The effect of camouflage on the escape behaviour of common shore crabs.

Volume 1 of 1

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

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Abstract

For any prey animal, avoiding and fleeing from predators is one of the most important tasks in the key to survival. In order to avoid predation, a prey on detecting the predator must make appropriate decisions on how to respond to the threat. According to the optimal escape theory, initiation of escape should be a balance between the costs of staying (such as predation risk or death) and costs of fleeing (such as loss of feeding and reproductive opportunities and energy expenditure). Camouflage, particularly background matching, is also an important and widespread antipredator strategy, and it is commonly predicted that the likelihood of fleeing from a predator should depend on the level of camouflage of a prey animal. Moreover, most organisms encounter multiple predators rather than a single predator. Each predator possesses a different visual system and demonstrate various means of foraging and capture behaviour. Consequently, it becomes difficult for the prey to use the same antipredator strategy for multiple predators so prey species may evolve to form predator-specific responses as a counter strategy. Although a number of recent studies have considered the camouflage aspect in the common shore crab, research on how it works in combination with escape behaviour and whether it is affected by the type of predator remains unanswered. Although the association between camouflage strategies and escape behaviour has been researched in the past literature, many of these studies focus on the vertebrate taxa or do not directly measure camouflage and only few of them explore multiple predators. Within this thesis, I aim to address these gaps through a series of field-based experiments using background and predator treatments in the common shore crab (*Carcinus maenas*).

In chapter 2, I investigate the effects of camouflage on the escape response of shore crabs using mismatched backgrounds. Individuals were placed on the mismatched backgrounds and exposed to predator treatment and control of no predator treatment. The camouflage of crabs was measured in terms of brightness and colour difference using image analysis. Predator and background independently rather than their interaction had a significant influence on the escape behaviour. However, brightness and colour difference did not significantly affect their flight response. In addition, I also examined the effects of camouflage using substrate heterogeneity on the escape response of these crabs. Here, the camouflage based on brightness difference affected their escape behaviour on interaction with predator and substrate. Colour difference alone had a significant effect on the fleeing times and the interaction between predator and substrate was also found to be significant.

In chapter 3, I explore whether the escape responses of shore crabs are tailored to the type of predators using the same camouflage metrics. Here, predator and background when considered independently were found significant. Additionally, size of crabs was also significantly influencing the escape behaviour of crabs. Overall, this thesis indicates that camouflage influences the escape behaviour of prey species in complex heterogenous environments. Furthermore, it suggests that crabs and possibly other marine animals are capable of distinguishing between different predators and alter their escape responses based on the level of perceived threat.

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I dedicate this thesis to my parents – Saroj and Sunil Choudhary.

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Chapter 1: Introduction

Predator-prey relationships are ubiquitous in nature and have evolved over millions of years. While prey have developed certain morphological and/or behavioural strategies (Stevens and Ruxton, 2018) to escape from being eaten by the predators, predators have also evolved counteractive tactics to seek, detect and capture prey. This has started an evolutionary arms race whereby both the predator and prey constantly evolve and overcome each other's strategies, and thus driving evolution (Dawkins and Krebs, 1979; Abrams, 2000). This thesis explores the antipredator strategies, such as camouflage and escape behaviour, adopted by the shore crab (*Carcinus maenas*) and aims to determine if these two strategies are linked. Although the association between camouflage tactics and escape behaviour has been researched in several studies (Cuadrado *et al*., 2001; Eterovick *et al.*, 2008; Stuart-Fox *et al*., 2008; Martin *et al.,* 2009; Staudinger *et al*., 2013; Dugas *et al*., 2015; Wilson-Aggarwal *et al*., 2016). However, many of these studies either focus on the vertebrate taxa or do not directly measure camouflage and only few of them explore multiple predators. Within this chapter, I use the predatory sequence to explain predator-prey encounters and then describe the working of camouflage, and finally how the escape behaviour is initiated.

Predatory sequence

Endler (1986), describes predator–prey encounters as a sequence of stages from a predator's perspective, beginning with detection by the predator, identification, approach, subjugation, and consumption (Figure 1.1). A successful predation attempt begins with detecting a potential target; then identifying if the target is an actual prey; and completes with capturing the target/prey item. Similarly, prey species are equipped with defence mechanisms that aim to eliminate the interaction with the predator at each, or several, of these stages. Predation risk for prey increases as the predation sequence proceeds from one stage to the next so it is only optimal for the prey species to interrupt this chain in the initial stages, but it can be interrupted after subjugation as well.

Many species possess defences that act on several stages such as avoiding encounter, preventing detection, making identification difficult for the predator, deterring attacks and so on (Figure 1). If prey can avoid being detected from the eyes of the predator, the chances of survival of that prey increase to some extent. Thus, preventing detection can be thought of as the first line of defence. For example, Arias *et al*., (2019), tested four butterfly species exhibiting different degrees of transparency, and found the most transparent species were either never found or detected less by both wild birds and humans as compared to opaque species indicating that crypsis (through transparency) prevents detection and the chances of survival are increased. Additionally, a single prey species is subject to multiple predator species so survival should be maximum for prey defences that result in early detection of predators. The defences that interrupt the sequence at the earliest stage, i.e., before the subjugation stage, can reduce the chances of injury or energy loss from fighting or escaping and are therefore considered a valuable asset to the animal. For instance, California ground squirrel (*Otospermophilus beecheyi*) display predator-deterrent signals (tail-flagging) and communicate their knowledge of the rattlesnake's ambush location to the snake directly to show vigilance and minimise the likelihood of an attack (Barbour *et al.,* 2012).

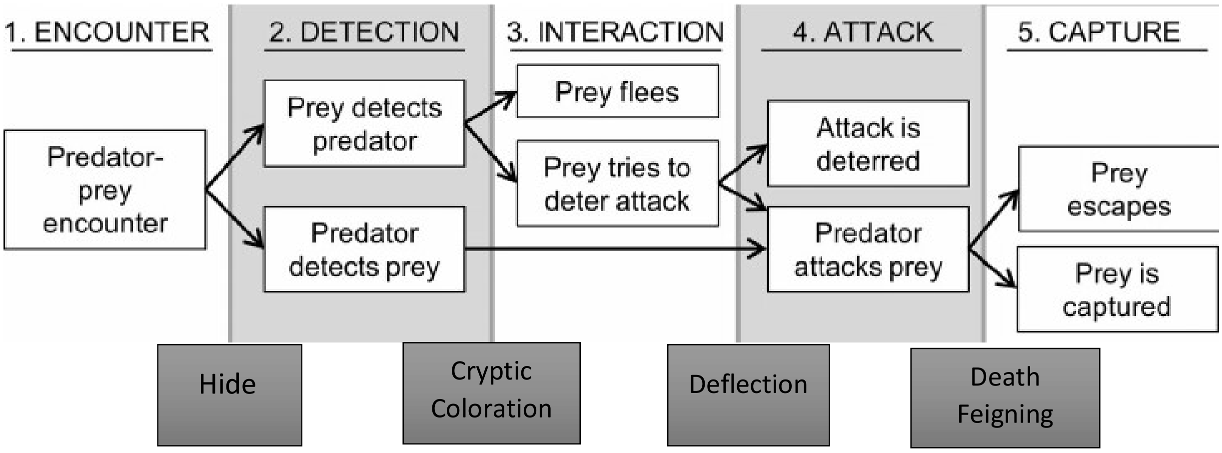


Figure 1.1: The sequence represents the five stages of predator-prey interaction and the antipredator behaviours used to prevent capture. An encounter occurs when both the prey and predator are in spatial and temporal proximity leading to detection, which is followed by interaction. Prey may choose to

flee or utilize pursuit-deterrent signalling to deter an attack or death feigning to prevent consumption. Flow chart adapted from Lima and Dill, 1990 and Ruxton *et al*., (2004).

Camouflage

Camouflage is a classic example of natural selection that hinders the detection and recognition of an animal (Stevens & Merilaita, 2011). This technique has been most commonly used by animals to prevent detection from visual predation. However, in addition to conferring protection against visually hunting predators, it is known to work against various other sensory modalities (Ruxton, 2009) such as chemical (Dettner & Liepert, 1994; Raffa *et al*., 2007), auditory (Deecke *et al*., 2005), mechanical signals (Zuk *et al*., 2001), and electric cues (Stoddard & Markham, 2008). Several authors express a range of opinions when defining visual camouflage strategies, for instance in topics such as classification based on appearance or usage of descriptive terminology (Stevens & Merilaita, 2009a; Stevens & Merilaita, 2011). However, camouflage refers to an umbrella term used to describe all forms of concealment and can be broadly segregated into three categories – crypsis, masquerade, and motion-based tactics (Stevens & Merilaita, 2009a).

Crypsis is a type of camouflage strategy that reduces detection using coloration, pattern and/or behavioural traits when the animal is potentially visible to an observer (Stevens & Merilaita, 2009a; Stevens & Merilaita, 2011). Several camouflage strategies fit under crypsis, including countershading, disruptive coloration, and disruptive markings (Stevens & Merilaita, 2011). Countershading, whereby the dorsal surface of an animal that faces light tends to be darker and vice versa, prevents detection by eliminating conspicuous shadows and obliterative shading hinders detection by removing the three-dimensional form of an animal caused by shadow/light cues (Stevens & Merilaita, 2009a). For instance, counter shaded prey such as lepidopteran larvae when present on branches assume an orientation that neutralises the effect of natural shadows so that the larvae become inconspicuous to avian predators (Rowland *et al*., 2007). Another strategy called disruptive coloration refers to creating an illusion of false edges that prevents detection or recognition of an organism (Stevens & Merilaita, 2009b). By breaking up edge information, the animal's true outline and shape are destroyed making it difficult for the predator to detect the animal (Stevens & Merilaita, 2009a). Disruptive markings, on the other hand, are markings that redirect the predator's attention from the salient features of the prey, even when some of these components are mismatched with the background (Ruxton *et al.*, 2004; Stevens & Merilaita, 2009b). Although these conspicuous markings would be detected, the true outline or other characteristics of the organism that facilitate recognition are not, thereby preventing detection from predators (Stevens & Merilaita, 2009a; Merilaita *et al.*, 2013).

Of these different types of camouflage, the most common example of crypsis is background matching and is found in a variety of species including moths (e.g., Kettlewell, 1955; Michalis *et al.*, 2017), carnivores, artiodactyls, lagomorphs (Caro, 2005), treefrogs (Choi and Jang, 2014), African jerboas (Boratynski *et al.*, 2014), and shrimp (Siegenthaler *et al.*, 2018). Background matching is a form of camouflage strategy where an animal's appearance (colour, lightness, and pattern) matches the substrate on which it is observed (Stevens & Merilaita, 2011). This can be achieved by numerous processes, such as populations evolving over time, so individuals resemble their local environment (Rosenblum *et al.*, 2010). Another method is behavioural choice, whereby individuals select backgrounds which are more effective at preventing detection as seen in the case of Japanese quail (*Coturnix japonica*), which choose to lay their eggs on substrates that provide improved background matching to avoid detection from visual predators (Lovell *et al.*, 2013). The morphs of the Pacific tree frog *Pseudacris (Hyla) regilla* use background coloration to prevent detection from predators. They choose substrates according to their appearances, so when preyed upon by garter snakes *(Thamnophis elegans),* mismatched frogs are likely to be attacked more than the camouflaged individuals, demonstrating that background matching offers greater survivorship (Morey, 1990; Wente and Phillips, 2003). Similarly, individuals readjust their orientation to effectively blend into the background as observed in moths, which align their cryptically coloured bodies according to patterning of the background, thereby decreasing the probability of visual detection and enhancing the camouflage effect (Kang *et al*., 2012). Furthermore, the organisms can also tune their camouflage to a particular background in their habitat through colour change (Stuart-Fox and Moussalli, 2009).

One certain attribute where colour change proves advantageous is camouflage, whereby animals use their coloration as a form of concealment strategy (Caro, 2005). Colour change is widespread in a diverse range of taxa from invertebrates such as cephalopods (Norman, 2000; Hanlon, 2007), crustaceans (Thurman, 1988), insects (Hinton and Jarman, 1972) to vertebrates such as amphibians (Garcia, 2003), fishes (Kodric-Brown, 1998), and reptiles (Cooper and Greenberg, 1992). There are mainly two types of mechanisms of colour change, physiological and morphological. Physiological colour change, occurs due to the dispersion or concentration of pigment granules within chromatophores and is a rapid process, taking milliseconds to hours. For example, cuttlefish are capable of rapid colour changes (Hanlon *et al*., 2007; Stuart-Fox and Moussalli, 2009). However, morphological colour change, is due to changes in the quantity and quality of chromatophores in the dermis and is a slower process taking weeks to months (Thurman, 1988; Stuart-Fox and Moussalli, 2009).

Colour change serves various functions such as thermoregulation, UV protection, communication, and signalling (Stuart-Fox and Moussalli, 2009; Umbers *et al*., 2014). For example, the fiddler crab, *Uca panacea*, has a circadian rhythm of colour change turning darker during the day and lighter at night to ensure protection from the intense UV radiations (Darnell, 2012). However, colour change for camouflage can be achieved by blending the overall coloration with the background of their local environment, mainly through background matching (Duarte, Flores, and Stevens, 2017). In this way animals change their colour in order to better adjust their camouflage to the substrate on which they are found (Stuart-Fox and Moussalli, 2009) (Figure 1.2). A substantial amount of work has been conducted on rapid colour changing organisms such as chameleons, cephalopods, and flatfish (Ramachandran, 1996; Kelman et al., 2006). In comparison, slower colour changing animals which are more common in nature have received little attention (Stevens *et al*., 2016). Examples include mammals and birds which demonstrate seasonal colour moulting by changing their fur and plumage for camouflage (Zimova *et al.*, 2018). Several crab species use colour change for camouflage as a protective device to prevent detection from visual predators. For example, shore crabs *Carcinus maenas*, are known to turn brighter on light backgrounds and darker on dark backgrounds by changing the brightness of their carapace. This increases their level of matching the substrate on which they live, reducing their visibility to predators (Stevens *et al*., 2014). Similarly, ghost crabs, *Ocypode ceratophthalmus*, adjust their brightness levels so as to become lighter during the day and changing to a darker shade during the night, hence, displaying a circadian rhythm of colour change. These individuals also increase their brightness when present on a more white background than black to increase the level of camouflage (Stevens *et al*., 2013). This study quantified the camouflage but did not include escape response of the species and only focussed on colour change. To bridge this gap, I have measured the individual crab camouflage using reflectance values and associated it with escape behaviour.

Colour changing animals also have the ability to exploit multiple camouflage strategies as evident from cephalopods (Stuart-Fox and Moussalli, 2009; Duarte, Flores, and Stevens, 2017). For example, *Sepia officinalis,* employs background matching by displaying uniform or mottled coloration when present on low contrast backgrounds (Hanlon *et al*., 2009). Conversely, this species produces coarse contrasting patterns consisting of spots of various colours, sizes, and shapes, when present on high contrasting patterns, indicating disruptive strategy (Hanlon *et al*., 2009). Additionally, most animals live in a multi-predator environment, potentially differing in their sensory systems and strategies of prey detection, so colour change is an important mechanism in providing concealment from different levels of predation risk (Stuart-Fox and Moussalli, 2009; Duarte, Flores, and Stevens, 2017). Colour-changing species can achieve this through facultative crypsis, whereby individuals tailor their camouflage in response to different predators and different backgrounds – mimic octopus (Norman *et al*., 2001), chameleon (Stuart-Fox *et al*., 2008).

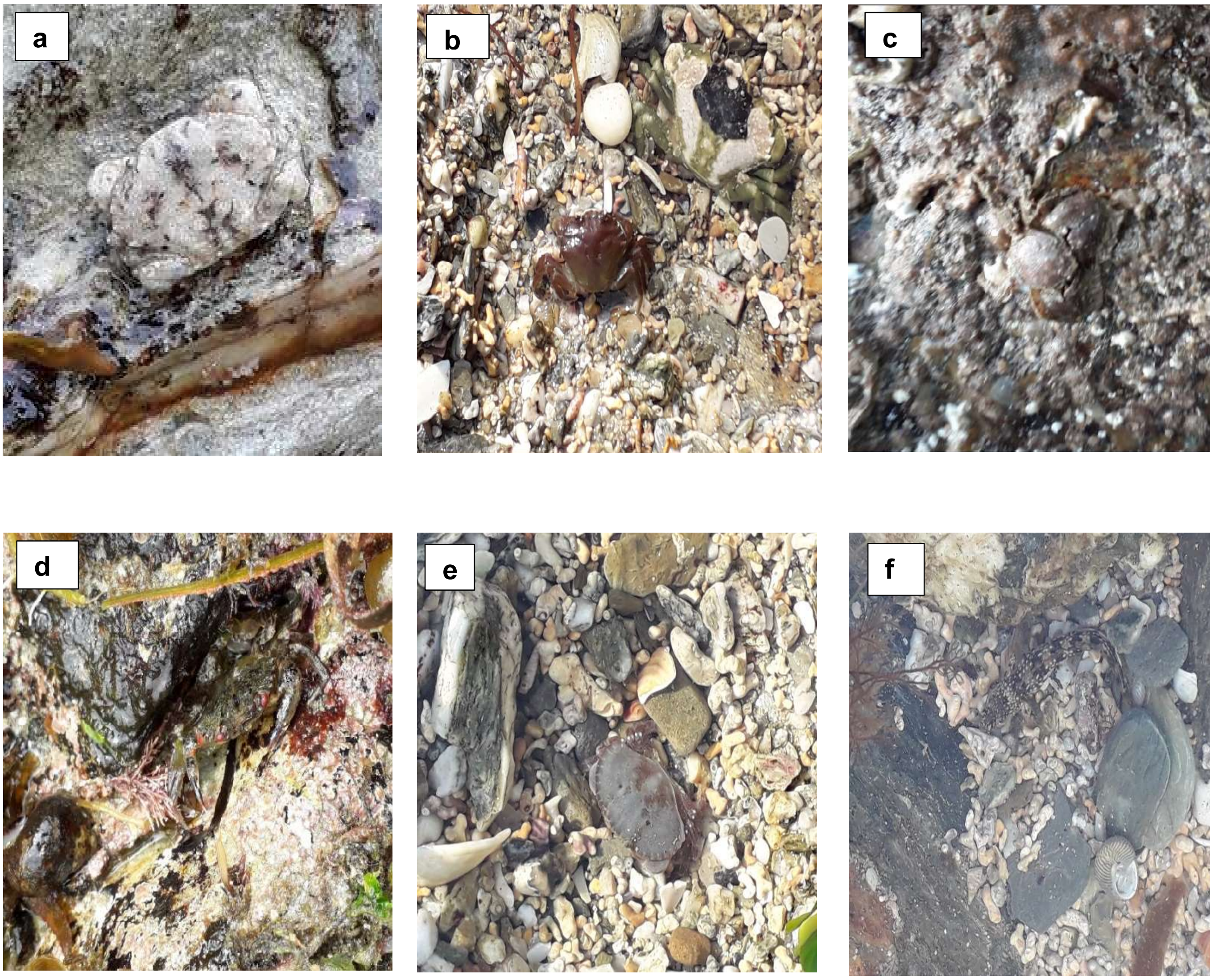


Figure 1.2: Examples of background matching in intertidal crabs and fish. Species shown include (a) a furrowed crab, *Xantho hydrophilus* (b) common shore crabs, *Carcinus maenas*; (c) a broad-clawed porcelain crab, *Porcellana platycheles*; (d) a juvenile velvet swimming crab, *Necora puber*; (e) a juvenile edible crab, *Cancer pagurus* and (f) a rock goby, *Gobius paganellus*. Shore crabs, furrowed crabs and rock gobies are known to change colour in response to their surroundings (Stevens *et al*., 2014; Bedini, 2002; Smithers *et al*., 2018). Capacity for colour change in the other species shown has not been documented although juvenile edible crabs are purple but change to brown/ red in adult stage.

Most animals live in an environment that continuously changes in space and time; therefore, background matching camouflage is challenging to achieve in backgrounds that are heterogeneous in colour and pattern to prevent predation (Caro *et al*., 2016). Individuals cope with heterogeneity either through physiological or morphological colour change and/or exhibit behavioural choices to resemble to the background (Duarte, Flores, and Stevens, 2017). Alternatively, animals can adopt a level of camouflage that is suboptimal on a specific background but confers protection on multiple backgrounds in the form of generalist camouflage (Houston *et al*., 2007; Hughes *et al*., 2019). In shore crabs (*Carcinus maenas*), variably coloured juveniles resemble their background at different spatial scales, however, they adopt an optimal cryptic strategy (turning to uniform dark green with increasing age) that confers crypsis against a suite of substrates instead of a single background; in other words, an imperfect background matching that provides some degree of resemblance to several habitats (Stevens *et al*., 2014; Nokelainen *et al*., 2019; Hughes *et al*., 2019). In addition, the likelihood of detecting an organism in a visually complex background is also difficult due to longer search times, so camouflage strategies can be effectively employed to prevent detection (Merilaita, 2003; Dimitrova & Merilaita, 2012).

Escape Behaviour

Animals engage in different defensive behaviours when faced with predatory threats to minimise chances of being harmed or killed and increase the probability of survival. These defensive behaviours can be morphological (such as camouflage) or behavioural and range from alarm calls, freezing, thanatosis, aggregation, attack to escape; however, which behaviour to implement, and in which sequence, is predator specific and dependent on the environment, internal state, and previous learning experience (Caro, 2005). For instance, rabbits freeze in response to an unexpected noise, or an anuran larva immobilizes in the presence of a threat. This strategy is especially useful when dealing with motion-oriented predators which fail to detect a static unmoving prey. This behaviour when coupled with another trait such as cryptic coloration, acts in accord to further make it harder for predators to detect prey species. Robinson *et al*., (1970), tested *Xanthodius sternberghii* crabs which effectively use their cryptic coloration in combination with ceasing movement when disturbed by human activity. Dilution effect is another means to increase the probability of survival; whereby, the prey species aggregate by forming schools (fish) or flocks (birds), reducing the per capita risk of predation (Ioannou, 2011; Yang, 2014). Though grouping increases the likelihood of being detected by the predator, it also decreases the predation rate of any given individual; thereby “diluting” its chances of being attacked.

In order to both avoid and respond to predation, animals firstly need to identify the difference between predator and non-predator based on sensory features such as shape, size, speed, and smell (Evans *et al*., 2019). It is extremely critical for prey to distinguish predators from non-predators or risky from safe situations as failure to make an effective decision could lead to either loss of fitness opportunities or worse being killed by predators. Juveniles are likely at a greater risk of predation as they are not only smaller than adults but also have a poor sense of coordination. Young adults tend to fly/run off either too quickly or too slowly and their manoeuvring ability is often less developed than adults. This makes the young prey vulnerable to predators and a great number of studies show that young suffer higher rates of predation-induced mortality than adults (Chivers, 2014). Thus, predator-naive young experience strong selection pressure on their first encounter with the predator (Ferrari, 2015).

Innate recognition of predators when prey have no experience of predator attack can be found in many species. In aquatic ecosystems, prey use chemosensory cues that signal the presence of a predator. As a result, antipredator responses such as hiding or schooling can be used to evade predators (Dupech, 2004; Brown, 2009). For example, Mezrai *et al*., (2018), found that embryos of cuttlefish, *Sepia pharaonis*, could recognize predator fish from non-predator fish when exposed to their chemical and visual cues. Similarly, Oulton *et al*., (2013), exposed the embryos of rainbow fish raised in isolated aquaria to several predator cues and found a substantial increase in heart rate in response to a native predator, spangled perch. Therefore, it is evident that certain prey species can react to predators appropriately even on their first encounter. This may be due to strong predation pressure after birth or hatching or where opportunities to learn about the predator are limited. Some animals depend on learning either through repeated encounters or through predator attacks on conspecifics, and predator recognition via learning has been shown in both vertebrates and invertebrates (Mirza, 2000; Mitchell, 2011). However, studies by Hanson and Coss, (1997), also show that predator recognition and response change with age, indicating maturation, experience and learning are equally important in avoiding predation. Thus, the expression of antipredator behaviour by prey, either employing tactics to reduce detection or avoiding being captured largely depends on predation.

Escape as a defensive behaviour has evolved to evade harm by increasing distance from a potential predator or conspecific threat. Animals that fail to escape from immediate threat will suffer death or reduced fitness, foraging abilities and weak social status when injured. Thus, individuals use various tactics such as when escaping fast-approaching predators using reflex-like actions such as C-start escape in fish (Zottoli and Faber, 2000) or make use of escape routes, trajectories and refuge locations when escaping from complex environments (Cooper *et al.*, 2015). Furthermore, the prey may be aware of the predator even before it decided to flee. For instance, most prey become attentive on detecting an approaching cheetah but only flee when it has approached within a certain distance (Ewer, 1968). According to Ydenberg and Dill (1986), optimal escape theory states that when encountered with an approaching predator, prey species should flee when the costs of fleeing (such as loss of foraging or mating opportunities) is equivalent to the costs of remaining (for example, the level of risk or being injured or predated). As the costs of remaining increases, flight distance increases; but as the costs of fleeing increases, flight distance decrease (Ydenberg and Dill, 1986; Cooper *et al.*, 2015). This means the prey must quickly assess the situation on detecting a predator and make an effective decision whether to stay or escape because an incorrect decision would lead to fitness costs or death (Figure 1.3).

Animals employ antipredator behaviour to avoid predation; however, it can incur significant cost even though increasing the chances of survival. Time spent to carry out daily activities-foraging, courting, basking and so on is lost when animals have to constantly monitor their surroundings for any threat. Some social species reduce these costs by taking turns in staying vigilant for predators. One such example can be seen in dwarf mongoose (*Helogale undulata*) which post sentinels on a rota basis to watch out for predators, and alert conspecifics of impending dangers using alarm calls (Rasa, 1987).

Prey can still deploy other secondary defences to prevent capture if the predatory sequence advances to the attack stage. Secondary defences can be chemical-olfactory deterrents or irritating repellents secretions (ejecting toxic chemicals and foul-smelling gases by Bombardier beetles and skunks, respectively), mechanical-body shape or spines (such as quills of the porcupine and spines of the hedgehog make it difficult for the predator to approach or attack), behaviour-aggressive retaliation, all working towards gaining sufficient time to escape (Caro, 2005). Another flight response that aids in escaping capture is startling the predator. The caterpillars of the North American walnut sphinx (*Amorpha juglandis*) when subjected to avian predators, produced a whistling sound causing the birds to flinch or fly away (Bura et al., 2011; Dookie et al., 2017). Similarly, the inking behaviour in cephalopods is another secondary defence that acts as a decoy and helps in escaping from predators. Hikidi (2020), pointed out that the Japanese pygmy squid (*Idiosepius paradoxus*) rapidly swims backward while ejecting ink intermittently and linearly in response to a predator. This confuses the predator which starts attacking the ink instead of the prey, giving the squid enough time to escape.

