminance JND between the carapace luminance of individuals and the background treatment luminance for both treatments at the start and end of the experiment. B) The average luminance JND between the carapace luminance of individuals and the background treatment luminance for individuals on either the patterned or uniform treatment and collected from either rock pool or mudflat habitats. The data in the graphs are modelled from avian vision.

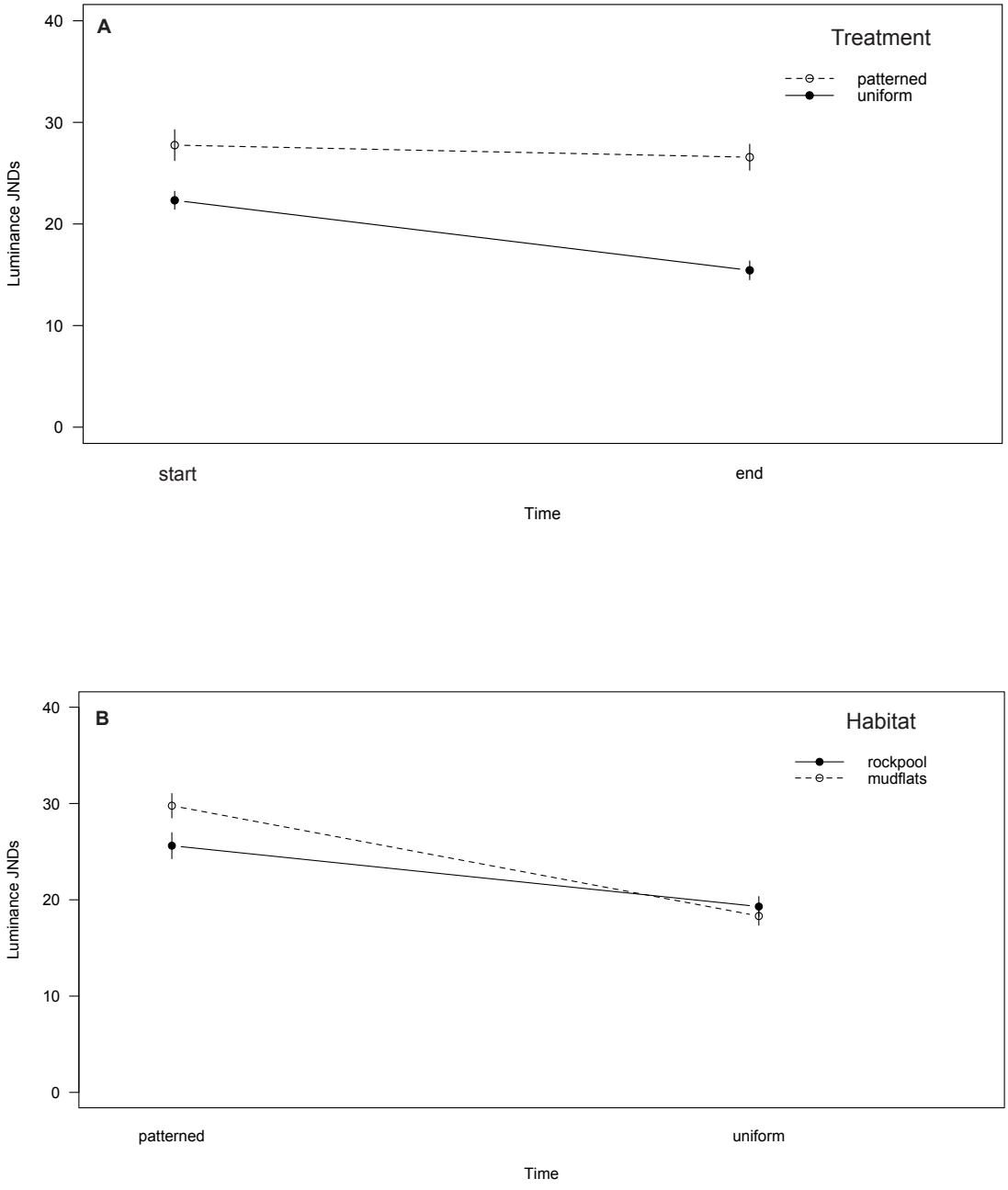


Figure 2.11: Graphs show the average carapace to background luminance JND values and the interaction between the treatment background the individual belongs to, the time at which the JND value was measured and the original habitat type the individual was collected from. A) The average luminance JND between the carapace luminance of individuals and the background treatment luminance plotted by treatment background and time. B) The average luminance JND between the carapace luminance of individuals and the background treatment luminance plotted by treatment background and habitat type. The data in the graphs are modelled from avian vision. SD error bars have been added.

	D.F	F	p
Treatment	196	29.65	< 0.001
Habitat	196	3.07	0.08
Time	196	6.81	0.009
Treatment: Habitat	196	4.00	0.046
Treatment: Time	196	8.07	0.004

Table 2.6: Results from the final GLMM model analysis for pollack vision. Showing the significant effect of the treatment background, and time, on the change in carapace luminance, as well as the significant interaction between the treatment background and time factors.

Moulting Frequency

Over the experimental period, individuals varied in the number of moults they went through, some individuals did not moult at all, others moulted up to three times. I tested whether the number of moults observed, affected the extent of carapace pattern and luminance change. Change in pattern was measured using pattern energy difference (PED) values of the difference in carapace pattern between the starting point and the end point of the experiment.

I found that, when using a GLMM model based on avian vision, the frequency of moults observed did effect the change in carapace pattern of individuals (GLMM, $F_{1,89}=5.97$, $p = <0.05$) from the start to the end of the experiment and that this was dependent on the treatment background (GLMM, $F_{1,88}= 6.50$, $p = < 0.05$; see figures 2.12 a and b). See table 2.7 for the final simplified model.

As can be seen in figure 2.12B, individuals that underwent two moults on the patterned treatment, changed carapace the most over the experiment and changed carapace pattern energy more than individuals who underwent the same number of moults but on the uniform treatment.

When assessing the change in carapace luminance, just noticeable difference (JND) values were used to measure change in luminance between the start and end. We found when modelling data under avian vision, moulting

frequency did significantly affect this change in luminance (GLMM, F1,85=4.21, p = < 0.05) but this effect was not dependent on the treatment background, (see table 2.8 for the final GLMM model). However this result was non significant for the fish vision model.

	D.F	F	P
Habitat	90	1.27	0.26
Treatment	91	0.87	0.35
Moult Freq	89	5.97	0.01
Treatment: Moult Freq	88	6.50	0.01

Table 2.7: Results from the final GLMM model analysis for peafowl vision. Showing the significant effect of moult frequency and the significant interaction effect of moult frequency and treatment background, on the change in carapace pattern over time.

	D.F	F	p
Treatment	88	1.29	0.257
Habitat	88	1.55	0.216
Moult Freq	88	4.21	0.043
Treatment : Moult Freq	88	4.10	0.045

Table 2.8: Results from the final GLMM model analysis for peafowl vision. Showing the significant effect of the moult frequency of individuals on the change in individual carapace luminance over time.

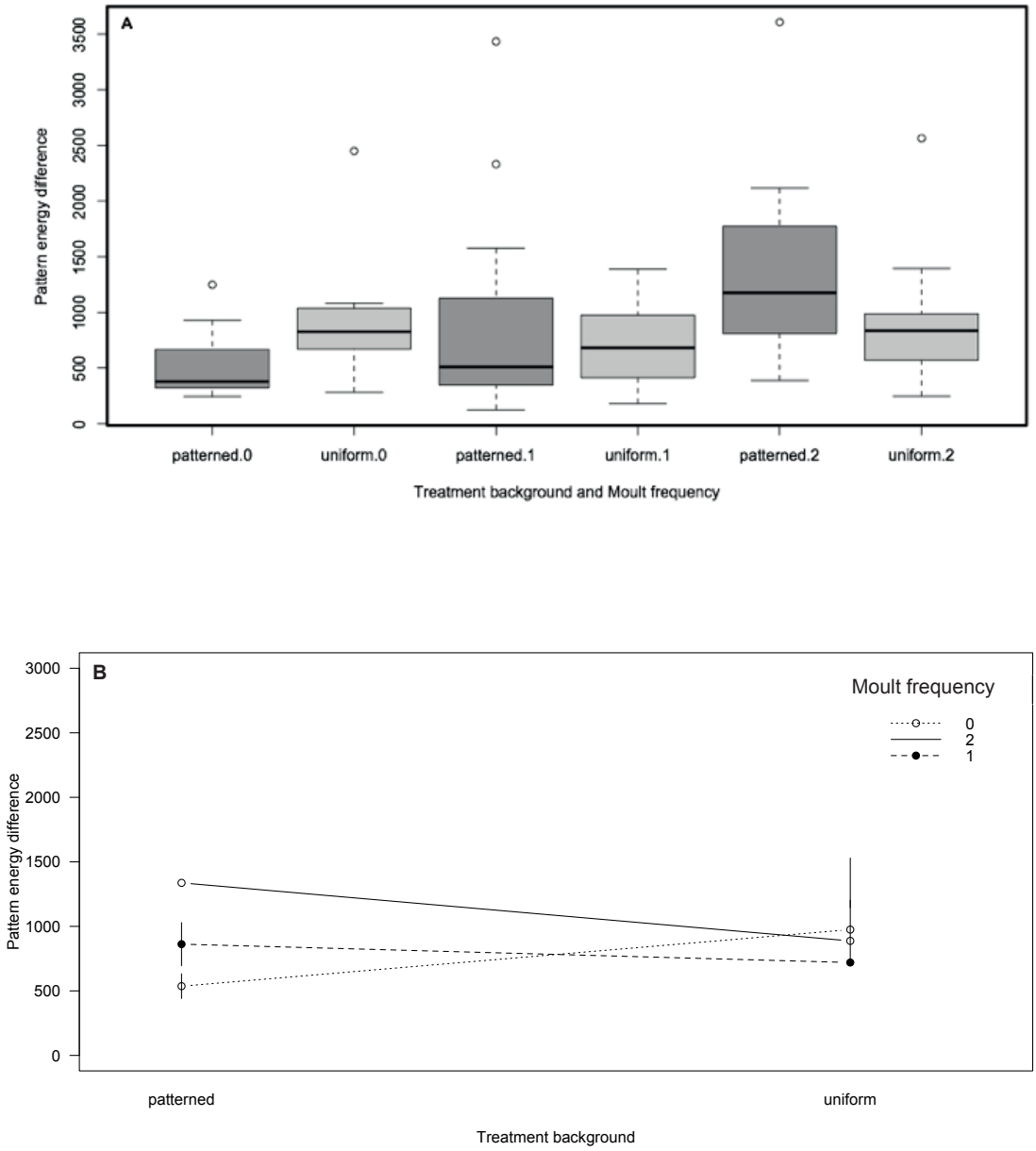


Figure 2.12: Graphs show the average carapace pattern energy difference values between 0 weeks and 12 weeks, for shore crabs from patterned and uniform treatments, categorised by the number of moults witnessed across the experimental period. A) The average carapace PED between the start and end categorised by treatment background and moult frequency. B) The interaction plot showing the difference in average carapace PED for individuals on different treatment background undergoing a different number of moults. The data in the graphs are modelled from avian vision. SD error bars added to 2.12B.

	Habitat	Treatment	Time	Comments
Total Pattern Energy	✓ ✓	✓ ✓	✓ ✓	Increase in the contrast of carapace pattern.
Dominant Marking Size	✗ ✗	✗ ✗	✓ ✓	Larger carapace pattern markings.
PED Carapace - Background	✗ ✓	✓ ✓	✓ ✗	An improvement in camouflage between the carapace and the background treatment.

Table 2.9: A summary table for all pattern analyses. Showing the significant and non significant results for each pattern metric (rows) and the main affects (columns) from GLMM analyses. Ticks indicate a significant main affect, crosses indicate no significant affect. Yellow colouration symbolises the results from bird vision whilst blue coloration symbolises fish vision.

	Habitat	Treatment	Time	Comments
Luminance	✗ ✗	✓ ✓	✓ ✓	Increase in carapace brightness.
JND Carapace - Background	✓ ✗	✓ ✓	✓ ✓	An improvement in camouflage between the carapace and the background treatment.

Table 2.10: A summary table for all luminance analyses. Showing the significant and non significant results for each luminance metric (rows) and the main affects (columns) from all GLMM analyses. Ticks indicate a significant main affect, crosses indicate no significant affect. Yellow colouration symbolises the results from bird vision whilst blue coloration symbolises fish vision.

Discussion

In this chapter, I tested the ability of European green shore crabs to change the pattern and luminance of their carapaces over time, and whether this change was in accordance with the treatment background they were subjected to. Individuals were placed on either a patterned treatment background, consisting of black, grey, and white gravel, or on a uniform treatment background, consisting only of grey gravel. Both treatments differed in pattern and luminance.

In the first instance, individuals' carapaces from both treatment backgrounds were analysed in terms of pattern at the beginning and end of the experiment. Only the shore crabs assigned to the uniform treatment background revealed a significant increase in the contrast of carapace pattern over the experimental period. This change was found to increase similarity on average, between the treatment background and the individuals carapace, providing a potential for reduced conspicuousness and camouflage, when viewed by both avian and fish predators. Our results also established that the habitat type individuals originated from, played a significant role in the effect of the treatment background on the pattern change. This is demonstrated by the increase in pattern contrast of mudflat crabs, but not of rockpool crabs. This result is to be expected, given that past work has found that rockpool crabs show more pattern diversity than mudflat crabs (Todd et al., 2016, 2009; Stevens et al., 2014) and are therefore likely to have started the experiment with more pattern variation than crabs collected from mudflats.

Our Wilcoxon tests indicated that changes in dominant marking size, were found for individuals on both treatment backgrounds. Although this was a very small change for individuals on the uniform treatment, individuals on the patterned treatment showed, on average a much larger increase in dominant marking size. This demonstrates that individuals on the patterned treatment did gain larger pattern markings over the experiment. These findings are in line with our predictions but are also the first to quantify this increase in carapace pattern marking size of shore crabs in relation to the background.

In the second part of this chapter, the same carapace and background images from the start and end of the experiment were analysed in terms of luminance. The disparity between patterned and uniform treatments was the same as that found for pattern analyses; on average, individuals on the uniform treatment increased their carapace brightness, becoming lighter over the experiment but individuals on the patterned treatment showed no significant change. Indeed, on average, the highest carapace luminance values came from individuals on the uniform treatment at the end of the experiment, despite the fact that on average this category also started off with the lowest carapace luminance. This change in brightness also resulted in an increase in similarity between carapace brightness and the brightness of the uniform background treatment, signifying in an improvement in camouflage over the experiment for individuals on the uniform treatment. As was the case with the pattern analyses, when analysing changes in carapace and background luminance over the experiment modelled through avian vision, a general linear model highlighted the significant dependency of the habitat type on the effect of the treatment background. Indeed, these results found the lowest difference between carapace and background (higher similarity and therefore camouflage) for crabs collected from mudlfats and subjected to a uniform treatment. Conversely, the largest difference was seen in crabs collected from a mudflat habitat and subjected to the patterned treatment. As expected, this confirms that crabs collected from mudflat habitats are more similar in luminance to the uniform treatment background that resembles the uniformity of their natural habitat. This finding is also in line with our predictions, given that rockpool and mudflat habitats comprise entirely different substrates, we would expect crabs collected from these two habitats to differ in brightness. We would therefore expect the extent of change in brightness to depend on the initial carapace brightness. Interestingly, this interaction was not found when using luminance change values, modelled through fish vision. Fish and birds are the main predators of shore crabs, however they have very different visual systems, the vision system of the Peafowl bird is tetrachromatic (Hart, 2002) and the pollack vision system is dichromatic (Shand et al, 1998). The non significant result for the interaction between habitat and treatment background for pollack vision, may be due to the reduced colour perception of the pollacks vision system. This distinction may reflect differences in predatory strategies for the two predators, for example birds predating on shore crabs from an arial perspective, may require a higher ability to distinguish between the crab and the

background. However, to fully understand the role of predator vision in the camouflage of shore crabs, further analysis is needed. Future studies should aim to quantify predator vision and undertake experiments to test how this affects detection probability.

Overall, results revealed that shore crabs are capable of changing their carapace pattern as well as luminance, supporting previous studies of plasticity in luminance in shore crabs (Stevens et al., 2014). However, to my knowledge this is the first study to quantify the ability of the shore crab to change carapace pattern under experimental conditions, both in general and in relation to different backgrounds. These findings also indicate that the habitat shore crabs live in, may affect both the pattern and luminance of their carapaces and the extent of flexibility in their ability to change and adapt to their surroundings. Whilst studies have shown that a shore crabs pattern and luminance provide a better camouflage to areas of their own habitat, over areas from other habitat types, no further studies have established how this phenotype specialisation affects the ability to change and adapt over time to artificial backgrounds.

In the luminance analyses, shore crabs from the patterned treatment background did not change brightness over the experimental period. Also, within the pattern analyses, most pattern metric results found similar non significant changes for this treatment, however we did find an increase in the dominant pattern marking size on the patterned treatment. The general lack of significant change found for individuals on the patterned treatment is unexpected given that shore crabs on the uniform treatment did develop more contrasting pattern markings and luminance by the end of the experiment. This result, however, may suggest that crabs on heterogeneous complex backgrounds (represented by the patterned treatment) do not show plasticity, changing brightness and pattern to match their backgrounds, but instead rely on the contrast of their carapace disrupting search image formation or providing camouflage through other mechanisms, such as disruptive patterning. Chapter three provides results in support of this suggestion, and is an exciting foundation for future research to focus on.

The results clearly show that shore crabs show plasticity - capable of changing their carapace pattern and luminance through moulting, over a long

term experiment. This increase in brightness for individuals on a uniform background resulted in an increase in similarity to the uniform substrate, providing potential for improved camouflage. The result that individuals on the uniform treatment increased in brightness and individuals on the patterned treatment did not, is not overly surprising given that previous studies have shown that shore crabs on white backgrounds, do become significantly brighter (Stevens et al., 2014; Easley et al., 2015). However, our finding that this change is also dependent on habitat, is more novel revealing possible insights into the camouflage strategy of shore crabs from these habitats. Whilst this supports previous findings for luminance change and background matching (Stevens, 2016 - unpublished data), the increase in carapace pattern energy of individuals placed on a uniform treatment is more perplexing. The apparent disconnection between this carapace pattern change and the treatment background could be due to one of several reasons. Firstly, individuals on the patterned treatment did show an increase in pattern energy but this result was not statistically significant. This may be due to the higher pattern occurrence and larger variation in the carapace pattern coverage of individuals collected from rockpool habitats (Stevens et al., 2014) prior to being placed on the patterned treatment. As seen in figure 2.5A, the large variation overlaps with the increase in pattern energy at the end of the experiment and this may have reduced the significance in the pattern change. Variation in phenotypic pattern for individuals from a heterogeneous habitat like rockpools, was expected, as the variability in substrates encountered is higher than that of individuals from mudflats. It is also worth exploring the possibility that for individuals from a heterogeneous habitat, such as rockpools, it is less beneficial to invest energy in phenotype - environment matching, as the environment is so variable and is in fact more useful to have a less plastic but more contrasting phenotype which provides camouflage across a wider range of substrates. For example, it may be more useful for rockpool individuals to invest in an alternative camouflage strategy such as disruptive camouflage, whereby pattern markings break up the outline of the body, hindering detection (Cuthill et al., 2005; Cott, 1940; Thayer, 1909) or perhaps, the high diversity in the background, hinders the predator's detection ability. Indeed, studies have long established that variation in shore crab pattern is linked to the natural background substrate they are found on (Hogarth, 1975, 1978; Todd et al., 2012; Stevens et al., 2014), and have even proposed that patterned crabs on polychromatic substrate have a selective advantage, whilst crabs on an open homogeneous

area, survive better if they are plain, indicating phenotypic specialisation (Todd et al., 2006; Easley, 2015).However, to fully analyse this hypothesis, an important factor to take into account would be how much crabs move around in their environment, across different substrates.

