Camouflage Strategies in Cryptic Predatory Fishes

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Summary

For a successful hunt, marine sit-and-wait predators such as the scorpionfishes need to be well camouflaged in the eyes of their prey. While scorpionfishes are indeed cryptic to the human eye, there is barely any research on the functionality of their camouflage in a prey-predator context. Therefore, my research investigated camouflage strategies in two scorpionfish species under consideration of the prey's visual perspective. I focussed on the question how scorpionfish can camouflage in a heterogeneous environment with a variety of backgrounds, such as different kinds of substrates. In camouflage research, three main strategies are discussed as a solution to this problem. First, animals could dynamically adjust their body colour and pattern depending on their background. Second, animals might choose to settle on backgrounds on which they are best camouflaged and avoid others. Third, animals could have a generalist body colouration that allows camouflage on many natural substrates and therefore mitigates the need to employ dynamic camouflage strategies such as colour change and background choice.

In two experiments, I placed scorpionfish on different backgrounds and documented changes in body colouration over one to five minutes using calibrated photography. I used visual modelling to process the images accounting for the visual system properties of prey fishes as naturally relevant observers. I confirmed that scorpionfish dynamically change their body colouration in response to their background, including body hue, luminance, and pattern contrast. In two behavioural choice experiments, I then tested whether scorpionfish prefer to settle on backgrounds that facilitate camouflage. Here, scorpionfish did not choose the background that provided the best background match for their average body colouration, but preferred the background that allowed disruptive colouration. Finally, using data of average scorpionfish body colouration and photographs of natural substrates, I calculated how well scorpionfish would match these substrates from the prey's perspective. I can show that even without adjustment, scorpionfish have low chromatic (colour) contrast to natural substrates, but high achromatic (luminance) contrast.

I demonstrate that scorpionfish show several strategies to camouflage in heterogeneous environments. I discuss how they might interact and interpret the importance of achromatic and chromatic cues for camouflage in these fishes.

Zusammenfassung

Für eine erfolgreiche Jagd müssen Lauerjäger wie die Drachenköpfe gut getarnt sein. Obwohl die Tarnung der Drachenköpfe aus menschlicher Sicht offensichtlich ist, gibt es wenig entsprechende Forschung zur Tarnung im ökologischen Räuber-Beute Kontext. Darum habe ich Tarnstrategien in zwei Drachenkopf-Arten untersucht, immer unter Berücksichtigung der visuellen Perspektive von Beutetieren. Dabei lag mein Fokus darauf, wie sich Drachenköpfe in einer heterogenen Umgebung mit unterschiedlichen Hintergründen, zum Beispiel verschiedenen Hartsubstraten, tarnen können. Dazu sind drei primäre Strategien bekannt. Erstens könnten Drachenköpfe sich durch dynamischen Farbwechsel and Hintergründe anpassen. Zweitens könnten sie sich nur auf Hintergründen niederlassen, auf denen sie am besten getarnt sind, und andere vermeiden. Drittens könnten Drachenköpfe eine generalistische Körperfärbung haben, die Tarnung auf vielen natürlichen Hintergründen erlaubt und dadurch den Bedarf an den dynamischen Strategien des Farbwechsels oder der Hintergrundwahl verringert.

In zwei Versuchen habe ich Drachenköpfe auf verschiedenen Hintergründen platziert und Veränderungen der Körperfärbung durch standardisierte Fotografie dokumentiert. Bei der Bildverarbeitung habe ich das visuelle System von Beutefischen als natürliche Beobachter der Drachenköpfe miteinbezogen. Drachenköpfe änderten dynamisch ihre Körperfärbung einschließlich Farbe, Helligkeit und Kontrast. In zwei Verhaltensversuchen habe ich außerdem getestet, ob Drachenköpfe sich bevorzugt auf Hintergründen niederlassen, die ihre Tarnung verbessern. Hier zeigten Drachenköpfe eine Präferenz für den Hintergrund der eine disruptive Färbung ermöglichte, statt des Hintergrunds der am ähnlichsten zur durchschnittlichen Körperfärbung war. Zuletzt habe ich mit Daten zur durchschnittlichen Drachenkopf-Färbung und Fotos von natürlichen Substraten berechnet, wie ähnlich Drachenköpfe ihrem Substrat aus der Perspektive ihrer Beute sind. Hier zeigte sich, dass Drachenköpfe einen geringen Farbkontrast, aber einen hohen Helligkeitskontrast zu ihren natürlichen Substraten haben.

Zusammengefasst haben Drachenköpfe mehrere Strategien zur Tarnung in einer heterogenen Umgebung. Ich diskutiere wie diese Strategien zusammenwirken könnten und welche Bedeutung Farbe und Helligkeit des Hintergrunds für die Tarnung der Drachenköpfe haben.

List of publications

Published or accepted publications

1. **John, L.**, Santon, M., & Michiels, N. K. (2023). Scorpionfish rapidly change colour in response to their background. *Frontiers in Zoology*, *20*, 10.

2. **John, L.**, Santon, M., & Michiels, N. K. (2024). Scorpionfish adjust skin pattern contrast on different backgrounds. *Ecology and Evolution*, 14:e11124.