Voluntary shedding of body parts or autotomy is another antipredator response shown by prey when entrapped by predators. Although it saves the individual, it comes with further implications or associated costs such as reduced locomotor ability, rendering more vulnerability to future encounters (Juanes and Smith, 1995; Flemming *et al*., 2007). Such individuals then must rely on other ways of reducing visibility until their body parts regrow. One such example can be seen in larvae of damselfly (*Ischnura elegens*). They are known to autotomize their lamella in unsuccessful predation, so they enter thanatosis more frequently than intact individuals in future encounters (Gyssels and Stoks, 2005). Wasson and Lyon, (2005), studied autotomy in porcelain crabs (genus *Petrolisthes*) observing that smaller crabs and females were more likely to autotomize than larger individuals or males. This is probably because (i.) larger crabs are better at defending themselves due to strong chelipeds and (ii.) casting off limbs would lead to more negative impacts such as loss of later opportunities. This thesis looks at the association of camouflage and escape behaviour of shore crabs, therefore, background matching camouflage, recognition between predator and non-predator, fleeing behaviour and predator-specific responses are most relevant areas that would explain the association of the two antipredator strategies.

Fleeing

Lastly, if none of the above tactics is sufficient in avoiding attack, the prey may choose to flee as a last resort because fleeing allows the prey to hide in a refuge until the threat level decreases. To minimise the chances of capture, prey species when exposed to approaching predator must assess the risk and make escape decisions and also decide how close to let the predator approach before initiating the fleeing response (Ydenberg and Dill, 1986; Cooper *et al.*, 2010; Cooper *et al.*, 2015). When prey exhibits a flight response to an approaching predator, escape can be measured by different means such as flight initiation distance, distance fled, latency to flee and type of flight (Cooper *et al.*, 2015). The most widely used measure is FID or flight initiation distance, which is the distance between prey and predator when the prey initiates escape (Ydenberg and Dill, 1986; Cooper *et al.*, 2015). In models where prey species turns immobile on detecting a predator, latency to flee (LF) which isthe time between detection of predation and initiation of escape, plays a crucial part. The immobility of prey may reduce the probability of getting detected at a given moment but the tendency of predator to detect the prey also increases over time; therefore, latency to flee is determined by a trade-off between cost of remaining immobile and cost of fleeing (Martin *et al.*, 2009; Cooper *et al.*, 2012). Furthermore, the decision of when to flee (LF) is dependent on the distance between the prey and predator, which is also referred to as the Standing distance (D) (Martin et al., 2009; Cooper *et al.*, 2015). Latency to flee is predicted to decrease when standing distance decreases because the closer the predator gets, the probability of getting detected and captured by the predator increases at a given time (Martin *et al.*, 2009; Cooper *et al.*, 2012). These models are similar to the FID models and support predictions that with increasing risk, latency to flee decreases (Ydenberg and Dill, 1986; Cooper *et al.*, 2007; Cooper *et al*., 2012).

FID including LF depends on various circumstances such as fast or slow predator approach, consistent approach, distance from refuge and so on (Cooper *et al*., 2012). As the risk increases, the prey species is expected to respond at larger predator distances. For example, Cooper, (2006), noted FID in *Dissosteira carolina* grasshoppers increases when the predator has a greater approach speed. Contrastingly, *Schistocerca alutacea* grasshoppers choose to increase the distance fled when repeatedly approached by a predator (Bateman and Fleming, 2014), suggesting fleeing to a greater distance keeps the predator from chasing the prey.

Retreating to shelter as an escape response in order to avoid predation is also significant in maximising the survival and this type of escape response is dependent on the availability and quality of shelter. Venzon *et al*., (2000), showed herbivorous/omnivorous thrips, *Frankliniella occidentalis*, seek refuge in response to odours associated with a generalist predatory bug, *Orius laevigatus*. However, the shelter used is that of the web of two-spotted spider mite, *Tetranychus urticae*, where thrips larvae experience lower predation risk because the predatory bug is hindered by the web. Research by Hemmi *et al.*, (2005), suggest that a species of fiddler crab, *Uca vomeris*, retreats towards its burrows in response to approaching predators and their FID increases when the distance to the burrow is large. Further research found that the crabs do not retreat fully into the burrow immediately, but visually monitor the predator from the entrance (Hemmi and Pfeil, 2010). Another species, *Uca pugilator*, keeps an eye on their conspecifics for cues about potential threat and retreats if they see their conspecifics reacting (Wong *et al*., 2005).

Multiple Predators

In nature, it is rare for a prey species to have a single predator so animals must ensure survival in the presence of multiple predators. This proves to be even more challenging when every single predator has its own prey foraging and subjugation strategy, so the prey species needs to be extremely cautious while choosing antipredator tactics. Hence, prey must adjust their defensive strategies depending on the predators that attack (Hanlon *et al*., 1999; Rundus *et al*., 2007; Blumstein *et al*., 2009). An antipredator strategy effective against one predator may not be effective against another predator, interfere with the defences against another predator enhancing the risk, and/or facilitate predation by another predator (Sih *et al*., 1998; Stankowich *et al*., 2014).

Various studies have been carried out in the past that show animals respond differently to attacks from different predators (Bushkirk, 2001; Sherbrooke, 2008; Boyero,2011; Staudinger *et al*., 2013; Guderley *et al*., 2015; Ventura *et al*., 2016). For instance, Montane lizards (*Tropidurus montanas*) are believed to make different escape decisions depending on the level of threat. When exposed to a non-natural predator, lizards fled to longer distances across open habitats while in the case of natural predator stimulus, distances fled were shorter and straight towards refuge (Ventura et al., 2016). Sherbrooke (2008), reported that the Texas horned lizard, *Phrynosoma cornutum* when subjected to two snake species, employed two distinct defensive behaviours. The lizards opt for relocation running in the presence of rattlesnake (*Crotalus atrox*) and stationary body orientation towards whipsnake (*Masticophis spp*.). This is probably because rattlesnakes are ambush venomous predators so fleeing is an appropriate escape response. In contrast, whipsnakes are rapid pursuit nonvenomous predators that use strong jaws to subjugate the prey so a stationary body conformation change is the most suitable response where prey can use its size and spiny skin to scare the snake. Cuttlefish, *Sepia officinalis*, also vary their behaviour when encountered by different teleost predators. When actively searching whether predators are nearby, cryptic behaviour seems appropriate while an interaction with sedentary predators, switching to swimming and startling display ensures survival (Staudinger *et al.,* 2013). For cryptic species, the degree of camouflage aids in the decision of staying or fleeing from a visual predator. This interesting behaviour has been explored in many species- mallards (Albrecht, Klvaňa*, 2004),* round-tailed horned lizard (Cooper & Sherbrooke*,* 2010), and frogs (Blanchette *et al*., 2017). Their findings confirmed the ability of prey species to significantly adjust their escape decisions in response to current effectiveness of crypsis. However, to my knowledge, the ability of tailored antipredator responses, has not been investigated in shore crabs previously. In chapter three, I use both fish and avian predator models to assess the ability of shore crabs to change escape behaviour in presence of these predators.

Purpose of this Thesis

To date, the majority of the research on animals is focused either on escape behaviour or camouflage as an antipredator behaviour. Very few studies have focused on linking camouflage and escape behaviour (but see Heatwole, 1968; Wilson-Aggarwal *et al*., 2016; Blanchett *et al.*, 2017; Moller *et al*., 2019). There also has been some research into measuring escape response and camouflage on heterogeneous substrates but not in a controlled manner.

The shore crab, a very common intertidal species is found along the coastline of the UK, most of Europe and other parts of the world. The carapace is variably coloured in the juveniles but with increasing age or size becomes more uniform (Stevens *et al*., 2014). This species of crab has multiple predators ranging from shore birds especially gulls to many species of fish (Todd *et al*., 2006), which makes it an ideal species to study camouflage and escape behaviour.

Previous literature suggests that shore crabs can indeed change their brightness to match the background, concealing them from predators (Powell, 1962; Stevens *et al*., 2014). However, whether or not this colour change and the complexity of background influences the escape behaviour of crabs is still unknown. In chapter 2 of this thesis, I test whether the escape behaviour of shore crabs is dependent on the level of camouflage or not. I use latency to flee (LF) which is similar to FID to measure the escape behaviour of crabs. In addition, I also investigate if the heterogeneity of the habitat also influences the escape response of crabs. I investigate this question using a seabird as a model predator to simulate attack and to quantify camouflage. Image analysis techniques are used to calculate the degree of match between the background and the carapace of crabs.

There has been extensive research on predator-prey interactions (Cooper, 2007; Blake, 2013) but little work focuses on the antipredator response in a multiple predator system. In chapter 3, I test whether the shore crabs can perceive different predators and tailor their antipredator response accordingly. I introduce another predator of the shore crab, a blenny fish model, along with a seabird model to explore the escape response of shore crabs and whether it is tailored according to different type of predators. All the experiments in this thesis measure camouflage in terms of brightness and colour difference and these values are calibrated through image analysis. In chapter 4, the research findings and implications in the shore and wider community are discussed along with some areas of future work.

Chapter 2: Are the escape responses of shore crabs (*Carcinus maenas*) dependent on the type of background and presence of predator?

Abstract

Fleeing is a common antipredator response when a predator is in close proximity. In order to avoid predation, a prey must foster an effective escape plan, encompassing detection of predators, avoidance, and flight. Camouflage, particularly background matching, is also an important and widespread defensive attribute, and it is commonly predicted that the propensity to flee from a predator should depend on the level of camouflage of a prey animal. However, the association between coloration and escape decisions to avoid potential predators has not been tested in many species. Although a number of recent studies have considered camouflage in the common shore crab, research on how it works in combination with escape behaviour and if it is affected by heterogeneous environments remains unanswered. I studied escape responses and camouflage in the common shore crab (*Carcinus maenas*) by testing whether crabs of different camouflage levels respond differently to a simulated predator attack when on light and dark backgrounds. I also explored whether heterogeneity of the background influences the escape behaviour of individuals by testing differently camouflaged crabs on patterned and uniform substrates in presence of a predator. Using image analysis, the study also considered whether brightness and colour-based background matching favours quicker escape times as, the closer the brightness and colour difference of crabs, higher the probability of crabs remaining than fleeing during a threat. The results show that crabs display a more effective escape behaviour in the presence of a predator, and that individuals are slower to move in the absence of a predator compared to when under predation pressure. The results suggest that crabs were slower and more likely to remain immobile when on a darker background in comparison to a lighter background. Shore crabs also showed a faster response on a uniform substrate compared to a heterogeneous substrate where individuals are more inconspicuous. Overall, crabs showed greater escape behaviour on a non-matching background and the escape response is dependent on the presence of a predator indicating that both factors are important in deterring predation. Furthermore, the escape behaviour of crabs is influenced by camouflage in a heterogeneous setting.

Introduction

Most organisms are exposed to strong predation pressure, and thus have been forced to evolve numerous protective responses commonly referred to as antipredator defences, which can be either morphological and/or behavioural (Caro, 2005; David *et al*., 2014; Blanchette & Saporito, 2016; Stevens & Ruxton, 2018). Camouflage is an extensively researched antipredator strategy to prevent detection from visual predation (Cott, 1940; Ruxton *et al*., 2004; Stevens and Merilaita, 2009a; Cuthill, 2019). However, the effectiveness of camouflage in avoiding detection from predators largely depends on how well camouflaged an individual is on the background on which it is observed (Merilaita, 2003). Background matching occurs when an animal's appearance resembles the colour, luminance/brightness and pattern of the background on which it is viewed thereby reducing detection (Ruxton *et al*., 2004; Stevens and Merilaita, 2009), and animals often match the colour and pattern of the environment (Stevens and Ruxton, 2018). Background matching camouflage can be achieved in many ways including individuals in a population evolving over time to better match the local environment (Rosenblum *et al*., 2010), employing behavioural choices to efficiently blend with the background (Stevens and Ruxton, 2018), and changing colour to effectively turn their camouflage to the specific background (Stuart-Fox and Moussalli, 2009). These close similarities with the environment, commonly known as phenotype-environment associations are a result of selection and can be advantageous against visual predation.

Phenotype-environment associations in the form of background matching can be widely seen in earlier studies in the peppered moth (*Biston betularia*) whereby pale and melanic morphs selected appropriate light and dark backrounds (Kettlewell 1955). Similar results were found in the colour forms of the Pacific tree frogs *Pseudacris (Hyla) regilla* which also chose backgrounds based on their appearance (Morey, 1990). Another example of background matching is western terrestrial garter snake (*Thamnophis elegans*), whereby dark and light colour morph snakes select basking sites that provide better camouflage against potential predators in comparison to choosing random backgrounds (Isaac & Gregory, 2013). Aegean wall lizards (*Podarcis erhardii*) are also known to behaviourally choose resting sites based on their appearance (their individual dorsal colorration) to predators which improves their camouflage and prevents detection (Marshall, Philpot & Stevens, 2016). Similar evidence has been found in the case of a crab spider (*M. vatia*), whereby the crabs' level of crypsis was dependent on the predator and substrate on which it was found (Defrize *et al*., 2010).

Many animals use phenotype-environment associations to increase their fitness. Habitat choice is one way to achieve matching and depends on the availability of shelter, food, and mates (Bostrom and Mattila, 1999) and plays a significant role in survival (Moksnes *et al*., 1998). For instance, juvenile shore crabs exhibit a diverse range of colour and pattern but turn to dull grey/green at adult stage and this phenotypic variation is associated with the habitats in which they live, so as to gain an advantage against visually guided predators (Crothers, 1966; Hogarth, 1975; 1978). This has been demonstrated in earlier studies by Hogarth, (1978) and Bedini, (2002) which tested variation in shore crab carapace pattern and habitat and showed individuals with less pattern were found in areas with high weed or mud cover and with increasing age/size, crabs became uniform. Furthermore, Todd *et al*., (2006), tested distinct crab phenotypes and determined their relationship with substrate types finding negative correlations between shore crab carapace and substrates, whereby plain crab carapace was associated with macro-algal cover and patterned crabs were found on mussel beds. Expanding on this study, Todd *et al*., (2012), later found associations between crab carapace and substrate types – rocks, algae, mussel bed) at different spatial scales (micro(<1m^2), meso (100s m^2) and macro (10,000s m^2). Further support for these results was evident when crab camouflage was compared with different backgrounds using predator vision models which showed that crabs are able to change their brightness based on the background (Stevens *et al.*, 2014). All these studies suggest that habitat choice is important in achieving phenotypic variation which confers protection through camouflage.

For effective background matching, individuals must greatly resemble the background against which they are viewed but animals inhabit a range of backgrounds and/or microhabitats, so it becomes increasingly difficult to perfectly blend into all those habitats (Thayer, 1909; Houston, Stevens and Cuthill, 2007). In a homogeneous habitat, enhancing the degree of crypsis against the background can reduce the probability of being detected by the predator. Conversely, in a heterogeneous environment (consisting of two or more visually different microhabitats), as the individual needs to match the section that provides the best match, the probability of detection becomes difficult due to spatial and temporal variation in both coloration and pattern (Merilaita, 2003; Stuart -Fox, 2009; Boratynski, 2014). Animals living in a heterogeneous environment can reduce the visual predation risk in a number of ways. An individual could avoid detection either by changing its colour to match the background on which it is observed, and this can be seen especially in rapid colour changing species such as cuttlefish (*Sepia officinalis*) (Allen *et al*., 2010). However, animals with slower colour changing are more likely to rely on their behaviour, choosing a background that closely matches its appearance and can be observed in a wide range of taxa (Wickler, 1968; Stevens and Ruxton, 2018). Earlier studies by Merilaita, (1999; 2001), suggest that to reduce detection and predation risk, adopting a compromising coloration whereby individuals imperfectly match to several different microhabitats, is an optimal strategy as compared to matching coloration to one specific microhabitat. This finding is also supported by Todd *et al*., (2012), whereby phenotypic environment associations, provides effective camouflage at specific spatial scales, with microscale (<1m^2) facilitating strongest phenotype environment associations because of effective fine-tuning for background matching. At the mesoscale (~100m^2), crabs were found to follow a more general approach in terms of coloration, becoming inconspicuous through compromise coloration whereby they imperfectly matched a range of backgrounds rather than a specific microhabitat. However, it should be noted that camouflage is not fixed, rather changes with animals modifying their appearance over time during their life span either as a result of ontogenetic changes or reversible plastic changes (Stuart-Fox and Moussalli, 2009; Duarte, Flores & Stevens, 2017). For instance, many crabs are strikingly diverse in colour and pattern as juveniles which declines with increasing age, and these developmental colour changes and this phenotypic variation has been suggested to provide camouflage against visual predators in different habitats as well (Todd *et al*., 2006; 2012; Stevens *et al*., 2014). Some early works by Wilson *et al*., (2007) although not directly, have attempted to explain the links between ontogenetic colour change, camouflage and behaviour using predator vision models. A more recent study by Nokelainen *et al*., (2019) addresses the association between ontogenetic colour change and camouflage efficacy in the common shore crab (*Carcinus maenas*) and shows that crabs display a generalist matching strategy by turning to a uniform dark green colour rather than matching to a specific microhabitat. They also found that dark green phenotype of adults was the hardest to locate on all backgrounds suggesting this generalist approach facilitates camouflage and promotes survival on a range of backgrounds.

In addition to providing camouflage, animals adopt methods that reduce predation risk and/or enhance their appearance. Several examples show that the presence of a predator reduces an animals' activity levels, as seen in case of zooplankton in the presence of predatory copepod (Li and Li, 1979), shrimp in the presence of pinfish (Main, 1987), daphnia in the presence of dragonfly nymph (Burks, Jeppesen & Lodge, 2001), and chamois in the presence of wolves (Baruzzi, Lovari, Fattorini, 2017). When a prey detects an approaching predator, the decision to flee or stay must be made quickly. If the prey chooses to flee, other than missing foraging opportunities, a major trade off is one of detection versus escape. If a prey waits then it relies on being hidden but the approaching predator may still see it, and then it might be too late for the prey to escape. On the other hand, the prey may flee earlier but this may attract the attention of the predator due to the movement involved (Martin and Lopez, 2005; Stevens *et al.*, 2011), whereas if the prey had been still, it may have been unnoticed. For instance, in a study on mice, a looming threat triggered the mice to flee to refuge whereas when exposed to a sweep stimulus, mice chose to freeze to avoid detection (Franceschi *et al*., 2016). Similarly, ants (*M. graminicola*) after detecting danger either adopt freezing behaviour or curl their bodies in a ball to roll away from predators (Grasso *et al*. 2020).

Background matching, however, becomes more complex in a heterogeneous habitat (Merilaita, 1999), and individuals must monitor and utilize their own degree of camouflage to modulate their escape behaviour appropriately. The decision to stay or flee from an approaching predator depending on the degree of individual background matching is of particular importance for cryptic species and their vulnerable offspring. Movement breaks the camouflage effect (Stevens *et al*., 2011; Hall *et al*., 2013), and increases the likelihood of the predator detecting the parents and/or young ones. Although not many studies have focused on this association between camouflage and escape behaviour, a recent study by Wilson-Aggarwal *et al*., (2016) has quantified the camouflage between eggs and plumage of ground-nesting birds and their background using image analysis and predator vision models. They found that the escape distance of coursers and plovers was dependent on the level of the eggs' pattern match to the background, whereas for nightjars, it was the degree of pattern and colour match of the adult's plumage to the background that determined the escape decisions. Additionally, escape responses such as whether the prey chooses to flee, are further dependent on the type of predator (Stankowich & Blumstein, 2005), direction and speed of approaching predator (Ydenberg & Dill 1986; Lima & Dill 1990; Martín & López 1995; Cooper & Whiting 2007), type of habitat (Martín & López 1995; Cooper & Whiting 2007), internal characteristics of prey such as body size (Shine *et al*., 2002), sex (King, 2002; Shine *et al*., 2002)) and body temperature (Goode & Duval 1989; Shine *et al*., 2002).

Here, I focus on the two main tactics employed by the prey species: avoiding detection through camouflage, and assessment of whether to flee to prevent capture. While camouflage is based on visual cues such as colour and shape to remain indistinguishable from the predator, escape strategy involves the prey's immediate behaviour. For a prey species, it is essential to choose an optimal escape strategy that involves detection of predator, avoidance, and flight. Flight initiation distance (FID), the distance between an approaching predator and prey at which the prey starts fleeing, is a well-documented means to assess escape behaviour in animals (Blumstein, 2003; Bjorvik, Dale, 2015; Moller *et al.*, 2016). Another measure of escape response includes latency to flee (LF), which is the time between detection of predator and initiation of flight in an immobile predator-prey model (Cooper *et al*., 2015). The latency to flee is determined by a trade-off between the cost of immobility and the cost of escaping, as remaining immobile on detecting a predator may reduce the chances of recognition at a given instance, but the likelihood of predator detecting the prey also increases over time (Martin *et al*., 2009; Cooper *et al*., 2012). In addition, the time to flee is also dependent on the standing distance or the distance between prey and predator (Cooper *et al*., 2015). The decision of when to flee is predicted to decrease with standing distance because with approaching predator, the chances of getting recognised and captured increases as well (Martin *et al*., 2009; Cooper *et al*., 2012). These LF models resemble the FID models in supporting predictions that with increasing risk, latency to flee decreases (Ydenberg and Dill, 1986; Cooper *et al*., 2007; Cooper *et al*., 2012). Furthermore, factors such as age, sex, reproductive state running speed and types of substrates also affect the FID. For instance, larger adult individuals may be better protected and have shorter FID than smaller juveniles due to their greater speed ensuring better escape. However, if juveniles are inconspicuous due to smaller size or being cryptic, the FID of juveniles would be shorter than adults. This is perhaps because if an animal is very well camouflaged, the chance of it being seen is lower, and so it may be able to stay still for longer as the probability of being seen is low (Martin and Lopez, 2005).

Further examples of utilizing FID to determine the escape response include the study on *Liolaemus* lizards that showed the use of flat grounds and tree trunks favoured greater FID compared to use of other perches (Schulte *et al*., 2004). Cuadrado *et al.* (2001) found that chameleons perching in denser shrubs had lower FID and allowed closer approach than individuals in open areas. Juvenile toads choose cryptic over non cryptic substrate suggesting habitat structure influences their escape behaviour. They are known to adopt a crawling movement when observed on cryptic background and hopping motion when on noncryptic substrates. The former could afford some protection from visual predators while a hopping motion is more conspicuous but can cover greater distance and still be advantageous on noncryptic substrates (Heinen, 1985). Therefore, it is suggested that FID increases in open vegetation as the occupants of such habitats are more likely to be wary of predators (Bulova, 1994; Martín & López, 1995).

The literature reviewed here has focused largely on the mechanisms underlying colour change, and few studies have investigated the adaptive value of this change or its ecological relevance. Few studies have experimentally tested the camouflage by quantifying the actual change in coloration in laboratory settings (Stevens *et al*., 2013; Stevens *et al*., 2014), creating a knowledge gap in the adaptive value of coloration in natural habitat. Additionally, only fewer studies have experimentally quantified and explored the association of camouflage to escape behaviour (Wilson-Aggarwal *et al*., 2016). Therefore, it is necessary to expand our knowledge of animal camouflage in marine ecosystems where predation is intense (Roslin *et al*., 2017), so as to understand how the camouflage benefits an organism in avoiding predation. For this study, the common shore crab (*Carcinus maenas*) which is an intertidal species, widely found along the coastlines of the UK and other parts of Europe is an ideal species (Crothers 1966; 1968; Todd *et al.*, 2006). It is known to have variable carapace patterns which could be driven by matching of individuals to different substrates (Bond & Kamil, 2006; Stevens *et al*., 2014). Both juvenile and adult crabs have numerous predators ranging from bird species such as gulls and shore birds to various fish species, and due to these potential predators', selection from visual predation is severe (Powell, 1962; Crothers, 1968; Hogarth, 1978; Stevens *et al.*, 2014). Roff (2009) describes how some life history stages of prey are more vulnerable to predators. In marine invertebrates, this includes juveniles which are more vulnerable to fish and avian predators than adults (Crothers, 1978; Todd *et al*., 2006; Stevens *et al*., 2014 a;b). This is perhaps because the adults are well equipped with strong armor and can better evade predators (Hogarth, 1975; 1978). The work of Stevens *et al.,* 2014, also provides valuable basis for this experiment but is limited because they did not measure the escape response of crabs and focussed on colour change rather than individual appearance and camouflage.

In this chapter, through a series of field-based experiments, I tested the escape behaviour of shore crabs in response to artificial backgrounds using a simulated predator attack. In the first experiment, crabs from rock pools were used to investigate whether the mismatched background colour and the presence of a predator affected the escape response of individuals. Escape response was analysed using a simulated model seagull as a predator. I predicted predator presence would increase the probability of fleeing, but that individuals which closely matched the background would elicit a slower response than mismatched individuals. In the second experiment, I explored whether the heterogeneity of the substrate influenced the escape decision by monitoring the response of crabs on patterned (heterogeneous) and uniform (homogeneous) achromatic substrates. This is important because shore crabs from a more visually heterogeneous environment, such as rock pools, have lower background matching than a homogeneous habitat, such as mudflats. However, crabs from rock pools tend to show a higher disruptive effect than mudflat crabs (Price *et al*., 2019) indicating that crabs adopt different camouflage strategies between different habitats. This would suggest that the escape response would also differ between the patterned and uniform habitats. This study used crabs from rock pools and predicted that a slower escape response would be demonstrated on a heterogeneous substrate than a homogeneous one.

Methods

Ethical note

Collection of individuals was carried out with the approval of the University of Exeter Biosciences ethical committee (application eCORN000605 v3.3 2018). For the pilot experiment, all individuals were used for a short period of time, approximately one hour and returned to their original rockpool area unharmed following the experiment. Those used in the main experiment were kept for longer periods, approximately six hours, but measures were adopted to minimize the stress to the individuals. These included providing natural seawater, minimizing photography time (see below and chapter 3 for details) before being released back to the rock pools after completion. Shore crabs are not endangered or protected invertebrate species therefore no additional licenses were required.

Crab Collection Methods

Crabs were sampled from rockpool sites at Gyllyngvase in Falmouth, Cornwall. Gyllyngvase beach (50° 8’ 39.42” N, -5° 4’ 5.244” W) was chosen as its substrate composition is a heterogeneous habitat for the shore crabs. This site primarily comprises large rocky clusters, deep gullies filled with stony/gravel and sandy substrates, a few mussel beds and increasing amounts of seaweed near the low water mark.

Sampling was conducted in 2019 from late March to early October taking the weather and tides into account. The field site is a publicly accessible land, again not requiring any further permits. Crabs were collected within 2-3 hours before the low tide. Rockpools were searched for shore crabs in low, middle, and high tidal zones for a time period of five minutes or less if the sampling area was small or bare. 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 their lack of swimming paddles. Movable rocks and sea weeds were turned over, sand and gravel raked using fingers, crevices were checked during the search to avoid missing any likely crabs. During the collection, sex was not taken into account as it is difficult to measure in juveniles (Mohamedeen & Hartnoll, 1990). Individuals measuring less than 7mm or greater than 35mm were classified as too small or big and were avoided in the study as extremely small crabs would be difficult to handle and the large crabs would not fit in the acclimatisation pot causing stress to the individuals prior to the experiment. A total of 209 crabs were collected and tested during the sampling period.

Experimental Design

Experiment 1: Does the mismatched background affect the escape response of crabs?

The first experiment aimed to measure the escape response of shore crabs corresponding to two factors, background colour and presence of predator. To achieve this, a factorial experimental design was adopted with trials organized into blocks. Each block has four individuals with the following four trials carried out on each individual (4 x 4),

- Trial a = Predator (Bird) ‘attacking’ a crab placed on Black circle (P B)
- Trial b = No predator attacking a crab placed on Black circle (N B)
- Trial c = Predator (Bird) attacking a crab placed on Yellow circle (P Y)
- Trial d = No predator attacking a crab placed on Yellow circle (N Y)

The four trials were run in a different order on each of the four individuals to ensure every possible combination of treatment order (crab 1 = a,b,c,d; crab 2 = b,c,d,a; crab 3 = c,d,a,b; crab 4 = d,a,b,c). The different orders were to balance the order of presentation across treatment, and control for differences in response that might arise due to order.