An alternative explanation may be that the change in pattern by individuals on the uniform treatment background was in fact not to do with the treatment background but instead may be due to progressive ontogenetic changes in carapace pattern. Ontogenetic colour changes have previously been established in certain species of crab, such as the fiddler crab and are explained as non-reversible colour changes which occur naturally as part of an individual's development. Various factors have been linked to this ontogenetic change, including, reproductive status, metabolism, and changes in size (Booth, 1990). Increases in carapace size occur through moulting and analyses have shown that there were more individuals on the patterned treatment that either did not moult at all, or only moulted once throughout the experiment in comparison to the uniform treatment. In addition, a pattern that is cryptic on a small individual may well be conspicuous on a larger one (Endler, 1978), for example, crabs exhibit white spots on their carapace when small but lose these as they grow. It is thought that the spots enable them to blend into the habitat that suits them as juveniles but these become conspicuous when the adults move onto a different habitat (Todd et al., 2006). Further studies support this link between individual variation and life stage in shore crabs (Stevens et al., 2014) whilst past work suggests that above 20-25mm carapace width, patterned crabs are consistently rare (Powell, 1962). It is therefore likely that shore crabs go through a developmental stage where they achieve crypsis using different colours and patterns, depending on their age and size.

Studies have also found that in the fiddler crab, differences in colouration can be linked to sex (Detto et al., 2008), with females showing more variation in comparison to males of a similar size. Due to the small size of individuals on collection, it was considered too difficult to accurately identify the sex of each individual and so this is not something that could be accounted for in the study. Sex differences in the shore crab is something that has not been studied in detail, however, it may be worth assessing the link between sex differences in carapace colouration and pattern in future studies.

As part of the granularity analyses used in measuring pattern change, we measured the predominant marking size on the carapace of individuals, assessing if the size of the predominant marking increased or decreased over time. These descriptive statistics found that shore crabs on both the patterned and uniform backgrounds on average, increased the size of the predominant pattern marking over the experiment. Although the treatment background was not found to play a significant role in this increase, this does indicate that individuals on the patterned treatment did in fact show some change in pattern markings over the experiment. Whilst it may be reasonable to assume that shore crabs do not display the same flexibility and control over their pattern size and pattern contrast as other marine species like the cuttlefish (Hanlon & Messenger, 1988), the ability of shore crabs to change their carapace pattern sizes and distributions has not been explored in detail.

Results from our camouflage analyses found that the change in both pattern and brightness of individuals on the uniform treatment background did result in an increase in similarity between individuals and the background. Although this increase in similarity was not substantial enough to completely camouflage the individuals against the background, preventing detection, modelled through both avian and fish visual systems, it did make carapace detection more difficult and therefore could reduce the likelihood of predation. A possible explanation for the increase in pattern on the uniform background, is that shadows formed in the gravel, may have created a background of light and dark markings even on the uniform treatment, resulting in shore crabs attempting to match these patterns.

Although it has been established that shore crabs are capable of changing carapace luminance to better match their backgrounds (Easley, 2015; Stevens et al., 2014; Todd et al., 2006), currently, no direct support has been found in shore crabs for carapace pattern change as an adaptive means of camouflage to match distinct backgrounds. Most animals capable of this are able to change pattern over short periods of time; seconds or minutes, and mainly include cephalopods (Allen et al., 2010) and chameleons (Stuart-Fox et al., 2008). Pattern change in other species, including shore crabs, is thought to occur over much longer periods and through different physiological mechanisms (Stevens,

2016) and so it is likely that an alternative process is responsible for phenotype - environment associations in the wild. Indeed, recently a study found that a behavioural mechanism for camouflage is witnessed across several taxa, including several insects (Grant & Howlett, 1988; Kang et al., 2012), reptiles (Nafus et al., 2015), birds (Lovell et al., 2013), and crustaceans. The ghost crab actively chooses background substrates that best match its colouration (Uy et al., 2017).

The results from this study reveal exciting and novel findings which extend our knowledge on the phenotypic plasticity of shore crabs in terms of pattern change as well as luminance. However, unexpected findings such as the lack of change quantified in individuals on the patterned treatment, also leave gaps for future research to fully understand the extent of phenotypic plasticity in shore crabs, and exactly how much of a role this plays in camouflage across habitats. Below I outline potential explanations for the changes in carapace pattern and luminance observed in the experiment. One case could be that these changes are part of a natural progression in a shore crabs' stages of maturity, to match changes in their habitat. It is already known that between habitats such as rockpools and mudflats, shore crabs show variation in carapace colour and pattern and this is associated with substrate differences between these habitats both on a macro and micro scale (Todd et al., 2012; Todd et al., 2006), as well as an individual's life stage; juveniles are more variable in colour and pattern than adults (Stevens et al., 2014). This is further supported by the significant difference in pattern change ability between crabs from mudflat and rock pool habitats seen in this experiment. Indeed, distribution within these habitats linked to changes in maturity may explain these natural changes in pattern expression. As the crabs mature and become larger, they lose pattern (Powell, 1962) generally becoming darker and this may be linked to changes in the substrate they are found on as adults, perhaps spending more time under seaweed and dark rocks than hidden in sand, where a mottled and diverse pattern would prove beneficial in avoiding detection. However, this hypothesised link is based on observation and speculation and is not currently supported by published data. A future study assessing the distribution of juveniles and adults within each habitat type, and quantifying the amount of carapace pattern, may reveal an interaction between life stage, carapace pattern and distribution on the shore. Interactions such as these could be critical in understanding the evolution of crypsis in a marine crustacean with such a large amount of carapace pattern diversity.

Alternatively, as I have briefly discussed already, the disparity in phenotypic change between treatments may also be explained by shadowing created by the lighting in the experimental conditions, which was not premeditated. Shadows created on the uniform treatment, may have resulted in the appearance of a less complex background than the patterned treatment but nonetheless, still patterned rather than uniform. To establish these differences more thoroughly, further studies could alter the experimental treatments to include patterns which more closely resemble the natural habitat, for example using contrasting red and green colours that are usually found in rock pools to create a patterned habitat and more brown and uniform colours for a uniform habitat.

For both pattern and luminance analyses in this chapter, the number of moults were taken into consideration and I found that the change in carapace pattern and luminance were both significantly affected by the frequency of moulting an individual went through over the experiment. This association was expected as it is already widely assumed that changes in crab appearance, both colour and pattern, occur when a crab moults through phenotypic plasticity (Hogarth, 1978; Styrishave et al., 2004). Whilst these studies have not directly quantified carapace pattern change through a model of the predators visionary system and in relation to artificial backgrounds, estimates have been made of around 30% small pattern changes and 10% large pattern changes in crabs allowed to moult (Hogarth, 1978). Interestingly, further analyses found that the effect of moult frequency on the change in a shore crab's pattern energy was dependent on the treatment background. We discovered that the most change in carapace pattern was observed by individuals on the patterned treatment background that went through two moults, and these individuals changed more in carapace pattern than individuals who went through two moults on the uniform treatment. This difference in the effect of moulting frequency on patterned and uniform treatments is the opposite for individuals going through only one moult throughout the experiment, with individuals on the uniform background changing more on this first moult than individuals on the patterned background on their first moult. It therefore appears, from this change, that the second moult had the largest effect on the change in carapace pattern of individuals on the patterned treatment and perhaps if more individuals had reached two or more moults on this treatment background, we would see a significant result in carapace pattern change of individuals on the patterned background. Future stud-

ies could build on this by extending the experimental period and ensuring that crabs are not larger than 10mm prior to the experiment. This would enable us to quantify pattern change over a longer scale and across more moults, more accurately revealing the effects of life stage, moulting frequency, and treatment background on this pattern change. A contributing factor to the lower pattern change for individuals on the patterned treatment at one moult, in comparison to the uniform treatment, may be due to the complexity of the patterned gravel. Future studies could increase the contrast of the pattern, using perhaps only black and white, rather than black, grey and white.

Very few studies assessing phenotypic plasticity and environment matching in crustaceans, have taken into consideration the visual system of the animals' predators (birds and fish), even fewer, have included ultraviolet vision in their visual model. This is important given that many predators are predominantly visual hunters (Merilaita & Stevens, 2011) and can see in UV light and therefore could pick up on changes in pattern and luminance that cannot be detected by the human eye. Any phenotypic changes would therefore only be selected for if they reduce conspicuousness when viewed by these predators. Whilst recent studies have incorporated the visual systems of predators of the sand flea (Stevens et al., 2015) and of shore crabs (Easley, 2015; Stevens et al., 2014) into their camouflage analyses, there have been no studies to date to actively quantify pattern change in addition to colour and luminance, using the visual systems of a shore crab's two main predators; birds and fish.

Interestingly, throughout this chapter, when running statistical analyses using pattern and luminance data quantified from bird (peafowl) and fish (pollack) visual systems, there appeared to be some contrasting results between the two predators. Although assessments of change in pattern and luminance over time were significant for both avian and pollack vision, further GLM analyses seemed to find differences in the interactions between the habitat type and other variables. For pattern analyses, there was a significant interaction between the habitat type (rockpool or mudflat) and the treatment background, when pattern change data was modelled through fish vision. The effect of the treatment background on any change in pattern over time was significantly dependent on the habitat type from which the individual shore crabs originated from. However, when using bird pattern change data, there appeared to be

no significant association with the habitat type. Conversely, when analysing luminance change, this was reversed, with a significant interaction between treatment and habitat for bird data and no such interaction found when using fish data. Indeed, we found that when modelled through a pollack's visual system, mudflat individuals on a patterned treatment had significantly lower pattern energy than rockpool individuals on a patterned treatment background. This was not detected when modelled through avian vision, suggesting that fish and birds differ in their sensitivity to detecting carapace pattern. The reversal found in sensitivity to luminance could provide a basis into further studies investigating whether avian predators are more sensitive to changes in carapace luminance and fish predators are more sensitive to changes in carapace pattern. These differences could be linked to the hunting strategy used by the predator, whilst we know that birds are capable of over turning rocks to predate on shore crabs, there may be more distinctions in the way in which these two predators hunt. Future studies could investigate background matching and camouflage across a wider range of fish and bird species, perhaps dogfish sharks and gulls could be included in the analyses, both of which also attack shore crabs and have slightly different visual systems. This would enable a closer investigation into differences in predator detection ability and comparisons between backgrounds upon which detection is easier for different predators.

Overall, the findings of this study provide support for previous work within the field of shore crab phenotypic plasticity. Specifically, I have provided further evidence for shore crab plasticity in terms of carapace luminance (Stevens et al., 2014; Easley et al., 2015). However, in addition, I have expanded these findings to include pattern, by showing that shore crabs are also capable of changing pattern, resulting in better camouflage on specific experimental backgrounds. I provide a platform for future work on these changes in pattern with regards to background matching in shore crabs. Whilst it would be worthwhile investigating the background matching ability of shore crabs over a longer experimental period on different patterned treatments and with a larger sample size, it would also be interesting to study alternative camouflage mechanisms, such as disruptive colouration, in regards to carapace pattern in shore crabs.

Chapter 3: Quantifying Background Matching and Disruptive Colouration and Testing the Habitat Dependency of these Camouflage Strategies in the Shore Crab *(Carcinus maenas)*

Abstract

It is suggested that many species defend themselves against the threat of predation through camouflage; an affective adaptation, reducing conspicuousness to predators. Camouflage through background matching is dependent upon the natural habitat substrate, in order for individuals to match phenotypic colours and patterns with that of their natural environment. However, in more complex, heterogeneous habitats, this strategy may become less affective due to difficulty over which colour or pattern to match. *Carcinus maenas,* despite facing several predators, is a widely distributed and invasive species found across a diverse range of homogeneous and heterogeneous habitats. Previous studies have highlighted the reduced carapace pattern diversity of shore crabs from homogeneous mudflats in comparison to heterogeneous rockpools. In this study, I propose that the success of shore crabs distributed across these habitat substrates, is due to the use of two different camouflage strategies; background matching and disruptive colouration, which I suggest are dependent on habitat. I tested this by quantifying the match in pattern between carapace and background substrate and the level of edge disruption for crabs collected from rockpool and mudflat habitats. Findings from the results showed that there is a significant difference in strategy between habitats. Using an avian predator model for vision, results for individuals from rockpools highlighted significantly higher edge disruption than shore crabs from mudflats and conversely, shore crabs from mudflat habitats were found to have significantly better carapace - background match than rockpool crabs. In addition to this, our findings indicated differences between adults and juveniles.These findings provide support for differences in camouflage strategy between habitats and suggest that the effectiveness of the strategy may change as crabs mature. This study highlights how variations in shore crab pattern may be specific to each habitat and furthermore, to a specific camouflage strategy; background matching or disruptive coloration.

Introduction

Many animals exhibit visual or other similarities with their environment, commonly referred to as 'phenotype - environment associations' (Todd et al., 2006). It has long been understood that these associations are a product of natural selection (Cott, 1940), enabling the animal to become better camouflaged and therefore avoid detection or recognition by predators (Stevens & Merilaita, 2009). Indeed, selection has driven correlations in colouration between individual phenotypes and backgrounds in, for example, African desert jerboas *(Jaculus jaculus)* (Boratynski et al., 2014), as well as variation in colour and pattern morphs amongst several marine invertebrates, particularly crabs (Harvell, 1994 ; Todd et al., 2006; Krause - Nehring et al., 2010; Nasir & Faulkes, 2011; Stevens et al., 2014; Jensen & Egnotovich, 2015). While these examples of phenotype environment associations are suggestive of a camouflage function, past work has rarely demonstrated or quantified the camouflage resulting from any match to the environmental background (but see Stevens et al., 2015).

Frequently, phenotype-environment associations are thought to be in the form of background matching (Endler, 1984; Merilaita & Stevens, 2011), a widely found and successful form of camouflage (e.g. Troscianko et al., 2016). Background matching is an adaptation that reduces the deviation in the local features between the appearance of an animal and its surroundings. Therefore, for an individual to camouflage itself through background matching, it must possess body colours or patterns that resemble those in the surrounding environment (Stevens & Merilaita, 2011), hindering detection by predators. One example of background matching is of the colour polymorphic isopod *Idotea baltica*, which exists in a heterogeneous habitat, whereby the risk of predation in this species is dependent on the background colouration (Merilaita, 2001), providing indirect support for the effect of phenotype environment matching on the threat of predation. Further evidence to support the adaptive function of background matching (Stevens & Merilaita, 2009) as a successful method of camouflage includes early studies such as the famous peppered moth (Kettlewell, 1955). Additional studies directly assessed the ability of predators such as the blue jay *(Cyanocitta cristata)*, to detect moths when camouflaged against different backgrounds

(Pietreicz & Kamil, 1977). More recently, evidence for background matching has been provided in species such as the crab spider *(M. vatia)*, when it was discovered that crypsis was dependent on the receiver and the substrate on which the spider was found (Defrize et al., 2010). Furthermore, another study assessed the survival benefit of background matching in bird egg colouration and pattern, and found that clutches were more likely to survive when the contrast of the eggs matched the surroundings (Troscianko et al., 2016). Stevens et al. (2015) also provide direct evidence for site specific background matching, showing that an intertidal crustacean, the sand flea, matched the colour and luminance of their own beaches more closely than neighbouring beaches. Recently, background matching has also been suggested in another marine crustacean - the yellow shore crab *(Hemigrapsus oregonensis)* (Jensen & Egnotovich, 2015). However, the study lacked direct quantification of the match between individuals and the environment.

Many crab species exhibit a large amount of variation in carapace colour and pattern, which appear to be anti-predator mechanisms, often providing protection by camouflage (Palma & Steneck, 2001). As a result crabs are commonly studied taxa for testing colour change, camouflage, and phenotype - environment matching. For example, the species *Charybdis annulata* exists in two colour morphs, a generalist brown and a more conspicuous orange which is found in areas with more protective cover (Trivedi & Vachhajani, 2012). Differences in colour morphs have also been recorded amongst juveniles of the common red rock crab *(Cancer productus)*, with 30 phenotypes varying in colour and pattern described, potentially the result of frequency-dependent selection in which rarer forms are favoured (negative frequency dependence) (Krause-Nehring et al., 2010). It has been suggested that this polymorphism amongst juveniles, may impede the formation of a search image amongst visual predators, decreasing the risk of predation (Krause-Nehring et al., 2010). Other demonstrations of crab polymorphism include the jaguar round crab (Reuschel & Schubart, 2007) and the marine rock crab *(Cancer irroratus)* (Nasir & Faulkes, 2011).

For many years, studies have assessed the variation of colour and pattern in the most common temperate species of crab, the shore crab *(Carcinus maenas)* (Powell, 1962; Hogarth, 1978; Crothers, 1967). Powell (1962)

discovered a direct link between size and pattern (larger shore crabs have a reduced amount of patterning in comparison to smaller crabs). In addition, the appearance of larger crabs varied depending on the site they were collected from. Hogarth (1978) also discovered a negative correlation between shore crab carapace pattern and algae cover, whereby sites with a larger amount of algae or mud cover in the habitat appeared to contain individuals with less carapace pattern.

To examine the above correlation in more detail, Todd et al. (2006) placed shore crabs into eight categories depending on colouration (the amount of white, brown, or grey carapace colour) and the level of spotting or pattern on the carapace. They then tested whether these morphs varied across three study sites in Scotland each representing a range of habitat types, including mussel beds, rock pools, seaweed, sandy beach and rocks. Significant differences were found between habitat sites and morphs; plain carapace crabs were associated with macro - algal cover and patterned morphs were associated with mussel beds. Expanding on this finding, Todd et al. (2012) later found evidence of an association between the proportion of patterned and unpatterned morphs at different spatial scales (micro (<1m2), meso (100 s m2) and macro (10,000 s m2)), depending on the substrate type (the amounts of rocks, algae and mussel bed cover).

Most recently, Stevens et al. (2014) built upon these earlier findings (Powell, 1962; Hogarth, 1978; Todd et al., 2006, 2012), examining the variation in shore crab colour and pattern across different habitats around Cornwall, UK. Their findings confirmed clear differences in crab appearance between habitat environments. Whilst homogeneous environments harboured lower diversity in shore crab phenotypes, environments comprising different substrate backgrounds showed substantial differences in pattern and colour. These findings suggested phenotype - environment matching, as it is widely assumed that shore crabs are able to change brightness over a period of days, and probably longer (Stevens, 2016), and these changes are often associated with background colouration (Stevens et al., 2014; Easley, 2015; Chapter 2).

Some studies have assessed how this match between carapace and habitat may have arisen, using colour change experiments (Stevens et al., 2014; Eas-

ley et al., 2015) to investigate the phenotypic plasticity of the shore crab. Other studies have proposed alternative explanations for the phenotype - environment matching of shore crabs, such as ontogenetic changes in habitat use, and even behavioural suggestions such as substrate choice (Todd et al., 2006, 2012; Stevens et al., 2014). However, very few studies to date have actively quantified whether differences in carapace pattern and colour between sites, directly reduces the threat of predation through habitat specific camouflage. For example, those that have quantified shore crab appearance objectively did not directly assess the camouflage match and therefore the survival benefit of the variation between habitats (Stevens et al., 2014) and did not use the visual system of the predators (birds and fish) (Todd et al., 2006, 2012), when quantifying variation between sites.