Publications to be submitted

3. **John, L.**, Santon, M., & Michiels, N. K. (2024). Generalist camouflage and background choice in scorpionfish. In preparation.

Declaration according to § 5 Abs. 2 No. 8 of the PhD regulations of the Faculty of Science

-Collaborative Publications-

Nr.	**Accepted publication yes/no**	**List of authors**	Position of candidate in list of authors	Scientific ideas by the candidate (%)	Data generation by the candidate (%)	Analysis and Interpretation by the candidate (%)	Paper writing done by the candidate (%)
1	yes	3	1	60	100	80	90
2	yes	3	1	90	100	90	90
3		3	1	80	100	90	90

Introduction

In nature, deception of hetero- or conspecifics is widespread (Stevens 2016). Between heterospecifics, deception is most commonly used to increase foraging success or to avoid predation, making predation probably the strongest selective force driving the evolution of deceptive strategies (Stevens 2016; Pembury Smith and Ruxton 2020). Camouflage, as one example of deception, is meant to prevent the deceiver from being detected or recognized as itself by an observer, and can be achieved with a multitude of strategies (Endler 1981; Stevens and Merilaita 2009a). What camouflage strategies animals employ and how this is related to their ecology is a diverse field of research (see Cuthill 2019 for a recent summary). My work focusses on visual camouflage while there might be other senses and modalities mediating camouflage (Ruxton 2009).

Camouflage in predators

While a large body of literature deals with camouflage in prey (Duarte et al. 2017; Ruxton et al. 2019; Galloway et al. 2020; Moreno–Rueda 2020), camouflage in predators has received much less attention. Discussing camouflage of prey and predators separately can help to understand which camouflage strategies evolved under which constraints (Pembury Smith and Ruxton 2020). These can differ between prey and predators because predators need to get very close to their prey before they attack, while prey does not intend such proximity. Moreover, there might be differences in the visual systems of predator and prey. The observer's visual system properties, such as spectral sensitivity and visual acuity, define how specific visual features are perceived. For instance, while the red coat of a tiger appears conspicuous to a human observer when seen in its natural, green-dominated habitat, tigers are well camouflaged for their prey that typically cannot distinguish between chromatic signals in the long wavelength range well (Fennell et al. 2019). This example shows how visual modelling of the prey visual perspective is essential to understand predator camouflage.

While there are some studies on terrestrial predators (Théry and Casas 2002; Ings and Chittka 2009; Brechbühl et al. 2010; Loos et al. 2011; Pembury Smith and Ruxton 2020), many camouflaged predators are aquatic. Since the light environment and backgrounds of aquatic and terrestrial habitats differ considerably (Jerlov 1976; Johnsen 2014), different specializations are known (e.g. Johnsen 2001, 2014), and more can be expected. Several families of fishes are commonly considered to be strictly relying on their cryptic appearance to capture prey, including the Orectolobidae (Corrigan et al. 2008), Platycephalidae (Coulson et al. 2015) and Scorpaenidae (Greenwell et al. 2018). My thesis focusses on two species within the Scorpaenidae.

Scorpionfish camouflage

Scorpionfishes belong to the family Scorpaenidae and share an ambush predation style. They can be found in all tropical and temperate marine waters (Nelson et al. 2016). Most species are strictly benthic, with the exception of the benthopelagic lionfishes. In the following, I will use the term ‘scorpionfish’ for benthic fishes within this family only. For their sit-and-wait hunting, scorpionfish settle on a substrate and remain motionless until prey is close enough to be caught with rapid suction feeding. The abundance of these and other cryptic, benthic fishes is underestimated, as they are easily overlooked during biodiversity surveys (Kruschel and Schultz 2012; De Brauwer et al. 2017; Brandl et al. 2018). This is reflected in a lack of knowledge about many aspects of their biology. For instance, little is known about scorpionfish ecology and behaviour. In the following, I will introduce camouflage strategies and address their diversity that is evident in benthic scorpionfishes to highlight the knowledge gap that forms the incentive for my research.

Background matching and disruptive colouration

To prevent being visually detected by other organisms, one way is to blend in with the background by matching its colour and pattern (Stevens and Merilaita 2009a). In fact, most animals are somewhat coloured like their environment to become inconspicuous. Many scorpionfishes seem to match not only colour, but also pattern of their background, i.e. the substrate (Figure 1A). Disruptive colouration on the other hand is characterized by high contrast markings that disrupt the body outline and shape, impeding detection or recognition of the fish (Figure 1B) (Stevens and Merilaita 2009b). Here, only some parts of the body colouration need to be similar to the background to assure differential blending (Stevens and Merilaita 2009b). Disruptive patterns are therefore expected to be especially valuable in a heterogeneous environment, where background matching on a variety of substrates would be difficult to achieve, and when backgrounds in themselves are complex (Cuthill et al. 2005; Phillips et al. 2017; Robledo-Ospina et al. 2017; Price et al. 2019).