Table 2.1: An overview of the block design showing a small fragment of all the possible combinations of the treatments in a set of 8 individuals. Crabs were tested in this treatment order starting from treatment 1 on all 24 individuals and moving to treatment 2, 3 and 4. This was to ensure all crabs had equal rest time prior to beginning of the next trial.

Individual	Treatment 1	Treatment 2	Treatment 3	Treatment 4
1	a	b	c	d
2	b	c	d	a
3	c	d	a	b
4	d	a	b	c
5	a	b	c	d
6	b	c	d	a

7	c	d	a	b
8	d	a	b	c

The experimental setup consisted of an arena designed to record movement, specifically the escape behaviour of the crabs. The texture of a sandpaper was found to best resemble their natural gravel substrate option as it was rough enough to create friction for running and smooth enough to allow for an even movement. Yellow and black colours were chosen for the sandpaper as they provide a good match with both the natural background and colour of crabs. A fine grade yellow sandpaper (of dimensions 230x280mm) was arranged in a grey tray (of dimensions 37x21x9cm) and a circular arena of fine grade black sandpaper (of diameter 10 cm) placed on top of it secured with two-sided tape. Similarly, black sandpaper (of dimensions 140x230mm) with a yellow circle (of diameter 10 cm) on top was arranged in another grey tray of same dimensions (Figure 2.1B).

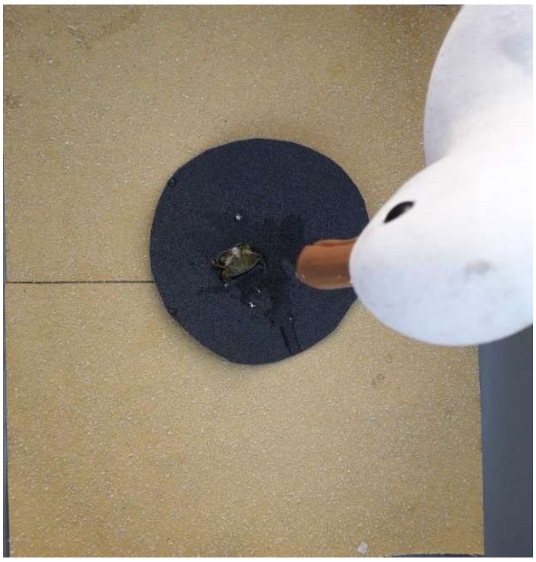
Crabs collected were in the range of 0.7 – 3.5 cm and housed in separate transparent plastic pots to prevent cannibalism or antagonism and each pot was randomly numbered from 1 to 24. To further minimise the stress levels of crabs, each pot was filled with natural sea water collected from nearby rockpool area to wash their gills and were also provided with a refuge in form of a large pebble again collected from around the site (Figure 2.1A). These pots were further arranged according to their trial turn to increase efficiency of the experiment and minimise the stress to the crabs. The crabs were then photographed to analyse the coloration and camouflage of crabs against the artificial background.

Experimental Procedure

For 1st treatment, trial ‘a’ was conducted on all the crabs that fall in ‘a’ category (Table 2.1). The crab was placed on black circle and covered with a black pot (of diameter 5cm and height 5cm) to let it acclimatise for 3 min. Following this, the pot was gently lifted so as to create minimum disturbance and a model sea gull (of dimensions 30cm x 28cm x 8cm) simulated as predator was slowly lowered at speed of 10cm/s from a distance of 0.3 m at an angle 45 degrees to simulate a predation attempt as it would encounter in a natural setting. The model predator tapped the crab to derive a response (Figure 2.1C) and crabs were observed for a maximum of 5 min. If the crab started fleeing, the initial time to move (FID) was recorded and the time to leave the black circle and onto the yellow background was noted. In case the crab failed to move with one tap, tapping was continued at an interval of 10 sec. For trial ‘b’, the arrangement remained same, but the predator simulation was removed. For trial ‘c’ and ‘d’, yellow sandpaper cut-out was used with and without predator simulation, respectively. This was followed by 2nd, 3rd, and 4th treatment, where the next following trial was carried out. In case the crab did not move or respond or failed to leave the circle within the time limit, it was recorded as ‘300s’ meaning did not move or were too slow to move.

A)

B)



C)

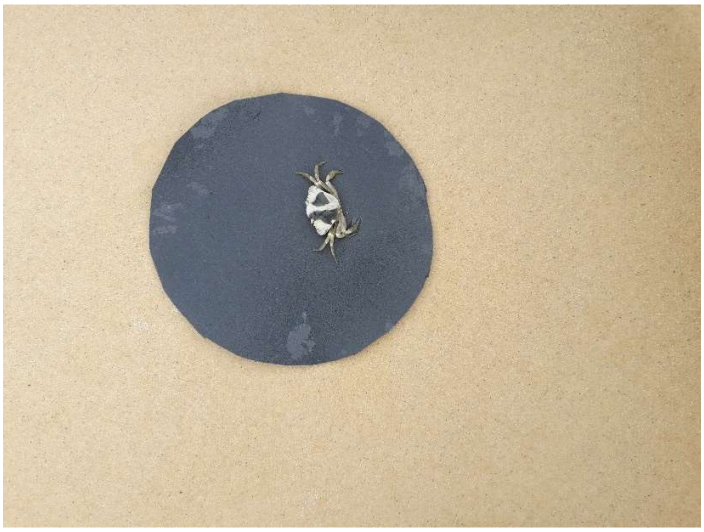


Figure 2.1: Experimental setup used in the experiment showing A) Arenas used for examining the escape behaviour. B) Trial ‘a’ in progress (top view). C) Trial ‘b’ in progress (top view).

Experiment 2: Does the substrate heterogeneity affect the escape response of crabs?

In the second experiment, I aimed to test whether crabs when placed on different substrate types show changes in escape response based on the level of brightness or colour match. Here, I test the hypothesis that crabs with different camouflage respond differently to a predator when present on homogeneous and heterogeneous backgrounds.

Experimental Procedure

For this, the experimental setup was slightly modified by introducing special gravel boards which functioned as arena to capture the movement of crabs instead of the previous plain sandpapers. Six white plastic chopping boards of dimensions (38cm x 25cm) boards were used and layered with uniformly grey or patterned gravel, three versions, to represent the uniform or homogeneous and patterned/heterogeneous substrates to ensure the artificial background closely resembled the natural environment. The different boards were randomly used in the treatments so as to prevent any learning behaviour in crabs.

The patterned gravel included black and white gravel pieces, from a black and white bag (Swell Harlequin Gravel, Swell, Cheshire, UK 3-8 mm in size). Uniform grey gravel consisted of purely grey gravel (Swell Harlequin Gravel, Swell, Cheshire, UK 3-8 mm in size); see figure 2. The texture and colour of gravel resembled their natural gravel substrate so was suited for this experiment. To keep the brightness of both backgrounds same, the gravel of all three colours was weighed and photographed individually and in a mixture with different ratios of black and white. The 70:30 ratio of black and white respectively was used in the mix after comparing with the grey gravel. The same volume of gravel was used for both patterned and uniform treatments to ensure all conditions were the same for each board except the colour of the gravel. The grey gravel was simply glued to the board in an even layer to create the uniform boards while black and white gravel was weighed using measuring spoons to create blotches of different sizes crating a patterned background. A boundary of diameter 10 cm was marked in the centre of the board to differentiate between initial time to move and total time taken. Crabs collected were in the range of 0.7 – 3.5 cm and housed in separate transparent plastic pots and each pot was randomly numbered from 1 to 28. To minimise the stress levels of crabs, each pot was filled with natural seawater collected from nearby rockpool and also were provided with a refuge in form of a large pebble again collected from around just as in case of first experiment (Figure 2.2. These pots were further arranged according to their trial turn to increase efficiency of the experiment and minimise the stress to the crabs. The crabs are then photographed in visible region as a measure camouflage keeping the photography time as short as possible.

This experiment aimed to measure the escape response of shore crabs corresponding to two factors, background type and predator. To achieve this, same factorial experimental design was adopted with following four trials are done on each individual (4 x 4),

- Trial a = Predator attempt simulation on Patterned/Heterogeneous background (P H)
- Trial b = No predator attack on Patterned background (N H)
- Trial c = Predator attempt simulation on Uniform/Homogeneous background (P U)
- Trial d = No predator attack on Uniform background (N U)

Each crab experienced the trials as in experiment 1. For trial ‘a’ exactly same procedure as the first experiment was followed except here the model predator gave the impression of gazing at the crab to derive a response rather than actual tapping to check if crabs could see the predator. Individuals were observed for a maximum of 3 min. If the crab started fleeing, the initial time to move (FID) was recorded and the time to leave the circle was noted. In case, the crab failed to move, no response or ‘3’ was noted down. For trial ‘b’, the arrangement remained same, but the predator simulation was removed. For trial ‘c’ and ‘d’, uniform or grey board was used with and without predator simulation, respectively.

Figure 2.2: Experimental setup used in the experiment showing Boards/Arenas – Heterogeneous or Patterned (top) and Uniform or grey (bottom), used for examining the escape behaviour.

Photography

A tripod was set up with a digital Nikon D7000 camera, which had undergone a quartz conversion to facilitate UV sensitivity (Advanced Camera Services, Norfolk, UK). The UV reflectance in both background and crabs and the UV reflectance to visible light are similar, and so crab coloration was analysed only in the visible range of spectrum (400-700nm) (Stevens *et al*., 2013). In addition, the exposure times for taking UV images are longer so to reduce stressing the crabs, photography was undertaken as quickly as possible. A filter (Baader UV/IR Cut filer) was slid in front of a Nikon 105mm lens that blocked UV and infrared light, transmitting wavelengths between 400-700nm (visible region) only, to enable capturing human visible images.

Crabs were taken out from their individual pots only before photographing and carapaces were dried by gently dabbing with blue roll to remove surface water to avoid any further stress. The crab was then gently placed underneath the camera in the arena. A black and white reflectance standard was placed adjacent to 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 5% and 95% of all wavelengths, respectively. Including a standard in every image allows controlling the variations in lighting conditions as all the photography was conducted under natural light (Stevens *et al*., 2007; Troscianko & Stevens., 2015). Photographs were taken with a fixed aperture setting in RAW format. To avoid over exposure as a result of specular reflectance in images, several photos were taken of the same individual at a range of exposures. Carapace width was used to determine crab maturity and was measured from the digital photographs using the ruler included in the photos. A baseline value of 25mm was used to categorize the crabs into smaller (immature) juveniles and larger (mature) adults based on their size (Hogarth, 1975; Stevens *et al*., 2014b). Photography was kept to approximately 1 min per individual to prevent any additional stress.

Image analyses

The Multispectral Image Calibration and Analysis Toolbox (Mica Toolbox version 1.22) developed by Troscianko & Stevens, (2015) was used for all the image calibration analyses and all the work was carried out in program ImageJ (version 1.8.0_112). All the photographs were sorted, and the best image of an individual was chosen by viewing the RGB histograms in the photo screening mode of the package. Any overexposed images due to specular reflectance were removed before starting the analyses multispectral images were created each individual by selecting the 95% and 5% reflectance standards in order to analyze the camouflage metrics. For this, within each image an outline of the individual's carapace (covering as much area as possible) was selected manually using the polygon tool as a region of interest (ROI) for the crab and rectangular section of yellow and black sandpapers as ROI for background. Images were calibrated with regards to the reflectance standard, with the image wavelength channel scaled so as a value of 65535 on a 16-bit scale equals 100% reflectance. Individuals should ideally be viewed and analyzed using the visual system used by their potential predator groups to gain camouflage advantage but in this case because shore crabs have many predators from shore birds to fish, it was difficult to choose one visual system, therefore objective measures were used instead of any specific visual system (Crothers 1966; Hogarth 1978).

Calculation of reflectance values

Image data from the multispectral images were used to calculate two metrics through batch image analysis. Overall reflectance is a measure of the overall brightness across the visible spectrum and was calculated as (LW+MW+SW)/3. The second metric, hue was measured with regards to yellow (LW+MW) versus blue (SW) light was calculated as (LW+MW)/SW. A value of 1 means that a crab is grey whereas a value of less than 1 indicates the crab or substrate is blue and above 1 means it is yellow. I also analyzed how well the brightness of crabs matched their background and if it affected their escape behaviour. I calculated a brightness match by taking the average difference in brightness between each crab and the two artificial substrates. Values closer to 0 mean a good match. The formula used was:

Absolute Brightness = $ABS(B_c – B_b)$,

where B is Brightness of the crab c and Background b. I also measured color camouflage by taking the average Euclidean distance in a trichromatic reflectance color space between each individual and their two backgrounds. To eliminate absolute variation, the LW, MW and SW reflectance values are standardized to relative proportions (Endler & Mielke, 2005) which are converted into a trichromatic colour space with each point represented by an X and Y coordinate. Low Euclidean distance values between crab and background means a good colour match. The equation used was:

$= \sqrt{} ((X_c - X_b)^2+(Y_c - Y_b)^2$

where X and Y are the X and Y coordinates for crab (c) and background (b) being compared, respectively (Kelber, Vorobyev and Osorio, 2003; Stevens *et al.*, 2009). All calibrations and analyses were undertaken in ImageJ.

Statistical Analyses

The time data collected showed that many of the crabs responded very fast, within a difference of 3 to 4 sec and this shifted the data to one side creating a skew since other crabs were much slower to respond (Figure 2.4 and 2.9). To deal with this, the time data was categorized on the basis of an average scale into two categories:

1. Fast (Individuals moving within 10s – experiment 1 and 50s – experiment 2),
2. Slow (Individuals moving after 10s – experiment 1 and 50s – experiment 2)

As the majority of the crabs responded to the first predator attack (which is at an interval of 10s) it was set as cut-off and this was considered a FAST response. The individuals that required second or third predator attack was placed under SLOW response. Individuals that responded quickly were assigned the number 1, and those that were slow to move were assigned 0.

All statistical analyses were carried out in RStudio (R v 3.5.1). GLMMs of the family binomial were used to test the escape response (fast or slow behaviour) of differently camouflaged crabs on matching light and dark backgrounds with individual id as random factor to control for repeated measurements on the same individual. The predator, circle/background, size, overall brightness, and colour were chosen as main effects and whether the interaction of their main effects influenced the flight response of the crabs was also considered as in case of GLMM, Binomial. The mixed-effects model was fitted using the 'lmer' function in the 'lme4' package (Bates *et al.,* 2015) and the associated significance tests through the 'anova' function in the 'lmerTest' package (Kuznetsova *et al.,* 2017). In addition to GLMMs, a chi-square test of independence was performed to examine the escape response of individuals to the different predators and different background treatments. Crabs were predicted to show greater escape response on a non-matching background, where the likelihood of being detected by the predator is higher. While analysing it was found that 13 individuals were outliers as they had extremely high values of brightness and colour match which were skewing the data. The high values seemed unlikely after rechecking the photographs and could perhaps be a result of overexposed spots that were not visible enough at the time of image analysis so had to be removed leaving to 196 individuals. Movement of individuals was timed in each of the four treatments.

In case of non-normal data (initial time, total time, brightness, and colour values), skewness test was performed (using moments package in R). These values were either greater positively skewed or showed moderate skewness so were transformed using square root or log transformation or non-parametric tests (Kruskal Wallis, Wilcoxon rank sum in the "dplyr" package) were used as an alternative. This mainly happened with the difference in brightness and colour variables so non-parametric test such as Kruskal Wallis, Wilcoxon rank sum were used. Crabs were also compared to the colour and brightness of all the background types. Crabs when placed on black background matched more closely to the black background compared to the yellow background in terms of brightness. On the contrary, crabs better matched the yellow background when placed on yellow than when placed on a black background in terms of colour. Crabs when placed on heterogeneous background closely matched the patterned background compared to when placed on uniform background in colour. Kruskal Wallace test was used to compare the standard of match between each individual and its background. For my second experiment, size interaction was not included in the GLMM because the model failed to execute the interaction. After removing the non-significant size and predator interaction, the model showed error.

Results

Experiment 1: Does the mismatched background affect the escape response of crabs?

Did individuals respond to the treatments?

The crabs responded to the different treatments by displaying a fleeing response. A chi-square test of independence was performed to examine the escape response of individuals to the predator and mismatched background treatments. There was a significant relationship between the escape response and treatments, X^2 (3, N = 196) = 114.36, $p < 0.001$. The association between predator and escape response was also examined and a significant relationship was found, X^2 (1, N = 196) = 108.6, $p < 0.001$, indicating that the individuals with a predator treatment responded faster compared to the control (Fig 2.5).

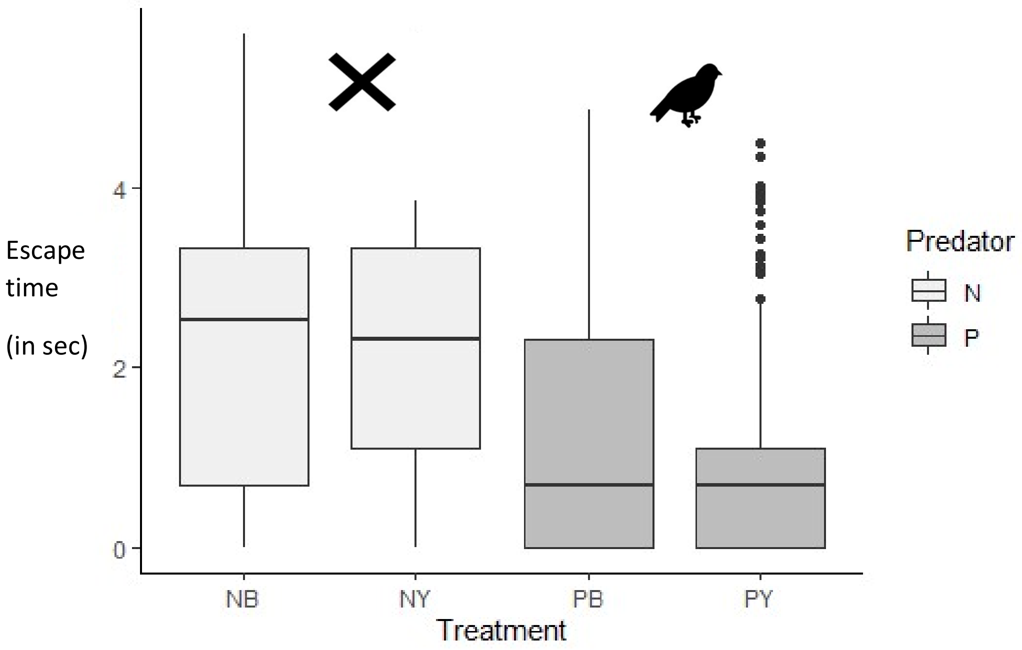
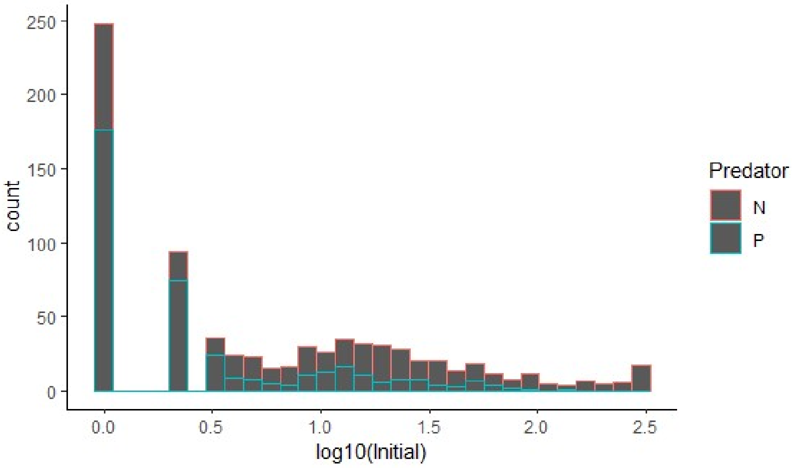


Figure 2.3: Escape time of shore crabs when exposed to Predator (P) or No Predator (N) and Background Black(B) or Yellow (Y) treatments as shown. Escape time is greater in the absence of a predator and faster in the presence of a threat. All values are log transformed. Plot shows median and interquartile range (IQR), dots indicate outliers.



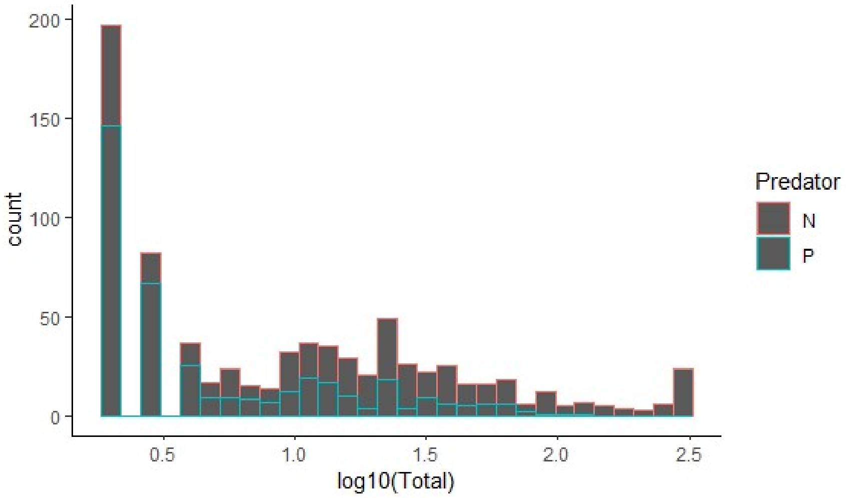


Figure 2.4: Time to move, Initial time (A) and Total time (B) of shore crabs in presence of Bird (P) and no predator (N). The majority of crabs moved within 10 to 20 sec showing willingness to move increases with level of threat. The initial and total time to flee were both greater positively skewed. All values are transformed using log transformation.

A)

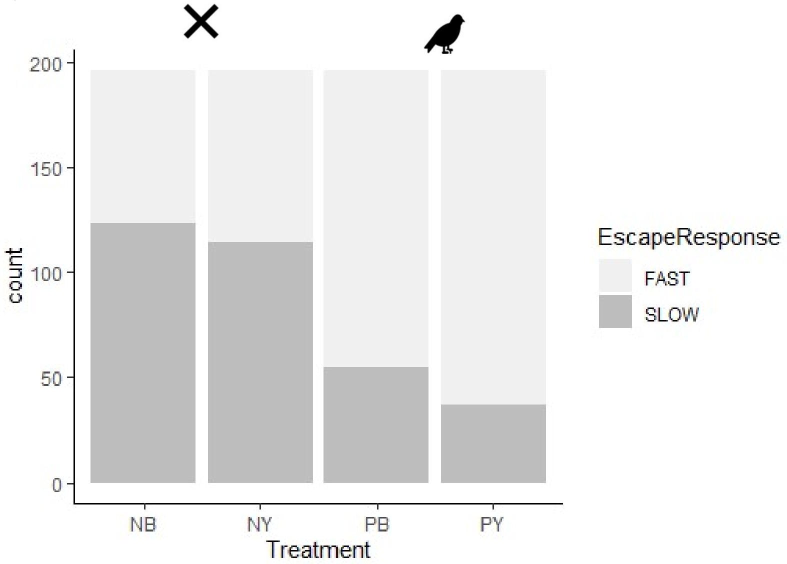


Figure 2.5: More individuals responded fast to the predator attack then no predator treatment. Proportion of crabs moving from the simulated predator attack when exposed to A) each predator and background treatments. Plots indicate the response was greater in the predator treatment, especially on the yellow background ($p<0.001$).

Brightness Difference

The mean difference in brightness of individuals indicated significant differences between the background groups (Kruskal-Wallis; $X^2 = 607.25$, df = 1, $p < 0.001$) showing crabs more closely matched the black background than the yellow one (Figure 2.6).

Colour Difference

The mean difference in colour of individuals indicated significant differences between the background groups (Kruskal-Wallis; $X^2 = 503.65$, df = 1, $p < 0.001$) showing crabs better matched the yellow background than black background (Figure 2.7).

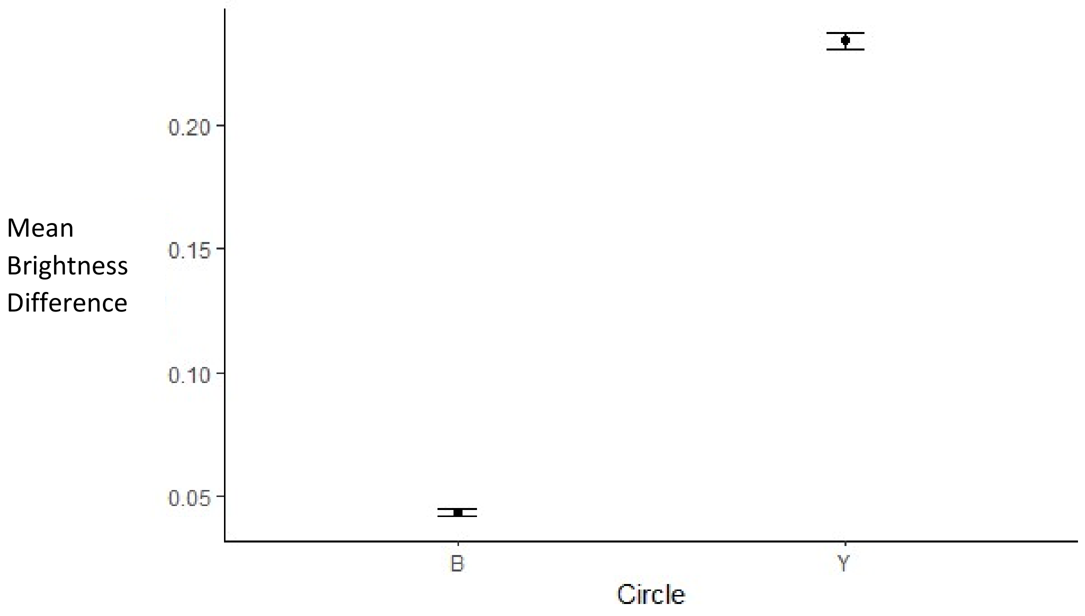


Figure 2.6: Mean brightness difference with 95% confidence interval of individuals exposed to each background treatment, black (B) and yellow (Y). The difference in brightness of the crab and background indicate that crabs were generally closer to the black background.