Of the few studies that have directly quantified the phenotype - environment matching of shore crabs, this technique was found to be less effective in the heterogeneous environments that shore crabs are found. For example, one study directly quantified the camouflage of shore crabs against their own background and compared this to how well camouflaged they were against other locations, using the visual system of shore birds (Easley et al., 2015). This study found that crabs from homogeneous environments such as mudflats, were better camouflaged against their own backgrounds than all other site backgrounds, showing a specific match to that background. However, crabs from heterogeneous rock pool environments had generalised camouflage to all other sites. A possible explanation for this is that shore crabs found in heterogeneous environments are unable to specialise in a generic phenotype that matches the background better than others because the environment is so complex in terms of pattern and colour. This specialisation would limit accessibility to other areas within the heterogeneous environment, due to the risk of becoming conspicuous to predators. In this respect, background matching would prove a less effective mechanism of camouflage for shore crabs inhabiting heterogeneous environments, than shore crabs inhabiting homogeneous environments.

Based on current evidence, variation in shore crab pattern and colour is dependent on habitat complexity (Hogarth, 1978; Todd et al., 2006, 2012; Stevens et al., 2014; Easley et al., 2015). To date, the majority of studies have focussed on assessing how this variation may enable camouflage to be achieved

through background matching (Easley et al., 2015; Stevens et al., 2014; Todd et al., 2006, 2012), despite evidence that this method is likely to be less effective in heterogeneous environments (Merilaita, 1999; Sherratt et al., 2007; Bond & Kamil, 2002; Houston et al., 2007). To date, none of these studie have fully quantified and compared the effectiveness of both background matching and disruptive colouration camouflage strategies in any animal, including shore crabs, across habitat complexities. In this chapter I test the prediction that the carapace patterns exhibited in shore crabs from heterogeneous environments predominantly provide camouflage through disruptive colouration, as opposed to shore crabs from homogeneous habitats where the reduced level of pattern and uniform colouration facilities camouflage through background matching. This will be the first study to quantify the presence and characteristics of these camouflage strategies based on live animals, and using the visual system of one of the main predators to shore crabs, shore birds.

It has been suggested that background matching as a strategy for camouflage has the potential to be limited due to the outline of an animal's body creating discontinuities with the background, thus rendering the animal more conspicuous to predators (Thayer, 1909; Cott, 1940). In addition, Merilaita et al (1999) created a model indicating that there may be situations where animals in a heterogeneous environment should bear some markings from a range of different backgrounds, instead of optimising camouflage with respect to a single background type . As a result, over the last decade or more, disruptive colouration has become more widely appreciated as a key method of camouflage across several taxonomic groups, in particular cephalopods (Chiao et al., 2005; Kelman et al., 2007), but also mammals (Stoner et al., 2003), invertebrates (Webster et al., 2013) and crustaceans (Merilaita, 1998; Reuschel & Schubart, 2007). Very few studies have quantified disruptive colouration in live animals (but see Merilaita, 1998) and we know very little about its tuning and characteristics in live animals against natural substrates (Stevens & Merilaita, 2009). However, recently a study assessed behavioural choice of a resting position in two species of moths. Their results found that both disruptive colouration and background matching concealing mechanisms were used to provide camouflage (Kang et al., 2015). However, evidence for the use of these two mechanisms within the same species has been limited.

Disruptive colouration theory suggests that because edges and boundaries play a central role in visual recognition (Tovee, 1996), highly contrasting markings near the edge of the body and adjacent to the background substrate break up the outline of the animal. This results in the appearance of apparently unrelated objects and therefore makes it more difficult for predators to identify their prey (Thayer, 1909; Cott, 1940). In the case of real animals like shore crabs, individuals that live in highly variable backgrounds may be unable to match many samples of the background well, and instead may benefit from disruptive markings to break up the body shape (Todd et al., 2006; Stevens et al., 2014). Indeed, studies assessing the survival performance of cryptic and disruptively coloured artificial moths, found that survival performance was higher for disruptively coloured moths when in heterogeneous backgrounds, potentially allowing them to exploit a wider range of habitats (Schaefer & Stobbe, 2006).

A considerable amount of work on disruptive coloration has investigated if and how it may work using artificial (human-made) prey, presented to either birds or humans. For example, a study by Stevens & Cuthill (2006) used a computational vision model of edge detection to establish the mechanism by which disruptive colouration reduces detectability. Their results found that disruptive colouration results in 'false' edges being detected within the body rather than at the periphery, confusing predators by inhibiting the successful detection of the animal's body outline. In addition, Webster et al (2013) used target moths and human predators in a controlled experiment and established that the number of intersecting edge patches on a target, significantly reduces the likelihood of detection. An earlier study using moth-like targets, had already established that highly contrasting colours should enhance this disruptive effect (Cuthill et al., 2005). In addition, a study using moths and human subjects under controlled trials, found support for the effectiveness of this strategy, as moths with edge extended disruptive markings survived at significantly higher rates and had longer detection times than all other moth phenotypes (Fraser et al., 2007). More recently, Troscianko et al. (2017) assessed exactly how disruption works and can be quantified using better methods. However, as stated above, it is important to note that currently, no previous studies have actively quantified the camouflage effect of disruptive colouration, using real life animals and quantifying the likelihood of detection through the visual system of the animal's predators.

Shore crabs inhabit heterogeneous environments, being a common intertidal species they are found in a wide variety of coastal environments (Amarel et al., 2009; Crothers, 1966), two of which are highly distinct visually; mudflats and rock pools. Mudflats are homogeneous habitats and rock pools are heterogeneous. Across these habitats, shore crabs exhibit a range of carapace colour and patterns, an attribute often closely associated with environmental heterogeneity (Jormalainen et al., 1995) and also common in cryptic prey species (Bond & Kamil, 2006; Bond, 2007). This is probably due to the complexity of the heterogenous environment, often with several coloured and patterned substrates, against which individuals must reduce conspicuousness by remaining cryptic. It has been suggested that variation in shore crab colouration and pattern and relationships between these morphs and the environment provide defence against visual predators (Todd et al., 2006; Stevens et al., 2014; Easley et al., 2015).

Whilst there is evidence to suggest that both background matching and disruptive colouration are effective means of camouflage for animals in heterogeneous environments, there are conflicting views as to whether these theories are independent mechanisms of camouflage or whether they are interrelated (Schaefer & Stobbe, 2006; Merilaita & Lind, 2005; Hanlon et al., 2009; Michelins et al., 2017). Indeed, some studies suggest that maximum camouflage is achieved when both strategies are used in conjunction. For example, disruptive cryptic colouration has been suggested in the jaguar round crab *(Xantho poressa)*, through which variable colour morphs match the underground and white transverse stripes on the crab's legs and frontal carapace, disrupt the outline (Reuschel & Schubart, 2007) to minimise the risk of detection by predators. However, it is important to note that these suggestions were based on subjective comparisons as no direct analyses were made to quantify the match to the background or disruption of the carapace. It is possible however, that markings on the carapaces of shore crabs, which first appear to match the background, could be inhibiting detection through alternative methods, such as the location on the body, breaking up the outline (disruptive colouration) rather than the similarity to the background, or indeed, through a combination of both. For example, Todd et al., (2006) observed white spots along the edges of patterned crab carapaces (less than 0.1% of these white markings were found on non-edge parts of the carapace) and suggested that this may well be breaking up the outline, providing crypsis through disruptive colouration.

In this study, I propose that the camouflage strategy that is predominant in providing camouflage in the European shore crab *(Carcinus maenas)* is dependent on the habitat background where individuals are found. Using quantitative image analysis through an avian predator's visual system, I test the predictions that shore crabs from heterogeneous rock pool environments, are predominantly camouflaged by disruptive colouration. I also predict that shore crabs from homogeneous mudflat environments, benefit predominantly from background matching. Furthermore, it is possible that these two strategies are adjusted between life stages. For example, based on observation rather than direct analysis, it has been suggested that the Caribbean spiny lobster *(Panulirus argus)* exhibits disruptive patterning as juveniles but not in adulthood (Anderson et al., 2013). Indeed amongst other species of crab, juveniles tend to be associated with heterogeneous substrates (Eggleston & Armstrong, 1995). In addition, studies have shown that shore crabs lose their patterns around the same time that they move from polychromatic heterogeneous areas to more open homogeneous areas (Hogarth, 1975, 1978; Todd et al., 2012). Therefore, to assess the distinction between camouflage strategy, habitat, and life stage, we include juveniles and adults in our analyses.

Finally, it has proven difficult to assess how disruptive prey are against a given background, and previous methods have focused on using models of visual edge detection (Stevens & Cuthill, 2006), measuring the number of edges in the prey's outline relative to either of the surroundings (Lovell et al., 2013) or the centre of the object (Kang et al., 2014). Neither of these methods have directly taken into consideration the angle and direction of the edges versus the true body outline. In my analyses, I used a new method developed that measures false edges (markings running at angles to the animals outline - maximising disruption) and coherent edges (markings that run parallel to the animals body outline) as key features of disruption (Troscianko et al., 2017). Angle sensitive gabor filters are used to measure the ratio of false edges to coherent edges around a target's outline, a high ratio should increase disruption resulting in a decrease in detection ability due to the outline of the animal becoming less visible (Troscianko et al., 2017). Indeed, a comparison of the existing measures of camouflage, including this new method and previously used approaches, found this novel method of measuring disruptive camouflage to be most af-

fective in predicting detection times by humans (Troscianko et al., 2017). This highlights the importance of false edges in concealment, improving our understanding of the best way to measure this method of camouflage and enabling us to compare disruptive coloration with measures of camouflage through background matching. For the latter, I used a modified granularity approach (Troscianko & Stevens, 2015), whereby I compared the energy spectra of crab and background patterns to one another, measuring the difference in marking size, contrast, and diversity following past studies (Troscianko et al., 2016, 2017).

Method

Photography in the Field

Throughout the project, 94 images were taken of rock pool and mudflat habitats, across the same six sites that were used in chapter one; Gyllyngvase, Kennack Sands, Perranuthnoe, Hayle, Penryn, Helford Passage. Three of these sites were rock pool habitats, totalling 47 photographs and the other three were mudflats, also totalling 47. Although there was some variation in features between rock pool sites, in general the background substrate was very similar, consisting of small pebbles and sand, with small pools. Conversely, the mudflat habitats were consistent in substrate across all three sites (See figure 3.1). Images were taken using a digital Nikon D700 camera which had undergone a quartz conversion to allow for UV sensitivity (Advanced Camera Services, Norfolk, UK). All photographs were taken in RAW format with the same fixed aperture settings. Several photos were taken of the same subject at a range of exposures to avoid over exposure resulting in images which then cannot be used in analyses. The camera was held in position using a tripod and all photographs were taken at

the same height on the tripod (approximately one metre from the ground). Each image was taken twice using both a visible (Baader UV/IR Cut filter) and UV (Baader Venus U filter) filter, to block UV and infra red light (human visible images) and allow UV between 300 - 400 nm but block infra red and human visible (UV images). When taking images in the field, light fluctuates and so to try and keep lighting conditions as uniform as possible, images were only taken on over cast, cloudy days. A photographic umbrella was also used for each photograph, and finally, a black and white reflectance standard with a scale bar incorporated, was placed in the corner of each image, to control for fluctuations in lighting conditions. The standard was made from 10 X 10mm sections of zenith diffuse sintered PTFE sheet (Labsphere, Congleton, UK) and was calibrated to reflect 8.2% and 94.8% of all wavelengths respectively. Placing a standard into each image allows for any changes in lighting conditions between images to be controlled for (Troscianko and Stevens, 2015; Stevens et al, 2007).

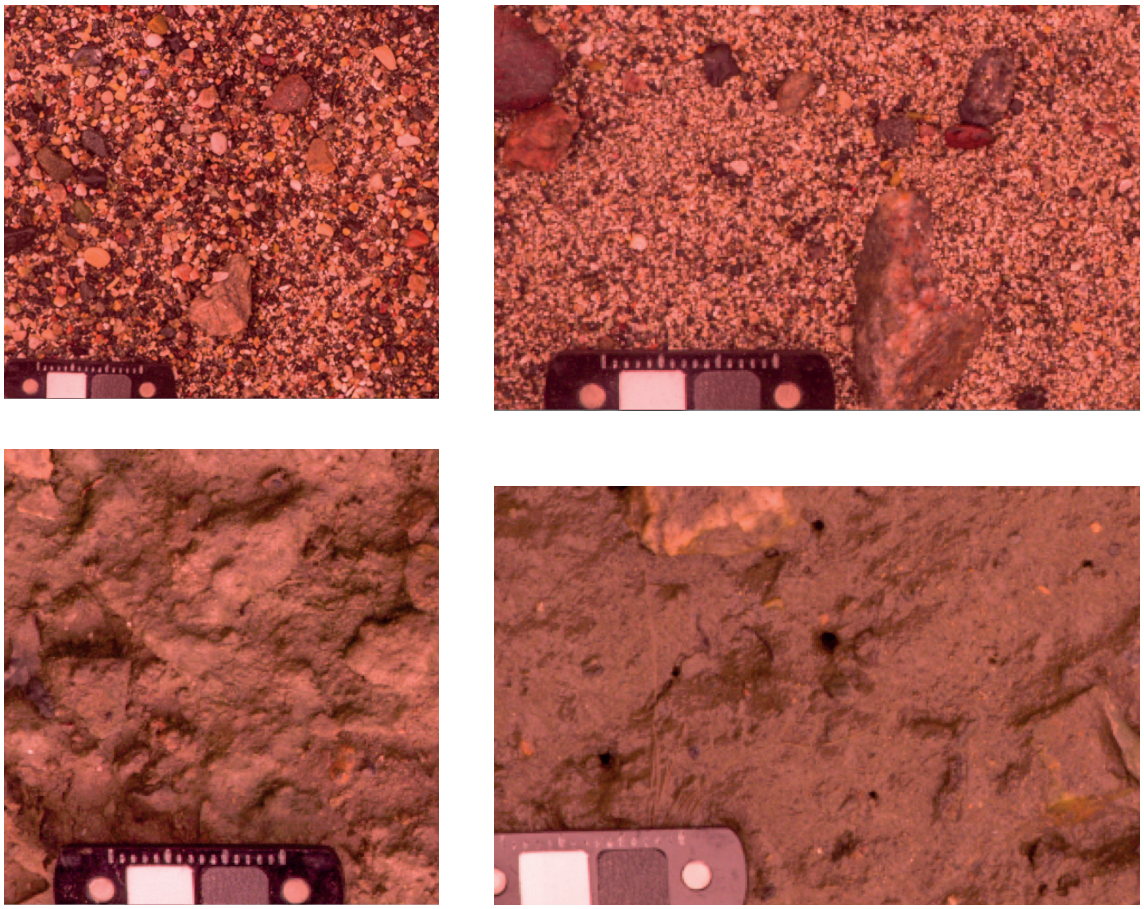


Figure 3.1: Sample background images taken from two different rockpool sites (top left and right), and two different mudflat sites (bottom left and right).

Crab Carapace Photography

To measure disruptive pattern markings in shore crabs, photographs were taken of both juvenile and adult crabs, collected from rock pool and mudflat habitats. In total, 134 crabs were collected (48 adults, 86 juveniles). Shore crabs were identified by their carapace shape, the distinct five spines either side of the eyes with three spines in between the eyes and a lack of swimming paddles (Crothers,1968). Collection of crabs was indiscriminate, at low tide all zones at each site were scanned and any individuals that fitted the juvenile or adult categorisation was collected. Individuals were measured using a 15cm ruler from the fifth pointed spine at the widest part of the carapace to the same point on the other side. Individuals measuring less than 15mm were classified as juveniles and individuals between 15 - 25mm were also used, but classified as adults. The crabs were then transported back to the laboratory in clear tanks containing salt water from the natural habitat and enough background substrate from the site to cover the bottom of the tank, providing refuge to avoid inflicting stress during transportation.

Back at the laboratory, individuals were gently dried and placed underneath a tripod set up in a dark photography room. The crab was placed on a spectrally flat sheet of black 2 mm thick foam with a reflective cylinder surrounding the individual. A black and white reflectance standard was placed by the side of the crab with an identification number. The standard was made from 10 X 10mm sections of zenith diffuse sintered PTFE sheet, (Labsphere, Congleton, UK) and was calibrated to reflect 8.2% and 94.8% of all wavelengths respectively, with a scale bar alongside the PTFE to enable pattern measurements to be made. Including a standard in every image allows changes in lighting conditions to be controlled for (Troscianko and Stevens, 2015).

A series of images were taken in human visible light and then immediately afterward in ultraviolet light, after being refocused. The images were taken with a digital Nikon D7000 camera, which had undergone a quartz conversion to allow for UV sensitivity (Advanced Camera Services, Norfolk, UK). A filter (Baader UV/IR Cut filer) was placed in front of a Nikon 105mm Nikkor lens that blocked UV and infrared light and only transmitted wavelengths between 400-

700 nm, this was to capture human visible images. For capturing UV images, a different filter (Baader Venus U filter) was placed in front of the lens, allowing UV transmission between 300-400 nm and blocking infrared and human visible light through. Photographs were taken in RAW format with fixed aperture settings. Several photos were taken of the same subject at a range of exposures, to avoid over exposure. This is important because overexposure causes pixel saturation which results in data being lost as images cannot be correctly measured when pixels are saturated (Troscianko & Stevens, 2015).

Image Analysis

The programme RawTherapee was used to view the RGB histograms of all images taken, one human visible and one UV image with optimal exposure was then selected for each individual at each timescale. Multispectral images were then created using custom codes from the 'multispectral image calibration and analysis toolbox' in the program Image J (Troscianko & Stevens, 2015). During this process, UV and visible images are manually aligned to form one image, which is then split down resulting in a stack of images broken down to relative wavelengths, shortwave (SW), mediumwave (MW), longwave (LW) and UV. Alignment is vital to ensure that any movement captured between filters is rectified without false colour being formed. During production of multispectral images, the white and grey standards were selected to allow images to be linearised with respect to radiance and standardised to control for effects of light conditions (Stevens et al., 2007, Troscianko & Stevens,2015).Once these images had been normalised, regions of interest (ROIs) were then selected for measurement. For the purpose of this study, the crab's carapace, excluding legs and pincers were selected as ROI's. Legs and pincers were excluded because subjective field observations found that in both rockpool and mudfalt habitats, the carapace is usually the largest part of the shore crab which is revealed to potential predators, as often the legs are tucked underneath the carapace or buried beneath the habitat substrate (sand or mud). The scale bar on the grey standard was also measured and saved alongside the ROI as an spec, to allow pattern measurements and comparisons to be made.

An important aspect of background image analysis was to ensure that

the image provided an accurate example of substrate for potential camouflage from predators and so this involved scanning each image for any possible areas that a crab would not likely be found on in the wild, these were labelled exclusion zones and examples include mainly large rocks sticking out of the substrate background and pieces of seaweed. These areas in each image were selected using tools in imageJ, and then labelled as exclusion zone ‘a’. A scale bar was placed into every background image when photographs were taken in the field, to allow pattern sizes to be scaled and measured accurately, and so the basis for selection of exclusion zones was any substrate object which had a width larger than half the length of the scale bar. These individual exclusion zones were then combined into single background images labelled ‘b’ using tools from the image analysis toolbox. A measurement of scale bar size across all background images allowed us to resize backgrounds to the same scale.