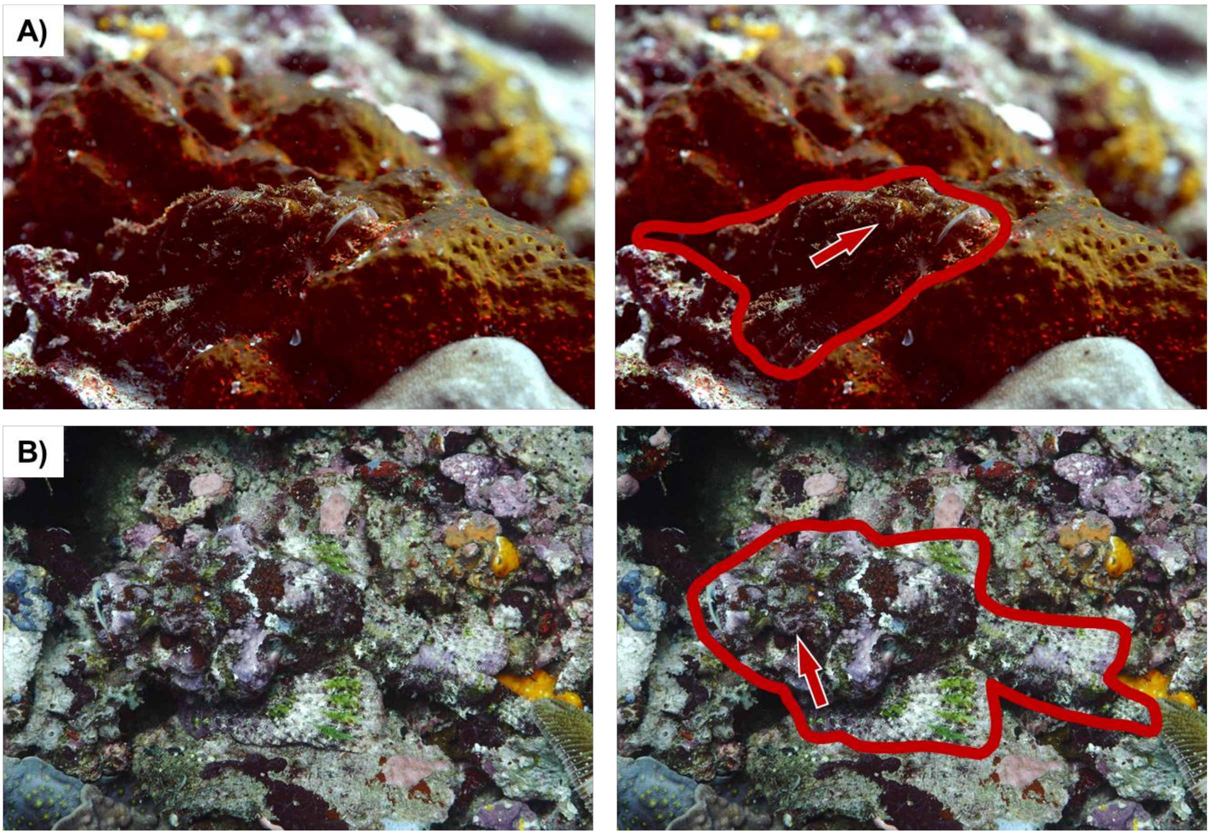


Figure 1: Examples of A) background matching (Scorpaenopsis cf. oxycephalus) and B) disruptive colouration (Scorpaenopsis cf. diabolus). Left: the original images, right: the same images with the rough fish outline highlighted in red and an arrow pointing at the eye. Photos taken by Leonie John.

Phenotypic variation

A fixed phenotype that is fine-tuned for background matching or disruptive colouration on a specific background can also bring challenges. Even when fish are concealed well, time and experience can help prey to form a search image that increases predator detection (Stevens et al. 2014; Galloway et al. 2020; Troscianko et al. 2021). A search image might be based on specific patterns or structures such as symmetrical patches that are similar between many individuals in a population of predators, but are not found in such regularity and frequency on the substrate. This process can favour negative frequency dependent selection, and promote phenotypic variation within a species, because variation can help to disrupt search image formation (Bond and Kamil 2002; Troscianko et al. 2021). Phenotypic variation seems common in scorpionfishes (personal observations, Figure 2, compare also Figure 1B and 6C), but it remains unclear how much of the variation can be attributed to inter-individually varying, fixed phenotypes and how much is due to intra-individual colour change (John et al. 2023). It is also uncertain how different environments shape phenotypic variation between populations.

Figure 2: Phenotypic variation in Scorpaena porcus. The two individuals show different patch sizes of their lateral pattern. Photos taken by Matteo Santon.

Fluorescence

In water, longer wavelengths are absorbed faster than blue-green wavelengths, thus leading to a light environment with little or no red light with increasing depth (Jerlov 1976; Meadows et al. 2014). However, many organisms on marine substrates fluoresce and therefore can display long wavelength colour despite its lack in the remaining sunlight. This is best known from algae and cnidarians (Salih et al. 2000; Mazel et al. 2003; Gruber et al. 2008; Michiels et al. 2008; Zawada and Mazel 2014). Red fluorescence has been demonstrated to be prevalent in cryptic, benthic fishes (Michiels et al. 2008; Anthes et al. 2016), and is therefore proposed to be part of their camouflage (Sparks et al. 2014; Anthes et al. 2016). A survey by Anthes et al. (2016) detected red fluorescence in almost all benthic fish families that were assessed, with the remarkable similarity that the red fluorescence in cryptic fishes such as scorpionfishes occurs in irregular patches across the body, and at least in some cases matches the fluorescent emission of chlorophyll typical for most substrates. Considering their benthic lifestyle, this seems like a perfect strategy to match the fluorescence of patchy algae on the substrate for these fishes and provide background matching, disruptive colouration or other camouflage at a depth where red reflectance is not possible (Figure 3). Although these observations are highly suggestive and the idea that red fluorescence could be important for camouflage at depth seem plausible, we lack experimental confirmation.