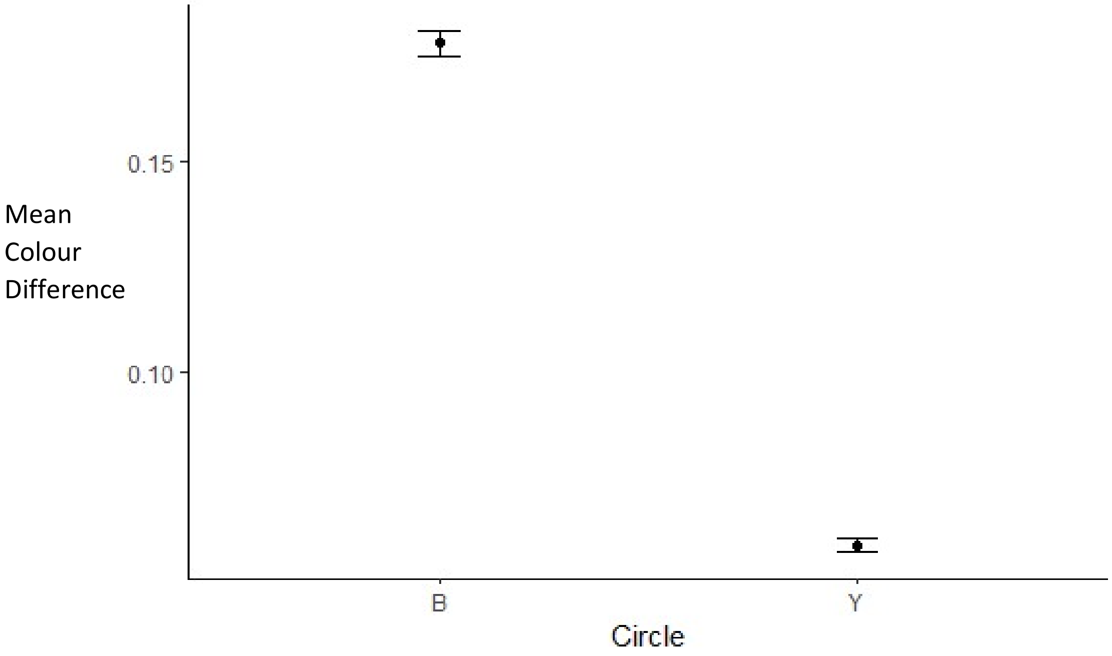


Figure 2.7: Mean color difference with 95% confidence interval of individuals exposed to each background treatment, black (B) and yellow (Y). The difference in color of the crab and background indicate that crabs were generally closer to the yellow background.

Brightness difference

The full model included an interaction between predator, background (circle), size and overall brightness but was found to have no significance (GLMM, Binomial, $X^2_0 = 0$, $p = 1$); hence, was removed from the model. An interaction between size and overall brightness and predator and overall brightness was initially included but found to have no significance (GLMM, Binomial, $X^2_1 = 1.45$, $p = 0.2$) and ($X^2_1 = 3.51$, $p = 0.06$) respectively. Therefore, these interactions were removed from the model. An interaction between size and background was also removed as it was not significant (X^2_1= 0.01, p =0.9) and similarly, size and predator interaction had no significant effect (X^2_1= 0.09, p = 0.7). An interaction between background (circle) and overall brightness (X^2_1= 0.4, p =0.5) as well as predator and background (X^2_1= 1.41, p =0.2) were also removed as they were not significant. Similarly, brightness had no significant effect (X^2_1= 0.33, p = 0.5). Background significantly affected the escape behaviour of crabs (X^2_1= 6.83, p =0.009) indicating that the chance of a slower response on a yellow background in the absence of predator is 55% as compared to 72% chances of being slower on a black background. This finding suggests that crabs showed a slower response on black background than yellow background. Predator was also significant (X^2_1= 158.69, $p < 0.001$) where the probability of being slow on a black background with no predator is 72%; however, in presence of predator this probability decreased to 32%. When crabs were on yellow background in the presence of predator, the probability was even lower with only 12% chance of a slow response. Size was also significant (X^2_1= 13.03, $p < 0.001$) suggesting that the probability of a slow response shown by crabs is 7.7% due to size. The results from the final model show the escape response was dependent on both predator and background (circle) (see Table 2.2).

Table 2.2: Results of the final GLMM with family binomial on 196 individuals showing the significant effect of predator, size and background (Circle) on the escape behaviour of crabs. Significant terms are indicated with '*'.

	D.F	**X^2**	**p**
Predator	1	158.69	<0.001***
Circle	1	6.83	0.009**
Size	1	13.03	<0.001***
Brightness	1	0.33	0.5
Pred:Brightness	1	3.51	0.06

Circle:Brightness	1	0.4	0.5
Pred:Circle	1	1.41	0.2
Pred:Size	1	0.09	0.7
Size:Brightness	1	1.45	0.2
Circle:Size	1	0.01	0.9

Colour difference

The full model included an interaction between predator, background (circle), size and colour camouflage but was found to have no significance (GLMM, Binomial, $X^2_0 = 0$, p =1); hence, was removed from the model. An interaction between background and colour and size and colour was also included but found to have no significance (GLMM, Binomial, $X^2_1 = 0.05$, p =0.8) and ($X^2_1 = 0.45$, p =0.5) respectively. Therefore, were removed from the model. An interaction between predator and circle was also initially included but removed as it was not significant ($X^2_1 = 1.41$, p =0.2) and similarly, colour had no significant effect ($X^2_1 = 0.47$, p = 0.5). The results from the final model show the escape response was dependent on both predator, size and background (circle) as well as size same as that of brightness difference (see Table 2.3).

Table 2.3: Results of the final GLMM with family binomial on 196 individuals showing the significant effect of predator, size and background (circle) on the escape behaviour of crabs. Significant terms are indicated with '*'.

	D.F	**X^2**	**p**
Predator	1	158.69	<0.001***
Circle	1	6.83	0.009**

Size	1	13.03	<0.001***
Colour	1	0.47	0.5
Pred:Colour	1	0.36	0.5
Size:Colour	1	0.64	0.4
Pred:Circle	1	1.41	0.2

Experiment 2: Does the substrate heterogeneity affect the escape response of crabs?

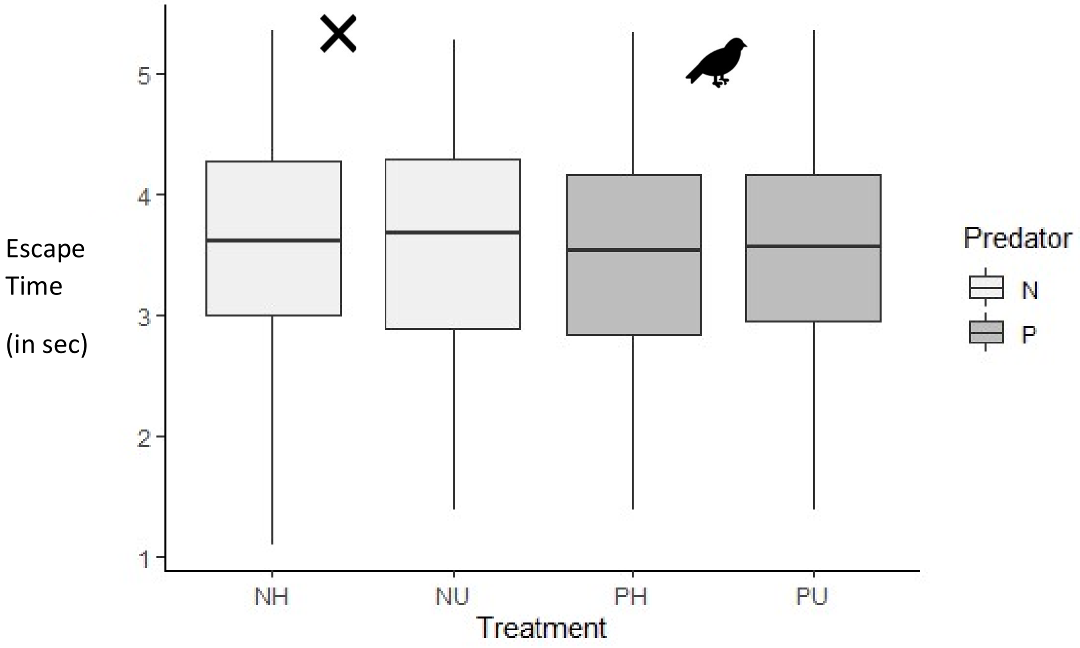


Figure 2.8: Escape time of shore crabs when exposed to Predator (P) or No Predator (N) and Background Uniform (U) or Heterogeneous (H) treatments as shown. Escape time is longer in absence of predator and a heterogeneous background. All values are log transformed. Plot shows median and interquartile range (IQR).

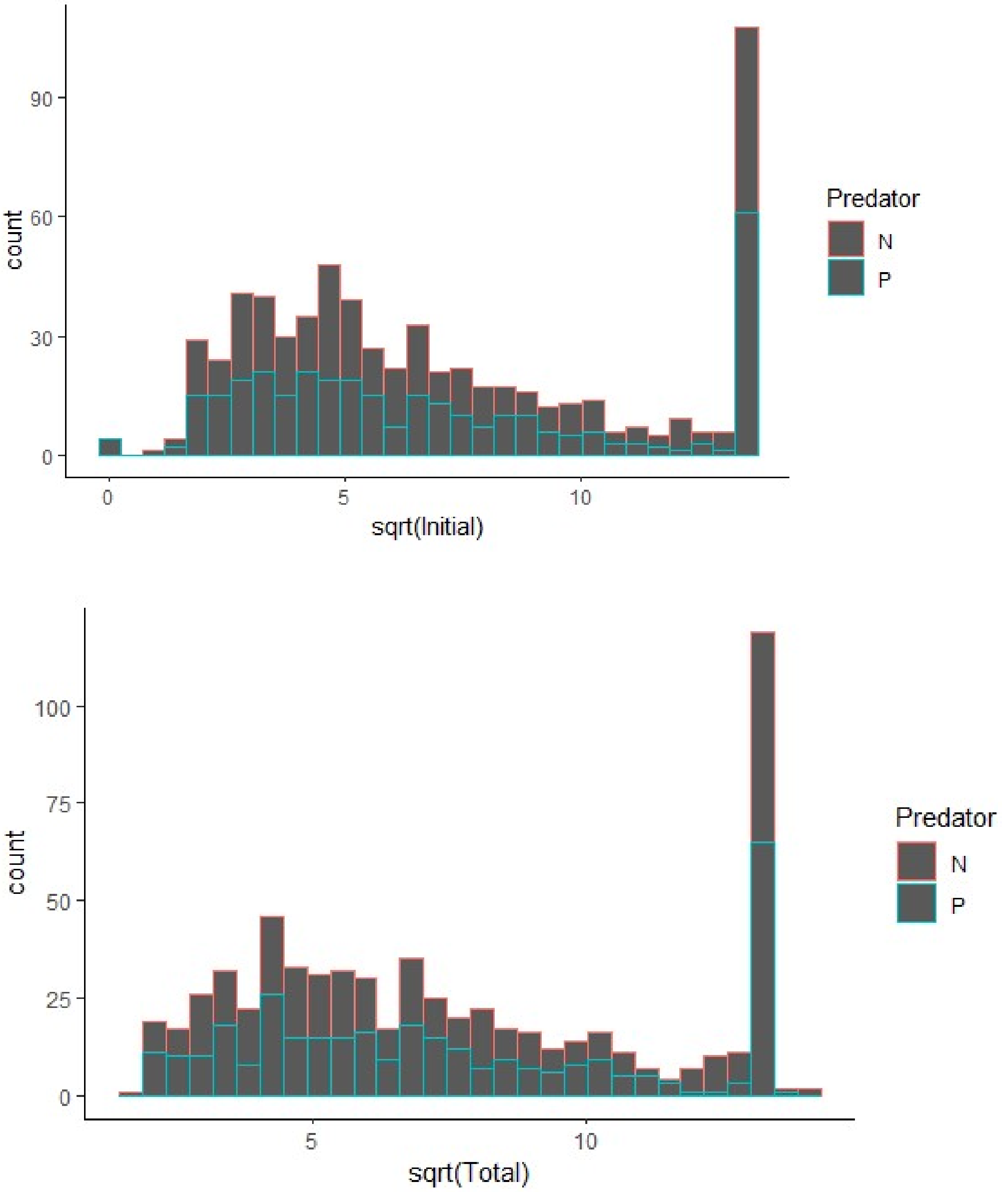


Figure 2.9: Time to move, Initial time (A) and Total time (B) of shore crabs in presence of Bird (P) and no predator (N) on Uniform and Heterogeneous backgrounds. Majority of them moved within 50 sec showing willingness to move increases with level of threat. The initial and total time to move were moderately positively skewed. All values are transformed using square root transformation.

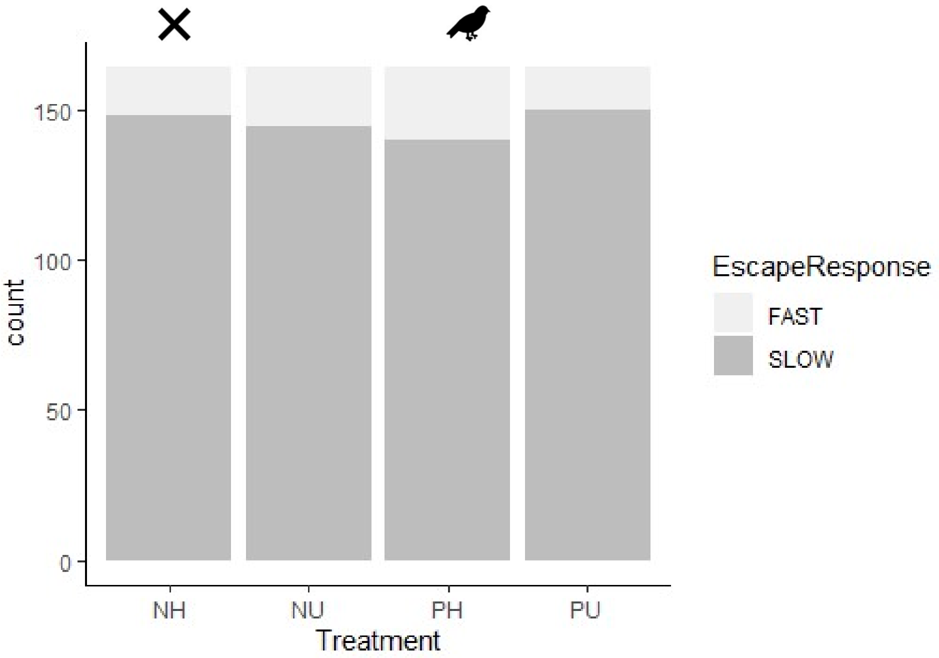


Figure 2.10: More individuals responded slower than the first experiment irrespective of the predator and no predator treatment. Proportion of crabs fleeing when exposed to each predator simulation and background treatments indicates that crabs had a slow response to all four treatments.

Brightness difference

The mean difference in brightness of individuals differed between boards (Kruskal Wallis; $\chi^2_{(1)}$ =36.2, $p<0.05$) showing crabs were closer to the uniform board (Figure 2.11).

Colour Difference

The mean difference in colour of individuals differed between boards (Kruskal Wallis; $\chi^2_{(1)}$ =35.2, $p<0.05$) showing crabs were closer to the heterogeneous board (Figure 2.12).

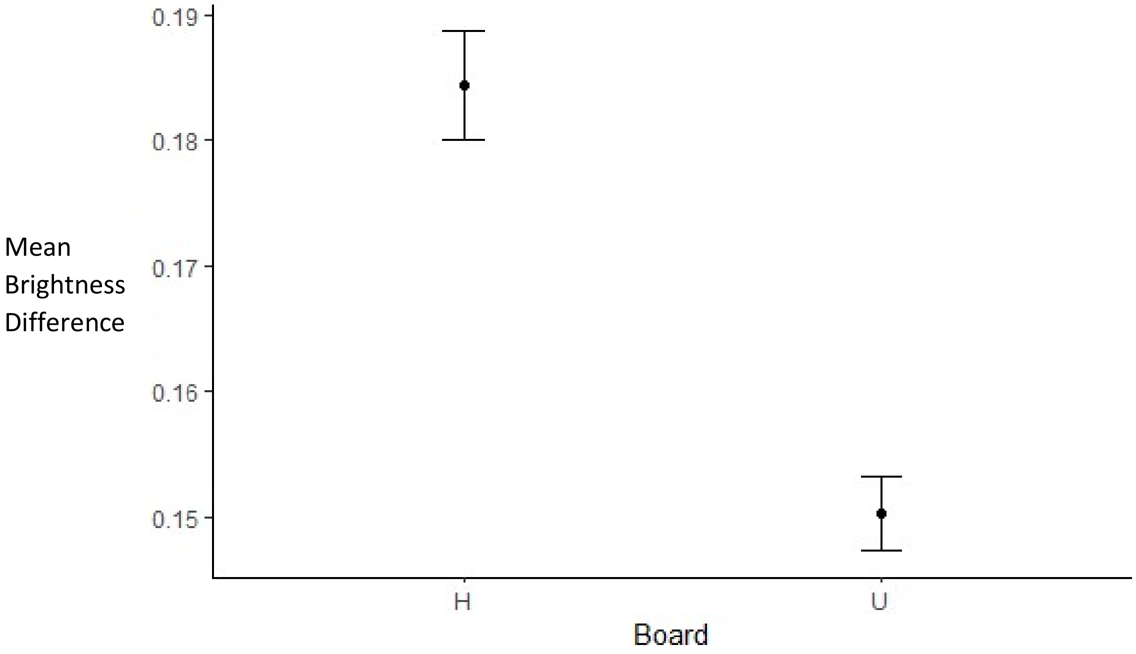


Figure 2.11: Mean brightness difference with 95% confidence interval of individuals exposed to each background treatment, Uniform (U) and Heterogeneous (Y). The difference in brightness of the crab and background indicate that crabs were generally closer to the uniform background ($p<0.05$).

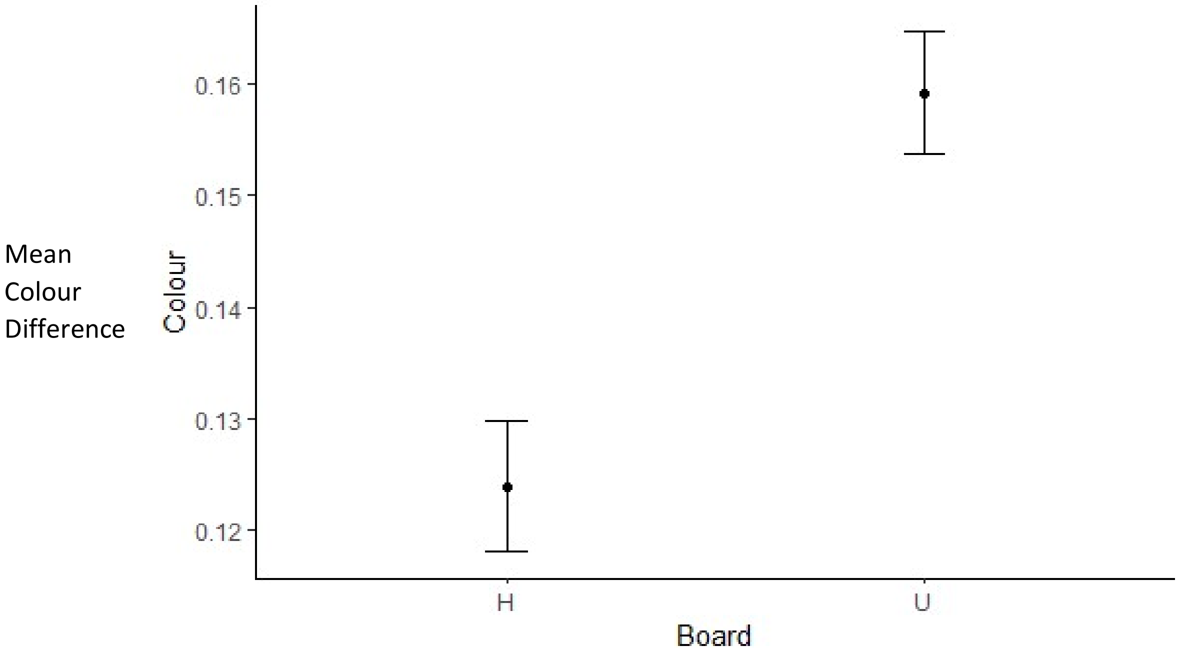


Figure 2.12: Mean colour difference with 95% confidence interval of individuals exposed to each background treatment, Uniform (U) and Heterogeneous (Y). The difference in colour of the crab and background indicate that crabs were generally closer to the Heterogeneous background ($p<0.05$).

Brightness Difference

The maximum model included an interaction between predator, substrate (board) and overall brightness and this 3 way-interaction was found to be significant (GLMM with family Binomial, $X^2{}_1 = 5.97$, $p = 0.01$). Therefore, the maximum model was the minimal adequate model. The probability of being slow on a heterogeneous substrate in the presence of predator is 96.5%, whereas in absence the probability was increased to 98%. The individuals which closely matched the brightness of the substrate displayed a slower response. (Figure 2.10). The results from the final model show the escape response was dependent on all three factors predator, substrate (board) and overall brightness (see Table 2.4).

Table 2.4: Results of the full GLMM with family binomial on 164 individuals and variables include Predator, Substrate (Board), overall brightness difference (BD). The model shows the significant effect of substrate (Board), Predator and significant effect of interaction of predator and substrate (board), substrate (board) and overall brightness (BD) and Predator, substrate, and overall brightness on the escape behaviour of crabs. Significant terms are indicated with '*'.

Variable	Estimate	Std. Error	z-value	p-value
Intercept	5.634	1.286	4.379	<0.001***
Predator	-2.397	1.225	-1.957	0.050*
Board - Uniform	-3.506	1.330	-2.636	0.008**

Brightness	-0.078	0.050	-1.587	0.112
Pred*Uniform	5.110	1.829	2.794	0.005**
Pred*Brightness	0.080	0.056	1.431	0.152
Uniform*Brightness	0.187	0.074	2.520	0.012*
Pred*Uniform*Brightness	-0.233	0.102	-2.279	0.023*

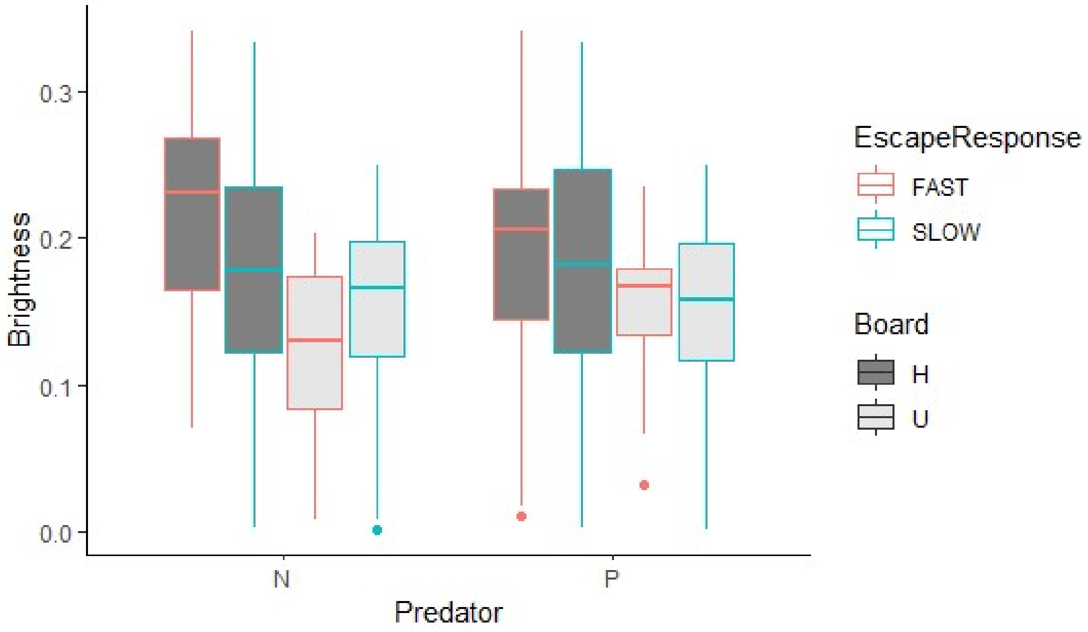


Figure 2.13: Boxplot showing brightness difference (y axis) influencing the fast or slow escape response with respect to Predator and substrate (Board) on x axis. The plot indicates with increasing brightness difference, escape response is faster but if on heterogeneous substrate, escape is slower. Plot shows median and interquartile range (IQR), circles indicate outliers.

Colour difference

The full model included an interaction between predator, substrate (board) and colour camouflage but was found to have no significance (GLMM, Binomial, X^2_1 = 0.43, p =0.5); hence, was removed from the model. An interaction between predator and colour was originally included but was removed as it was not significant (X^2_1= 0.15, p =0.7) and similarly, substrate and colour interaction had no significant effect (X^2_1= 1.59, p = 0.2). Colour significantly affected the escape behaviour of crabs (X^2_1= 4.36, p =0.03) (Figure 2.11). Crabs with greater colour difference were faster in escaping as opposed to the closely matching crabs. The interaction between predator and substrate was also significant (X^2_1= 4.32, p =0.03) where the probability of being slow on a heterogeneous substrate in the presence of predator is 96.2%. The results from the final model show the escape response was dependent on both predator and substrate (board) (see Table 2.5).

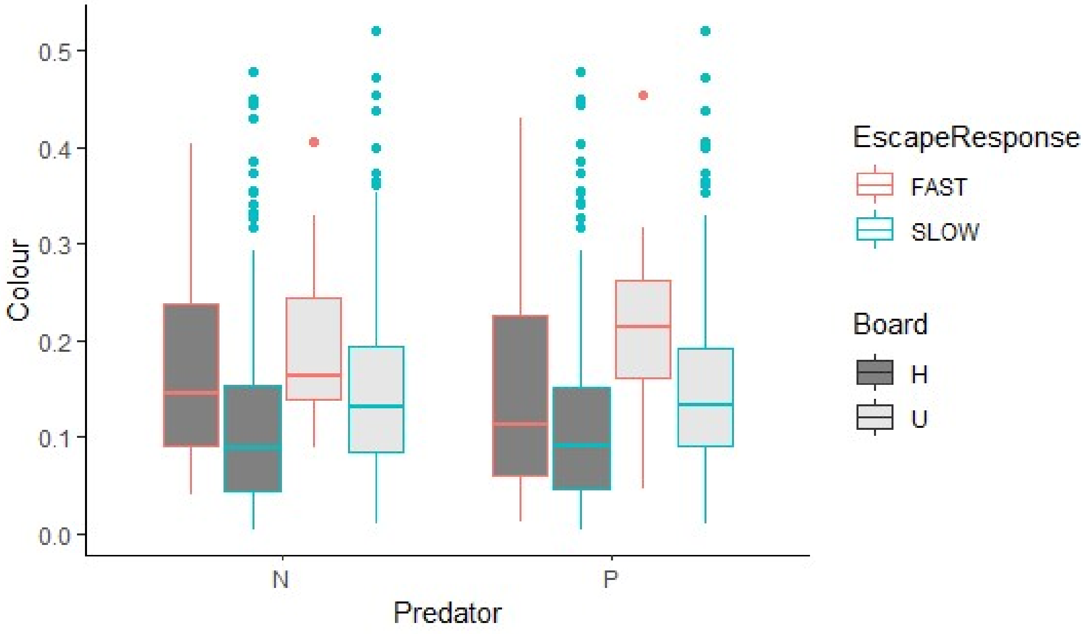


Figure 2.14: Boxplot showing colour difference (on y axis) influencing the fast or slow escape response with respect to Predator and substrate (Board) on x axis. The plot indicates an increasing colour difference results in faster escape**.** Plot shows median and interquartile range (IQR), outliers are indicated by circles.

Table 2.5: Results of the final GLMM with family binomial on 164 individuals showing the significant effect of colour and significant effect of interaction of predator and substrate (board) on the escape behaviour of crabs. Significant terms are indicated with ‘*’.