The main predators of shore crabs are shore birds such as oyster catchers and turnstones (Crothers, 1968), these birds are more sensitive to longer violet wavelengths than other UV sensitive birds and they are part of the avian violet group (VS) system (Odeen et al., 2010). Human vision is very different to avian vision, as birds are tetrachromats, using four cone types in their colour vision for LW, MW, SW and UV light whereas humans are trichromatic (Cuthill, 2006) . More specifically, in terms of pattern processing, humans rely on the luminance channel rather than double cones, which are used in birds (Osorio and Vorobyev, 2005). Therefore, when analysing the edge disruption of shore crab carapaces, it is vital that the visual system of the predator is accounted for. So for this project, the peafowl (Pravo cristatus) visual system was chosen due to its wide application and regard as a model species of avian vs vision (Hart, 2002).

For disruptive patterning analysis, images were then exported as binary mask images (the crab carapace white against a black background) in the TIF format, and a Gabor filter was applied to each of the pixels around the edge of the carapace. The size of the filter can be selected and for this experiment, we used a sigma level (filter size) of 5, and this remained constant to ensure comparisons were made at the same spatial frequency. The filter size is dependent on the px/mm of the carapace and controls the size of the pattern markings which are detected, larger sigma levels would detect larger disruptive patterns. The edge disruption of each crab was then measured against a neutral back-

ground, using the sigma level above. This enabled the angle of the crab's outline at each point of the carapace to be measured.

This process was then repeated against all rock pool and mudflat image backgrounds, using the same sigma level. This function in imageJ randomly places the crab onto a background and measures the carapace edge disruption by applying the Gabor filter. This measured each point around the crab's outline at an angle parallel to and at right angles to the edge, enabling measurements of the interaction between the crab and its background to be made. The disruption ratio was then calculated at each point on the crab's outline, and the mean of these ratios across the whole carapace outline, was calculated, resulting in the final Gabor edge disruption ratio (GabRat). GabRat values range from 0 - 1, with < 0.2 being considered low edge disruption and > 0.4 high edge disruption (Troscianko et al, 2017). Each crab was randomly placed in 50 different positions that did not overlap with each other or any exclusion zones. This was repeated on all 94 backgrounds, each juvenile and adult crab was randomly placed into 50 different positions on 47 rock pool backgrounds and 47 mudflat backgrounds using custom tools from the image analysis program ImageJ, resulting in a total of 4700 edge disruption measurements per individual crab. This was in order to increase sample size, accounting for variation in positioning of crabs in the wild and to increase the accuracy per image background by ensuring that the average edge disruption value taken from these 50 positions would be an accurate representation of how crabs would appear in the wild on that background. See figure 3.2 for an example of each background and crab category. See figure 3.3 for an example of individuals from all four crab categories (adults, juveniles, rockpool and mudflat collection), superimposed onto a rockpool and mudflat background, to assess background matching and disruptive colouration.

Edge disruption was calculated using a new method devised using custom tools in imageJ. This method is the first to take into account direction of perceived edges versus actual edges, enabling us to distinguish 'false edges' (markings that run at right angles to the prey's outline and are maximally disruptive) from 'coherent edges' (markings that match the outline of the animals body, potentially making the prey's shape easier to detect by predators). The novel edge disruption metric called 'GabRat' uses angle sensitive filters to measure

the ratio of false edges to coherent edges around the prey's outline and when compared with older, existing methods used to assess disruptive markings, has outperformed all existing measures (Troscianko et al, 2017). A high ratio of false edges to coherent edges should be more disruptive, and may therefore indicate that prey are more difficult to detect, whilst lower values suggest salient coherent edges, likely associated with an alternative method of camouflage, for example background matching.

To assess the level of background matching between carapace and habitat background images, a granularity analysis was conducted, a method which has previously been used to analyse patterns in other animals, including cuttlefish (Barbosa et al., 2008 ; Chiao et al., 2009) and the patterns found on cuckoo eggs (Stoddard & Stevens, 2010). During granularity analysis, each image is filtered using Fast Fourier bandpass filtering, at multiple spatial frequency scales, resulting in each filter catching information at different spatial scales. The energy at each of these scales is measured as the standard deviation of the filtered pixel values (Troscianko and Stevens, 2015), and so larger markings of low spatial frequency are captured by smaller filter sizes and larger filter sizes capture smaller markings of higher spatial frequency. For camouflage purposes specifically, pattern energy difference values outputted from this granularity analysis were used. These were generated by a custom made difference calculator in imageJ (Troscianko and Stevens, 2015) as part of the granularity analysis process, which works out the absolute difference between the spectra of the two images, across the spatial scales measured (Troscianko and Stevens, 2015). Any two patterns with similar amounts of energy across the spatial scales will produce low pattern difference values, indicative of background matching. In this instance, the absolute difference between the spectra of crab carapaces and habitat backgrounds was assessed (both rock pool and mudflat separately).

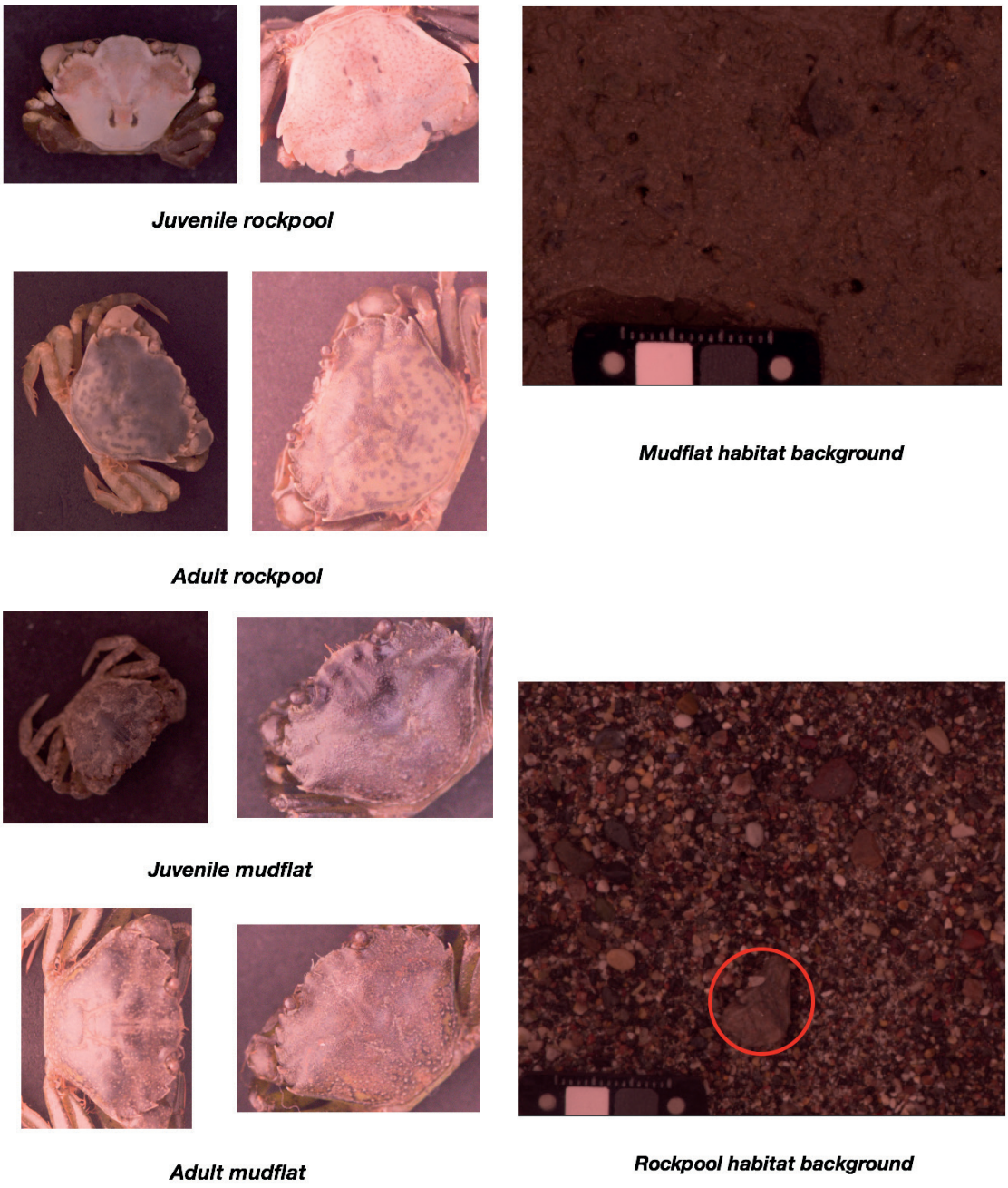


Figure 3.2: Showing examples of rockpool and mudflat habitat background images and the categories of crab age and collection habitat on the left. Each crab from the categories on the left was placed randomly 50 times in each of the 47 rockpool and 47 mudflat background images, after scaling appropriately. The red circle in the rockpool habitat shows an exclusion zone that would have been identified during the processing stage.

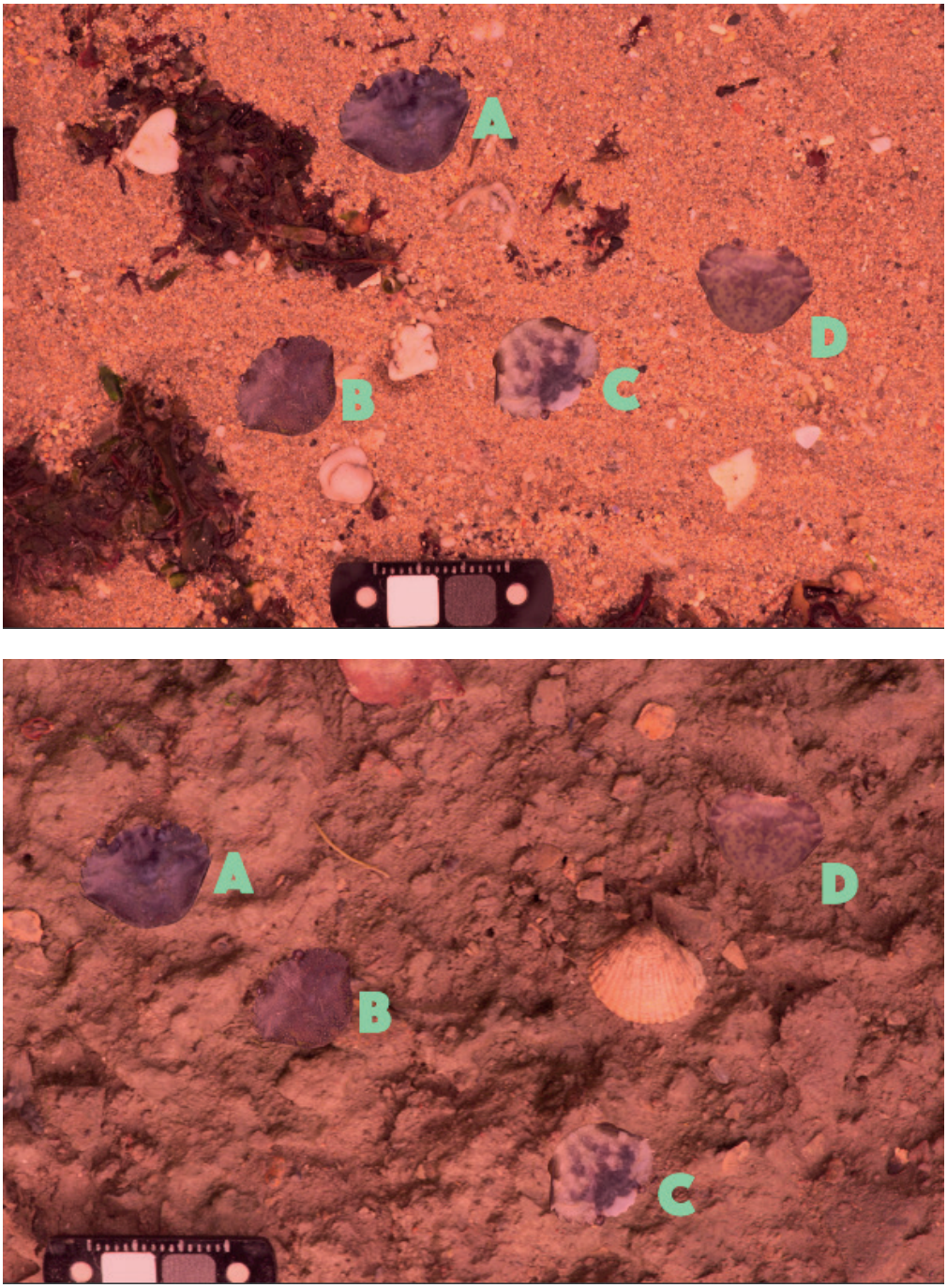


Figure 3.3: Showing a rockpool image background (top) with an example individual from all four categories superimposed onto the background. A) an adult collected from a mudflat site B) a juvenile collected from a mudflat site C) a juvenile collected from a rockpool site D) an adult collected from a rockpool site. The bottom image shows the same four individuals superimposed onto a mudflat example background. In imageJ each crab was superimposed into 50 random positions per background image. Edge disruption and the carapace to background match was then quantified.

Predictions

We already know that there is a large amount of variation in shore crab carapace pattern (Powell, 1962; Crothers, 1968) and that this is linked to both age (Hogarth,1978) and habitat (Todd et al, 2006), indeed shore crabs from homogeneous habitats have on average less pattern than crabs from heterogeneous habitats. However, studies have not tested the possibility that different camouflage mechanisms may exist between these habitats, explaining the differences in appearance. We hypothesise that shore crabs from heterogeneous habitats camouflage themselves through disruptive mechanisms, using pattern markings to break up the outline of the body and reduce detectability by predators and conversely shore crabs from homogeneous habitats use background matching to provide protection from predation through camouflage. We therefore predict that crabs from rock pool habitat sites will have higher GabRat edge disruption values than crabs from mudflat habitats and that this will also differ depending on the background image; specifically, rock pool collected crabs on rock pool background images will have the highest edge disruption and mudflat collected crabs on mudflat background images will have the lowest edge disruption. The larger amount of carapace pattern variation observed in juvenile shore crabs in comparison to adults (Hogarth, 1978), may be associated with a higher level of vulnerability to predation and it has been suggested that this variation may confer some protection against predators (Todd et al., 2006). Indeed, juvenile individuals undergo several moults as they grow, during which they are highly vulnerable to predation, whilst adults undergo significantly fewer moults (Crothers,1967). Based on this, we also predict that juveniles from rock pool sites will have more edge disruption than adults from rock pool sites. Conversely, we predict that when analysing the match in pattern energy between shore crab carapaces and the background image pattern, crabs from mudflat habitats will better match the background and therefore have lower PED values than crabs from rock pool habitats. We also predict that the lowest PED and therefore closest background to carapace match will be found from mudflat individuals on a mudflat background and that both adults and juveniles from mudflats will better match this background than adults and juveniles from rock pool habitats.

Statistical Analyses

The average GabRat value of the total 50 positions was calculated for each background, so that one value was generated per crab/image combination. Averages were then calculated across all rock pool and mudflat backgrounds so that each crab had an average edge disruption value for both habitat types. This study was a repeated measures analysis as all individuals were placed onto both habitat types, resulting in two average values per individual. A split plot 2 x 2 repeated measures mixed factorial ANOVA was used to assess the affect of individuals collected from one of two habitats on the edge disruption and background matching when superimposed onto both rock pool and habitat background images. This test was used because individuals were categorised into two different groups (rock pool or mudflat collection sites) but subjected to the same treatments (rock pool and mudflat background images), and we wanted to make comparisons of individuals between those groups and within the treatments. Therefore our within subjects factor was image background and the between subjects factor was the collection habitat. The model was carried out on juveniles and adults separately and ran twice using edge disruption data (for disruptive colouration analyses) and PED data (for background matching analyses). Homogeneity of variance was assessed using levene's test and the assumption of sphericity was not necessary due to only two levels of repeated measures.

The main effects were analysed and if a significant interaction was found then this was further analysed using simple effects procedures. Assumptions of normality and homogeneity of variance were met in both cases and the assumption of sphericity was non applicable due to only two repeated measures levels. All analyses were conducted in the statistical program R (R Core Team, 2014).

Results

Camouflage: Disruptive Patterning

Is the level of carapace edge disruption linked to the habitat shore crabs are collected from?

To assess how disruptive shore crabs' carapaces were, GabRat values were calculated for every image of the carapace against a rock pool or mudflat substrate. Crabs were collected from homogeneous mudflat or heterogeneous rock pool habitats. A larger GabRat value indicates more disruptive edges, whilst lower values suggest salient coherent edges, likely associated with an alternative method of camouflage, such as background matching (see granularity analyses results for background matching). GabRat values ranged from very low (0.04) to values above what is considered as highly disruptive edges (> 0.40) (Troscianko et al, 2017), this overlap can be seen from figure 3.4. A split plot 2 x 2 repeated measures mixed factorial ANOVA was used to assess the effect of individuals collected from one of two habitats on the level of disruptive colouration when superimposed onto both rock pool and habitat background images.

Juveniles

When analysing the disruptive colouration of juvenile individuals from rock pool and mudflat habitats, we found that there was a significant difference in the level of edge disruption between crabs collected from rock pools and crabs collected from mudflat habitats. Crabs collected from rock pool habitats, had significantly higher levels of edge disruption when superimposed onto both heterogeneous rock pool and homogeneous mudflat background images than juvenile crabs collected from mudflat habitats and superimposed onto the same backgrounds (see figure 3.4; results table 3.1 for split plot ANOVA results and mean edge disruption values).

However, analyses between treatments gave more unexpected results. As can be seen from figure 3.4, the edge disruption of crabs collected from rock

pool sites was not significantly different when superimposed onto a rock pool or a mudflat background. Although it is clear that rock pool crabs on both backgrounds have more disruptive edges than crabs from mudflats, rock pool crabs had slightly more disruptive edges on mudflat backgrounds than on their own habitat substrate. Likewise, the same can be seen from juvenile mudflat crabs, when superimposed on a rock pool background, they have higher edge disruption than when superimposed onto their own substrate background, presumably this is due to the principle of differential blending (Cott, 1940), where some colour patches of the pattern blend into the broken rock pool background, disrupting the edges of the carapace.

The split plot ANOVA also revealed a significant interaction between collection site and image background (see table 3.1 for results), as can be seen from figure 3.4B, the edge disruption is higher on mudflat backgrounds for crabs collected from rock pools and higher on rock pool backgrounds for crabs collected from mudflats habitats.