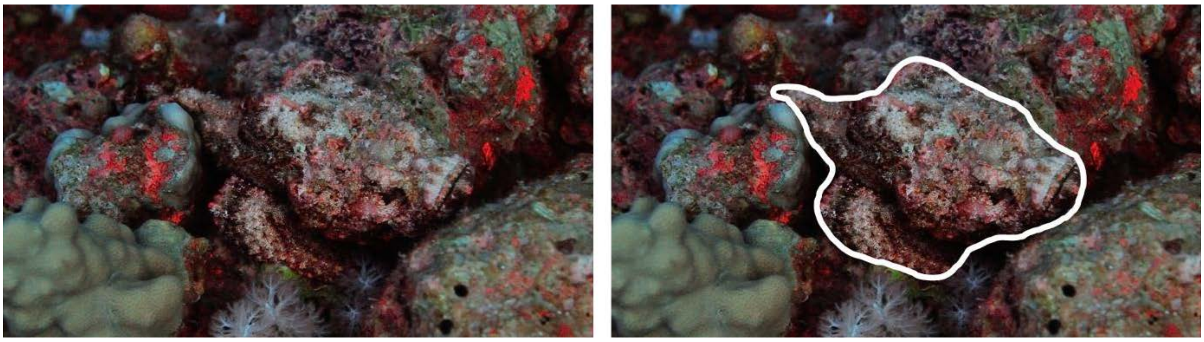


Figure 3: Red fluorescence in a scorpionfish and its background. Scorpaenopsis diabolus photographed in around 20 m depth, through a filter that reduces blue light and emphasises red fluorescence of both the fish and the background. Left: the original image, right: the same image with the rough fish outline highlighted in white. Photo taken by Nico Michiels.

Outline disruption

Skin flaps and other dermal structures are a way to disrupt the body outline of a fish and therefore conceal the typical fish shape (Allen et al. 2015). Disruption of the body outline can also be achieved by disruptive markings found close to the edge of the body (Stevens and Merilaita 2009b). Both three-dimensional skin structure and disruptive markings at the edge of the body are common in scorpionfishes and therefore likely important for their camouflage (Figure 4).

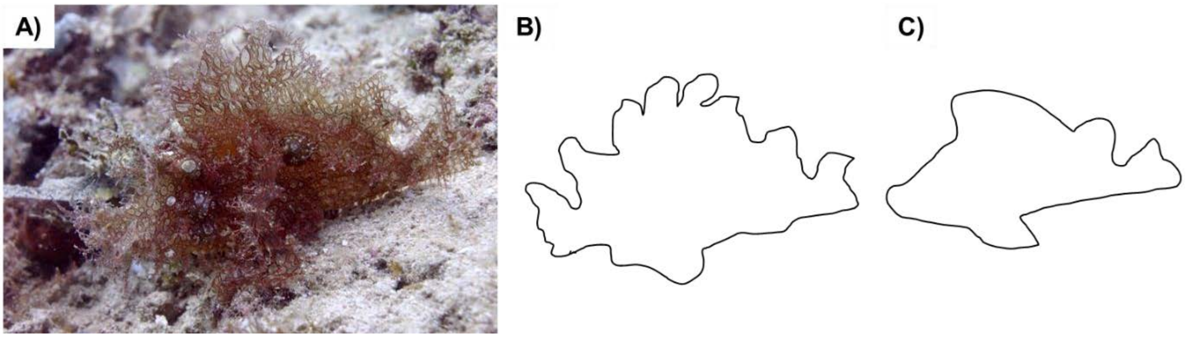


Figure 4: Outline disruption in *Rhinopias aphanes*. A) Photograph of the fish (photo taken by Matteo Santon), B) the fish outline drawn with dermal flaps, C) the outline drawn without dermal flaps. The outline in C) makes it easy for a human observer to recognize a fish, while for the outline in B), the typical fish-shaped outline is disrupted.

Eye camouflage

Eyes are arguably a conspicuous structure that draws attention through the dark colour and round shape of the pupil (Cott 1940). Not surprisingly, a whole suite of solutions for eye camouflage has evolved in fishes (Cott 1940; Neudecker 1989). For instance, the round shape can be disrupted by altering pupil shape, often through eye covers or parts of the iris partially covering the pupil (Figure 5A) (Douglas et al. 2005; Mäthger et al. 2013; Youn et al. 2019). A different way to hide the round shape of eyes it to mask it with a colour pattern or with disruptive markings, known from many reef fishes (Marshall et al. 2019), and also found in scorpionfishes (Figure 5B). To alter colour of the pupil, scorpionfishes show two types of eyeshine (Fritsch et al. 2017b; Santon et al. 2018). Here, side-welling light that enters the eye is reflected by the retroreflective *stratum argenteum*. Additionally, down-welling light is transmitted into the eye through a translucent choroid and adds to the eyeshine. These two mechanisms turn the otherwise black pupil into a pale brown that has a low contrast to the surrounding tissue of the iris (Figure 5C) (Santon et al. 2018). This principle is assumed to be used for eye camouflage by several cryptic, benthic predators (Santon et al. 2018; Marshall et al. 2019).