	D.F	X^2	P
Predator:Board	1	4.32	0.03*
Colour	1	4.36	0.03*
Pred:Colour	1	0.15	0.7
Board:Colour	1	1.59	0.2

Discussion

In this chapter, for experiment one, I explored whether the escape decisions of the common shore crab (*Carcinus maenas*) are influenced by brightness and colour-matching camouflage. The escape response was assessed using a simulated model of a predator bird (in this chapter - seagull). Crabs were placed on mismatched black and yellow treatment backgrounds and their escape was measured in terms of latency to flee, which is similar to Flight Initiation Distance (FID), the distance at which a prey species flees in response to an approaching threat. The second experiment tested whether the heterogeneity of the treatment background (here patterned or uniform artificial substrates) affected the escape response of the crabs. Individuals were placed on patterned substrate, consisting of black and white gravel and uniform substrate, consisting of only grey gravel with both substrates having approximately same levels of brightness. The escape behaviour of the crabs was measured in the same way as experiment one using time to flee.

In experiment one, the results find that the escape decisions of shore crabs are greatly influenced by both the predator and background as well as size. The individuals showed a greater escape response by fleeing faster in the predator treatments as compared to no predator treatments indicating that presence of predator plays a key role in escape decisions as predicted. This is perhaps because when in danger, a prey must decide whether, when and how far to flee as failure to do so results in injury or death (Stankowich & Blumstein, 2005; Cooper *et al*., 2010). Previous studies indicate that several factors such as direct or indirect approach, type of predator, proximity and speed of the predator influence the risk assessment and plan of action (Ydenberg & Dill, 1986; Lima & Dill, 1990; Broom & Ruxton, 2005; Martin *et al.*, 2009; Martin and Lopez, 2009). For example, in wall lizards, *Podarcis muralis* populations exposed to higher predation risk exhibit longer FID as opposed to low predation pressure (Diego-Rasilla, 2003). Another study on pea aphids observed that dropping behaviour in aphids increased in presence of foliar-foraging predators and decreased in absence of predators (Losey and Denno, 1998). Therefore, it seems likely that shore crabs considered the predator as threat and their time to flee was shorter in presence of a predator as opposed to its absence. Furthermore, the model predator chosen for this study greatly resembled the seagull, which is one of crabs' main predators (Crothers 1966). As a result, most crabs started fleeing after the first simulated attack which indicated detection by the predator demonstrating flight-response was probably the best course of action in this situation. In addition, this study implemented direct approach in the predator treatments which is in line with the study on Balearic lizard (*Podarcis lilfordi*), where FID of lizard was greater and the lizards were quick to flee in case of a direct approach than slower indirect approach (Cooper *et al*., 2009). This suggests that crabs are quicker to respond when predator uses a direct approach as opposed to an indirect approach when attacking.

The probability of a predator detecting a cryptic prey is generally low, therefore, in most predator-prey interactions, prey chooses not to flee. However, in certain circumstances such as being present on a mismatched background, the likelihood of prey being detected is higher which results in prey having to decide to stay or escape in case an attack occurs. In this study, the crabs were exposed on the mismatched backgrounds without any cover or refuge to hide suggesting the likelihood of being detected by the predator increased. The FID increases with distance to refuge (Kramer and Bonefant, 1997; Cooper *et al*., 2015); however, in case of absence of refuge, the prey moves away from the threat as seen in gerbils (Ellard and Goodale, 1988). This supports the crabs fleeing away from the predator in absence of refuge suggesting the probability of being recognized and attacked.

The background also significantly affected the flight-response of crabs, with black background eliciting a slower response compared to the yellow background. This is perhaps because majority of crabs were darker in appearance and crabs are at lower perceived risk on the dark background (Nokelainen *et al*., 2019). This finding concurs with a similar recent study on tadpole (*Ololygon machadoi*) which exhibits a flight response to rest on the background that provides an appropriate match when under predation pressure (Eterovick *et al*., 2018). Here, the crabs prefer the dark background and display a slower response suggest that the dark background provides an enhanced camouflage. Substrate selection in Japanese quail for egg laying (Lovell *et al*., 2013) is another example that prevents detection from visual hunters. Another study on Aegean wall lizard had similar findings, whereby lizards chose to rest on backgrounds that provide better match and enhance the level of camouflage (Marshall *et al*., 2016). Several studies suggest that for cryptic prey, because the probability of being detected by a predator is less, cryptic prey would have shorter FID. This has been demonstrated in experiments on cryptic *Anolis* lizards by Heatwole, (1968). Similarly, FID was shorter in case of Round-tailed Horned Lizard when matching the rocky substrates compared to the mismatched sandy area (Cooper & Sherbrooke, 2010). Another study by Lee *et al*., 2010 also quantified the egg colour match and nest background in Black-tailed Gulls (*Larus crassirostris*) using RGB image analysis and found the closer match between eggshell and nest was linked with increased egg survival. This is in contrast to my findings, where neither the overall brightness nor the colour made any significant difference in affecting the escape response of crabs. I predicted the closer the brightness and colour difference between the crab carapace and background, the slower the escape response; however, brightness and colour difference had no effect on escape response independently or on interacting with background colour, size and predator. The interaction of the background colour and predator also did not influence the escape response of crabs. This could be due to the colour, and texture of the background chosen (black and plain yellow sandpaper) which despite best efforts to give a natural look gave an artificial view. The size of crabs also significantly affected the flight-response. Schmidt *et al.,* 2008, found smaller scallop (*A. opercularis*) individuals closed their shells faster and for longer periods than larger adults; however, bigger adults were more resilient to higher water currents. This supports that crabs were fleeing based on the their carapace width with large adults taking longer to escape in the predator treatments.

For my second experiment on substrate heterogeneity, colour significantly influenced the escape response of crabs, whereby the individuals with a closer colour match with the patterned substrate showed a slower response. This aligns with my prediction as crabs that better match the background would have greater FID. This is supported by previous studies involving colour matching (Yahner and Mahan, 1996; Blanco *et al*., 2002; Lee *et al.,* 2010). For instance, stone curlew *Burhinus oedicnemus* clutches were more likely to survive if their egg colour matched the background colour (Solis *et al*., 1995). The interaction between predator and substrate (board) was also significant indicating that crabs were relying of their conspicuousness and showed a slower response when matching the background even in presence of predator.

For brightness match, the interaction between all three terms, predator, substrate, and brightness difference was significant. In scenario 1, no predator and patterned substrate treatment, with increasing brightness match, the individuals were 99.6% slower indicating greater level of background matching as compared to scenario 2, whereby in predator and heterogeneous substrate treatment, predator, brightness difference and their interaction affected the flight-response with 96.2% individuals being slow. In scenario 3, no predator and homogeneous substrate, substrate, brightness difference and their interaction influenced the escape response, with 90.3% individuals being slow as opposed to the last scenario 4, whereby in predator and homogeneous substrate treatment, all three -predator, substrate, brightness difference and their interaction affected the escape response with

99.1% individuals showing a slower response. This is perhaps because crabs were aware of their current level of conspicuousness and acted accordingly. This is in validation with several studies that demonstrate that escape decisions are dependent on the coloration as seen in chameleons (Cuadrado *et al.*, 2001), mountain hares (Stoner, Bininda-Emonds, & Caro, 2003), hermit crabs (Briffa, Haskell & Wilding, 2008). Similarly, shore crabs are known to change their brightness by becoming darker in appearance on a black background and lighter on a white background and adjust their camouflage over time. This reduces the likelihood of being detected by potential predators (Stevens *et al*., 2014). Some crabs were also observed repositioning in patches that better matched their carapace again indicating that they are vary of their surroundings. This was perhaps to increase the level of camouflage and ties in with the findings by Kang *et al*., (2013), whereby moths are reported to reposition themselves to a more cryptic position if they land on less cryptic spots.

Furthermore, in case of no predator treatments, where the distance between crab and the model predator was constant and no attack had been launched but most crabs still choose to flee after some time. This is probably because the risk of being detected and attacked increases with time (Broom & Ruxton, 2005). This finding is in line with a similar study on rock lizards, whereby lizards chose to flee after some time, even if the predator was immobile and no attack was initiated (Martin *et al*., 2009).

This study suggests that crabs escape when either poorly matching the background or after being detected by a predator. A limitation in this study could be the ecological relevance of the artificial backgrounds (sandpapers). While selecting my background colours, I had four options – black, sand-coloured (pale-yellow), red and green sandpapers. Previous study on shore crabs by Stevens *et al.,* 2014 incorporated red and green coloured gravel in their colour change experiments because of red encrusting algae and green algae/seaweed, which are both common at the rockpool (study) site. However, they found that crabs showed very little changes for these colour stimuli and no evidence was found that any changes in colour or luminance/brightness equated to an improvement in camouflage.

Additionally, crabs that had been placed on either a red or green background showed no differences in their level of match to either background type. This is why I opted for black and sand-coloured background. Although these backgrounds may at first be seen as relatively artificial in the context of the coloration of shore crabs and where they live, the habitat where individuals were collected does have a range of relatively dark and light-coloured rocks, stones and sand with a varied hue. It is also possible that in the wild, however, background mismatch would be lower and perhaps not eliciting a response. More work with other colour types, background mismatch and/or more information about shore crab vision is needed to investigate this further. Another limitation is that I did not model camouflage to predator vision because in this study, the crabs could not be immobilized (to minimize stress) so UV photography could not be conducted which is required to model predator vision. However, future research should involve predator vision modelling when investigating how camouflage mismatch influences escape responses as this will improve our knowledge of flight response from a predator's perspective. The results of both the experiments suggest that the escape behaviour of prey species is not entirely dependent on a single factor instead several factors are involved when making escape decisions on encountering threat. The animals adjust their escape response according to background they are present on and their own body coloration when a predator approaches so as to reduce the predation risk without incurring excessive costs.

Chapter 3: Are the escape responses of shore crabs (*Carcinus maenas*) tailored to different predators?

Abstract

Most prey animals deal with multiple predators and each predator possesses a different visual system, use diverse modes of prey detection, and demonstrate various means of capture behaviour. Therefore, it becomes difficult for the prey to use the same antipredator strategy for multiple predators. Prey species may adjust their antipredator behaviour in response to different predators as a counter strategy. Although a number of recent studies have considered the camouflage aspect in the common shore crab, research on how it works in combination with escape behaviour and whether it is affected by the type of predator remains unanswered. Here, in a field-based study I examined the escape responses and camouflage in the variable common shore crab (*Carcinus maenas*), by testing whether crabs of different camouflage respond differently to simulated bird and fish attack when on matching light and dark backgrounds. Using multispectral image analysis, I also considered whether background matching in terms of brightness difference and colour difference favours longer escape time in response to a specific predator because with a better match the prey would be harder to detect by the predator. Therefore, the prey is more likely to stay rather than immediately fleeing. brightness and colour matching did not have any observed significance on the escape response of crabs. However, during these simulated predation trials, crabs showed a quicker escape behaviour in the presence of a bird model and the escape response was faster on a lighter background. The response was slightly slower in case of a fish predator and longest in the absence of a predator. These findings suggest that crabs may consider birds as a greater threat than the fish and the crabs modulate their escape behaviour in terms of intensity of fleeing when exposed to different predators.

Introduction

One of the main challenges that most animal species face during their lifetime especially as juveniles is predator avoidance. Failure to detect a predator or fend off an attack can be lethal, therefore, for survival and future reproductive success an efficient escape is crucial. The decision of when or how strongly to respond to predators is based on animal’s ability to assess the threat, and individuals may choose to flee, stay, and monitor the predator from a distance or fight to prevent capture (Caro, 2005). In addition, the strongest escape responses are observed in cases of higher predation encounters in comparison to lower risk as stated by the threat sensitivity hypothesis (Helfman, 1989). This level of risk assessment is based on several factors such as multiple cues from the predator including size and speed, defensive armour and/or strategies and distance to the refuge (Amo *et al.*, 2004; Stankowich & Blumstein, 2005).

Animals use either primary or secondary defences to respond to predators (Cott, 1940). Primary defences work towards decreasing the probability of detection by predators, and chiefly involves cryptic behaviour among many others. Secondary defences on the other hand, are advantageous once predator has detected and recognized the prey, and prey must decide between “staying” or “fleeing” to escape an imminent predation (Edmunds, 1974; Hanlon and Messenger, 1996; Staudinger *et al*., 2013). Cryptic prey employs various strategies to thwart detection including transparency (as seen in Ctenophora, Johnsen, 2001), countershading (for example, lepidopteran larvae, Rowland *et al.*, 2007), disruptive colouration (as observed in cuttlefish, Hanlon *et al*., 2007). Another way is background matching whereby matching morphological traits to resemble the local environment, can be advantageous in preventing detection from predators (Relyea, 2001; Stevens, 2016). Prey may also use behavioural choices to achieve background matching as observed in *Ambystoma texanum* which preferred substrates that match its own body colour whereas *A. barbouri* preferred dark substrates in risky situations (Garcia and Sih, 2003).

Among cryptic behaviours, burying also minimizes the risk of detection from predators providing quick and temporary concealment. This behavioural trait has been observed in a range of taxa such as flatfish, cephalopods (cuttlefish), crabs (*Carcinus maenas*) (Bellwood, 2002). The vulnerable juveniles in particular benefit from hiding in the sand due to this concealment tactic. Burying behaviour in shore crabs is often seen in conjunction with background matching and/or disruptive coloration (Detto *et al*., 2008; Stevens *et al*., 2014). Alternatively, fleeing is the most common antipredator response employed by animals under natural conditions as it uses rapid locomotion to move away from threat (Humphries and Driver, 1970; Edmunds, 1974).

The decision of whether a prey chooses to flee, or stay is made after analyzing the overall costs (Ydenberg and Dill, 1986). If prey decides to flee after detecting the predator, there is a potential cost of losing foraging and mating opportunities as well as energy expenditure (Cooper, 2007). Moreover, in most cases, fleeing also alerts the predator and the chances of evoking an attack are increased. However, if the prey chooses to rely on its crypsis and stay, there is a possibility of being undetected by the predator but if detected it may be too late to flee (Broom and Ruxton, 2005; Staudinger, 2013). Therefore, it becomes essential to assess the risk and respond appropriately to minimize the costs.

One way to adjust to different levels of risk is to vary the Flight Initiation Distance (FID), the distance between the prey and threat when the prey flees (Ydenberg and Dill, 1986). The Flight Initiation distance is further influenced by several traits of both the prey and predator. The less experienced individuals such as juveniles may increase their FID to escape predators if they have slower sprint velocities, thereby decreasing the need of energetically expensive burst of speed required during rapid locomotion. Conversely, juveniles of cryptic species may have lower FID and might benefit from remaining stationary to decrease the likelihood of detection (Ioannou and Krause, 2009; Martin *et al*., 2009). In contrast, adults may display increase in FID to conserve energy expenditure during rapid locomotion to escape predation risk. However, if the cost of locomotion is low, FID may be decreased, and prey can escape using high sprint velocity. This would also be advantageous for cryptic individuals when staying immobile (Cooper, 2009). The FID of prey individuals increases with the distance to refuge (Bonefant and Kramer, 1996), for example, fiddler crab (*Uca vomeris*) decreases their FID when are closer to refuge Hemmi, (2005). Predator approach can also affect the Flight Initiation Distance, such that sparrowhawks showed significantly longer FID when the predator approached directly than when it approached tangentially (Møller *et al.*, 2014). Another measure of escape response is the time between detection of predator and initiation of flight in an immobile predator-prey model, also known as latency, to flee (LF), (Cooper *et al*., 2015). The latency to flee is determined by a trade-off between the cost of immobility and the cost of escaping, as remaining immobile on detecting a predator may reduce the chances of recognition at a given instance, but the likelihood of predator detecting the prey also increases over time (Martin *et al*., 2009; Cooper *et al*., 2012). In addition, the time to flee is also dependent on the standing distance or the distance between prey and predator (Cooper *et al*., 2015). The decision of when to flee is predicted to decrease with standing distance because with approaching predator, the chances of getting recognised and captured increases as well (Martin *et al*., 2009; Cooper *et al*., 2012). These LF models resemble the FID models in supporting predictions that with increasing risk, latency to flee decreases (Ydenberg and Dill, 1986; Cooper *et al*., 2007; Cooper *et al*., 2012).

Prey often encounter multiple types of predators, each with unique features of shape, size, chemical cues, and foraging tactics, and accordingly, prey respond with an array of defensive strategies, each best tailored against a particular predator (Aguilera *et al*., 2019). For example, *Liolaemus* lizards when in presence of two ambush predators, raptor and snake, reduce their activity levels in presence of both the predators. However, their antipredator defences are different for both predators such that, time to move/chemical exploration is more for *L. chiliensis* in presence of raptor while tail waving and autotomy ae mostly observed in *L. nitidus* in presence of snake (Constanzo-Cháveza, Penna & Labra, 2018). Predator type shaping the antipredator response has been observed in many marine invertebrates such as snails (Marko, 1991; Turner *et al*., 2006) and scallops (Guderley *et al*., 2015). Some studies have also tested that cuttlefish adjust their coloration to different predators. For instance, cuttlefish relies on cryptic behaviour in presence of active searching bluefish while uses swimming and startle displays in presence of sedentary seabass (Staudinger *et al*., 2013). Iwasaki (1993) observed limpet (*S. sirius*) to clamp tightly to its home scar in presence of sea star but fleeing from whelks, indicating that gastropods perceive predator signals and also adjust their response to different predators. Similar observations were seen in a recent study on the behavioural responses of limpet species to their main predators, seastar and crabs (Aguilera *et al*., 2019). They found the species escaped from sea stars but did not flee in presence of crabs rather displayed clamping behaviour because the velocity of limpet was insufficient in case of the fast-running crabs.

Exposure to multiple predators can put the prey in a conflicting situation (DeWitt *et al*., 2000). The prey may respond to one predator, but this may simultaneously increase vulnerability to another predator. Models suggest that if the predators are of approximately equal predation risk, then prey should take a general defensive approach as no antipredator response would work. In contrast, when threat from one predator outweighs another then most prey species respond to the more risky predators (McIntosh and Peckarsky, 1999; Ferrari *et al*., 2010). For instance, tadpoles when presented with the nonlethal fish and lethal dragonfly larvae simultaneously, responded by increasing tail fin depth, which was similar to that expressed in the presence of dragonfly larvae alone, suggesting a hierarchy of response corresponding to the predation risk (Teplitsky *et al*., 2004). Similarly, dwarf chameleons (*Bradypodion taeniabronchum*), when in presence of bird and snake, each possessing a different visual system and colour discrimination, show better background matching in response to birds than snakes. This indicates that birds are seen as greater predation threat than snakes (Stuart-Fox *et al*., 2008).

Various crab species are preyed upon by gulls and other birds and their behavioral aspects of predator avoidance have been extensively investigated in the field studies, whereby they dig individual burrows to which they run and hide if they assess a risk to escape predators as seen in fiddler crabs (Hemmi, 2005) and *Neohelice* crabs (Fathala and Maldonado, 2011). Some studies have even explored the visually elicited escape behaviour in these crabs using moving dummies to simulate predator stimuli (Hemmi, 2005; Smolka *et al*., 2013; Tomsic *et al*., 2017). Here, upon perceiving an approaching object, the crab's first observable response is to freeze, which likely increase the crab's chances of remaining undetected by the predator and helps in stabilizing the image and improve visual information (Hemmi and Tomsic, 2012). However, if the predator continues to approach, a second strategy consisting of a ‘home run’ is initiated which ends at the burrow entrance sometimes followed by retreat into the burrow (Hemmi, 2005; Hemmi and Pfeil, 2010; Fathala and Maldonado, 2011; Hemmi and Tomsic, 2015). In the absence of a burrow as sometimes observed in *Neohelice*, the behavioral response comprises first freezing, followed by running directly away from the threat and, ultimately, raising the claws and pointing them towards the predator (Tomsic *et al*., 2017).

At low tide, crabs recognize their predators by visual cues (Layne *et al.,*1997; Layne 1998). In the aquatic environment, most crustaceans are known to recognise predators through chemical cues captured through the chemosensory organs present on their antennae and bristles (Thiel & Breithaupt 2011). Therefore, this sensory cue cannot help in detecting predators in aquatic environments. A recent study by Rafael De Grande *et al*., 2019 observed that fiddler crab (*Leptuca thayeri)* detected the presence of chemical cues from the predator fish (*Sphoeroides greeleyi*) but neglected the non-predator fish (*Mugil curema*). Adult crabs remained within their burrows and avoided predator exposition because experienced adults recognise the predator itself, while juveniles increased their activity perhaps to allow them to gather information about the possible risk of different predatory species. To bridge this gap, in my study I investigate antipredator response from both land and water predator which would employ the use of visual and chemical cues, respectively.

The visual system of crabs consists of two compound eyes located at the tip of movable eyestalks, each containing spherically distributed ommatidia which are oriented in such a way that imparts monocular vision of 360 degrees to crabs (Zeil and Al-Mutari, 1996; Smolka and Hemmi, 2009). Additionally, the visual nervous systems of decapod crustaceans and insects are thought to be homologous (Sinakevitch et al., 2003; Sombke and Harzsch, 2015), containing the retina and a series of nested retinotopic neuropils that are organized in vertical columns, so that each column brings information from a particular part of the visual field (Sztarker et al., 2005; Berón de Astrada *et al.,* 2013). Visual information is processed with the help of motion-sensitive LG neurons that regulate the run speed and direction when the crab escapes from a visual danger (Berón de Astrada *et al.,* 2002). Therefore, a crab's response to visual threats is more complex than performing a single escape run. It adopts various strategies such as freezing, running and raising its claws towards the threatening stimulus (Scarano and Tomsic, 2014). The decision to implement any of these defensive strategies depends on the risk assessment made by the animal on the basis of the visual information available on the stimulus.

For this study, the common shore crab (*Carcinus maenas*) is used which is an intertidal species widely found along the coastlines of the UK and other parts of Europe (Crothers 1966; 1968; Todd *et al.*, 2006). It is known to have variable carapace patterns which could be driven by matching of individuals to different substrates (Bond & Kamil, 2006; Stevens *et al*., 2014). Both juvenile and adult crabs are an important prey source of numerous predators ranging from bird species such as gulls and shore birds to various fish species (Powell, 1962; Crothers, 1968; Hogarth, 1978; Stevens *et al.*, 2014). Gulls feed in the rocky intertidal and shallow subtidal zone and capture prey by paddling on or flying close to the water, then diving up to 1m below the water (Good, 1998). The feeding behaviour of gulls adopt a generalist foraging strategy and their principal diet includes earthworms, bivalves, fish, lobsters, and crabs of which green shore crabs form a major portion (Goethe, 1956; Harris, 1965; Spaans, 1971; Pennycott *et al*., 2021). Fish predators such as blenny is a small shallow-water predator abundant in the rocky intertidal zones around the UK coast. It searches for small prey species such as prawns, lugworms and crabs when the tide is in but uses cryptic coloration to hide in crevices when the tide is out (British sea fishing).

To my knowledge, there are several studies on responses of various organisms to different predators (Semlitsch, 1993; Relyea, 2001; Thompson III and Burhans, 2003; Storfer and White, 2004). For instance, cuttlefish responses were recorded to three different predator types – bluefish (active predator), flounder (ambush predator) and seabass (intermediate hunter) (Staudinger *et al.,* 2013). The results suggested that cryptic behaviours were preferred in case of active searching bluefish while swimming and startle displays were prevalent in case of the bottom-dweller seabass. Another study that measured the antipredator responses to different predators is that of striped skunks, *Mephitis mephiti*s (Fisher and Stankowich, 2018). Striped skunks when exposed to the audio recording of their main predators' owls (aerial) and coyotes (terrestrial), were observed to flee sooner in response to owl vocalizations suggesting that owls are considered as a greater threat than coyotes. The primary and secondary defences of Longfin squid, *Loligo pealeii* has also been tested in response to cruising bluefish and ambush flounder predators (Staudinger *et al.,* 2010). The squid exhibited immobility in presence of bluefish while fleeing was detected in case of flounder. The camouflage was measured in the study on escape responses of dwarf chameleons in response to bird and snake predators (Stuart-Fox *et al*., 2005). The chameleons became more achromatically contrasting in the presence of snake as opposed to birds due to the differences in their visual systems. However, these studies suggest that camouflage is not directly measured and knowledge of diverse marine species that face intense predation from multiple predators is still lacking. In the present study, I investigated the escape responses shown by the European shore crab (*Carcinus maenas*) to two different predators: herring gull and blenny and if the response is adjusted to specific predator. I used model seabird and fish as looming predators and hypothesized that crabs would elicit predator specific responses after assessing the risks posed by these different predator species. I expect the individuals to respond slowly, as in the time to flee to be longer, in case of fish predator but a quicker response in presence of bird because increase in predator size is associated with greater escape responsiveness (Seamone *et al*., 2014). I also predict the crabs would be slower on the black background because the likelihood of being detected on matching background is lower as compared to the yellow background. For this, I used multispectral images to calculate brightness and colour difference between the crab carapace and background to determine the level of background matching (Stevens *et al.,* 2014).

Methods

Ethical note

All individuals collected for both preliminary and main experiment trials was done with the approval of the University of Exeter Biosciences ethical committee (application eCORN000605 v3.3 2018). For conducting the pilot experiment, all individuals were used for a short period of time and returned to their original rockpool area unharmed following the experiment. Those used in the main experiment were kept for longer periods, but measures were adopted to minimize the stress to the individuals (see chapter 2 and 3 for details) before being released back to the rock pools after completion. Shore crabs are not endangered or protected invertebrate species therefore no additional licenses were required.

Crab Collection Methods

Crabs were sampled from the closest rockpool site. The Gyllyngvase rockpools (50° 8’ 39.42” N, -5° 4’ 5.244” W) in Falmouth, Cornwall, were chosen as a rockpool site because its substrate composition was found to be a heterogeneous habitat for the shore crabs. This site primarily comprises large rocky clusters, deep gullies filled with stony/gravel and sandy substrates, few mussel beds and increasing amounts of seaweed near the low tide.

Sampling was conducted in 2019 from late March to early October taking the weather and tides into account. The field site is a publicly accessible land, again not requiring any further permits. Crabs were collected within 2-3 hours before the low tide. Rockpools were searched for shore crabs in low, middle, and high tidal zones for a time period of 5 minutes or less if the sampling area was small or bare. Shore crabs were identified by their carapace shape, the distinct five spines on either side of the eyes with three spines in between the eyes and the lack of swimming paddles. Movable rocks and sea weeds were turned over, sand and gravel raked using fingers, crevices were checked during the search to avoid missing any likely crabs. During the collection, sex was not taken into account as it is difficult to measure in juveniles (Mohamedeen & Hartnoll, 1990). Individuals measuring less than 7mm or greater than 35mm were classified as too small or big, respectively for the experiment and were avoided in the study. A total of 209 crabs were collected and tested during the sampling period.

Experimental Design

My second experiment aimed to measure the escape response of shore crabs corresponding to two factors, background colour and predator type. To achieve this, a factorial experimental design was adopted with trials organized into blocks. Each block has six individuals and following six trials are done on each individual (6 x 6),

- Trial a = Bird attempt simulation on Black circle (B B)

- Trial b = No predator attack on Black circle (N B)
- Trial c = Bird attempt simulation on Yellow circle (B Y)
- Trial d = No predator attack on Yellow circle (N Y)
- Trial e = Fish attempt simulation on Black circle (F B)
- Trial f = Fish attempt simulation on Yellow circle (F Y)

Each of the six crabs experience the six trials in a different order to ensure different possible combinations of treatment order (crab 1 = a,b,c,d,e,f; crab 2 = b,c,d,e,f,a; crab 3 = c,d,e,f,a,b; crab 4 = d,e,f,a,b,c); crab 5 = e,f,a,b,c,d; crab 6 = f,a,b,c,d,e and so on) created using a random number generator in R. The different orders were to balance the order of presentation across treatment, and control for differences in response that might arise due to order.