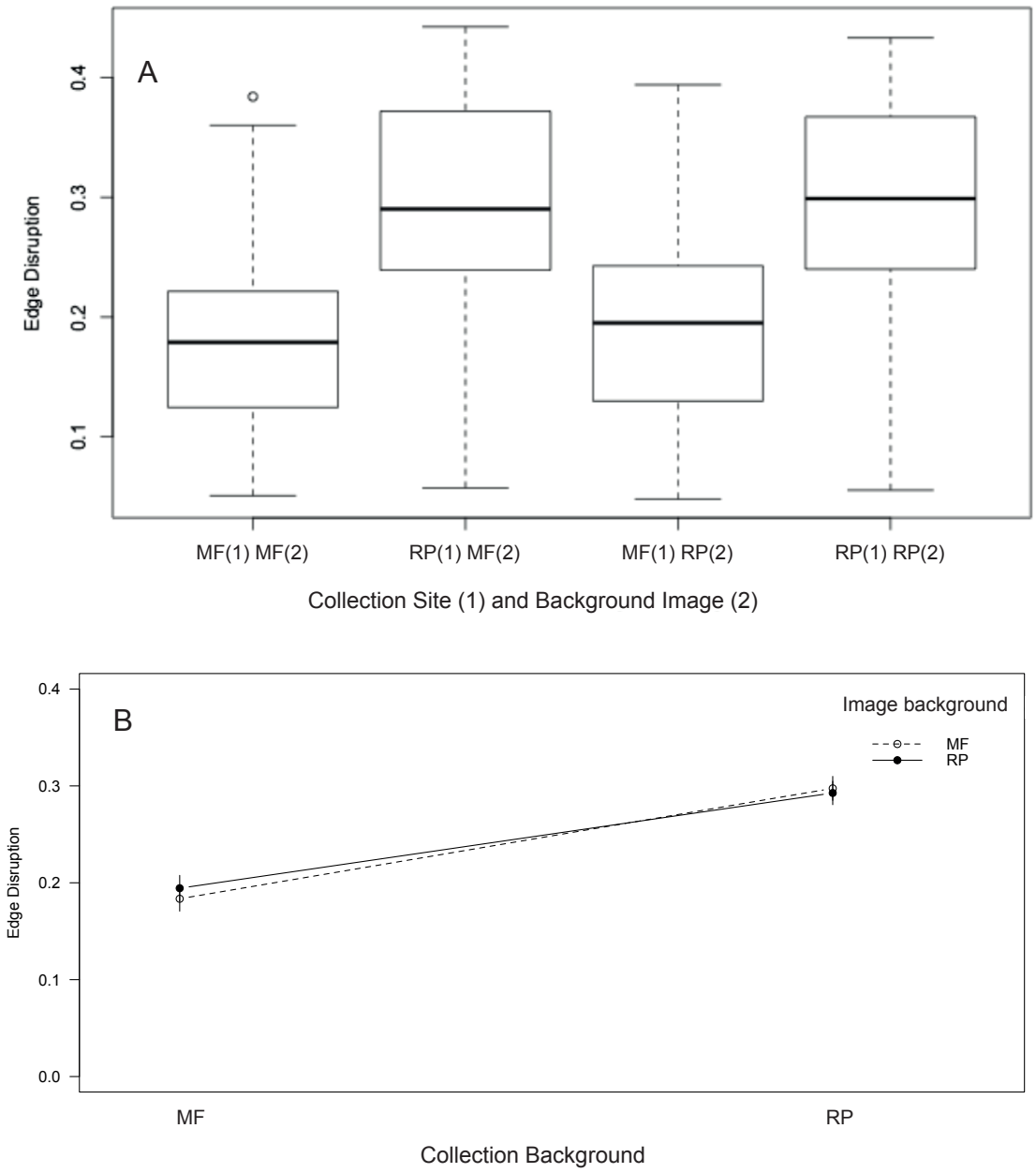


Figure 3.4: Plots show medians plus inter-quartile range(IQR), outliers are shown by a circle. SD error bars have been added to 3.4B. RP stands for rock pool and MF stands for mudflat for both collection habitat and image background. Graphs show the carapace edge disruption averages of juvenile crabs collected from either a rock pool or mudflat habitat and superimposed onto both background images - rock pool and mudflat. A) A box plot showing the range of data values in each data group B) An interaction plot showing the significant difference in edge disruption between collection sites on the X axis and the non significant difference between the image background levels. The significant interaction effect of collection background and image background can be seen between the lines plotted.

Adults

When performing the same analysis but on adult crabs, we found the same result. Indeed, adult rock pool crabs had significantly higher edge disruption than adult mudflat crabs. Adult crabs collected from rock pools superimposed onto a homogeneous mudflat background had the highest level of edge disruption, followed by adult rock pool crabs superimposed on a heterogeneous rock pool background. Likewise, for adults collected from mudflat sites, the edge disruption was higher on a rock pool substrate background than on a mudflat background (See figure 3.5; results table 3.1 for split plot ANOVA results). No significant interaction was found between the collection site and background image (See table 3.1).

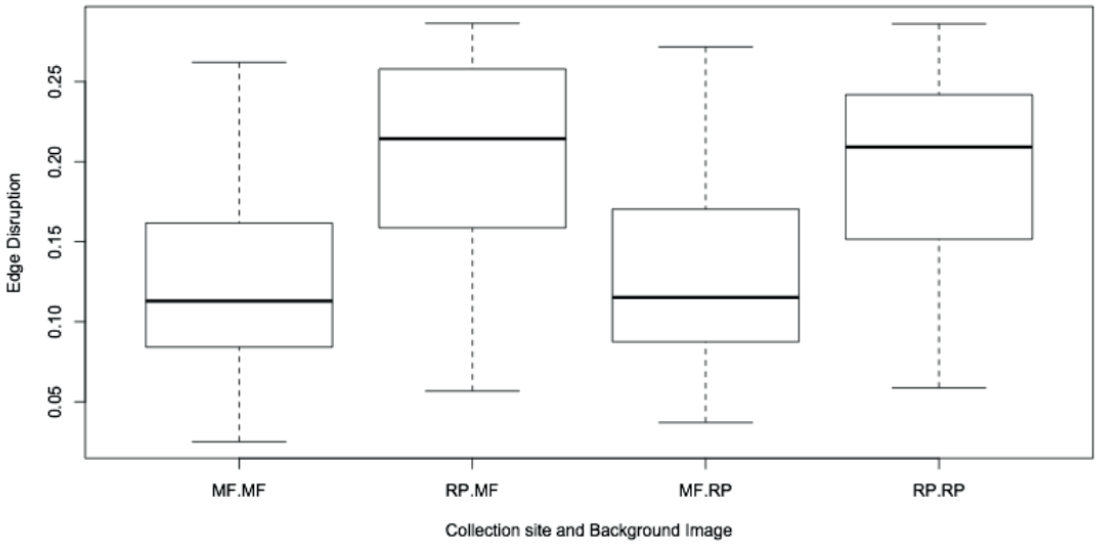


Figure 3.5: Boxplot shows the medians plus inter-quartile range (IQR). RP stands for rock pool and MF stands for mudflat, for both collection habitat and background image. The graph shows the carapace edge disruption averages of adult crabs collected from either a rock pool or mudflat habitat and superimposed onto both background images - rock pool and mudflat.

Camouflage: Background Matching

Is the level of background matching related to the habitat shore crabs are collected from?

To assess how well crabs matched their backgrounds, pattern energy difference (PED) values were used to determine the difference in pattern between two images, in this instance, the carapace of an individual and the background habitat image (rock pool or mudflat). This enabled a comparison of how well crabs from rock pools matched rock pool habitat backgrounds, and how well crabs from mudflats matched mudflat habitat backgrounds. Larger values indicate less similarity in pattern between the crab and its background, indicating a poorer camouflage match. A split plot 2 x 2 repeated measures mixed factorial ANOVA was used to assess the effect of individuals collected from one of two habitats on the level of background matching when superimposed onto both rock pool and habitat background images.

Juveniles

When assessing the match between the crab and the background of all juveniles from both mudflat and rock pool habitats, a significant interaction was found between the collection habitat of crabs and the image background ($F_{1,88} = 4.66$, $p < 0.05$). Indeed, the level of background matching between the carapace of individuals and the background was dependent on both the habitat the individual was collected from, and the background image it was superimposed onto. As illustrated in figure 3.6, the lowest pattern energy difference, and therefore the closest match to the background, was seen in crabs collected from mudflats and superimposed onto mudflat habitat images. Conversely, the largest difference between carapace and background, and therefore the lowest background matching camouflage, was seen in shore crabs collected from rock pools and superimposed onto a rock pool background, providing further support for our predictions that rock pool crabs are better camouflaged through disruptive patterning and mudflat crabs are better camouflaged through background matching.

We can also observe that there was a significant difference in the PED between image backgrounds ($F_{1,88} = 1209$, $p < 0.001$). For example, interestingly, rock pool individuals on mudflat backgrounds had significantly lower PED values and therefore better match the background than rock pool crabs on a rock pool background (see table 3.2 for ANOVA results).

Combined with the results for edge disruption, these findings demonstrate that, when superimposed onto their own background, shore crabs from mudflat habitats have less disruptive edges, and are better camouflaged through background matching. However, shore crabs from rock pool habitats, when superimposed onto their own habitat background, have more disruptive edges but lower background matching.

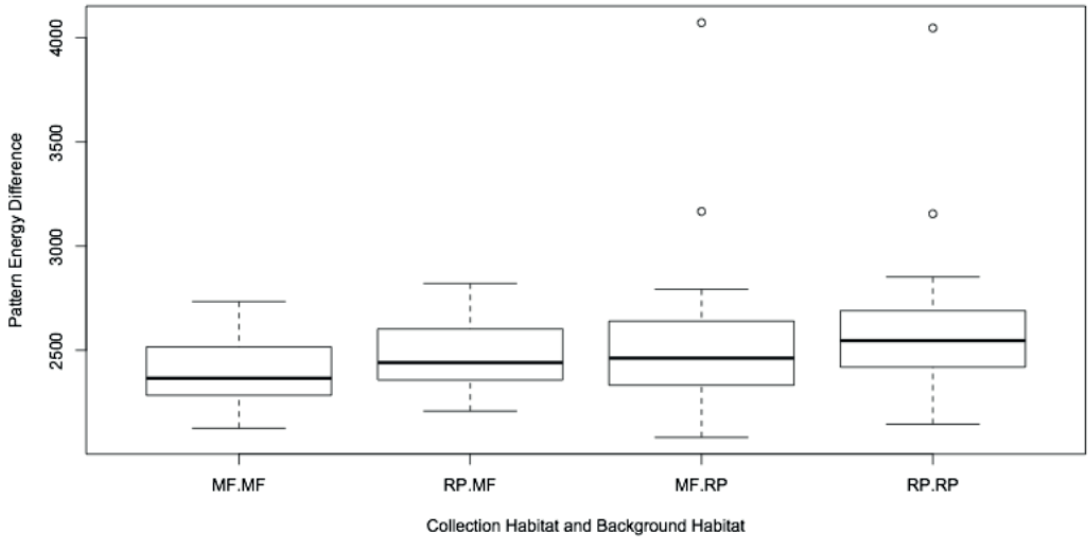


Figure 3.6: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. RP stands for rock pool and MF stands for MF, for both collection habitat and background habitat. A box plot graph to show the spread of data for the pattern energy difference (PED) values of juvenile crabs collected from either a rockpool or mudflat habitat and superimposed onto both background images - rockpool and mudflat.

Adults

Using the same analysis but for adult shore crabs, no significant interaction was found between the collection habitat and the background. However, independently as main effects, both collection habitat and background were found to have a significant effect on PED (see table 3.2 for ANOVA results). As demonstrated in table and figure 3.7, all adult crabs collected from mudflats had significantly lower PED when on both habitat backgrounds, than crabs collected from rock pool habitats. Furthermore, all crabs regardless of collection habitat, on the mudflat background had significantly lower PED than all crabs on the rock pool background. As you can see from figure 3.7, similar to juveniles, for adults, the lowest pattern energy difference, and therefore the closest match to the background was seen in crabs collected from mudflats and superimposed onto mudflat habitat images. Conversely, the largest difference between carapace and background, was seen in shore crabs collected from rock pools and superimposed onto a rock pool background.

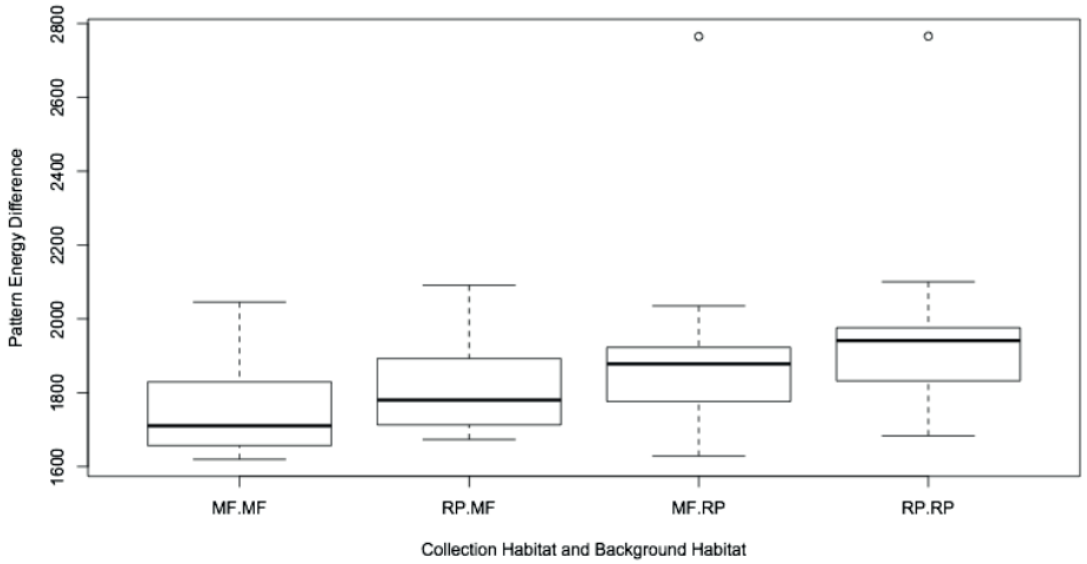


Figure 3.7: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. RP stands for rock pool and MF stands for MF, for both collection habitat and background habitat. A box plot graph to show the spread of data for the pattern energy difference (PED) values of adult crabs collected from either a rock pool or mudflat habitat and superimposed onto both background images - rock pool and mudflat.

Age and Habitat: Disruptive Patterning

Do juvenile and adult crabs from the same habitat differ in the amount of edge disruption?

Rockpool collection habitat

A split plot repeated measures ANOVA was used to assess whether juvenile and adult shore crabs collected from a rock pool habitat have significantly different amounts of edge disruption and whether this is also dependent on the background image they were superimposed onto. Homogeneity of variance was assessed using Levene's test and the assumption of sphericity was not necessary due to only two levels of repeated measures.

Results showed a significant difference in the level of edge disruption between juvenile and adult rock pool crabs ($F_{1,54} = 15.44$, $p < 0.001$). In line with previous research showing more pattern diversity in juvenile shore crabs, juveniles had significantly higher levels of edge disruption when superimposed onto both the heterogeneous rockpool and homogeneous mudflat background images than adult rock pool crabs on the same background images (see figure 3.8 & table 3.3 for mixed ANOVA results).

However, the split plot ANOVA found no significant difference between image backgrounds ($F_{1,54} = 0.21$, $p = 0.65$), as can be seen from figure 3.7, juvenile and adult rock pool crabs are significantly different to each other on both rockpool and mudflat backgrounds. However, no significant difference is shown between juvenile rock pool crabs on a rockpool and a mudflat background image. Likewise, no significant interaction was found ($F_{1,54} = 0.08$, $p = 0.77$) and this again can be seen in figure 3.8, as for both mudflat and rockpool background images, juvenile rockpool crabs have significantly higher edge disruption than adult rockpool crabs. The highest edge disruption values were collected from rockpool juveniles on mudflat backgrounds and the lowest values from rockpool adults on rock pool backgrounds.

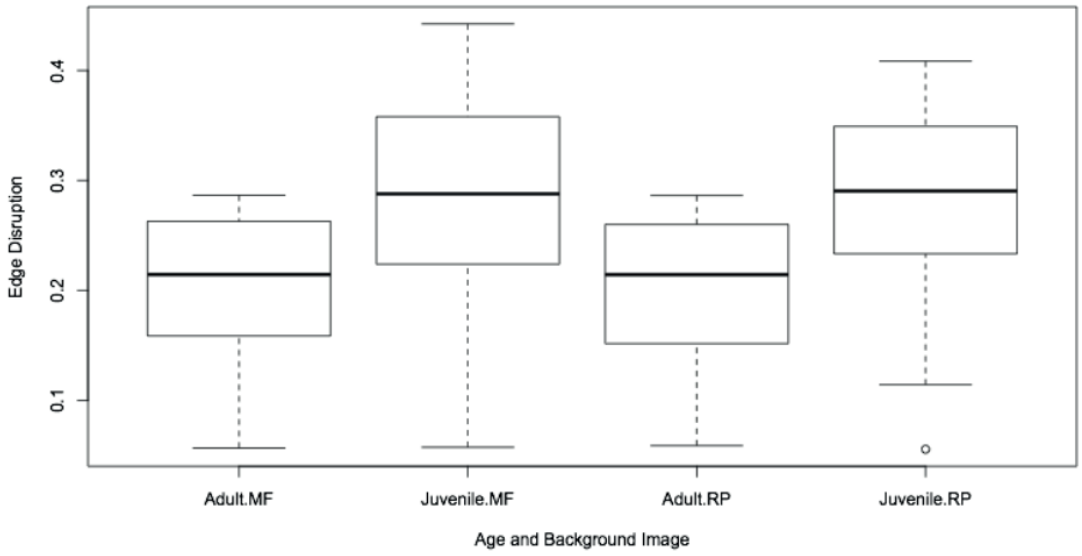


Figure 3.8: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. RP stands for rock pool and MF stands for mudflat, for the background habitat images. The box plot graph shows the carapace edge disruption averages of juvenile and adult crabs collected from a rock pool habitat and superimposed onto both background images - rock pool and mudflat.

Mudflat collection habitat

The same tests and assumptions were used in a separate 2 x 2 mixed factorial ANOVA model for juveniles and adults collected from mudflat habitats.

As expected, in line with our theory of alternative camouflage strategies existing between habitats, our results found no significant difference in the level of edge disruption between juvenile and adult crabs collected from mudflat habitats ($F_{1,48} = 1.82$, $p = 0.18$). This result supports the significantly lower edge disruption found when comparing rock pool and mudflat crabs, suggesting that both adult and juvenile shore crabs collected from mudflats, achieve camouflage through alternative mechanisms such as background matching, through which pattern markings do not change significantly between juvenile and adulthood stages of maturity.

However, a significant difference was found between all mudflat crabs on

rock pool and mudflat backgrounds ($F_{1,48} = 8.27$, $p < 0.01$), regardless of the age of individuals. Indeed, both adult and juvenile mudflat crabs had more disruptive patterning when superimposed onto rockpool background images than on mudflat background images, providing further support for earlier results and the theory of differential blending (Cott, 1940).

Age and Habitat: Background Matching

Do juvenile and adult crabs from the same habitat differ in the extent to which they match the habitat substrate (rock pool or mudflat)?

Rockpool collection habitat

We performed the same analysis for background matching, testing to see if juveniles and adults from the same rock pool habitat, differ in the amount to which they match the habitat substrate. Our results found a significant difference in the level of background matching between juvenile and adult rock pool crabs ($F_{1,79} = 95.44$, $p < 0.001$). Rockpool juveniles had significantly higher PED values on average, and therefore were not as well matched the their backgrounds, when superimposed onto both the heterogeneous rock pool and homogeneous mudflat background images, as adult rockpool crabs on the same background images (see figure 3.9 & table 3.4 for mixed ANOVA results). This finding corresponds to previous literature reporting a significant decline in carapace pattern in adult shore crabs in comparison to juveniles (Hogarth, 1978). As can be seen from figure 3.9, the individuals that best matched their background were adult rock pool crabs on a plain homogeneous mudflat background. However, even crabs from rockpools develop improved background matching as they mature, suggesting a switch in camouflage strategy with size.

The split plot ANOVA also found a significant interaction between the age of rockpool crabs and the image background they were superimposed onto ($F_{1,79} = 6.68$, $p < 0.005$). Indeed, although on both backgrounds juveniles have larger PED values than adults, both adult and juvenile rockpool crabs

have larger PED values when on rock pool backgrounds than when on mud-flat backgrounds (figure 3.9). The individuals that have the lowest match to the background are juvenile rock pool crabs on a rockpool background. This finding supports earlier results highlighting more edge disruption in juvenile rockpool crabs and suggests a difference in camouflage technique between rockpool and mudflat shore crabs.