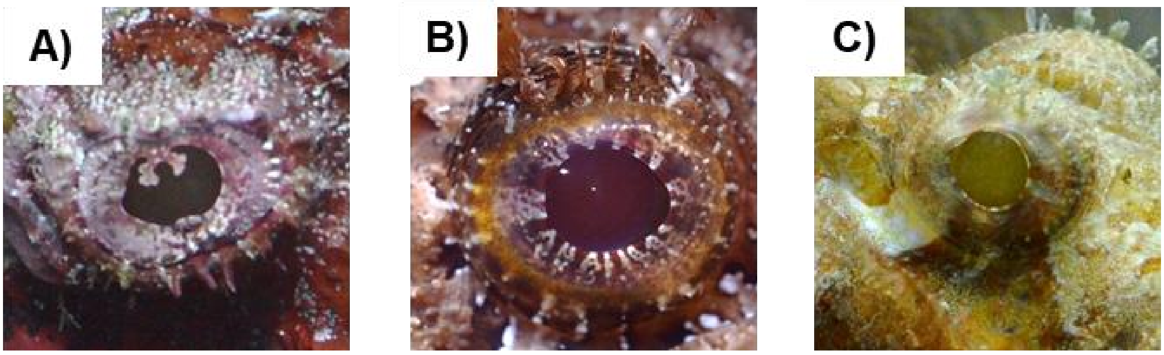


Figure 5: Eye camouflage in scorpionfishes. A) The round pupil shape is altered by a part of the iris extending onto the pupil (*Scorpaenopsis* cf. *diabolus*). B) Dark and light alternating markings on the iris disrupt the sharp-edged, round outline of the pupil (*Scorpaena porcus*). C) Eyeshine alters the colour of the pupil, which as a result is similar to the surrounding tissue (*Scorpaenopsis* cf. *oxycephalus*). Photos taken by Leonie John.

Masquerade

A type of camouflage that allows detection but prevents recognition of the fish is masquerade (Stevens and Merilaita 2009a). Here, the fish is perceived as an uninteresting object, for example a rock or algae. To date, it has been challenging to discern the function of a phenotype as masquerade. Only few studies can show direct evidence for animals to be camouflaged via masquerade, as the distinction between detection and recognition of an object can be difficult (Skelhorn et al. 2010; Skelhorn

2015). However, there are many animals that evidently look like regular objects in their environment (Skelhorn 2015). Among them are some scorpionfishes which show phenotypes that closely resemble algae or leaves (Figure 4 and 6A, B). Notably, most scorpionfishes seem to have some degree of masquerade, as they can usually be detected and still be misclassified as rocks and part of the substrate (Figure 6C, D). It is likely that in scorpionfishes, cryptic strategies such as background matching and disruptive colouration go hand-in-hand with masquerade as a second level strategy to prevent recognition when crypsis fails. While the success of background matching and disruptive colouration can rely on a specific colouration or pattern of the background, masquerade could allow a less background-dependent camouflage. Certainly, masquerade should be restricted to backgrounds on which the mimicked object can be found, but in the case of scorpionfishes, the overgrown rocks that they resemble can be found in almost every marine benthic microhabitat. Masquerade might have an increasing importance with increasing size of an animal, as for larger animals, it should be more difficult to remain undetected (Cuthill 2019). Therefore, masquerade might benefit scorpionfishes, as some species grow up to 50 cm (Eschmeyer and Dempster 1990). However, the effect of body size on camouflage and the potentially differential use of camouflage strategies related to size remains largely unexplored (Cuthill 2019; Barnett et al. 2023).

An interesting implication when thinking about masquerade employed by predators is that it could well function as a luring mechanism. In the specific case of scorpionfishes, it can be speculated that their appearance as a rock or something similar, potentially placed on a substrate that otherwise lacks three-dimensional structures (as seen e.g. in Figure 6C, D), might be attracting small animals seeking shelter (Skelhorn 2015). This could increase the fish’s prey capture success.