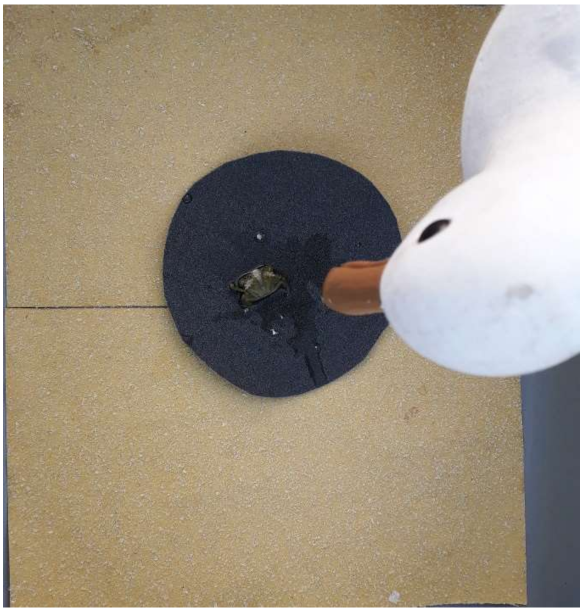
The experimental setup consisted of an arena designed to record the movement specifically escape behaviour of the crabs (Figure 3.1C). The texture of a sandpaper was found to be the best option as it was rough enough to create friction for running resembling their natural gravel substrate and smooth enough to allow for an even movement. Yellow and black colours were chosen for the sandpaper as they provide a good match with both the natural background and colour of crabs. A fine grade yellow sandpaper (of dimensions 230x280mm) was arranged in a grey tray (of dimensions 37x21x9cm) and a circular arena of fine grade black sandpaper (of diameter 10 cm) placed on top of it secured with two-sided tape. Similarly, black sandpaper (of dimensions 140x230mm) with a yellow circle (of diameter 10 cm) on top was arranged in another grey tray of same dimensions (Figure 3.1 B). The trays were filled with water up to a depth of 7 cm to provide a natural setting and for easy manoeuvring of the fish. Crabs collected were in the range of 0.7 – 3.5 cm and were randomly housed in separate transparent plastic pots and each pot was randomly numbered from 1 to 18. To minimise the stress levels of crabs, each pot was filled with natural seawater collected from nearby rockpool area to wash their gills and were also provided with a refuge in form of a large pebble again collected from around the site (Figure 3.1A). These pots were further arranged according to their trial turn to increase efficiency of the experiment and minimise the stress to the crabs. The crabs were then photographed to analyse the colouration and camouflage of crabs against the artificial background.

Experimental Procedure

For the 1st treatment, trial 'a' was conducted on a crab. The crab was placed on black circle and covered with a clay pot (of diameter 8cm and height 10cm) to let it acclimatise for 3min. Here, I used three-dimensional realistic models of the bird and fish predators as these models evoke similar responses as real predators and eliminate the risk of injuring the crabs (Stuart-Fox *et al*., 2005; Carlile *et al.,* 2006; Ito and Mori, 2012; Catano *et al*., 2016; Constanzo-Cháveza, Penna & Labra, 2018). Following this, the pot was gently lifted so as to create minimum disturbance and a model sea gull (of dimensions 30x28x8) simulated as predator was slowly lowered at speed of 10cm/s from a distance of 0.3 m at an angle 45 degrees to simulate a predation attempt as it would encounter in a natural setting. The model predator (bird) tapped the crab's carapace with its beak to derive a response and crabs were observed for a maximum of 5 min. If the crab started fleeing, the initial time to move (FID) was recorded and the time to leave the black circle and onto the yellow background was noted. In case, the crab failed to move with one tap, tapping was continued at an interval of 10 sec. For trial 'b', the arrangement remained same, but the predator simulation was removed. For trial 'c' and 'd', yellow sandpaper cut-out was used with and without predator (bird) simulation, respectively. For trial 'e', a rubber fish lure (10x3cm) acted as the second predator on black background and touched the crab's carapace and similar tapping at an interval of 10 s was observed. Lastly, for trial 'f', the background was switched to yellow and the time for crabs were noted in the absence of fish. In case the crab did not move or respond or failed to leave the circle within the time limit, it was recorded as '300s' meaning did not move or were too slow to move.

A)

B)



C)

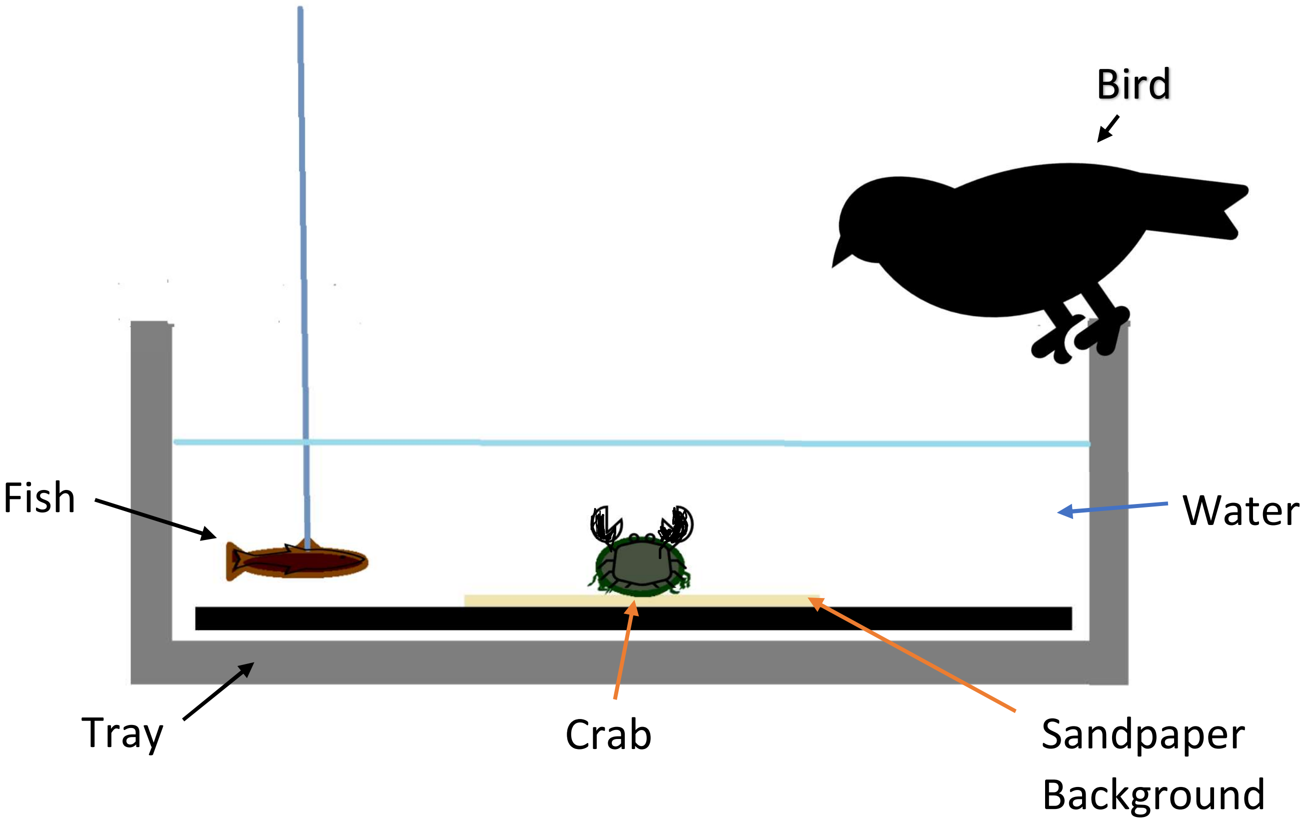


Figure 3.1: **Experimental setup used in the experiment -** A) Arenas used for examining the escape behaviour. B.) Trial ‘b’ in progress (top view) (Same as Chapter 2). C.) Cross section diagram of arena used during the experiment.

Photography

A tripod was set up with a digital Nikon D7000 camera, which had undergone a quartz conversion to facilitate UV sensitivity (Advanced Camera Services, Norfolk, UK). The UV reflectance in both background and crabs and the UV reflectance to visible light are similar, and so crab coloration was analysed only in the visible range of spectrum (400-700nm) (Stevens et al., 2013). In addition, the exposure times for taking UV images are longer so to reduce stressing the crabs, photography was undertaken as quickly as possible. A filter (Baader UV/IR Cut filer) was slid in front of a Nikon 105mm lens that blocked UV and infrared light, transmitting wavelengths between 400-700nm (visible region) only, to enable capturing human visible images.

Crabs were taken out from their individual pots only before photographing and carapaces were dried by gently dabbing with blue roll to remove surface water to avoid any further stress. The crab was then gently placed underneath the camera in the arena. A black and white reflectance standard was placed adjacent to 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 5% and 95% of all wavelengths, respectively. Including a standard in every image allows controlling the variations in lighting conditions as all the photography was conducted under natural light (Stevens *et al*., 2007; Troscianko & Stevens, 2015). Photographs were taken with a fixed aperture setting in RAW format. To avoid over exposure as a result of specular reflectance in images, several photos were taken of the same individual at a range of exposures. Carapace width was used to determine crab maturity and was measured from the digital photographs using the ruler included in the photos. A baseline value of 25mm was used to categorize the crabs into smaller (immature) juveniles and larger (mature) adults based on their size (Hogarth, 1975; Stevens *et al*., 2014b). Photography was kept to approximately 1 min per individual to prevent any additional stress.

Image analyses

The Multispectral Image Calibration and Analysis Toolbox (Mica Toolbox version 1.22) developed by Troscianko & Stevens, (2015) was used for all the image calibration analyses and all the work was carried out in program ImageJ (version 1.8.0_112). All the photographs were sorted, and the best image of an individual was chosen by viewing the RGB histograms in the photo screening mode of the package. Any overexposed images due to specular reflectance were removed before starting the analyses multispectral images were created each individual by selecting the 95% and 5% reflectance standards in order to analyse the camouflage metrics. For this, within each image an outline of the individual’s carapace (covering as much area as possible) was selected manually using the polygon tool as a region of interest (ROI) for the crab and rectangular section of yellow and black sandpapers as ROI for background. Images were calibrated with regards to the reflectance standard, with the image wavelength channel scaled so as a value of 65535 on a 16-bit scale equals 100% reflectance. Individuals should ideally be viewed and analysed using the visual system used by their potential predator groups to gain camouflage advantage but in this case because shore crabs have many predators from shore birds to fish, so it was difficult to choose one visual system, therefore objective measures were used instead of any specific visual system (Crothers 1966; Hogarth 1978).

Calculating reflectance values

Image data from the multispectral images were used to calculate two metrics through batch image analysis. Overall reflectance is a measure of the overall brightness across the visible spectrum and was calculated as (LW+MW+SW)/3. The second metric, hue was measured with regards to yellow (LW+MW) versus blue (SW) light was calculated as (LW+MW)/SW. A value of 1 means that a crab is grey whereas a value of less than 1 indicates the crab or substrate is blue and above 1 means it is yellow. I also analyzed how well the brightness of crabs matched their background and if it affected their escape behaviour. I calculated a brightness match by taking the average difference in brightness between each crab and the two artificial substrates. Values closer to 0 mean a good match. The formula used was:

Absolute Brightness = $\mathrm{ABS}(B_c - B_b)$,

where B is Brightness of the crab c and Background b

I also measured color camouflage by taking the average Euclidean distance in a trichromatic reflectance color space between each individual and their two backgrounds. To eliminate absolute variation, the LW, MW and SW reflectance values are standardized to relative proportions (Endler & Mielke, 2005) which are converted into a trichromatic colour space with each point represented by an X and Y coordinate. Low Euclidean distance values between crab and background means a good colour match. The equation used was:

$$= \sqrt{}\ ((X_c - X_b)^2+(Y_c - Y_b)^2$$

, where X and Y are the X and Y coordinates for crab (c) and background (b) being compared, respectively (Kelber, Vorobyev and Osorio, 2003; Stevens *et al*, 2009). All calibrations and analyses were undertaken in ImageJ.

Statistical Analyses

The time data collected showed that many of the crabs responded very fast, within a difference of 3 to 4 sec and this shifted the data to one side creating a skew since other crabs were much slower to respond (Figure 3.3). To deal with this, the time data was categorized on the basis of an average scale into two categories:

2. Fast (Individuals moving within 10s),
3. Slow (Individuals moving after 10s)

As the majority of the crabs responded to the first predator attack (which is at an interval of 10s); hence, it was set as cut-off and this was considered a FAST response. The individuals that required second or third predator attack were placed under SLOW response. Individuals that responded quickly were assigned the number 1, and those that were slow to move were assigned 0.

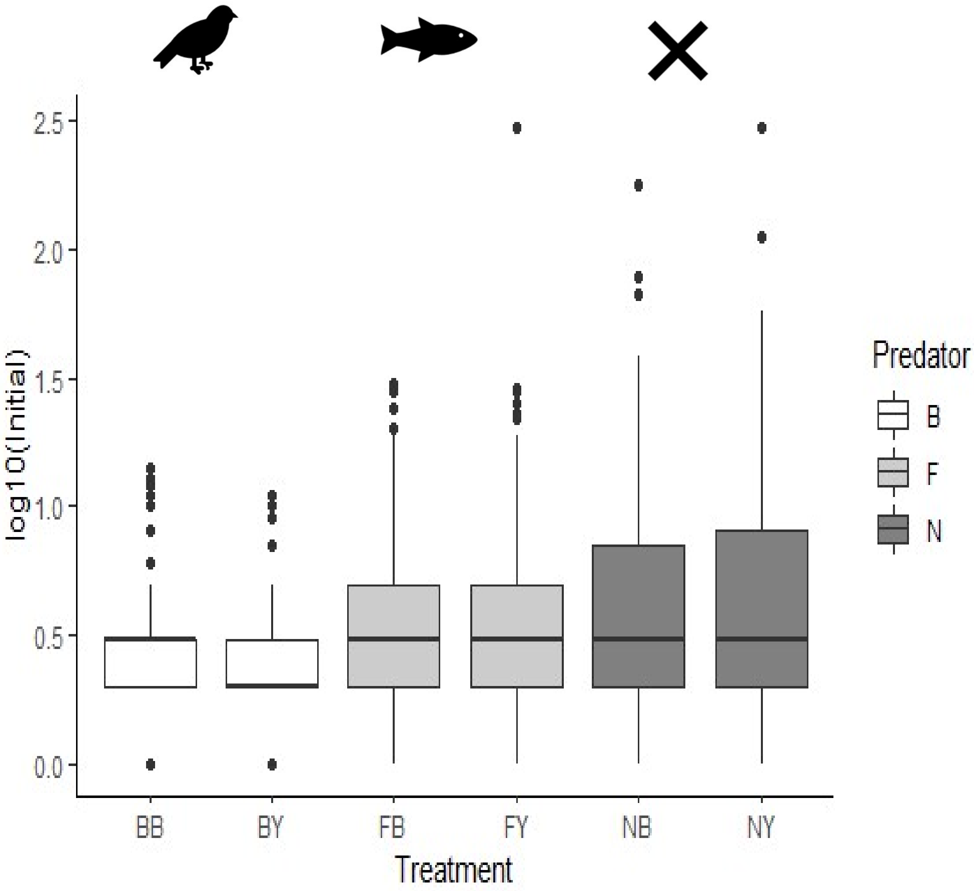
All statistical analyses were carried out in RStudio (R v 3.5.1). GLMMs of the family binomial were used to test the escape response (fast or slow behaviour) of differently camouflaged crabs on matching light and dark backgrounds with individual id as random factor to control for repeated measurements on the same individual. The predator, circle/background, overall brightness, and colour were chosen as main effects and whether the interaction of their main effects influenced the flight response of the crabs was also considered as in case of GLMM, Binomial. The mixed-effects model was fitted using the 'lmer' function in the 'lme4' package (Bates *et al.,* 2015) and the associated significance tests through the 'anova' function in the 'lmerTest' package (Kuznetsova *et al.,* 2017). In addition to GLMMs, a chi-square test of independence was performed to examine the escape response of individuals to the different predators and different background treatments. Crabs were predicted to show greater escape response on a non-matching background, where the likelihood of being detected by the predator is higher. While analysing it was found that 14 individuals were outliers as they had extremely high values of brightness and colour match which were skewing the data. The high values seemed unlikely after rechecking the photographs and could perhaps be a result of overexposed spots that were not visible enough at the time of image analysis so had to be removed leaving to 188 individuals. Movement of individuals was timed in each of the four treatments.

In case of non-normal data (initial time, total time, brightness, and colour values), skewness test was performed (using moments package in R). These values were either greater positively skewed or showed moderate skewness so were transformed using square root or log transformation values were log transformed or non-parametric tests (Kruskal Wallis, Wilcoxon rank sum) were used as an alternative. This mainly happened with the difference in brightness and colour variables so non-parametric test such as Kruskal Wallis, Wilcoxon rank sum were used. Crabs were also compared to the colour and brightness of both the background types. Crabs when placed on black background should closely match the black background compared to when placed on a yellow background. On contrary, crabs should better match the yellow background when placed on yellow than they had been placed on a black background. Results were analysed with Kruskal Wallace test (in the “dplyr” package) to compare the standard of match between each individual and its background. Finally, in the case of significant effects, post hoc test was applied to compare mean differences between different predators using the ‘lsmeans’ function from the ‘lsmeans’ package.

Results

Did individuals respond to the treatments?

The crabs responded to the different treatments by displaying a fleeing response. A chi-square test of independence was performed to examine the escape response of individuals to the different predator and different background treatments. There was a significant relationship between the escape response and treatments, X^2 (5, N = 188) = 43.00, p < 0.001. The association between predator and escape response was also examined and a significant relationship was found, X^2 (2, N = 188) = 40.72, p < 0.001, indicating that the individuals with a predator treatment responded faster compared to the control (Figure 3.4).



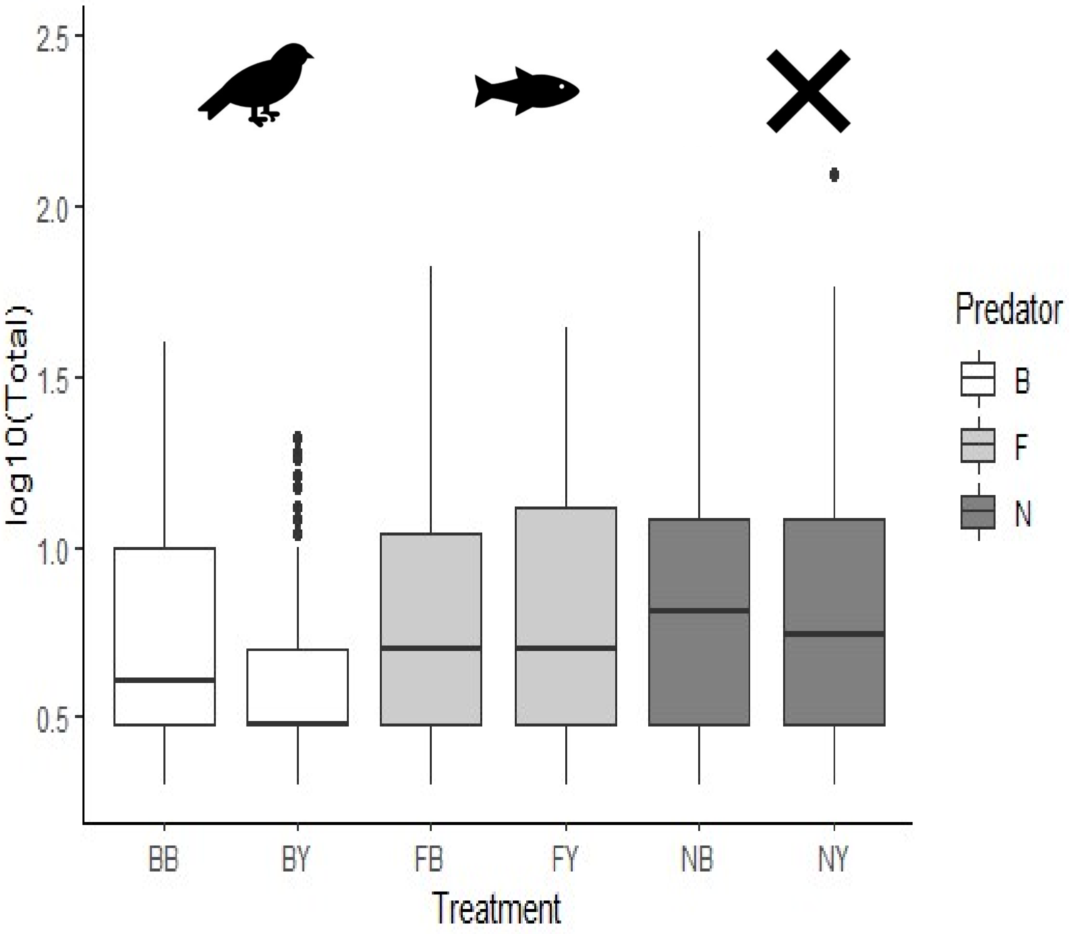


Figure 3.2: Escape time of shore crabs – initial time to flee (top) and total time taken to flee (bottom), when exposed to Predators, Bird (B), Fish (F) or No Predator/control (N) and Backgrounds, Black (B) or Yellow (Y) treatments as shown. Escape time is longest in absence of predator. In case of predator, crabs are quicker in responding to bird compared to fish. All values are log transformed. Dots indicate outliers.

Time to move.

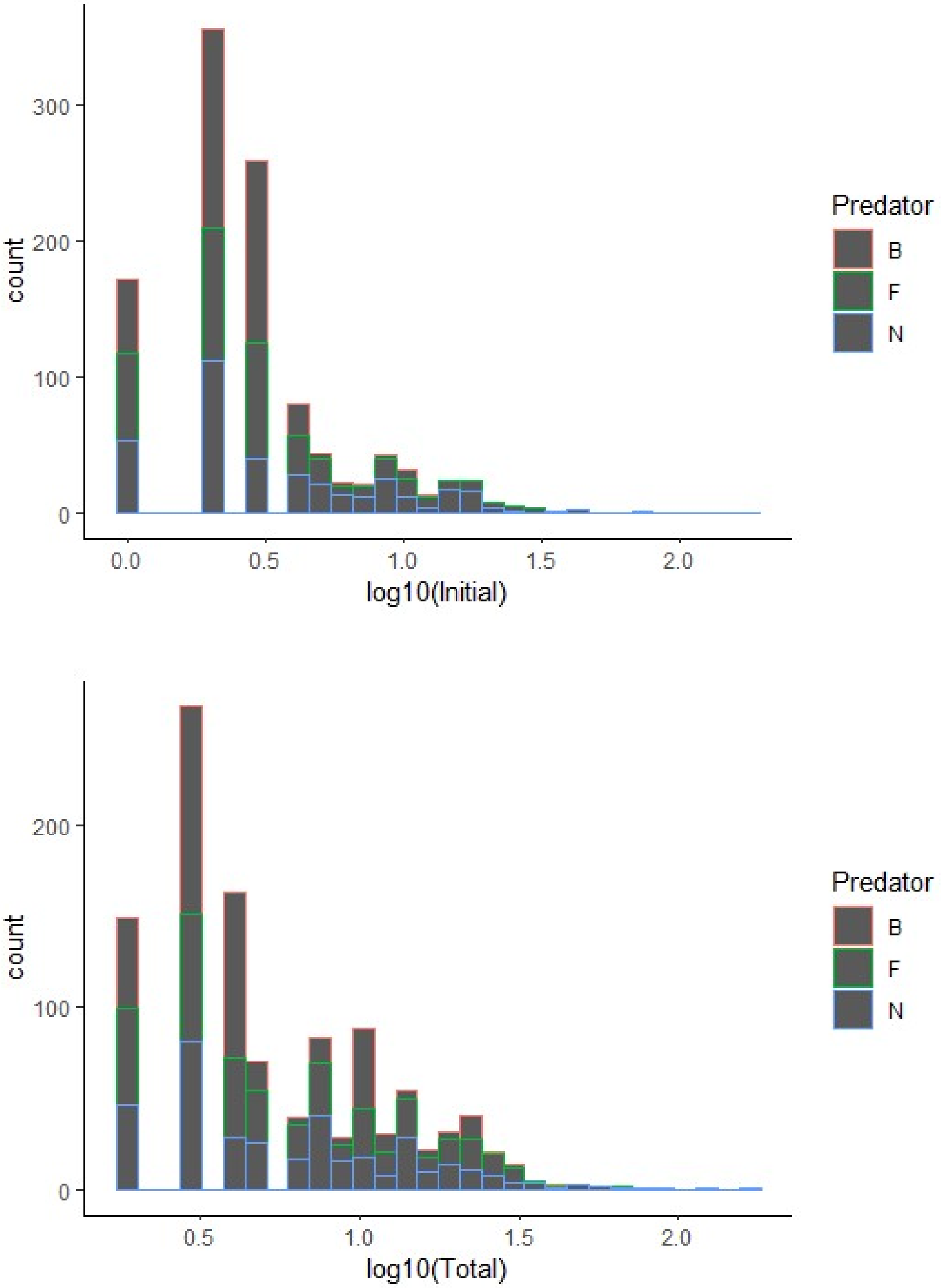


Figure 3.3: Time to move, Initial time (top) and Total time (bottom) of shore crabs in presence of Bird (B), Fish (F) and no predator (N). Majority of them moved within 25 sec showing willingness to move increases with level of

threat. The initial and total time to flee both had greater positive skew so log transformation was done on all values.

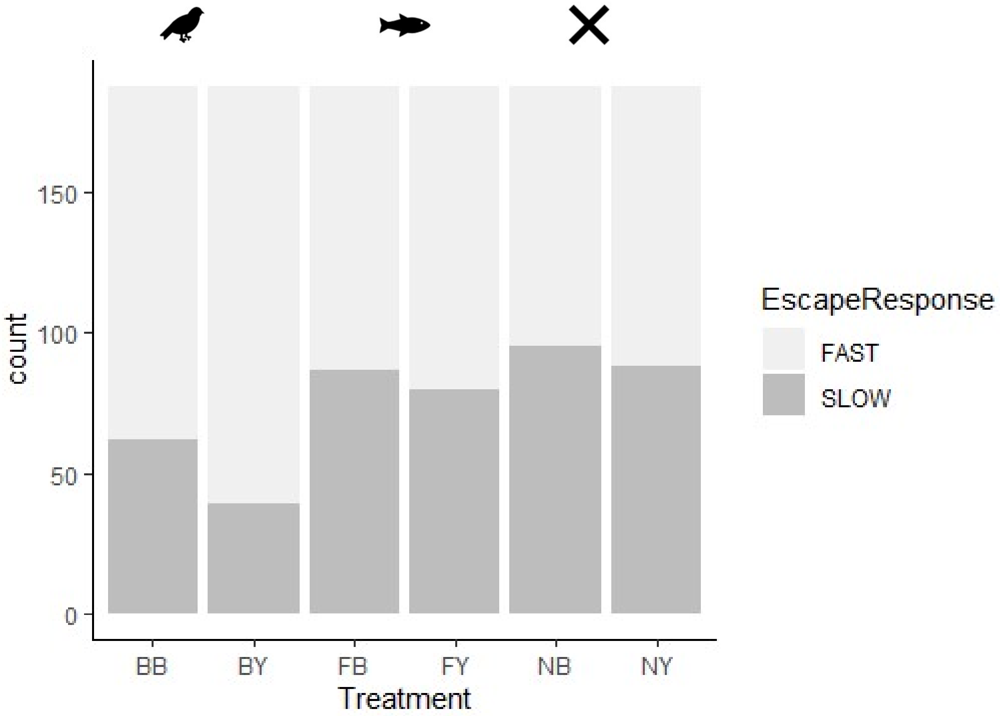


Figure 3.4: More individuals responded to the predator attack than no predator treatment on Black and Yellow backgrounds. Proportion of crabs fleeing when exposed to each predator simulation and background treatments showing the faster responses were greater in the predator treatment especially on yellow background ($p<0.001$).