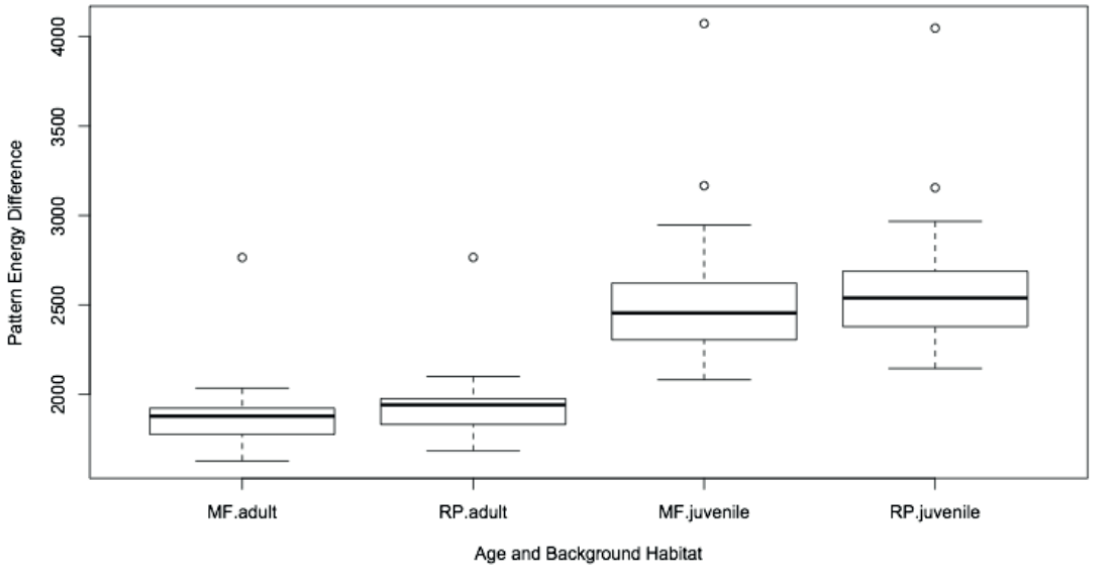


Figure 3.9: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. RP stands for rockpool and MF stands for mudflat with re-spect to background habitat image. Graphs show the PED averages of juvenile and adult crabs collected from a rock pool habitat and superimposed onto both background images - rockpool and mudflat. This box plot shows the range of data values in each age and treatment group.

Mudflat collection habitat

Our results also for mudflat crabs also found a significant difference in the level of background matching between juvenile and adult mudflat crabs ($F_{1,56} = 271.41$, $p < 0.001$). Mudflat juveniles had significantly higher PED values on average, and therefore were not as well matched to their backgrounds when superimposed onto both the heterogeneous rockpool and homogeneous mudflat background images as mudflat adult crabs on the same background images (see figure 3.10 & table 3.4 for mixed ANOVA results). This disparity amongst juveniles and adults is consistent across both rockpool and mudflat shore crabs, and therefore suggests that camouflage in shore crabs may improve with age. This is consistent with research showing that shore crabs are capable of changing pattern (chapter 2) and luminance (Easley et al, 2015 ; Stevens et al., 2014) over time.

However, the split plot ANOVA also found a significant interaction between the age of mudflat crabs and the image background they were superimposed onto ($F_{1,56} = 123.62$, $p < 0.001$). Indeed, although on both backgrounds, juveniles have larger PED values than adults, as can be seen from figure 3.10, both adult and juvenile mudflat crabs have larger PED values when on rock pool backgrounds than when on mudflat backgrounds. The individuals that have the lowest match to the background (highest PED value) are juvenile mudflat crabs on a rockpool background. Conversely, the best camouflage match to the background can be seen from mudflat adults on a mudflat background. This provides further support for our predictions that both the age of individuals and the habitat they originate from, affects how well disruptive patterning and background matching strategies enable camouflage, suggesting that differences in carapace pattern between juveniles and adults and rock pool and mudflat habitats are associated with the camouflage strategy used.

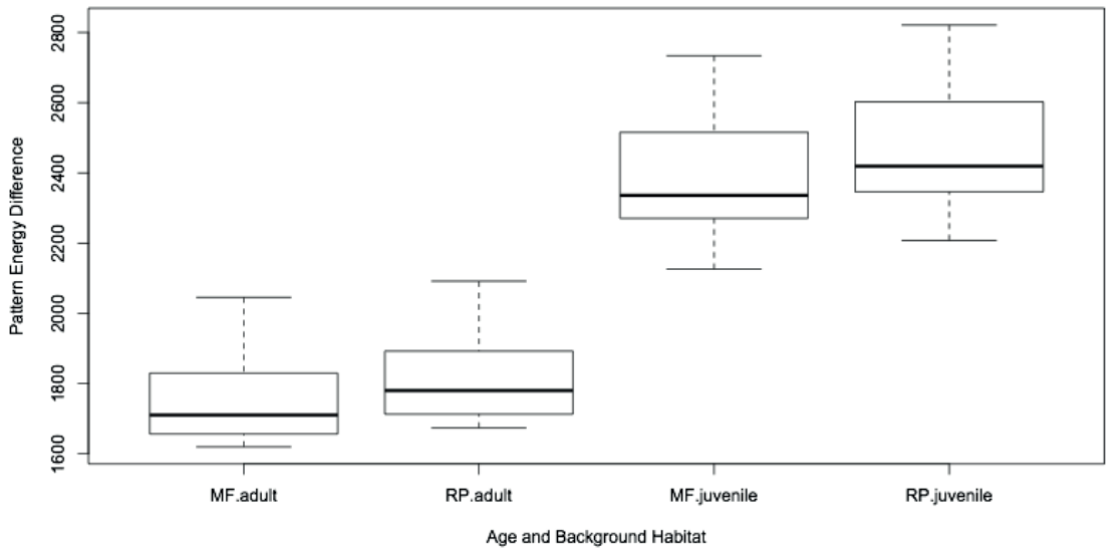


Figure 3.10: Plots show medians plus inter-quartile range (IQR), outliers are shown by a circle. RP stands for rock pool and MF stands for mudflat, with regards to background habitat image. Graphs show the PED averages of juvenile and adult crabs collected from a mudflat habitat and superimposed onto both background images - rockpool and mudflat. This box plot shows the range of data values in each age and treatment group.

Age	Effect	DFn	DFd	F	P
	Collection Site	1	84	29.99	**< 0.001**
Juveniles	Background Image	1	84	2.37	0.127
	Collection * Background Image	1	84	9.96	**< 0.005**
	Collection Site	1	46	16.65	**< 0.001**
Adults	Background Image	1	46	0.02	0.88
	Collection * Background Image	1	46	0.59	0.44

Table 3.1: F and P values for the main effects and interaction effect between collection site and background image for the two separate disruptive patterning mixed ANOVA models we ran on juvenile shore crabs and adult shore crabs.

Age	Effect	DFn	DFd	F	P
	Collection Site	1	88	3.02	0.085
Juveniles	**Background Image**	1	88	1209.76	**< 0.001**
	Collection * Background Image	1	88	4.66	**< 0.05**
	Collection Site	1	56	7.94	**< 0.01**
Adults	**Background Image**	1	56	1443.85	**< 0.001**
	Collection * Background Image	1	56	0.26	0.60

Table 3.2: F and P values for the main effects and interaction effect between collection site and background image for the two separate background matching mixed ANOVA models we ran on juvenile shore crabs and adult shore crabs.

Collection Site	Effect	DFn	DFd	F	P
	Age	1	48	1.82	0.18
Mudflat	**Background Image**	1	48	8.27	**< 0.01**
	Age * Background Image	1	48	0.23	0.62
	Age	1	54	15.43	**< 0.001**
Rock pool	Background Image	1	54	0.20	0.65
	Age * Background Image	1	54	0.08	0.77

Table 3.3: F and P values for the main effects and interaction effect between Age and background image for the two separate edge disruption mixed ANOVA models we ran on crabs collected from mudflat habitats and crabs collected from rock pool habitats.

Collection Site	Effect	DFn	DFd	F	P
	Age	1	56	271.41	< 0.001
Mudflat	Background Image	1	56	4135.51	< 0.001
	Age * Background Image	1	56	123.62	< 0.001
	Age	1	79	95.44	< 0.001
Rock pool	Background Image	1	79	483.49	< 0.001
	Age * Background Image	1	79	6.68	< 0.005

Table 3.4: F and P values for the main effects and the interaction effect between age and background image for the two separate background matching mixed ANOVA models we ran on crabs from mudflat habitats and crabs from rock pool habitats.

Discussion

In this chapter I assessed the use of two different camouflage strategies (background matching and disruptive patterning) between adult and juvenile shore crabs collected from rock pool and mudflat habitats. I used edge disruption values and pattern energy differences between the crabs and the backgrounds to quantify these strategies.

In line with our predictions, there was a clear difference in background similarity and edge disruption, depending on whether crabs were collected from rock pools or mudflats. When analysing the edge disruption of juvenile and adult shore crabs through avian vision, I found that both adult and juvenile crabs collected from heterogeneous rock pools, had a higher ratio of false edges when superimposed onto both rock pool and mudflat backgrounds, than crabs collected from homogeneous mudflat habitats. Amongst juvenile shore crabs, the ratio of false edges was also significantly different between background images. For example, juvenile rock pool individuals superimposed onto a mudflat background had more false edges than those same individuals on a rock pool background. A higher ratio of false edges increases the disruption around the outline of the crab carapace, and is predicted to reduce detection by predators through the camouflage strategy of disruptive colouration (Troscianko et al, 2017).

The background matching analyses found that both adult and juvenile crabs collected from homogeneous mudflat habitats were better matched to the mudflat background they were collected from than rock pool adults and juveniles were to both rock pool and mudflat backgrounds. I found that rock pool adult and juvenile crabs superimposed onto a rock pool background, had the lowest level of background matching and mudflat crabs (adults and juveniles) superimposed onto mudflat backgrounds had the highest match to the background. Interestingly, these results also indicated that adults from mudflat habitats superimposed onto mudflat backgrounds, were significantly better matched than juveniles from the same habitat, superimposed onto the same background.

From these results we can clearly see that rock pool individuals do not

match their own habitat or mudflat habitats as well as mudflat individuals and also, mudflat individuals do not have as disruptive edges as rock pool individuals. These findings support our predictions that differences in camouflage strategy exist between shore crabs from mudflat and rock pool habitats.

These results support earlier work investigating the cryptic function of pattern polymorphism in shore crabs, such as that of Todd et al. (2006) and Stevens et al. (2014) who established phenotype - environment associations in shore crabs and discovered that shore crabs from plain homogeneous habitats had less carapace pattern than crabs from heterogeneous habitats. However, neither of these studies directly measured camouflage itself or the camouflage strategies, specifically background matching and disruptive colouration.

Shore crabs are a widely distributed species, found amongst habitats with very different substrate backgrounds. It is possible that shore crabs experiencing heterogeneous environments, with several different patterned and coloured substrates, such as rock pools, would benefit from a less specialised camouflage strategy which may not fully require the matching of a particular colour or pattern (Stevens et al. 2006; Schaefer & Stobbe 2006; etc). The disruption of carapace edges could provide the main mechanism of camouflage for rock pool crabs, reducing the ability of predators to detect individuals across several substrates. This mechanism would allow for temporal and spatial changes in a heterogeneous environment, by breaking up the outline of the crab carapace rather than focusing on matching a particular colour or pattern. White spots have been observed on the edges of patterned crab carapaces (Todd et al, 2006) previously, resulting in the suggestion that these markings may provide crypsis through disruptive patterning. However, there has previously been no quantification of this camouflage method in shore crabs and its association to heterogeneous habitats.

The significant difference in the ratio of false edges between rock pool collected individuals and mudflat individuals, supports our theory that the difference in pattern diversity between these two habitats could be explained by a difference in camouflage strategy used by shore crabs from these different habitats. However, the ratio's for rock pool crabs, obtained from these results are not extremely large. It must therefore be considered that although it is clear

that rock pool crabs have more disruptive edges, the disruptive effect might not be the only or main method reducing conspicuousness for these individuals . Diverse pattern markings contribute significantly to the background matching of shore crab carapaces (Todd et al, 2015) and it is possible that these diverse pattern formations also reduce the ability of predators to detect individuals, by hindering the formation of a search image (Bond & Kamil, 2002, 2006; Krause - Nehring et al, 2010; Surmacki et al, 2013). The higher levels of pattern diversity found on rock pool crabs may disrupt edges and inhibit predatory birds from easily detecting the shape and colour of the crabs as prey items.

It has been suggested that disruptive patterning has evolved as a successful form of camouflage because the edge detecting neurons of predators are unable to process and register the true form of the organism (Osorio and Srinivasan, 1991; Stevens and Cuthill, 2006; Troscianko et al., 2009). However, it is also likely that crabs collected from rock pools, with a higher diversity in pattern, remain cryptic due to both disruptive edges and an element of carapace pattern that matches the background. Research does suggest that patterned crabs are cryptic on polychromatic, complex backgrounds (Palma & Steneck, 2001). Indeed, experiments using humans as predators and computer-generated ‘crabs’ discovered that patterned morphs were recorded as more difficult to detect and revealed better survivorship even when the spot sizes did not match the background entirely (Todd et al, 2015) or spot sizes were larger than background spot (Ben Toh & Todd, 2017). This indicates that even a small degree of background matching or perhaps more patterning in general regardless of the match to the background, may provide camouflage benefit for crabs from heterogeneous environments.

Disruptive coloration has been suggested to act independently of background matching in some species, for example in predation experiments with paper moths (Schaeffer & Stobbe, 2006). However, here we use a more advanced method for quantifying disruptive patterning and a real prey species, and it is possible that in the highly diverse shore crab, that background matching and disruption are interdependent, and a morph with generic colouration to partially match the background substrate, alongside disruptive edges is required in order to provide optimum camouflage in a heterogeneous environment.

Nevertheless, this study is the first to objectively quantify disruptive edges in shore crabs as a method of camouflage, using some of the most advanced methods available. To further demonstrate the relative impact of these disruptive edges as a camouflage mechanism, it would be interesting for future studies to carry out predation experiments to test the effectiveness of disruptive edges on the survival of shore crabs.

The results regarding crabs collected from mudflats also fit our hypothesis. The association between shore crab pattern and habitat background has already been examined (Todd et al, 2006; Stevens, Lown & Wood, 2014), with results showing that shore crabs from homogeneous habitats show much less diversity in carapace pattern. None, however, have directly quantified the level of background matching in comparison with heterogeneous habitats, or compared both background matching and disruptive edges in the same individuals. Indeed, quantification of camouflage match in real animals is still very rare, with most studies undertaking subjective assessments or working with model prey (but see for example Stevens et al., 2015; Stevens et al., 2017).

In these analyses, I discovered that there was a clear difference in the match between carapace and habitat substrate for individuals collected from mudflats in comparison to individuals collected from rock pools. Indeed, these results build upon the association studies of Todd et al (2006) and Stevens et al (2014), demonstrating that the less patterned appearance of shore crabs collected from homogeneous habitats (Todd et al, 2006; Stevens et al, 2014) better matches the mudflat substrate than crabs with more patterned carapaces from rock pool habitats. This result is very exciting as it suggests that the differences in carapace colouration and pattern of shore crabs are not only specific to different habitats but are also related to a difference in camouflage strategy adopted. This finding is novel and extremely important in gaining a deeper understanding of the purpose of shore crab pattern markings.

The amount to which shore crabs from mudflats matched their background and had significantly fewer false edges (causing disruption) in comparison to rock pool crabs, could lead us to assume that individuals from this homogeneous environment have a much more specialist phenotypic appearance, which may have arisen due to factors in the environment, such as a lack of pro-

tective cover. Subjectively, when collecting crabs from both habitats there are some quite clear differences. For example, mudflats are uniform in colour, with very few areas to hide other than to submerge themselves in the mud and blend in. The threat of predation could therefore have selected for a specialist uniform phenotype, using camouflage solely, due to the inability to seek protection from rocks, crevices and sand, as found in rock pool habitats. However it could also be explained by the uniformity of the mudflat habitat, indeed for mudflat habitats there is no ambiguity over the best colour or pattern to match. Conversely, in heterogeneous habitats, the complexity of the substrate would likely make it difficult to blend in with the background. It appears that in these heterogeneous environments, the appropriate response would be to match the most common substrate (Michalis et al, 2017).

These findings show that shore crabs collected from homogeneous mudflats better match their habitat background than crabs from rock pools, and also have less disruptive patterning. Overall, mudflat crabs were significantly better matched to mudflat backgrounds than rock pool backgrounds and that rock pool crabs had the lowest match to rock pool backgrounds. What is surprising, however, is that rock pool crabs had the most disruptive edges when superimposed onto mudflat backgrounds. Given that background complexity is thought to affect disruption and therefore detection by predators (Merilaita et al, 1999, 2001; Merilaita, 2003; Dimitrova & Merilaita, 2010, 2014; Xiao & Cuthill, 2016), we would expect disruption to be highest for rock pool crabs on a heterogeneous, more complex, rock pool background. A possible explanation for this may be that rock pool crabs in homogeneous habitats, cannot match the substrate well and so disruptive colouration could possibly be more effective in both hindering the search image of predators and disrupting the outline of the carapace. In addition, this finding does provide further support for the theory that disruptive patterning is a distinct camouflage strategy that is not reliant on matching the background (Webster et al, 2013) and therefore assists our hypothesis that crabs from heterogeneous environments with complex substrates would benefit from disruptive patterning to break up the outline of the carapace rather than attempting to match the background.

When analysing the effect of the background image on edge disruption, we also found that whilst crabs collected from mudflats did have significantly

lower edge disruption than rock pool crabs, the disruption of edges was higher for mudflat crabs on rock pool backgrounds than on mudflat backgrounds. This finding provides further support for the suggested theory of a specialist phenotype in mudflat crabs and the effectiveness of background matching in a homogeneous environment. We suggest that the increase in disruptiveness for mudflat crabs on rock pool backgrounds is probably explained by differential blending (Cott, 1940), a sub principle of disruptive colouration (Stevens & Merilaita, 2009) where at least some of the carapace markings will blend into the complex heterogenous background, and others will stand out, destroying the carapace outline more than on a homogeneous background.

When interpreting these results however, the invasiveness and movement ability of shore crabs should be addressed. Shore crabs are highly mobile, and can travel up to 2km in a short amount of time. The extent to which crabs move on different backgrounds, will therefore influence the effectiveness of the camouflage strategy employed. Mobility will affect the camouflage strategy on both habitats, and so further support for differences in camouflage strategy between these habitats would involve building on studies similar to that of Todd et al (2012), quantifying the movement of individuals across microhabitats within each rockpool and mudflat habitats.

Throughout our analyses, there were significant differences found between adult and juvenile shore crabs. In our disruptive colouration analyses, our results revealed that rock pool juvenile crabs, had significantly higher edge disruption than adult crabs and in our background matching analyses, mudflat juveniles were not as well matched to their mudflat backgrounds as adult mudflat individuals were. In common with other decapod crustaceans (Palma & Steneck, 2001; Palma et al. 2003), research has previously shown that carapace patterns in the shore crab become less distinct with age (Hogarth, 1978) and therefore this reduction in pattern could be responsible for the increase seen in the match between carapace and background in mudflat adults in comparison to mudflat juveniles. This improvement in background matching with age, suggests that colour change and plasticity, even with growth, allows improved camouflage.