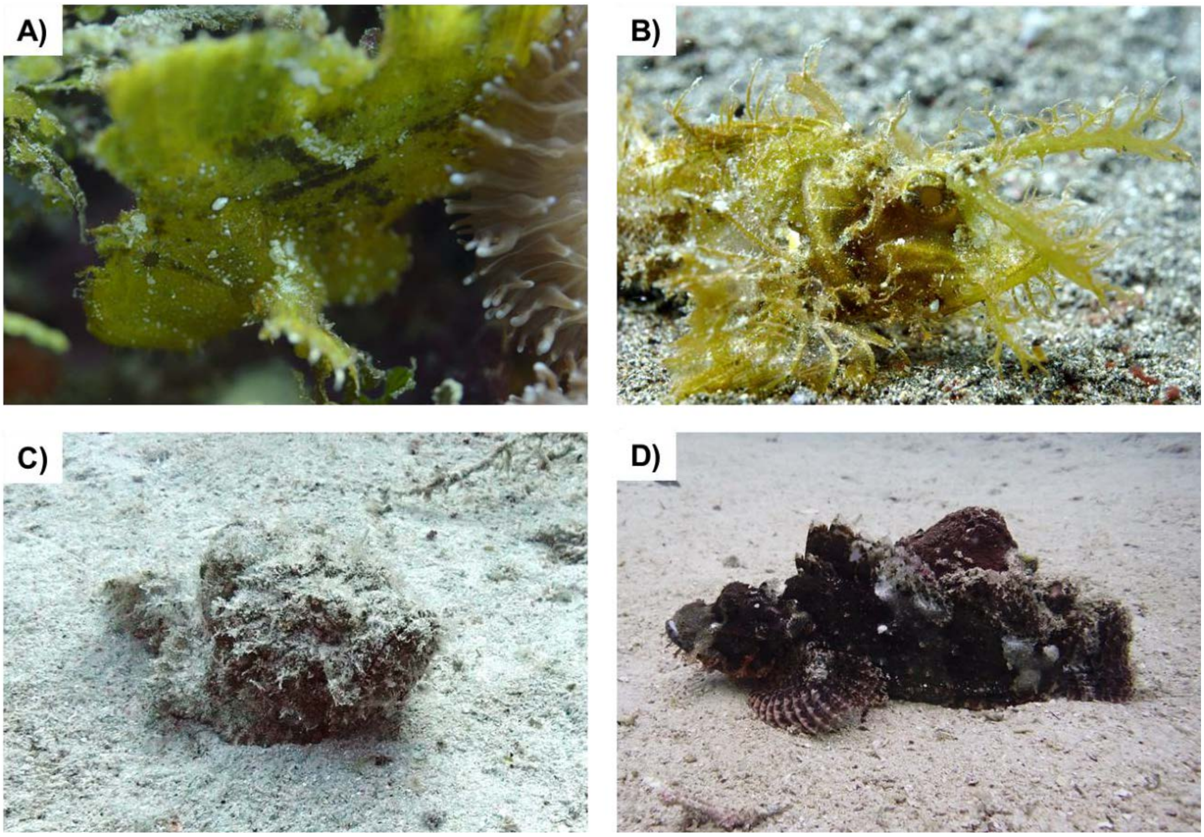


Figure 6: Examples of masquerade in A) Taenianotus triacanthus, B) Pteroidichthys amboinensis, C) Scorpaenopsis diabolus and D) Scorpaenopsis cf. oxycephalus. A, B) Both fishes show a phenotype that resembles algae or leaf-like structures. C, D) Both fishes are easily detected on the plain sand, but difficult to recognise as a fish. The fish in D) has a strong contrast to the background, but resembles a rock directly behind it. Photos taken by A, C) Leonie John, B) Matteo Santon, and D) Robin Kraft.

Behaviour

Behavioural adaptations that improve camouflage can provide flexibility to adjust to different backgrounds (Stevens and Ruxton 2019). For instance, fish might employ behavioural preferences on which background they settle, or how they orient their body. Background modification and partial burying or covering with substrate can be part of behavioural camouflage. While many scorpionfishes are partially covered with substrate and overgrown with epiphytes (Figure 6C), this is unlikely behavioural camouflage but rather a (probably beneficial) by-product of their motionless lifestyle and possibly promoted by their skin mucus chemistry (Millstein 1998). When a fish masquerades as an object that shows a particular movement, mimicking this movement can be part of behavioural camouflage as well. A characteristic swaying movement that resembles the movement of a dead leaf drifting in the water can be observed in the leaf scorpionfish *Taenianotus triacanthus* (personal observation). However, movement is usually considered as a potential disruption of camouflage, as it can increase detectability of an object or organism (Ioannou and Krause 2009; Zylinski et al. 2009; Hall et al. 2013, 2017). Motionlessness is therefore a crucial part of scorpionfish camouflage. This is reflected in their ‘sit-and-wait’ predation strategy, where motion is restricted to the rapid attack of prey that is caught via suction feeding, and occasional relocation. Often, the only movement that can be detected from above when observing a scorpionfish sitting in ambush is the movement of their gill covers while breathing. Interestingly, while exhaling, water is directed out from the gills dorsocaudally (personal observation, Figure 7). A strategy where water is exclusively released dorsocaudally and not laterally potentially improves the scorpionfish’s motionless appearance, as even the water flow created while breathing would be redirected in such a way that prey approaching from the side cannot sense it and potentially see less movement of the gill openings from their position. It was shown that the Black Scorpionfish *Scorpaena porcus* can suppress cardiac and respiratory activity for up to 50 seconds after exposure to a sound stimulus (Kolesnikova et al. 2021), potentially a mechanism adding to extreme motionlessness.