Brightness difference

The mean difference in brightness of individuals differed between boards (Kruskal Wallis; $X^2{}_1$ = 611.03, $p<0.05$) showing crabs were closer to the black background (Figure 3.5).

Colour Difference

The mean difference in colour of individuals differed between boards (Kruskal Wallis; $X^2{}_1$ =753.42, $p<0.05$) showing crabs were closer to the yellow background (Figure 3.6).

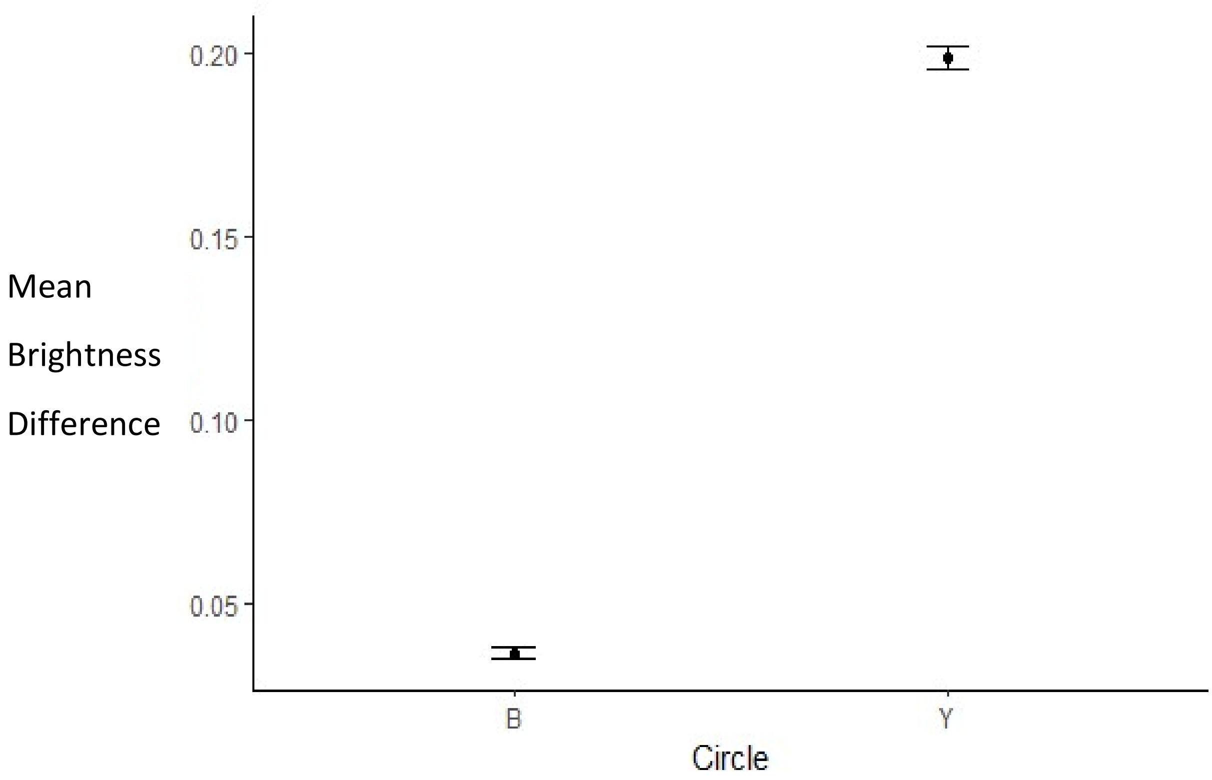


Figure 3.5: Mean brightness difference with 95% confidence intervals of individuals exposed to each background treatment, Black (B) and Yellow (Y). The difference in brightness of the crab and background indicate that crabs were generally closer to the black background ($p<0.05$).

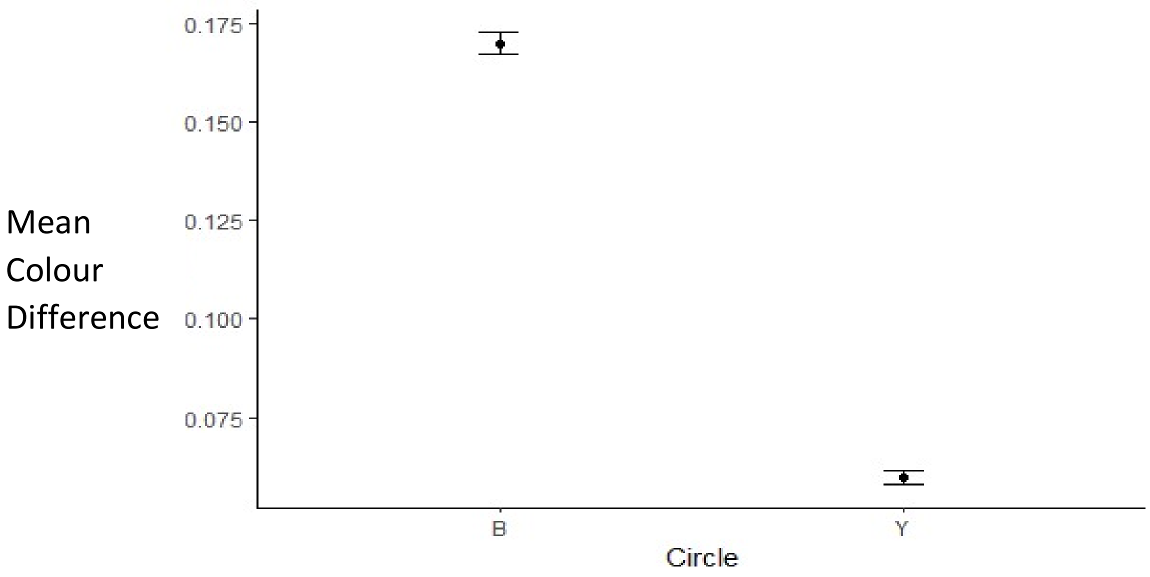


Figure 3.6: Mean colour difference with 95% confidence intervals of individuals exposed to each background treatment, Black (B) and Yellow (Y). The difference in colour of the crab and background indicate that crabs were generally closer to the yellow background ($p<0.05$).

Table 3.1: The pairwise comparison between types of predators when interacting with different treatments on crabs using lsmeans function. The comparison estimate, standard error (SE), z-ratio and p-value of the pairwise comparison of different predators (bird – B, fish – F and no predator – N).

Contrast	**Estimate**	**SE**	**z-ratio**	**P**
B - F	-0.1902	0.0361	-5.267	<0.0001*
B – N	-0.2414	0.0371	-6.508	<0.0001*
F - N	-0.0513	0.0407	-1.260	0.4181

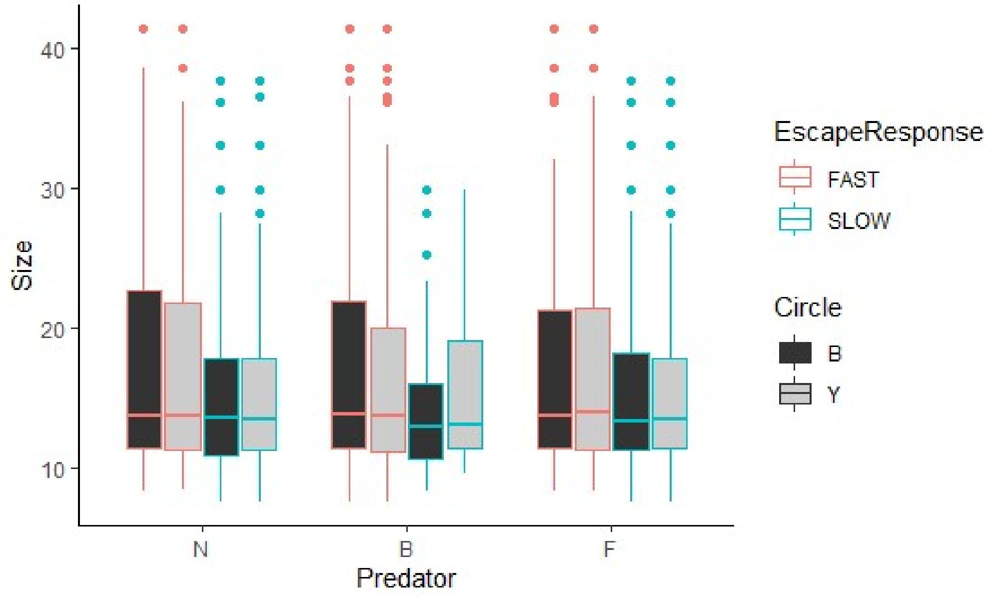


Figure 3.7: Graph showing size affecting the fast or slow escape response with respect to Predator - No Predator (N), Bird (B) and Fish (F) and background (circle) – Black (B) and Yellow (Y). Plot shows median and interquartile range (IQR), circles indicate outliers. Smaller crabs show a slower response.

Brightness Difference

The full model included an interaction between predator, background (circle), size and overall brightness but was found to have no significance (GLMM, Binomial, $X^2_9 = 4.61$, p =0.87); hence, was removed from the model. An interaction between predator and overall brightness and predator and size was initially included but found to have no significance (GLMM, Binomial, $X^2_2 = 0.14$, p =0.93) and ($X^2_2 = 0.94$, p =0.63) respectively. Therefore, were removed from the model. An interaction between size and overall brightness was also removed as it was not significant ($X^2_1 = 0.13$, p =0.72) and similarly, circle and brightness had no significant effect ($X^2_1 = 0.34$, p = 0.56). Brightness did not have any significant effect ($X^2_1 = 0.12$, p =0.73) so was not included and an interaction of background (circle) and size was also found to be non-significant ($X^2_1 = 1.15$, p =0.28). Lastly, interaction between background and predator was also removed due to being non-significant ($X^2_2 = 3.50$, p =0.17). Size significantly affected the escape behaviour of crabs (X^2_1= 6.41, p =0.01) indicating smaller crabs were more lethargic. The chances of a slower response on a yellow background in absence of predator is 58.49%. Background (circle) and Predator both were also significant (X^2_1= 6.54, p = 0.01) where the probability of being slow on a black background with no predator is 67.6%. However, in presence of fish predator this probability decreased to 62% and was lowest in presence of bird predator to only 39% indicating slower responses towards fish than bird (Table 3.1). The probability of a slower escape response was even lower on a yellow background with 58.43% in case of no predator, 54.13% in presence of fish and only 31% in presence of bird. The results from the final model show the escape response was dependent on both predator and background (circle) as well as size (see Table 3.2).

Table 3.2: Results of the final GLMM with family binomial on 188 individuals showing the significant effect of Predator, background (Circle) and size on the escape behaviour of crabs.

	D.F	X^2	P
Predator	1	6.54	0.01*
Circle	1	6.54	0.01*
Size	1	6.41	0.01*
Brightness	1	0.12	0.73
Pred:Brightness	2	0.14	0.93
Circle:Brightness	1	0.34	0.56
Size: Brightness	1	0.13	0.72
Pred: Size	2	0.94	0.63
Circle: Size	1	1.15	0.28
Pred: Circle	2	3.50	0.17

Colour difference

The full model included an interaction between predator, background (circle), size and colour camouflage but was found to have no significance (GLMM, Binomial, X^2_9 = 12.38, p =0.19); hence, was removed from the model. An interaction between predator and colour as well as predator and size were initially included but found to have no significance (GLMM, Binomial, X^2_2 = 0.64, p =0.73) and (X^2_2= 0.91, p =0.63) respectively. Therefore, were discarded from the model. An interaction between background (circle) and colour was also removed as it was not significant (X^2_1= 0.27, p =0.60) and similarly, colour and size had no significant effect (X^2_1= 0.48, p = 0.49). An interaction of background and size was also found to be non-significant (X^2_1= 0.81, p =0.37) and background and predator was also removed due to being non-significant (X^2_2= 3.47, p =0.18). Colour did not have any significant effect (X^2_1= 3.14, p =0.08) so was not included. Size significantly affected the escape behaviour of crabs (X^2_1= 6.41, p =0.01). indicating that chances of a slower response on a yellow background in absence of predator is 58.49%. Background and predator were also significant (X^2_1= 6.54, p = 0.01) as seen above in brightness difference. The results from the final model show the escape response was dependent on size, predator, and background (circle) (see Table 3.3).

Table 3.3: Results of the final GLMM with family binomial on 188 individuals showing the significant effect of size and significant effect of predator and background (circle) on the escape behaviour of crabs.

	D.F	**X^2**	**P**
Predator	1	6.54	0.01*
Circle	1	6.54	0.01*
Size	1	6.41	0.01*
Colour	1	3.14	0.08

Pred: Colour	2	0.64	0.73
Circle: Colour	1	0.27	0.60
Size: Colour	1	0.48	0.49
Pred: Size	2	0.91	0.63
Circle: Size	1	0.81	0.37
Pred: Circle	2	3.47	0.18

Discussion

In this chapter, I investigated whether the escape behaviour of the common shore crab is dependent on the type of predator and if the brightness and colour of crab carapace influences the escape decision. Crabs were placed on mismatched black and yellow treatment backgrounds and their escape was measured in terms of Latency to flee (LF) which is similar to Flight Initiation Distance (FID), using model bird and fish as predators. In general, the response of shore crabs was slowest in the control trial with no predators, which suggests that active reactions such as escape response and antipredator behaviour are costly and require energy expenditure so are only implemented at greater risk. In addition, background matching in terms of both brightness difference and colour difference did not significantly affect the escape behaviour of crabs in presence of different predators. This finding suggests that background matching camouflage may not affect the escape response of marine organisms or at least crabs when exposed to multiple predators.

Most animals deal with multiple predators, which usually differ in their sensory modalities, hunting strategies or prey detection and the level of threat. The escape responses employed by animals are not effective against all predators. As a result, prey species needs to adjust their antipredator strategies including appearance and/or defensive tactics in response to different predators to prevent predation.

The shore crab has an array of predators ranging from bird species such as gulls and shore birds to various fish species (Powell, 1962; Crothers, 1968; Hogarth, 1978; Stevens *et al.*, 2014), and they all use different methods to attack crabs. The crabs possess only a limited number of escape strategies to prevent predation, the most common being the option of fleeing. The shore crabs showed a greater escape response by fleeing faster in the predator treatments as compared to no predator treatments indicating that presence of predator plays a key role in escape decisions as predicted. The escape response was quickest in case of the bird while it was moderate during the interactions with fish whereby most crabs did not run immediately rather avoided the fish at first, indicating the likelihood of a slower response was close to that of no predator and fish was perceived less of a threat as compared to bird. This could be because avian attacks are fast compared to other potential predators so running seems to be a more efficient antipredator tactic under high predation risk (Cuadrado *et al*., 2001; Carretero *et al*., 2006; Constanzo-Cháveza *et al*., 2018). Another reason for a faster response in case of a bird predator could be due to the predator size and attacking strategy. The escape responsiveness increases with the size of the predator as demonstrated in the fathead minnow (*Pimephales promelas*) where the frequency of responding was higher when presented with a predator of larger frontal profile as opposed to a smaller predator (Webb, 1982). Similar findings were observed in the escape responses of the dogfish shark (*Squalus acanthias*) when exposed to increased sizes of predator (Seamone *et al*., 2014). In my experiment, the fish was moving at ground level which resulted in avoidance behaviour in the crabs; however, this was not observed in the case of the bird. Here, the bird flying above the ground elicited an immediate fleeing response. Therefore, crabs could be using the general rule of thumb to categorise objects as high risk (if the visual object is large and moves overhead) and low risk (if the visual object is small and moves at ground level) (Daleo *et al*., 2003; Tomsic *et al*., 2017).

The increased carapace size of shore crabs should limit predation by small predatory fish because of the small mouth gape of the fish. This may have resulted in the crabs demonstrating a lethargic response when presented with a fish predator as opposed to a bird, indicating that fish posed a lower risk to adult crabs.

This finding concerts with a study on the escape response of giant scallop (*Placopecten magellanicus*) in response to their principal predators, seastars and crustaceans (Guderley *et al*., 2015). The increased shell thickness of older scallops reduced predation by mobile predators, so the individuals were slower to respond to predators than their smaller counterparts. The time to move for crabs was longer in presence of a fish than a bird. This suggests that for crabs' bird may be a more dangerous predator than the fish as in natural settings crabs can easily escape from the bird predators by hiding in crevices or under rocks. The fish is also an active forager, pursuing and striking repeatedly instead of grabbing, so the prey is likely to elicit more avoidance behaviour or require strong swimming skills. These behaviours were observed in the fish treatments whereby the crabs either avoided the repeated strikes or swiftly swam to the corners of arena to escape the attacks. This finding aligns with a similar study on larval anurans, where the tadpoles showed increasing swimming speed in the presence of stickleback predators (Teplitsky *et al*., 2004). A few juvenile crabs also exhibited other antipredator behaviour such as raising claws when confronted with a fish predator but was not observed in case of bird. This suggests that crabs use this behaviour as a pursuit-deterrent signal and like several animals warn the approaching predators of being detected to evade predation before fleeing (Woodland *et al*. 1980; Hasson, 1991; Ito and Mori, 2012).

This experiment was conducted during the summer months and the quick escape behaviour of crabs could be linked to the warm conditions. In the earlier studies done by Meijering (1954), after analysing pellets of gulls from March to October found that the most common food consumed by gulls in the summer months was the European shore crab (*Carcinus maenas*). Another study by Ellis *et al*., (2005) in the intertidal sites of the Gulf of Maine found that a large proportion of gull predation on crabs occurred during the summer period. Therefore, the crabs may view gulls as a greater threat during the summer season and their quick fleeing response could be a reaction to higher rates of gull encounter.

The time to flee was longer on black background as compared to the yellow background (67% to 58%). This is perhaps because the dark green colour of crab carapace became cryptic on the black background and decreased the probability of detection by predators. This can be seen in a study by Dumas and Witman, 1993 as well. They found that *C. maenas* was less vulnerable to predation from gulls in treatments with high percent cover of mussel but suffered greater mortality in treatments where percent cover of mussel was low, or substrate was changed to bleached coral. This was because the dark-coloured carapace of shore crab was cryptic on the dark background of mussel habitat but immediately became less cryptic on the lighter coral algae, suggesting that vulnerability to predation is dependent on cryptic coloration. This background matching as a means of defensive strategy in a multiple predator system has also been observed in vertebrates. For instance, one study on dwarf chameleons (*Bradypodion transvaalense*) showed that chameleons can adjust their brightness in response to multiple predators. When tested in presence of bird and snake models, chameleons become brighter in presence of snake as compared to the bird due to the differences in the visual acuities and achromatic contrast sensitivity of the two predators (Stuart-Fox *et al.,* 2005). Further study on chameleons found that background matching was more effective in case of a bird than a snake predator because birds have better colour discrimination ability so in order to achieve a similar level of camouflage, chameleons need to match the background more closely (Stuart-Fox *et al*., 2008). The abundance of avian predators is also a strong driver of natural selection towards crypsis which suggests birds are considered as a greater threat by prey species. In this study, the crabs on black background demonstrated a slower fleeing response even with a bird predator (62% probability) suggests that background is an important factor when determining the escape response. However, the brightness difference and colour difference did not affect the escape behaviour of crabs. The crabs that closely matched the backgrounds were expected to stay longer in the predator treatments.

It is still not fully clear whether crabs can distinguish between different potential predators. However, the results suggest individuals can at least identify fish from bird predator and alter their escape responses accordingly as each predator possesses a different hunting strategy. It could be possible that crabs only recognize small visual objects in water as fish and large flying objects as avian predators but nonetheless, this study shows that shore crabs can identify one of their most common predators and tailor their escape behaviour, as prior to initiating a flight response and weighing the cost of staying, it is important to recognize the type of predator.

Chapter 4: General Discussion

Research Findings and Implications

Predation is a strong selective force that influences the behaviour of an organism, and the prey species employ a diverse range of antipredator strategies to overcome predation (Cooper & Blumstein, 2015). Animals make appropriate escape decisions when encountered with predators and flee only when the cost of staying (predation risk or death) exceeds or balances the costs of fleeing (such as losing foraging and mating opportunities, energy expenditure) (Ydenberg & Dill, 1986; Cooper & Fredrick, 2007). In cases where both prey and predator have detected each other, the prey can initiate fleeing after considering the risks and costs of fleeing (Lima & Dill, 1990; Stankowich & Blumstein, 2005). However, in a scenario involving cryptic prey which has detected the predator, the prey must quickly decide on whether to remain or flee because staying immobile increases the likelihood of detection with time yet fleeing instantly alerts the predator of the prey's location (Broom and Ruxton, 2005). Camouflage is an important antipredator strategy and the association between camouflage tactics and escape behaviour has been researched in several studies (Zuberbühler, 2000; Cuadrado *et al*., 2001; Eterovick *et al.*, 2008; Stuart-Fox *et al*., 2008; Martin *et al.,* 2009; Staudinger *et al*., 2013; Dugas *et al*., 2015; Wilson-Aggarwal *et al*., 2016). However, many of these studies focus on the vertebrate taxa or do not directly measure camouflage and only few of them explore multiple predators.

This thesis aims to address these gaps by examining the escape behaviour of shore crabs through the effects of background types and presence of one predator (Chapter 2) and multiple predators (Chapter 3) on prey with mismatched backgrounds. Using brightness difference and colour difference as metrics for camouflage, investigating the effects on escape decisions shows how animals use the self-assessed camouflage information to make behavioural decisions in response to predators. Furthermore, predators can also use their degree of camouflage to assess when to initiate an attack. Thus, a better understanding of other marine species can be achieved, and broader predator-prey dynamics can be predicted.

Camouflage as an antipredator strategy

Camouflage is a powerful means of preventing detection and recognition from visual predators and can be achieved through a diverse range of mechanisms (Ruxton *et al.*, 2004; Stevens and Merilaita, 2009a; Cuthill, 2019). A widespread camouflage strategy, whereby an organism closely matches its local environment in colour, brightness/luminance and pattern, is referred to as background matching (Ruxton *et al.*, 2004; Stevens and Merilaita, 2009a). This is perhaps the most common type of camouflage strategy and has been observed in a range of taxa including invertebrates, fish, reptiles and birds (Stuart-Fox *et al*., 2004; Caro, 2005; Stevens and Ruxton, 2018). Examples of background matching have been observed from earlier studies in the peppered moth (*Biston betularia*) whereby pale and melanic morphs selected appropriate light and dark backgrounds (Kettlewell, 1955) to more recent studies of colour polymorphisms in cichlids (Sowersby *et al*., 2014), or refining nest-site choices in wild birds that appropriately camouflage their eggs (Stevens *et al*., 2017). Colour change for camouflage aims to prevent detection or recognition from predators and is important in species capable of extremely rapid changes (within seconds) such as cephalopods (Hanlon *et al.*, 2009; Chiao *et al.*, 2011), and in species exhibiting comparatively slow colour change such as crabs (Stevens *et al*, 2013), prawns (Green *et al*., 2019), and fish (Clark and Schluter, 2011). Therefore, with the help of background matching, the probability of colour changing species being detected by the predators decreases and animals can evade predation.

Exposure to predators involves a quick risk assessment and making appropriate escape decisions (Lima & Dill, 1990). As per the optimal escape strategy, prey species should flee when the cost of remaining is either more or equal to the costs of fleeing such as lose of foraging and reproducing opportunities as well as energy expenditure (Ydenberg & Dill, 1986; Cooper & Fredrick, 2007). For cryptic individuals, their degree of background matching should influence the escape decision (of staying or fleeing immediately on detecting an approaching predator). If the prey decides to stay hidden, the predator may eventually discover it but fleeing would break the camouflage (Stevens *et al*., 2011), and the prey’s presence and location may be revealed. This suggests that escape response is linked with the camouflage of an animal. The association between camouflage and escape behaviour has been supported in recent studies of Wilson-Aggarwal *et al*., 2016, who found that poor background matching of the eggs influenced the escape distances of plovers and coursers.

Background matching camouflage in terms of brightness difference and colour difference did not significantly affect the escape response of shore crabs when present on mismatched backgrounds across both chapters 2 and 3. I predicted that crabs closely matching the brightness and colour of the background would take longer to flee when exposed to predators, but this was not the case. This finding contradicts with previous research, which suggests that poor level of camouflage initiates fleeing behaviour at greater distances (Cooper *et al*., 2008; Wilson-Aggarwal *et al*., 2016; Atmeh *et al*., 2018). However, in studies suggesting background matching influences escape response, the predator is either only approaching the undetected or already detected prey species. In my experiments, crabs were attacked by the predator after detection. It is possible that background matching is ineffective once the prey has been detected by the predator, then the individual's decision of staying or fleeing immediately becomes more important. This is because an early fleeing strategy although would incur costs such as loss of opportunities but would increase the probability of survival. In addition, the predator might be considered as a greater threat simply because it had already attacked, so fleeing is more appropriate rather than relying on background matching. This has been supported by Martin *et al*., 2009, who found that the effect of remaining cryptic was not observed when the predator had already attempted an attack. The lizards in this situation considered the predator as high-risk and more dangerous and exhibited shorter fleeing times. Thus, background matching might not significantly affect fleeing response when the risk of prey detection increases, or the prey has been already detected or in cases where predators are highly dangerous. However, in scenarios involving more cryptic individuals, much greater threat might be required to break their camouflage strategy (Staudinger *et al*., 2013).

Background matching was however found to significantly affect the escape response of crabs on heterogeneous substrates in chapter 2. The escape response of crabs was significantly affected by the colour difference whereby a closer matching with the heterogeneous substrate displayed a slower response. Colour matching has been previously employed to measure the effectiveness of camouflage (Solis *et al*., 1995; Yahner and Mahan, 1996; Blanco *et al.*, 2002). For example, the surviving probability of Black-tailed gull *Larus crassirostris* clutches increased when the eggs had a better colour match to their background (Lee *et al*., 2010). Brightness matching also significantly affected the fleeing behaviour of crabs on heterogeneous substrates. My prediction of longer escape times in crabs with better brightness matching to the substrate was in line with our findings. The significant interaction between brightness difference and background heterogeneity suggests that crabs are relying on their degree of conspicuousness and show a slower response when matching the background even when exposed to predator. This aligns with the similar findings in the ground-nesting birds that are able to assess the camouflage of their eggs against their nesting background (Wilson-Aggarwal *et al.*, 2016). In addition, predator did not launch an attack on crabs in this experiment rather was immobile at a constant distant from the prey. Here, the crabs did not flee immediately but delayed their fleeing after detecting the predator. It is possible that crabs are using the background matching strategy and do not consider the predator as a greater risk initially and flee when the costs of remaining increases with time as the predator might succeed in recognising the prey. This is supported by fleeing response of lizards even when the distance between prey and predator remained constant and no attempt to attack was made (Martin *et al.*, 2009).