It has been suggested that the loss of pattern with maturity could be

caused by differential visual predation removing patterned crabs progressively (only less patterned juveniles survive), movement of older crabs into new habitats, or due to ontogenetic changes, with crabs naturally losing pattern with age and an increase in size (Hogarth, 1978 ; Todd et al, 2006 ; Crothers, 1968 ; Bedini, 2002). These results, combined with previous studies showing plasticity in shore crab pattern and luminance (Easley, 2015 ; Stevens et al. 2014 , chapter 2) I believe, provide support for the latter. Indeed, the disruption of the carapace outline as quantified through predatory bird vision should increase survival and therefore be selected and so patterned crabs should not be removed from the population. However, as crabs mature and grow, we would expect the susceptibility to predation to decrease (Todd et al., 2009) as adults become less vulnerable and are more robust to attacks from predators. The necessity to invest energy in the expression of carapace pattern for disruptive camouflage may therefore not be as important in adults as in juveniles, this may explain the more disruptive edges in juvenile shore crabs than in adults. Or perhaps, a trade off may exist between camouflage strategy and other functions. This is certainly an area that would benefit from further research.

It is clear from our results that background matching is most efficient for crabs from homogeneous habitats such as mudflats and that crabs from complex heterogeneous habitats (rock pools) are not as well matched to their backgrounds and instead, have more disruptive edges. This distinction in camouflage strategy and connection with habitat type could have arisen through several factors. Background choice is one suggestion given that shore crabs have been known to move up to 2km in a short space of time (Ameyaw-Akumfi & Naylor, 1987). Indeed, shore crabs may be moving to a habitat that resembles their general appearance, i.e. less patterned crabs moving to homogeneous mudflat habitats and more patterned crabs moving to more complex habitats where their patterns will enable the carapace outline to become disrupted. However, it is more likely that the distinction is a result of more than one factor, possibly a combination of phenotypic plasticity, which has been demonstrated in shore crabs, through colour and pattern change in controlled experimental conditions (Stevens et al., 2014; Easley et al., 2015) and potentially background choice.

Aside from cephalopods (Hanlon et al., 2008), there has been limited research into the relationship between body pattern variation, environmental background, and camouflage mechanism. This study used predator vision to provide the first quantitative analysis of both disruptive patterning and background matching in shore crabs, revealing a significant association between camouflage mechanism and habitat type.

Chapter 4: General Discussion

Collecting crabs - Helford mudflats

This thesis has explored the mechanisms behind which variation in European green shore crab *(Carcinus maenas)* appearance may reduce conspicuousness across habitats. Specifically, I first tested one of the proposed theories to explain colour change in shore crabs and several other animals ; phenotypic plasticity, based on the proposition that shore crabs may also show plasticity in carapace pattern as well as colour. I then applied these findings to shore crabs in their natural habitat, to test the difference in effectiveness of two different camouflage strategies; background matching and disruptive colouration, in providing concealment from predators, across rockpool and mudflat habitats.

In chapter 2, I found that under controlled experimental conditions, shore crabs exhibit phenotypic plasticity which extends beyond the previously discovered ability to change luminance to better match black and white backgrounds (Stevens et al., 2014; Easley et al., 2015). I found that shore crabs collected from mudflat habitats exhibited the most significant increase in carapace pattern energy when subjected to a uniform background treatment, indicating that the change in pattern is affected by habitat, treatment background, and of course, time. This increase in pattern energy was also found to increase the level of background matching camouflage from the start to the end of the experiment, for those individuals on the uniform backgrounds. An increase in pattern contrast resulting in improved background matching has been studied under experimental conditions in other species, but this has mainly been short term plasticity in species such as cephalopods. Long term pattern change and plasticity for camouflage has not previously been studied in shore crabs and therefore this result is exciting. However, what is intriguing is the lack of change quantified from shore crabs on the patterned background treatment. In my opinion, the best way to answer this would be to focus on understanding how shore crabs sense their environment. Animals process visual information differently, and this is largely dependent on the visual system the animal possesses. The little work that has been done on shore crab vision is inconclusive (Wald, 1968; Bruno et al., 1973) and therefore we currently do not know how crabs perceive their surroundings. It is possible that the visual system of *Carcinus maenas* does not include colour constancy and as a result the perception of the gravel colours are changed between different illuminants (Hulbert, 2007; Chitka et al., 2014) or perhaps that they do not see in colour. Indeed, animals capable of achieving

outstanding camouflage, for example cuttlefish, respond to changes in brightness in the environment rather than colour (Barry et al., 2014). I suggest in the discussion of chapter 2, that the artificial lighting under which the experiments were conducted, may have resulted in the appearance of shadows amongst the gravel background. It is possible that shore crabs do not respond to colour cues, and instead they use achromatic contrasts or markings in their natural habitat. The presence of artificial light creating shadows may have disrupted the natural response to these achromatic backgrounds, particularly on the patterned treatment background which may have appeared much more complex and difficult to become camouflaged against through background matching.

In addition, our analyses found a significant link between the increase in pattern contrast and the number of moults. Significant change in brightness during moulting has been subjectively recorded in shore crabs in previous studies, but rarely has this link been quantified, particularly with regards to pattern. Shore crabs that moulted two or more times showed a larger increase in pattern contrast than those that moulted once or not at all. Due to the variation in the starting size of juveniles used in this study, it is possible that individuals which were larger and therefore only moulted once, were limited in their capacity for change in pattern. A repeated experimental study over a longer period, allowing more moults to occur per individual, would potentially reveal more about shore crab plasticity and background matching associations. Colour change is facilitated by moulting in many species including other species of crabs (Stevens, 2016) and caterpillars (Noor & Parnell, 2008), understanding more about the capacity for change through morphological processes like moulting could enable us to understand its pertinence and discover if certain environmental pressures may induce moulting for camouflage purposes.

In chapter 3, I found that the diversity in shore crab pattern, results in different camouflage strategies between crabs of different ages and from different habitats. Rock pool crabs, both adults and juveniles, displayed pattern markings which broke up the outline of the carapace, resulting in more false edges than crabs from mudflat habitats. Conversely, mudflat crabs, both adults and juveniles had a significantly better match to their natural mudflat background than crabs from rock pools.

Shore crabs are widely distributed across habitats which differ substantially in substrate, as a result, crabs distributed across these habitats are likely to require difference camouflage strategies. The heterogeneity of rock pool habitats, suggests that background matching would make it difficult to achieve camouflage accurately (Merilaita et al., 1999, Merilaita, 2003). Studies assessing the difference in pattern of shore crabs between habitats have already shown that rock pool individuals have more diversity in pattern (Todd et al., 2006, 2009; Stevens et al, 2014b) than mudflat individuals. Our findings suggest that these markings on rock pool individuals are helping to create the perception of false edges, in order to break up the outline of the carapace and inhibit detection (Cuthill et al., 2005, Stevens and Cuthill, 2006).

I suggest that false edges have been selected for in shore crabs inhabiting rock pool areas, due to the variety of colours and patterns found in both the rock pool substrates and the carapace of shore crabs, which may hinder the detection ability of predators, causing them to focus on alternative identification cues such as the body outline and shape of the crab. As a result, patterns that break up this outline and hinder detection would be selected for. Indeed, in some animals, for example primates, studies have shown that fast visual detection does not depend upon colour but instead information on the edge outline of the body is far more important (Delorme et al., 2000 ; Elder and Velisavljevic, 2009).

It is likely that the patterns exhibited amongst rock pool crabs, do provide an element of background matching. However, this is probably not the primary mechanism of camouflage compared to that of mudflat crabs. The specific background matching mechanism and lack of disruptive edges in mudflat crabs indicates that a specialist phenotype (plain with less pattern markings) is advantageous in this environment in order to remain camouflaged and reduce detection.

Our chapter 3 analyses are based on the most advanced and accurate (Troscianko et al., 2017) approach for quantifying disruptive colouration through the eyes of the predator. Given that several animals habituate complex heterogeneous environments, it is likely that disruptive colouration is more common than we originally thought and that indeed, background matching is less ef-

fective in these environments (Merilaita, 1999). In conjunction with predation experiments, these techniques and additional studies like this, could be vital in helping us understand the function of phenotypic variation and the evolution of two very different camouflage strategies.

Future Research

The most exciting and novel finding from this thesis is the difference in camouflage strategy found between shore crabs from rock pools and mudflats. Prior to this work, few studies have quantified background matching using live animals and even fewer have quantified disruptive colouration. Our findings provide an exciting platform for further study, to address the survival advantage gained from each strategy across different habitats. Specifically, I suggest that future work builds on these results by using predation experiments to quantify the camouflage benefit of these two strategies, in order to better understand the evolution of such differences between habitats and between the ages of individuals using these strategies.

It would also be extremely useful to build upon the findings from our first chapter (2), by extending the amount of time studying shore crabs in the laboratory. This study is one of the longest experimental studies of shore crabs, as very few have kept subjects in experimental conditions for durations longer than 12 weeks. To gain a deeper understanding of phenotypic plasticity in shore crabs, the next step would be to study them from birth to adulthood, on samples of their own natural habitat, on different habitat backgrounds, and under more natural lighting conditions. This would allow us to quantify changes in pattern and luminance from birth, across several moults, in order to establish if there is a connection between this change over time, the habitat of individuals and their stage of maturity.

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Bibliography

Abramowitz, A. (1937) The Chromatophorotropic Hormone of the Crustacea: Standardization, Properties and Physiology of the Eye-Stalk Glands. *The Biological Bulletin.* 72(3):344-365.

Allen, J. J., Mathger, L. M., Barbosa, A., et al. (2010) Cuttlefish dynamic camouflage: responses to substrate choice and integration of multiple visual cues. *Proc R Soc B.* 277:1031–1039.

Amaral, V., Penha-Lopes, G., Paula, J. (2009) Effects of vegetation and sewage load on mangrove crab condition using experimental mesocosms. *Estuarine, Coastal and Shelf Science.* 84(3):300-304.

Ameyaw-Akumfi,C. & Naylor, E. (1987) Spontaneous and induced components of salinity preference behaviour in *Carcinus meanas. Mar. Ecol. Prog. Ser.* 37:153-158.

Anderson, J., Spadaro, J., Baeza, J.A., & Behringer, D. (2013) Ontogenetic shifts in resource allocation: colour change and allometric growth of defensive and reproductive structures in the Caribbean spiny lobster (Panulirus argus). *Biological Journal of the Linnean Society.* 108(1):87–98.

Bagnara, J. T., & Hadley, M. E. (1973) Chromatophores and color change; the comparative physiology of animal pigmentation. *Englewood Cliffs, N.J., Prentice-Hall.*

Bagnara, J.T., Matsumoto, J. (2006) Comparative anatomy and physiology of pigment cells in nonmammalian tissues. In The pigmentary system: physiology and pathophysiology. *2nd edn (eds Nordlund JJ, Boissy RE, Hearing VJ, King RA, Oetting WS, Ortonne J-P.). Oxford, UK: Blackwell.*

Barbosa T.M., Garrido M.F., & Bragada J.A. (2007) Physiological adaptations to

head-out aquatic exercises with different levels of body immersion. *Journal of Strength and Conditioning Research.* 21:1255-1259.

Barbosa, A., Mathger, L., Buresch, K., Kelly, J., Chubb, C., Chiao, C., & Hanlon, R. (2008). Cuttlefish camouflage: The effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. *Vision Research.* 48(10):1242–1253.

Barry, K. L., White, T. E., Rathnayake, D. N., Fabricant, S. a., & Herberstein, M. E. (2014) Sexual signals for the colour-blind: cryptic female mantids signal quality through brightness. *Functional Ecology.* 29:531–539.

Bedini, R. (2002) Colour Change and Mimicry from Juvenile to Adult: Xantho poressa (Olivi, 1792) (Brachyura, Xanthidae) and Carcinus maenas (Linnaeus, 1758) (Brachyura, Portunidae). *Crustaceana*. 75(5):703-710.

Belk, M. and Smith, M. (1996) Pelage coloration in oldfield mice (Peromyscus polionotus): Antipredator adaptation?. *Journal of Mammalogy.* 77(3):882-890.

Ben Toh, K. and Todd, P. (2017) Camouflage that is spot on! Optimization of spot size in prey-background matching. *Evolutionary Ecology.* 31(4):447– 461.

Bond, A.B. (2007) The evolution of color polymorphism: crypticity, searching images, and apostatic selection. *Annual Review of Ecology, Evolution, and Systematics.* 38:489–514.

Bond, A.B., & Kamil, A.C. (2002) Visual predators select for crypticity and polymorphism in virtual prey. *Nature.* 415(6872):609-13.

Bond, A.B., & Kamil, A. C. (2006) Spatial Heterogeneity, Predator Cognition, and the Evolution of Color Polymorphism in Virtual Prey. *Proceedings National Academy of Science.* 103:3214-19.

Booth, C. (1990). Evolutionary significance of ontogenetic colour changeinanimals. *Biological Journal of the Linnean Society.* 40(2):125-163.

Boratyński, Z., Britto, J., Campos, J., Karala, M. and Mappes, T. (2014) Large spatial scale of the phenotype-environment color matching in two cryptic species of African desert jerboas (Dipodidae: Jaculus). *PLoS ONE.* 9(4).

Briffa, M., Haskell, P., & Wilding, C. (2008) Behavioural colour change in the hermit crab Pagurus bernhardus: reduced crypticity when the threat of predation is high. *Behaviour.* 145(7):915-929.

Brown, F.A., Jr. and Sandeen, M.I. (1948) Responses of the chromatophores of the fiddler crab, Uca, to light and temperature. *Physiological Zoology*. 21(4):361-371.

Bruno, M.S., Mote, M.I., & Goldsmith, T.H. (1973) Spectral absorption and sensitivity measurements in single ommatidia of the green crab Carcinus. *J. comp. Physiol.* 82.

Caro, T. (2005) Antipredator Defenses in Birds and Mammals. *University of Chicago Press.*

Caro, T. (2005). The Adaptive Significance of Colouration in Mammals. *BioScience.* 55(2):125-136.

Caro,T. (2016) Wallace on Colouration: Contemporary Perspective and Unresolved Insights. *Trends in Ecology & Evolution*. 32 (1):23-30.

Carvalho, L., Zuanon, J. and Sazima, I. (2006). The almost invisible league: crypsis and association between minute fishes and shrimps as a possible defence against visually hunting predators. *Neotropical Ichthyology.* 4(2):219-224.

Chiao, C.C., Chubb, C., Buresch, K., Siemann, L., & Hanlon, R.T. (2009) The scaling effects of substrate texture on camouflage patterning in cuttlefish *Vision Research.* 49:1647-1656.

Chiao C.-C., Kelman E.J., & Hanlon R.T. (2005) Disruptive body pattern of cuttlefish (Sepia officinalis) requires visual information regarding edges and contrast of objects in natural substrate backgrounds. *Biol. Bull.* 208:7–11.

Cohen, A.N.,Carlton, J.T., & Fountain, M.C., (1995) Introduction, dispersal and potential impacts of the green crab Carcinus maenas in San Francisco Bay, California. *Marine Biology.* 122(2):225-238.

Cooper, W.E., & Greenberg, N. (1992) Reptilian coloration and behavior. In: Gans C, Crews D, editors. Biology of the Reptilia. *Chicago University Press.*

Cuthill I.C., Hiby E., & Lloyd E. (2006) The predation costs of symmetrical cryptic coloration. *Proc. R. Soc. B.* 273:1267–1271.

Cuthill, I.C., Stevens, M., Sheppard, J., Maddocks, T., Parraga, C., & Troscianko, T. (2005) Disruptive coloration and background pattern matching. *Nature*. 434:72-74.

Cuthill, I.C., Stevens, M., Windsor, A.M.M., & Walker, H.J. (2006) The effects of pattern symmetry on detection of disruptive and background-matching coloration. *Behavioral Ecolog.* 17(5):828–832.

Crothers, J.H. (1966) Dale Fort marine fauna, *2nd edition. Fld Stud*., 2(supplement):169.

Crothers, J.H. (1968) The biology of the shore crab Carcinus maenas (L.): The life of the adult crab. *Field Studies*. 2:579–614.

Darling, J., Bagley, M., Roman, J., Teolt, C., & Geller, J. (2008). Genetic patterns across multiple manipulations of the globally invasive crab genus Carcinus. *Mol Ecol.* 17(23):4992-5007.

Darst, C., Cummings, M., & Cannatella, D. (2006) A mechanism for diversity in warning signals: conspicuousness versus toxicity in poison frogs. *Proc Natl Acad Sci*. 103:5852–5857.

Defrize, J., Théry, M., & Casas, J., (2010) Background colour matching by a crab spider in the field: a community sensory ecology perspective. *Journal of Experimental Biology.* 213.

Delorme, A., Richard, G., & Fabre-Thorpe, M. (2000) Ultra-rapid categorisation of natural scenes does not rely on colour cues: a study in monkeys and humans. *Vision Res.* 40:2187–2200.

Detto, T., Hemmi, J., & Backwell, P. (2008). Colouration and Colour Changes of the Fiddler Crab, Uca capricornis: A Descriptive Study. *PloS ONE.* 3(2):1629.

Dimitrova, M. and Merilaita, S. (2010). Prey concealment: visual background complexity and prey contrast distribution. *Behavioural Ecology.* 21(1):176-181.

Dimitrova, M., & Merilaita, S. (2014). Hide and seek: properties of prey and background patterns affect prey detection by blue tits. *Behavioural Ecology.* 25(2):402-408.

Duarte, R.C., Flores, A.A.V., & Stevens,M. (2017) Camouflage through colour change: mechanisms, adaptive value and ecological significance. *Philos Trans R Soc Lon B Biol Sci.* 372(1724).

Easley, J. (2015) Phenotype-environment matching and colour change for camouflage in the shore crab Carcinus maenas. *unpublished.*

Eggleston, D.B., & Armstrong, D.B. (1995) Pre-and post-settlement determinants of estuarine dungeness crab recruitment. *Ecological Monographs.* 65(2):193-216.

Elder, J. H., & Velisavljević, L. (2009) Cue dynamics underlying rapid detection of animals in natural scenes. *J. Vis.* 9(7).

Endler, J. (1978) A predator's view of animal colour patterns. *1st ed. Springer U.* 319-364.

Endler, J. (1978) A predators view of animal colour patterns. E*volutionary Biology.* 11:319 - 357.

Endler, J.A. (1984) Progressive background matching in moths, and a quantita-

tive measure of crypsis. *Biol. J. Linn. Soc.* 22:187–231.

Espinosa, I. and Cuthill, I. (2014) Disruptive Colouration and Perceptual Grouping. *PloS ONE*. 9(1).

Fingerman, M. (1970) Comparative physiology: Chromatophores. *Annu.Rev. Physiol.* 32345-372.

Flux, J. (1970) Colour change of Mountain hares (Lepus timidus scoticus) in north-east Scotland. *Journal of Zoology.* 162(3):345–358.

Fraser, S., Callahan, A., Klassen, D., & Sherratt, T.N. (2007) Empirical tests of the role of disruptive coloration in reducing detectability. *Proc. R. Soc. B.* 274: 1325–1331.

Gamble, F. W., & F. W. Keeble. (1900) Hippolyte varians: a study in colour change. *Quarterly Journal of Microscopical Science.* 43:589-698.

Garcia, T.S., & Sih, A. (2003) Colour change and colour-dependent behaviour in response to predation risk in the salamander sister species Ambystoma barbouri and A. Texanum. *Oecologia (Berl.)*. 137:131-139.

Grant, B.S., & Howlett R.J. (1988) Background selection by the peppered moth (Biston betularia Linn.): individual differences. *Biol J Linn Soc.* 33:217–232.

Hanlon, R. (2007). Cephalopod dynamic camouflage. *Current Biology.* 17(11).