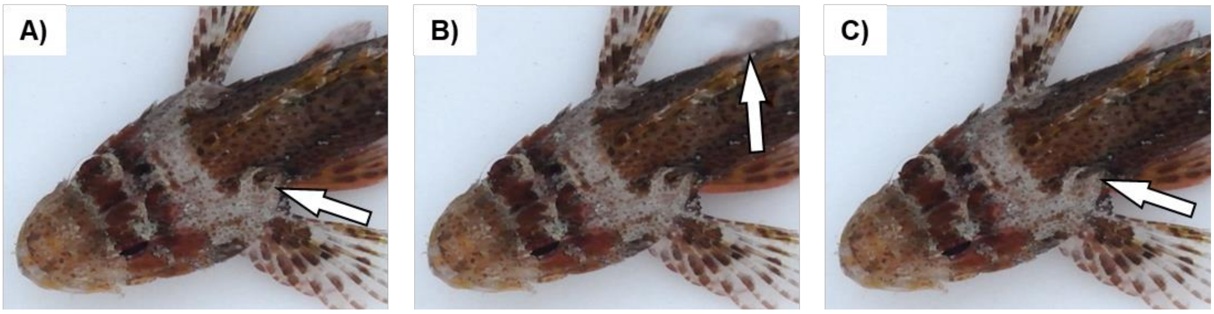


Figure 7: Sequential photos (within one second) of a scorpionfish (Scorpaena maderensis) breathing, in top view. The white arrows (A, C) indicate the opening though which water is directed out from the gills. A) The opening is largest while gills are opened, B) water is released and directed upwards during exhaling, seen as a small vortex (white arrow) at the surface 5 cm above the fish, and C) the opening is closed when the fish inhales through the slightly opened mouth. Photos extracted from video taken by Leonie John.

Camouflage in heterogeneous environments

Typically, a given phenotype will allow camouflage on a specific type of background and be less effective on others (Merilaita et al. 1999). Therefore, background diversity in colour, pattern and texture can be a challenge for camouflaged animals when navigating their natural environment. To maintain camouflage in such a heterogeneous environment with various microhabitats, different strategies might be employed, of which three are well established in camouflage research (Hughes et al. 2019). The first strategy is that animals stay restricted to a specific background or a limited range of backgrounds on which they move, meaning that they need to actively choose the most suitable background (Stevens and Ruxton 2019). Flatfish and rockpool gobies were shown to preferentially settle on backgrounds that match their own body colouration or pattern (Tyrie et al. 2015; Smithers et al. 2018). Such a preference can promote camouflage via background matching, disruptive colouration, or more (Stevens and Ruxton 2019). Another potential strategy is to prefer backgrounds of high complexity. Visual complexity can depend on variation in colour, shape and edge orientation (Dimitrova and Merilaita 2010; Rowe et al. 2021). Background variation in colour can be beneficial for animals with a disruptive body colouration, because it increases the chance of differential blending. In general, a more complex background can enhance disruption (Cuthill et al. 2005; Phillips et al. 2017; Price et al. 2019). Moreover, the more complex a background, the more visual information needs to be processed by an observer, which can increase the detection time of a target on the background (Merilaita 2003; Dimitrova and Merilaita 2010, 2012; Xiao and Cuthill 2016; Nokelainen et al. 2019). This strategy can even compensate for poor camouflage (Rowe et al. 2021). A study on the least killifish shows that complexity of the background can be an important cue for fish to choose a background, however choice also depends on predation risk and sex (Kjernsmo and Merilaita 2012). Regardless of what visual cues are driving background choice, there needs to be some kind of active and continuous assessment of available backgrounds while moving, making background choice for camouflage a dynamic strategy. Moreover, preferred backgrounds need to be frequent enough, which highlights how a background preference can limit the animal’s ability to use new microhabitats for foraging or other purposes, and the ability to deal with possible environmental changes (Caro et al. 2016).

Second, animals can dynamically change their appearance by changing their body colour and pattern to adjust to different substrates (Duarte et al. 2017). Here, colour change can be mediated by different morphological or physiological mechanisms, which also define how fast the changes happen. Slow (morphological) colour change, over several hours up to months, underlies the formation and loss of pigment cells in the skin, the chromatophores, or the change in amount of pigments they hold (Nilsson Sköld et al. 2016). Rapid (physiological) colour change on the other hand happens within minutes or even a few seconds and is regulated by the movement of pigment organelles within chromatophores (Nilsson Sköld et al. 2016). Rapid colour change for camouflage was shown in several terrestrial animals but also some fish species such as flatfishes (reviewed in Wuthrich *et al.*, 2022). Different flatfish species were shown to change average body colouration in response to background colouration, where the tropical flounder *Bothus ocellatus* only takes 2-8 seconds to achieve this change (Ramachandran et al. 1996). Moreover, flatfish colour change can include changes of body pattern in response to background pattern, especially background granularity (Ramachandran et al. 1996; Kelman et al. 2006; Tyrie et al. 2015; Akkaynak et al. 2017). This pattern change can involve the variable combination of up to six pattern components (Ramachandran et al. 1996). Other fishes like the Nassau grouper, the slender filefish and the rockpool goby can rapidly switch between two to three body patterns (Watson et al. 2014; Allen et al. 2015; Smithers et al. 2017). There is some limited knowledge on colour change in the order Scorpaeniformes. The northern sand flathead and the Coastrange sculpin have been shown to change colour, while the studies were limited to longer durations and therefore did not quantify the rapid colour change abilities of these fishes (Douglas and Lanzing 1981; Whiteley et al. 2009), although anecdotally at least in the flathead, the observed change seems to be rapid (Douglas and Lanzing 1981).