Table 4.1: Summary table showing trends from different studies and results from this study with respect to camouflage.

Prey Organism	Study	Reference
Peppered moth	Pale and melanic morphs selected appropriate light and dark backgrounds	Kettlewell, 1955
Ghost crab	Crabs became lighter during day and darker at night	Stevens *et al*, 2013
Ground nesting birds	Poor level of camouflage initiates fleeing behaviour at greater distances	Wilson-Aggarwal *et al.*, 2016
Shore crabs	Crabs with both good and poor level of camouflage took shorter	This study

	time intervals to flee when exposed to predators – Exp 1	
Black-tailed gull	Clutches survival increased when the eggs had a better colour match to their background	Lee *et al*., 2010
Shore crabs	Closer matching with the heterogeneous substrate displayed a slower response in crabs – Exp 2	This study
Shore crabs	Longer escape times in crabs with better brightness matching to the substrate – Exp 2	This study

Role of background

Background selection is an important aspect in animal camouflage and to ensure an effective camouflage resembling the background is essential. However, this may be difficult in a changing environment meaning the animal cannot perfectly match all or most backgrounds (Merilaita, 1999). To overcome this, several animals change colour for camouflage to fine tune their phenotype to the background. Although this is highly beneficial for animals that are capable of rapid colour change (within seconds) but also problematic for other slow colour changing animals which would exhibit mismatched appearance to the background during changes (Chiao *et al*., 2011; Stevens, 2016; Duarte *et al*., 2017). Another solution is, animals investing in ‘compromise’ appearance which matches to several backgrounds to some extent rather than perfectly matching one specific background (Merilaita, 1999; Houston *et al*., 2007). Lastly, animals using behavioural choices such as where to rest or position their orientation that blends in with their appearance (Stevens and Ruxton, 2018).

Background when considered independently significantly affected the escape behaviour of shore crabs across both chapters 2 and 3. This suggests that crabs favoured one background more than the other when making escape decisions. We predicted crabs that closely resembled the background would display longer escape times and my findings supported this. Similar results have been found in previous research such as metamorphosed American toads (*Bufo americanus*) showing preferences for dark soil and mixed sandy substrates over plain sand backgrounds because of higher predation risk on plain sand from snakes (Heinen, 1993). A recent study on western terrestrial garter snake (*Thamnophis elegans*) also observed the snakes were choosing sites that provided better camouflage against potential predators (such as mammals and birds) than resting on random backgrounds (Isaac & Gregory, 2013). In addition, the flight response of crabs present on black background was slower than the crabs on yellow background. This is perhaps because crabs resembled black background more than yellow background adopting a compromise coloration strategy. In shore crabs (*Carcinus maenas*), juveniles are variably coloured and resemble their background at different spatial scales, however, they turn to uniform dark green on maturation adopting an optimal cryptic strategy, that confers crypsis against several substrates instead of a single background to visual predators (Stevens *et al*., 2014; Nokelainen *et al*., 2019; Hughes *et al*., 2019). This is in line with the findings of Cuadrado *et al*., 2001, who observed chameleons that perched on more protected *Myoporum* bushes allowed closest approach distances by predator (human) as opposed to those perched on less protected *Retama* bushes. Therefore, the escape tactics of individuals are influenced by the background on which they are found.

The results from chapter 2 demonstrate that the escape behaviour of shore crabs is significantly influenced by substrate heterogeneity. The individuals on heterogeneous substrate demonstrated slower fleeing responses as compared to homogeneous (uniform) substrate as predicted. This is because heterogeneous habitats are visually more complex which improve camouflage and the probability of being detected by potential predators decreases in these environments (Merilaita, 1999; Merilaita, 2003; Dimitrova and Merilaita, 2009). This aligns with recent research on ground-nesting birds exhibiting shorter escape distances in high-contrast backgrounds (Wilson-Aggarwal *et al*., 2016). Further research on this showed that lower predation was observed when high contrast courser and plover eggs were laid on high contrast backgrounds. (Troscianko *et al*., 2016). Additionally, some crabs were also observed repositioning in patches that better matched their carapace. It is possible crabs may be evaluating their current level of camouflage and individuals reposition themselves to patches that provide better concealment so as to increase their level of camouflage. This ties in with the findings by Kang *et al*., (2012), whereby moths (*Hypomecis roboraria* and *Jankowskia fuscaria*) were reported to reposition themselves to a more cryptic spot if they landed on less cryptic areas and these new resting sites were difficult to detect by human observers. Further study found that repositioning behaviour was associated with the individuals' level of camouflage on their first landing (Kang *et al*., 2013). The well concealed moths were less likely to reposition themselves than individuals landing in more conspicuous orientations and positions indicating that moths are able to determine their degree of camouflage and can improve their camouflage if needed. This suggests that substrate heterogeneity is an important predictor and can aid in making escape decisions.

Table 4.2: Summary table showing trends from different studies and results from this study with respect to background.

Prey Organism	Study	Reference
American toads	Toads showed preferences for dark soil and mixed sandy substrates over plain sand backgrounds because of higher predation risk on plain sand from snakes	Heinen, 1993
Western terrestrial garter snake	Snakes were choosing sites that provided better camouflage against potential predators (such as mammals and birds) than resting on random backgrounds	Isaac & Gregory, 2013

Ground nesting birds	Poor level of camouflage initiates fleeing behaviour at greater distances	Wilson-Aggarwal *et al.*, 2016
Shore crabs	Crabs on heterogeneous substrate demonstrated slower fleeing responses as compared to homogeneous (uniform) substrate – Exp 2	This study
Moths	Moths were reported to reposition themselves to a more cryptic spot if they landed on less cryptic areas	Kang *et al.,* 2012
Shore crabs	Crabs were also observed repositioning in patches that better matched their carapace	This study

Role of predator

For most animals, predation is a constant source of risk and is an important factor in ecological systems. The study of antipredator mechanisms as an element of this process is therefore of equal significance. For a successful predation attempt, a predator must detect a potential target; then identify if the target is an actual prey; and complete the chain of predatory sequence with capturing the target/prey item (Endler, 1986). Prey species, on the other hand are also equipped with defence mechanisms including various camouflage strategies, that aim to eliminate the interaction with the predator at each, or several, of these stages (Stevens and Merilaita, 2009a; Cooper *et al*., 2015). However, these tactics can only confer protection until the predator has not detected the prey species. Once detected the prey must quickly assess the risk using different cues from predators in risk assessment such as size, speed, style of the predator’s approach, and the predator’s body condition to develop an escape strategy (Stankowich & Blumstein, 2005). For instance, kangaroo rats (*Dipodomys*) are able to discriminate predation risk by decreasing foraging and increasing vigilance in the presence of live snakes to assess risk and may approach and footdrum as a pursuit deterrent technique (Randall & Boltas King, 2001). When encountered with an approaching predator, prey species should flee only when the costs of fleeing (such as loss of foraging or mating opportunities and energy expenditure) is equivalent to the costs of remaining (for example, the level of risk or being injured or predated) (Ydenberg and Dill, 1986). Therefore, quickly assessing the situation on detecting a predator and make an effective decision of whether to stay or escape is crucial for the prey because an incorrect decision would lead to fitness costs or death. A popular and well-documented means to assess escape behaviour in animals is the flight initiation distance (FID), the distance between an approaching predator and prey at which the prey starts fleeing (Blumstein, 2003; Bjorvik, Dale, 2015; Moller *et al.*, 2016). Several traits of both the predator and prey further modulate the flight initiation distance such as approaching speed and direction of predator, body size of predator and prey, prey's detectability, distance to refuge, presence of conspecifics, previous experience of predators as well as internal state of the prey species (Stankowich & Blumstein, 2005; Bateman & Fleming, 2015). For instance, lizard (Anolis lineatopus) and fish (*Gadus morhua*) have been observed to show increased FID to predator speed, exhibiting greater FID when the predator approach accelerates and lower FID when the predator approach decelerates (Cooper, 2006; Meager *et al.*, 2006).

Across both chapters 2 and 3, predator when considered independently significantly affected the escape behaviour of shore crabs. I predicted the presence of a predator plays a key role in escape decisions and the results directly supported this. The flight response of crabs was quicker in the presence of a model predator than the control treatment of no predator. This is primarily because on detecting a threat, prey must assess the risk and make appropriate escape decisions of when and how far to flee as failure to do so may result in injury or death (Stankowich & Blumstein, 2005; Cooper *et al.*, 2010). For instance, greater flight distances were observed in red knots (*Calidris canutus*) when exposed to models of flying sparrowhawks (*Accipiter nisus*) than perching models (Mathot *et al.*, 2009), indicating that knots can assess the level of risk and elicit the escape responses accordingly. Another example is Columbian black-tailed deer (Odocoileus hemionus columbianus) fleeing at greater distances and displaying longer escape bouts when approached by humans when faster and more direct movements were used, suggests that these species also respond after assessing the level of risk (Stankowich & Coss 2006, 2007). Predation risk increases the flight initiation distance and can be affected by several factors (Ydenberg & Dill, 1986; Stankowich & Blumstein, 2005). The antipredator responses such as increased dropping behaviour in pea aphids (*Acyrthosiphon pisum*) (Losey and Denno, 1998) or longer FID in wall lizard (*Podarcis muralis*) (Diego-Rasilla, 2003) are a result of higher predation pressure. In my experiments, the crabs were exposed to maximal risk in such that no refuges were provided to take cover and the model predator simulated an attack on the crabs. Therefore, it is likely that the shore crabs considered the predator as threat and their escape time was shorter in presence of a predator as compared to its absence. In addition, the model predator chosen for this study resembled a live seagull, which is one of the main predators of shore crabs (Crothers, 1966). Consequently, majority of the crabs started fleeing after the first simulated attack which reflected detection by the predator demonstrating flight response was probably the best option.

The size of predator could be another reason for a quicker response in crabs. The prey species needs to be certain that the approaching animal is a predator and not another prey item by taking into account the size of the approaching individual (Stankowich, 2009). The FID is longer in response to larger predators because of increased risk associated with increasing predator size (Stankowich & Blumstein, 2005; Cooper and Fredrick, 2007). For instance, Cooper and Stankowich, 2010, found that the probability of fleeing in lizards is greater with increasing model predator sizes. Therefore, from the prey's perspective, the probability of an approaching animal to be a predator increases with increasing body size, and larger body size is perceived as greater threat (Stankowich & Blumstein, 2005; Cooper and Fredrick, 2007). This finding has been observed in the previous research by Web, 1982, whereby the escape responsiveness increases with the size of the predator as demonstrated in the fathead minnow (*Pimephales promelas*) where the frequency of responding was higher when presented with a predator of larger frontal profile than a smaller predator. Similar findings were observed in the escape responses of lizards (*Sceloporus jarrovii* and *S. virgatus*) (Cooper and Stankowich, 2010) and dogfish shark (*Squalus acanthias*) (Seamone *et al*., 2014) when exposed to increased sizes of model predators. So, the shore crabs in my experiments might also be categorizing the approaching large model as a predator and exhibiting shorter fleeing time. Another possibility for the shorter time to flee could be due to the model predator's approach. This study implemented direct approach in the predator treatments and several studies show FID is greater in response to a direct approach as opposed to an indirect one (Cooper *et al*., 2003; Cooper & Whiting, 2007; Møller *et al*., 2014). For example, FID was greater in Balearic lizard (*Podarcis lilfordi*), and the lizards were quick to flee in case of a direct approach than slower indirect approach (Cooper *et al*., 2010). This suggests that crabs are quicker to respond when predator uses a direct approach as opposed to an indirect approach when attacking.

The flight behaviour of shore crabs was significantly influenced by the model predator in substrate heterogeneity experiment as well in chapter 2. In the experiment, the model predator did not attack the crabs rather was stationary at a constant distant. Here, the time to flee was longer because the crabs did not flee immediately instead delayed their fleeing after detecting the predator. This is perhaps because after detection crabs are monitoring the risk posed by the predator and show a delayed response in order to balance the costs and benefits of flight (Ydenberg & Dill, 1986). In this species, stalked eyes are used for predator detection. During field work, it was apparent that shore crabs actually detected the model predator long before they moved, as they lifted their heads to observe the predator while remaining immobile. It would be advantageous for the prey to wait as long as possible before responding to a predator because predators might sometimes require the stimulus of a moving prey for prey detection or attack and fleeing immediately would only alert the predator. This is also supported by fleeing response of lizards even when no attempt to attack was made by the predator (Martin *et al*., 2009). There could also be a possibility of posture of the predator in affecting the escape response of crabs, whereby the posture acted as a cue in threat assessment. In this study, crabs were exposed to the model predator in a striking posture. This reason has been supported by a study on rhesus macaques (*Macaca mulatta*) which responded more strongly to a snake model in a striking posture than in a coiled posture (Etting & Isbell, 2014). There is evidence that gaze directness affects the flight initiation distance, whereby the probability of fleeing is greater if the predator’s gaze is averted than direct to prevent detection of the prey individual. For instance, the proportion of fleeing in the zebra-tailed lizard, *Callisaurus draconoides* is three times greater for averted as opposed to direct gaze (Cooper & Sherbrooke, 2015). In my study, crabs were under constant scrutiny of the model predator which also explains the longer latency to flee. The physical condition of an animal (good versus poor) influences its escape behaviour in terms of speed, agility, and endurance (Stankowich & Blumstein, 2005). For example, woodpigeons (*Columba palumbus*) in poor overall condition had longer flight initiation distances as compared to those in good overall condition (Kenward, 1978). In my experiment, the crabs were generally of good physical condition and excluded any crabs with autotomised limbs or newly moulted state and the crabs displayed longer escape times. The long flight response of crabs suggests that a good physical condition of crabs also affects the escape behaviour of crabs. In addition, the escape behaviour of shore crabs was significantly affected when the interaction between predator and substrate (board) was considered in the substrate heterogeneity experiment in chapter 2. The crabs showed a slower response when matching the substrate even in presence of predator. This is perhaps because individuals were relying on their level of conspicuousness to prevent predation and a quicker response on detection of predator on such substrates might alert the predator of the prey’s location. The probability of detection by a predator decreases when the prey is present on matching substrates and is consistent with previous studies on lizards (Marshall *et al.,* 2016) and spiders (Defrize *et al*., 2010) that choose a particular substrate when exposed to predators. A recent study on rainbow darter (*Etheostoma caeruleum*) also supports this, whereby the fish prefers a heterogeneous substrate in comparison to a homogeneous one and show decreased activity levels in presence of predators (White, 2021). Therefore, this finding suggests that the escape behaviour of shore crabs is influenced by the substrate heterogeneity.

Table 4.3: Summary table showing trends from different studies and results from this study with respect to predator.

Prey Organism	Study	Reference
Red knots	Greater flight distances were observed in red knots (when exposed to models of flying sparrowhawks than perching models	Mathot *et al*., 2009
Columbian black-tailed deer	Deer fleeing at greater distances and displaying longer escape bouts when approached by humans when faster and directly	Stankowich & Coss 2006, 2007
Shore crabs	Flight response of crabs was quicker in the presence of a model predator than the control treatment of no predator	This study
Lizards	The probability of fleeing in lizards is greater with increasing model predator sizes	Cooper and Stankowich, 2010
Shore crabs	Crabs might also be categorizing the approaching large model as a predator and exhibiting shorter fleeing time	This study
Balearic lizard	Lizards were quick to flee in case of a direct approach than slower indirect approach	Cooper *et al*., 2010
Shore crabs	Crabs are quicker to respond when predator uses a direct approach as opposed to an indirect approach when attacking	This study

Lizards	Fleeing response of lizards even when no attempt to attack was made by the predator	Martin *et al*., 2009
Shore crabs	Fleeing exhibited and time to flee was longer when the model predator was stationary	This study
Rainbow darter	The fish prefers a heterogeneous substrate in comparison to a homogeneous one and show decreased activity levels in presence of predators	White, 2021
Shore crabs	Crabs showed a slower response when matching the substrate even in presence of predator.	This study

Multiple Predators

In natural communities, it is rare for a prey species to encounter a single predator. Prey often encounter multiple predators, and each predator possesses different foraging and capture techniques (Sih *et al.*, 1998). As a result, many prey species have evolved predator-specific defences that are incorporated across the life-history, morphology, and behaviour of the prey individual (Krupa and Sih 1998; Kats and Dill 1998; McIntosh and Peckarsky 1999; Relyea, 2001; Aguilera *et al*., 2019). For instance, Wild Diana monkeys (*Cercopithecus diana*) display two distinct antipredator strategies, conspicuous alarm-calling and silent, cryptic behaviour to leopards and chimpanzees, respectively (Zuberbühler, 2000) because of different hunting tactics. Similarly, *Liolaemus* lizards when in presence of two ambush predators, raptor and snake, reduce their activity levels in presence of both the predators, however, their antipredator defences are different for both predators such that, time to move/chemical exploration is more for *L. chiliensis* in presence of a raptor while tail waving and autotomy are mostly observed in *L. nitidus* in presence of a snake (Constanzo-Cháveza *et al*., 2018). Predator-specific antipredator responses have also been recorded in several marine invertebrates, snails (Marko, 1991; Turner *et al*., 2006), scallops (Guderley *et al*., 2015), cuttlefish (Staudinger *et al*., 2013) and limpets (Iwasaki, 1993; Aguilera *et al*., 2019). Another example is Longfin squid (*Loligo pealeii*) that varies its defensive strategies when exposed to bluefish (*Pomatomus saltatrix*) and summer flounder (*Paralichthys dentatus*) (Staudinger *et al*., 2011). The squids used disruptive body pattern and motionless tactics on encounter with bluefish but changed fleeing with or without inking in case of flounder.

The escape behaviour of shore crabs was significantly affected by the different model predators in chapter 3. I predicted that crabs are able to distinguish between different predators and their escape responses are tailored to the type of predator. The results support the prediction in that the latency to flee was shorter when crabs were exposed to the bird model. The time to flee was slightly longer with the fish predator and some crabs were observed to exhibit avoidance behaviour. This is perhaps because avian attacks are fast compared to other potential predators so the perceived risk by the crabs is greater for birds than fish. Therefore, fleeing is likely to be a more efficient antipredator strategy under high predation risk (Cuadrado *et al.,* 2001; Carretero *et al*., 2006; Constanzo-Cháveza *et al*., 2018). This aligns with studies on dwarf chameleons (*Bradypodion transvaalense*) to multiple predators. When tested in the presence of bird and snake models, chameleons become brighter in the presence of a snake as compared to the bird, suggesting a bird posed a greater risk to chameleons either because birds possess better vision compared to snakes or due to the abundance of birds (Stuart-Fox, Whiting and Moussalli; 2005). Similar observation has been made in tit species when exposed to life like models of different potential predators, whereby the latency time of return was significantly longer after presenting the Sparrowhawk than the Siberian Jay, whereas the woodpecker aroused no specific reaction indicating that tits are capable to recognise the individual predator species and make decisions on their perceptions of the threat level (Hogstad, 2017). The ability to distinguish between different fish predators has also been recorded in the megalopae of the native mud crab (*Dyspanopeus sayi*) and the Asian shore crab (*Hemigrapsus sanguineus*) in flowing seawater in response to chemical cues from potential fish predators - cunner (*Tautogolabrus adspersus*), tautog (*Tautoga onitis*), and mummichog (*Fundulus heteroclitus*). In control seawater, megalopae of both species swam upstream more than inseawater withfish or crab cues. Mud crab megalopae were inactive in the presence of chemicals from all fish species whereas Asian shore crab megalopae were still active in the presence of cues from mummichog (Araujo *et al*., 2017). Therefore, the greater response of shore crabs with bird model in comparison to fish predator suggests that crabs are able to identify between fish and bird and make escape decisions based on the level of threat perceived.

The fleeing response of shore crabs was significantly influenced by the size of the crabs in chapter 2 and 3. The fleeing times were longer for juvenile crabs in comparison to adult crabs. This is likely because juvenile shore crabs are highly variable in colour and pattern (Stevens *et al*., 2014) and the FID of cryptic juveniles is shorter than adults. This is because if an animal is very well camouflaged, the chance of it being seen is lower, and so it may be able to stay still for longer as the probability of being seen is low (Martin and Lopez, 1995b). In addition, adult crabs have well developed claws and might be able to avoid a predator attack, whereas small crabs on fleeing too soon may increase their vulnerability by alerting an otherwise unaware predator. Body size affecting the escape response have also been seen in lizards (Martin and Lopez, 1995b) and frogs (Martin and Lopez, 2005). Furthermore, smaller species are inconspicuous and their probability of being detected by predator is also low whereas larger species get easily recognized by predators which makes them more vulnerable to predation as seen in birds (Blumstein, 2006; Piratelli *et al*., 2015). Diet selection is also influenced by prey size because gape-limited predators cannot swallow prey bigger than some maximum size (Webb and Shine 1993). The nutritive benefits increase with the size of prey, but energetic costs of subduing that prey and the risk of being injured increase. Thus, with smaller prey size, the probability of selecting the prey may increase with prey size, then reach a maximum (Curio, 1976). As profitability decreases beyond the maximum size, the prey should not be attacked (Curio, 1976; Forsman, 1996). In my study, increased carapace size of shore crabs should limit predation by small predatory fish because of the small mouth gape of the fish. This shows why crabs demonstrated a slow response when presented with a fish predator as opposed to a bird, suggesting that fish posed a lower risk to adult crabs. This finding concerts with a study on the escape response of giant scallop (*Placopecten magellanicus*) in response to their principal predators, seastars and crustaceans (Guderley *et al*., 2015). The increased shell thickness of older scallops reduced predation by mobile predators, so the individuals were slower to respond to predators than their smaller counterparts. Another possible reason could be the foraging techniques and experience of the predator. For example, western gulls (*Larus occidentalis*) forages optimally by selecting the most profitable size (larger) of purple sea urchins (*Strongylocentrotus purpuratus*). However, gulls when air-dropping chose smaller urchins being influenced by group size and age. Gulls when foraging in larger groups selected smaller urchins due to the risk of kleptoparasitism. Whereas adult gulls chose larger, and juvenile gulls chose smaller urchins when air-dropping, suggesting that juveniles are less experienced in foraging techniques. (Snellen *et al*., 2007). Therefore, it is likely that the hunting strategy and experience of both fish and bird predators could also be influencing the escape response of shore crabs and other marine species.

Table 4.4: Summary table showing trends from different studies and results from this study with respect to multiple predators and size.

Prey Organism	Study	Reference
Crab megalopae	Mud crab megalopae were inactive in the presence of chemicals from all fish species whereas Asian shore crab megalopae were still active in the presence of cues from mummichog	Araujo *et al*., 2017

Tit species	The latency time of return for tits was significantly longer after presenting the Sparrowhawk than the Siberian Jay	Hogstad, 2017
Shore crabs	Time to flee was slightly longer with the fish predator than bird	This study
Giant scallop	Older scallops reduced predation by mobile predators, so the individuals were slower to respond to predators than their smaller counterparts	Guderley *et al.*, 2015
Shore crabs	Fleeing times were longer for juvenile crabs in comparison to adult crabs.	This study

Limitations

Predator vision models can be utilised to examine how camouflage mismatch influences escape responses from a predator's perspective, thereby, getting a better insight of flight response of prey species. The experiments within this thesis only use visible light to calibrate reflectance values and did not model camouflage to predator vision. This is because shore crabs have a diverse array of predators which are highly visually guided, including birds such as corvids, gulls and shorebirds (all possibly tetrachromats), and numerous species of fish (such as gobies, blennies, pollack and wrasse, generally dichromatic or trichromatic), in addition to catsharks and cephalopods (which are monochromatic). This indicates that the crab camouflage is likely be subject to selection pressure from a wide range of visual systems and many use UV vision to detect prey species (Crothers, 1968; Troscianko *et al.*, 2021). Predator vision models could not be used because they require UV photography. Another reason to avoid taking UV photography was to minimise overstressing the crabs by physically restraining them which would be required while taking images in UV. The crabs collected for the experiments were only of good physical condition and crabs of poor condition such as newly moulted crabs or individuals with missing legs or claws were excluded from the study. However, in a natural setting, escape behaviour of crabs with poor physical condition is likely to be influenced by the level of their camouflage. As the experiments were conducted in a natural system, the abiotic conditions such as temperature, tide timings and light could not be controlled. Past research suggests that surface temperature affects the flight response of animals as seen in Namaqua dwarf adder (*Bitis schneideri*), whereby snakes that were active on surface were more likely to flee than buried snakes (Maritz, 2012), indicating that abiotic conditions could also be influencing the escape decisions of crabs.

Future research

This thesis highlights some of the knowledge gaps in the previous literature of the importance of camouflage in escape behaviour of species and gives rise to further research. The shore crabs have multiple predators with different visual systems and many of them use ultraviolet vision which my thesis does not examine. Despite UV brightness matching being the same across different visual systems, it still increases the efficacy of examining the visual systems. Therefore, future research should undertake an examination of the use of visual systems of different potential predators and test how this aim to break the camouflage in predator-prey interactions. The results from chapter 3 were based on the study of multiple predators including treatments with no predators, predator A and predator B but did not put the predators together. Future work should use combined predator treatments as well in escape behaviour studies. This is because the prey can be in a conflicting situation when exposed to multiple predators, and these can have risk-reducing or risk-enhancing effects if they cause lower or higher predation rates than expected (Sih *et al*., 1998; DeWitt *et al*., 2000). If the prey responds to one predator, this may simultaneously increase vulnerability to another predator. Models suggest that if the predators are of approximately equal predation risk then prey should use general defence as no antipredator response would be effective. In contrast, when predation threat from one exceeds another then most prey species respond to the more risky predators (McIntosh and Peckarsky, 1999;

Ferrari *et al*., 2010). For instance, tadpoles when presented with the nonlethal fish and lethal dragonfly larvae simultaneously, responded by increasing tail fin depth, which was similar to that expressed in the presence of dragonfly larvae alone, suggesting a hierarchy of response corresponding to the predation risk (Teplitsky *et al*., 2004). Another situation is where prey response against one predator increases the threat of being killed by the other predator and vice versa. For example, mortality of mayfly (*Ephemerella*) prey in the presence of both fish (*Cottus*) and stoneflies (*Agnetina*) was greater than expected, because stoneflies under rocks caused mayflies to emerge out from under rocks, thus resulting in greater exposure to fish (Soluk, 1993; Soluk *et al*., 1998). Lastly, the escape behaviour of prey species is likely to be affected with poor physical condition such as newly moulted stage or individuals with missing legs or claws and their poor level of camouflage would further make them vulnerable to predation. Therefore, future studies should also include the physical condition of animals in predator-prey research.

Concluding words

This thesis shows that the escape behaviour of shore crabs and other species capable of crypsis is influenced by background matching camouflage on more heterogenous substrates rather than mismatched substrates. However, an effective level of camouflage is not a sole factor when an animal is making critical escape decisions. Many other factors such as nature of background and several characteristics of the predator itself are involved in deciding if the prey species should stay or flee (Ydenberg & Dill, 1986; Stankowich & Blumstein, 2005). Indeed, natural systems contain more diverse predator communities of more than two predator species, so it is important to learn about the predator-specific responses as to better understand how animals assess increased predation risk. Therefore, an understanding of the prey defences including camouflage will greatly benefit our interpretation of evolution of animals in complex heterogeneous environments such as intertidal rockpools and how they cope with predators.

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