Hanlon, R. T., Chiao, C-C., Mäthger, L. M., Barbosa, A., Buresch, K. C., & Chubb, C. (2009) Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 364:429–437.

Hanlon, R. T., & Messenger, J. (1988). Adaptive coloration in young Cuttlefish (Sepia officinalis L.): the morphology and development of body patterns and their relation to behaviour. *Philos. Trans. R. Soc. Lond. B. Biol. Sci*. 320(1200):437–487.

Hart, N. S. (2002) Vision in the peafowl (Aves: Pavo cristatus). *J. Exp. Biol.* 205:3925–3935.

Hart, N.S., & Hunt, D.M. (2007) Avian visual pigments: Characteristics, spectral tuning, and evolution. *American Naturalist.* 169:S7-S26.

Harvell, C.D. (1994) The evolution of polymorphism in colonial invertebrates and social insects. *Q.Rev.Biol.* 69:155-185.

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

Hogarth, P.J. (1975) Pattern polymorphism and predation in the shore crab, Carcinus maenas (L.). *Crustaceana.* 28:316–319.

Hogarth, P. (1978) Variation in the carapace pattern of juvenile Carcinus maenas. *Marine Biology*. 44(4):337-343.

Houston, A.I., Stevens, M., & Cuthill, I.C. (2007) Animal camouflage: compromise or specialise in a two patch-type environment? *Behav. Ecol.* 18:769–775.

Hulbert, A. (2007) Color constancy. *Curr. Biol.* 17:906-907.

Hultgren KM, Mittelstaedt H. (2015) Color change in a marine isopod is adaptive in reducing predation. *Curr. Zool*. 61:739–748.

Hultgren, K.M., & Stachowicz, J.J. (2010) Size-related habitat shifts facilitated by positive preference induction in a marine kelp crab. *Behav. Ecol*. 21:329–336.

Jensen, G.C., & Egnotovich, M.S. (2015) A whiter shade of male: Color background matching as a function of size and sex in the yellow shore crab Hemigrapsus oregonensis (Dana, 1851). *Current Zoology.* 61(4):729-738.

Jormalainen, V., Merilaita, S., & Tuomi, J. (1995) Differential predation on sexes affects colour polymorphism of the isopod. Idotea baltica (Pallas). *Biol. J. Linn. Soc.* 55:45–68

Kang, C.K., Moon, J.Y., Lee, S.I., & Jablonski, P.G. (2012) Camouflage through an active choice of a resting spot and body orientation in moths. *J Evol Biol.* 25(9): 1965-702.

Kang, C., Stevens, M., Moon, J., Lee, S., & Jablonski, P. (2015). Camouflage through behavior in moths: the role of background matching and disruptive coloration. *Behavioral Ecology.* 26(1):45-54.

Kelman, E., Baddeley, R., Shohet, A., & Osorio, D. (2007) Perception of visual texture and the expression of disruptive camouflage by the cuttlefish, Sepia oficinalis. *Proc. Roy. Soc. Lond. B Biol.* 274(1616):1369-1375.

Kettlewell, H. (1955) election experiments on industrial melanism in the Lepidoptera. *Heredity*. 9:323-342.

Kiltie, R.A. (1988) Countershading: Universally deceptive or deceptively universal? *Trends Ecol Evol.* 3(1):21-23.

Kindermann C, Hero J-M. (2016) Pigment cell distribution in a rapid colour changing amphibian (Litoria wilcoxii). *Zoomorphology.* 135:197-203.

Kindermann, C., Narayan, E.J., & Hero, J.M. (2014) The Neuro-Hormonal Control of Rapid Dynamic Skin Colour Change in an Amphibian during Amplexus. *PloS ONE.*

Krause - Nehring, J., Matthias Starck, J. & Palmer, A. (2010) Juvenile colour polymorphism in the red rock crab, Cancer productus: patterns, causes, and possible adaptive significance. *Zoology*. 113(3):131-139.

Langerhans, R.B., Layman,C.A., Langerhans,A.K., Dewitt, T.J. (2003) Habitat-associated morphological divergence in two Neotropical fish species. *Biological Journal of the Linnean Society.* 80:689–698.

Levins R. (1968) Evolution in changing environments. *Princeton, NJ: Princeton University Press.*

Lovell, P.G., Ruxton, G.D., Langridge, K.V., Spencer, K.A. (2013) Egg-Laying Substrate Selection for Optimal Camouflage by Quail. *Current Biology.* 23(3): 260-264.

Mäthger L.M., Barbosa A., Miner S., Hanlon R.T. (2006) Color blindness and contrast perception in cuttlefish (Sepia officinalis) determined by a visual sensorimotor assay. *Vision Res.* 46:1746–1753.

Mäthger L.M., Chiao C., Barbosa A., Buresch K., Kaye S., Hanlon R.T. (2007) Disruptive coloration elicited on controlled natural substrates in cuttlefish, Sepia officinalis. *J. Exp. Biol.* 210:2657–2666.

Merilaita, S. (1998). Crypsis through disruptive colouration in an isopod. *Proc.R.Soc.B.* 265(1401):1059-1064.

Merilaita, S. (2003). Visual background complexity facilitates the evolution of camouflage. *Evolution: International Journal of Organic Evolution.* 57(6).

Merilaita, S., Lind, J. (2005) Background-matching and disruptive coloration, and the evolution of cryptic coloration. *Proc. R. Soc. B.* 272(1563).

Merilaita, S., Lyytinen, A., Mappes, J. (2001) Selection for cryptic coloration in a visually heterogeneous habitat. *Proc. R. Soc.* B.268:1925–1929.

Merilaita, S., Tuomi, J., Jormalainen, V. (1999) Optimization of cryptic coloration in heterogeneous habitats. *Biol. J. Linn. Soc*. 67:151–161.

Michalis, C., Scott-Samuel, N., Gibson, D., & Cuthill, I. (2017) Optimal background matching camouflage. *Proceedings of the Royal Society B.* 284(1858).

Nafus, M.G., Germano, J.M., Perry, J.A., Todd, B.D., Walsh, A., Swaisgood, R.R., (2015) Hiding in plain sight: a study on camouflage and habitat selection

in a slow-moving desert herbivore. *Behav. Ecol*. 26:1389–1394.

Nasir, U. and Faulkes, Z. (2011). Color polymorphism of sand crabs, lepidopa benedicti (decapoda: anomura: albuneidae). *Journal of Crustacean Biology*. 31(2):240-245.

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

Noor, M.A.F., Parnell, R.S., Grant, B.S. (2008). A reversible color polyphenism in American peppered moth (Biston betularia cognataria) caterpillars. *PLoS ONE.* 3.

Odeen, A., Håstad, O., & Alström, P. (2010). Evolution of ultraviolet vision in shorebirds (Charadriiformes). *Biology Letters*. 6(3):370–4.

Orr,M.R, Smith,T.B. (1998) Ecology and speciation. *Trends in Ecology and Evolution*. 13:502–506.

Osorio, D., & Srinivasan, M.V. (1991) Camouflage by edge enhancement in animal coloration patterns and its implications for visual mechanisms. *Proc. R. Soc. B.* 244:81–85.

Osorio D., Vorobyev M. (2005) Photoreceptor spectral sensitivities in terrestrial animals: adaptations for luminance and colour vision. *Proc. R. Soc. B.* 272:1745–1752.

Palma, A.T., Orrego, C., & Arriagada, M. (2003) Cripsis in early benthic phases of brachyuran decapod crustaceans in central Chile. *Revista Chilena de Historia Natural.* 76:149–156.

Palma, A.T., & Steneck, R.S. (2001) Does variable coloration in juvenile marine crabs reduce risk of visual predation? *Ecology.* 82:2961–2967.

Pietrewicz, A. T., & Kamil, A. C. (1977) Visual detection of cryptic prey by blue jays (Cyanocitta cristata). *Science*. 195:580-582.

Powell BL. (1962) The responses of the chromatophores of Carcinus maenas (L. 1758) to light and temperature. *Crustaceana.* 4:93–102.

Powell, B. (1966) The Control of the 24 Hour Rhythm of Colour Change in Juvenile Carcinus maenas (L). *Proceedings of the Royal Irish Academy. Section B: Biological, Geological, and Chemical Science.* 64:379-399.

Price, T.D., Qvarnstrom, A., Irwin, D.E. (2003) The role of phenotypic plasticity in driving genetic evolution. *Proc.R.Soc.B.* 270(1523).

R Core Team. 2013. R: a language and environment for statistical comput-ing. Vienna (Austria): R Foundation for Statistical Computing.

Ramachandran, V., Tyler, C., Gregory, R., Rogers-Ramachandran, D., Duensing, S., Pillsbury, C., & Ramachandran, C. (1996). Rapid adaptive camouflage in tropical flounders. *Nature*. 379(6568):815-818.

Rao, K. R., Fingerman, M., & Bartell, C. K. (1967) Physiology of the white chromatophores in the fiddler crab, Uca pugilator. *Biol. Bullet.* 133:606–617.

Reuschel, S. and Schubart, C. (2007) Contrasting genetic diversity with phenotypic diversity in coloration and size in Xantho poressa (Brachyura: Xanthidae), with new results on its ecology. *Marine Ecology.* 28(2):296-305.

Rosenblum EB (2006) Convergent evolution and divergent selection: Lizards at the White Sands ecotone. *Am Nat* 167:1–15.

Rowland, H., Speed, M., Ruxton, G., Edmunds, M., Stevens, M. and Harvey, I. (2007). Countershading enhances cryptic protection: an experiment with wild birds and artificial prey. *Anim. Behav.* 74:1249–1258.

Rudge, D. (2005). The Beauty of Kettlewell's Classic Experimental Demonstration of Natural Selection. *BioScience.* 55(4):369-375.

Russell, B. J., and Dierssen, H. M. (2015) Use of hyperspectral imagery to assess cryptic color matching in Sargassum associated crabs. *PLoS ONE*. 10.

Ruxton, G., Sherratt, T. and Speed, M. (2004). Avoiding attack: the evolutionary ecology of crypsis, warning signals, and mimicry. *Oxford: Oxford University Press.*

Salemaa, H. & Ranta, E., (1991) Phenotypic variability of the intertidal isopod Idotea granulosa Rathke) in the Irish Sea. *Crustaceana*. 61:155-174.

Schaefer, H. and Stobbe, N. (2006). Disruptive coloration provides camouflage independent of background matching. Proc R Soc B Biol Sci, 273, pp.2427–2432.

Shand, Julia. Partridge, J.C, Archer, S.N, Potts G.W., L. J. . (1988) Pollachius pollacbius. *Journal Of Comparative Physiology A Neuroethology Sensory Neural And Behavioral Physiology.* 699–703.

Sherratt, T.N., Pollitt, D., Wilkinson, D.M. (2007) The evolution of crypsis in replicating populations of web-based prey. *Oikos*. 116:449–460.

Siddiqi, A., Cronin, T., Loew, E., Vorobyev, M. and Summers, K. (2004). Interspecific and intraspecific views of color signals in the strawberry poison frog Dendrobates pumilio. *Journal of Experimental Biology*. 207:2471–2485.

Smithers, S., Stevens, M. and Wilson, A. (2016) Rock pool gobies change their body pattern in response to background features. *The Linnean Society of London, Biological Journal of the Linnean Society.*

Stachowicz, J. and Hay, M. (1999) Reducing predation through chemically mediated camouflage: indirect effects of plant defences on herbivores. *Ecology.* 80(2):495-509.

Stevens, M. (2005) The role of eyespots as anti-predator mechanisms, principally demonstrated in the Lepidoptera. *Biological Reviews.* 80(4):573-588.

Stevens M. (2016). Color change, phenotypic plasticity, and camouflage. *Front. Ecol. Evol.* 4:51.

Stevens, M., Broderick, A. C., Godley,B. J., Lown, A. E., Troscianko, J., Weber, N. & Weber, S. B. (2015) Phenotype-environment matching in sand fleas. *Biology Letters*. 11.

Stevens M, Cuthill I.C. (2006) Disruptive coloration, crypsis and edge detection in early visual processing. *Proc. R. Soc. B.* 273(1598).

Stevens, M., Cuthill, I.C., Windsor, A.M.M., & Walker, H.J. (2006) Disruptive contrast in animal camouflage. *Proc. R. Soc. B.* 273:2433-2438.

Stevens, M., Lown, A. and Wood, L. (2014). Colour change and camouflage in juvenile shore crabs (Carcinus maenas). *Frontiers in Ecology and Evolution.* 2(14).

Stevens, M., & Merilaita, S. (2008) Animal Camouflage: Current issues and new perspectives. *Philosophical Transactions of The Royal Society B Biological Sciences.* 364(1516):423-7.

Stevens, M., & Merilaita, S. (2009). Animal camouflage: current issues and new perspectives. *Philos.Trans.R.Soc.Lond.B. Biol.Sci.* 364(1516):423-427.

Stevens, M., & Merilaita, S. (2009) Defining disruptive coloration and distinguishing its functions. *Philos. Trans. R. Soc. B.* 364:481-488.

Stevens, M. & Merilaita, S. (2011) Animal Camouflage: Mechanisms and Function. *Cambridge University Press. Cambridge.*

Stevens, M., Párraga, C. A., Cuthill, I.C., Partridge, J.C. & Troscianko, T. (2007) Using digital photography to study animal coloration. *Biological Journal of the Linnean Society.* 90:211-237.

Stevens, M., Pei Rong, C. & Todd, P.A. (2013) Colour change and camouflage in the horned ghost crab Ocypode ceratophthalmus. *Biological Journal of the*

Linnean Society. 109:257-270.

Stevens, M., Troscianko, J., Wilson-Aggarwal, J., & Spottiswoode, C. N. (2017) Improvement of individual camouflage through background choice in ground-nesting birds. *Nature Ecology and Evolution.* 1:1325–1333.

Stoddard, M.C., Stevens, M. (2010) Pattern mimicry of host eggs by the common cuckoo, as seen through a bird's eye. *Proc Biol Sci.* 277(1686):1387-93.

Stoddard, M. and Stevens, M. (2011) Avian vision and the evolution of egg color mimicry in the common cuckoo. *Evolution.* 65(7):2004-2013.

Stoner, C., Bininda-Emonds, O., & Caro, T. (2003) The adaptive significance of colouration in lagomorphs. *Biological Journal of the Linnean Society.* 79(2):309-328.

Stuart-Fox, D. and Moussalli, A. (2008) Selection for Social Signalling Drives the Evolution of Chameleon Colour Change. *Plos Biology.* 6(1).

Stuart-Fox, D. and Moussalli, A. (2009). Camouflage, communication and thermoregulation: lessons from colour changing organisms. *Philosophical Transactions of The Royal Society B.* 364(1516):463-470.

Stuart-Fox, D., Moussalli, A., & Whiting, M. (2008) Predator-specific camouflage in chameleons. *Biology Letters.* 4(4).

Stuart-Fox, D., Whiting, M.J., Moussalli, A. (2006) Camouflage and colour change antipredator responses to bird and snake predators across multiple populations in a dwarf chameleon. *Biol. J. Linn. Soc*. 88:437–446.

Styrishave, B., Rewitz, K., & Andersen, O. (2004) Frequency of moulting by shore crabs (*Carcinus maenas*) (L.) changes their colour and their success in mating and physiological performance. *Journal of Experimental Marine Biology and Ecology.* 313(2):317-336.

Sudha Devi, A., & Smija, M. (2015) Growth and moulting pattern in the fresh-

water crab (Travancoriana schirnerae). *International Journal of Fisheries and Aquatic Studies.* 3(2):391-396.

Surmacki, A., Ożarowska A., & Rosin, Z.M. (2013) Color polymorphism in a land snail (*Cepea nemoralis*) (Pulmonata: Helicidae) as viewed by potential avian predators. *Naturwissenschaften.* 100:533–540.

Teyssier, J. Saenko, S.V., Van der Marel, D. Milinkovitch, M.C. (2015) Photonic crystals cause active colour change in chameleons. *Nature Comms.* 6(6368).

Thayer, G. (1909) Concealing-coloration in the animal kingdom: an exposition of the laws of disguise through color and pattern: being a summary of Abbott H. Thayer's discoveries. *In Macmillan New York.*

Thurman, C. (1988). Rhythmic physiological colour change in crustacea: A review. *Comparative Biochemsitry and Physiology Part C: Comparative Pharmacology.* 91(1):171-185.

Todd, P.A. (2009) Testing for camouflage using virtual prey and human "predators". *J Biol Educ.* 43:81–84.

Todd, P.A., Briers, R., Ladle, R., & Middleton, F. (2006). Phenotype-environment matching in the shore crab (*Carcinus maenas*). *Marine Biology.* 148(6):1357-1367.

Todd, P.A., Oh, J., Loke, L., & Ladle, R. (2012). Multi-scale phenotype-substrate matching: evidence from shore crabs (*Carcinus meanas*). *Ecological Complexity.* 12:58-62.

Todd, P.A., Phua, H., & Toh, K.B. (2015) Interactions between background matching and disruptive colouration:experiments using human predators and virtual crabs. *Curr Zool.* 61:718–728.

Tovee, M.J. (1996) An Introduction to the Visual System. *Cambridge University Press.*

Trivedi, J.N., Vachhrajani, K.D. (2012) Distribution and diversity of brachyuran crabs along the coastal region of Junagadh district, Gujarat. In: *Proceedings of the Biodiversity and Conservation of Coastal and Marine Ecosystems of India.* 8–14.

Troscianko, J., Skelhorn, J., & Stevens, M. (2017) Quantifying camouflage: how to predict detectability from appearance. *BMC Evolutionary Biology.* 17:7.

Troscianko, J. & Stevens, M. (2015) Image calibration and analysis toolbox – a free software suite for objectively measuring reflectance, colour and pattern. *Methods in Ecology and Evolution.* 6:1320–1331.

Troscianko, J., Wilson-Aggarwal,J., Stevens, M., Spottiswoode, C.N. (2016) Camouflage predicts survival in ground-nesting birds. *Scientific Reports.* 6(19966).

Troscianko,T., Benton, C.P., Lovell, P.G., Tolhurst, D.J., & Pizlo Z. (2009) Camouflage and visual perception. *Phil. Trans. R. Soc. B.* 364:449–461.

Umbers, K. D. L., Fabricant, S. A., Gawryszewski, F. M., Seago, A. E., and Herberstein, M. E. (2014). Reversible color change in Arthropoda. *Biol. Rev*. 89:820–848.

Uy, F.M.K., Ravichandran, S., Patel, K.S., Aresty,J., Aresty, P.P., Audett,R.M., Chen, K., Epple, L., Jeffries, S.F., Serein, G.N., Tullis-Joyce, P., J. Albert C. Uy, J.A.C. (2017) Active background choice facilitates crypsis in a tropical crab. *Biotropica.* 49(3):365.

Wald, G. (1968) Single and multiple visual systems of arthropods. *J Gen Physiol.* 51:125–156.

Wallersteing, B.R. & Brusca, R.C. (1982) Fish Predation: A Preliminary Study of its Role in the Zoogeography and Evolution of Shallow Water Idoteid Isopods (Crustacea: Isopoda: Idoteidae). *Journal of Biogeography*. 9(2):135-150.

Webster, R., Hassall, C., Herdman, C., Godin, J., & Sherratt, T. (2013) Disrup-

tive camouflage impairs object recognition. *Biology Letters.* 9(6).

West-Eberhard, M.J. (1989) Phenotypic plasticity and the origins of diversity. *Annu. Rev. Ecol. Syst*. 20:249-78.

Xiao, F., & Cuthill, I.C. (2016) Background complexity and the detectability of camouflaged targets by birds and humans. *Proc. Biol. Sci.* 283(1838).