Third, animals can have a generalist phenotype that allows camouflage on multiple substrates (Nokelainen et al. 2019; Briolat et al. 2021). A fixed generalist phenotype could therefore compensate the need to dynamically adjust to backgrounds or choose backgrounds. A generalist body colouration typically offers reasonable camouflage on most substrates in the natural environment but comes with the cost that on few or no substrates, the animal will be camouflaged perfectly (Hughes et al. 2019). Whether a generalist or a specialist camouflage strategy is more beneficial depends strongly on background composition, background availability, and predation risk or prey availability associated to the backgrounds (Merilaita et al. 1999). For instance, in a heterogeneous environment, the efficiency of a generalist strategy should have an inverse relationship to the degree of variation between the backgrounds. While there seem to be no studies that quantify a generalist body colouration in fishes, many fishes likely show this adaptation for camouflage.

In contrast to the two previous strategies, the generalist body colouration does not require dynamic assessment and response to different backgrounds. Instead, it allows animals to move freely in their environment. However, in nature, even a generalist colouration will not be matching all backgrounds that can be present in the natural environment. Therefore, a generalist body colouration alone might not be sufficient for animals to camouflage and should come with at least some degree of background choice or possible adjustment to different backgrounds. It is therefore likely that several strategies are interacting and employed at the same time (Caro et al. 2016), or can be employed depending on background properties and frequency, and ecological or social context (Kjernsmo and Merilaita 2012; Encel and Ward 2021). However, most studies on this topic test single strategies for a study organism and the interplay and relative importance of the strategies under certain conditions is therefore poorly understood (but see Magellan & Swartz, 2013; Smithers *et al.*, 2017, 2018). When considering literature and observations of scorpionfishes, it becomes evident that these fishes likely show several of the three introduced strategies. My research explores these strategies in the two scorpionfish species *Scorpaena maderensis* and *Scorpaena porcus*.

Model species

The Madeira rockfish *Scorpaena maderensis* and the black scorpionfish *Scorpaena porcus* (Figure 8) are species within the Scorpaenidae that, to the human eye, are well camouflaged in their natural environment. Both species are ambush predators that sit motionlessly between rocks or algae on natural hard substrates and occur mainly above 30-40 m (Louisy 2002; Neumann and Paulus 2005). They are generalists that feed on a variety of small fishes and invertebrates. Both species can be found along the North Eastern Atlantic coast. While *S. porcus* is common throughout the Mediterranean Sea (Whitehead et al. 1984), *S. maderensis* is common along the northwestern African coast and only in specific areas in the Mediterranean, predominantly the southern Mediterranean (La Mesa et al. 2005). The visual system of *S. porcus* is characterized by single cones with average sensitivity peaking at 455 nm and double cones with average sensitivity peaking at 530 nm (Govardovskii and Zueva 1988; Schweikert et al. 2018), and unknown visual acuity. Visual system properties of *S. maderensis* are unknown, but closely related species with a similar ecology often share similar properties (Caves et al. 2018; Schweikert et al. 2018).

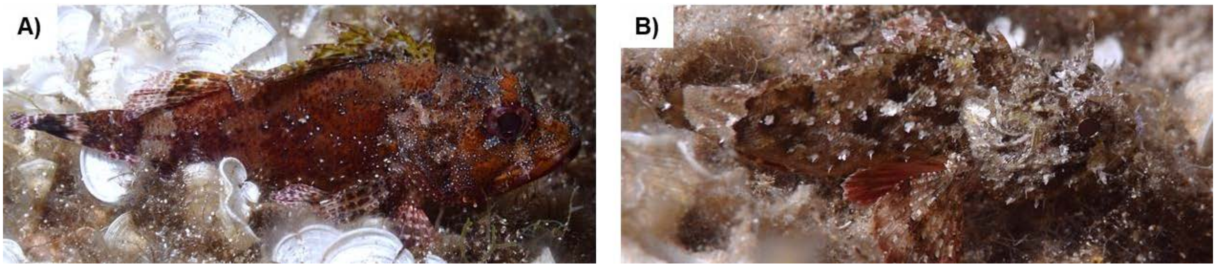


Figure 8: A) Scorpaena maderensis and B) Scorpaena porcus photographed in their natural environment. Photos taken by Leonie John.

Two prey fishes that occur in the geographic range of these two scorpionfish species are the black-faced triplefin *Tripterygion delaisi* and the two-spotted goby *Pomatoschistus flavescens*. *T. delaisi* is a small cryptobenthic fish that can be found on hard substrates. Its visual system is characterized by single cones with average peak sensitivity at 468 nm, and double cones with average sensitivity peaking at 517 and 530 nm (Bitton et al. 2017), and a visual acuity of 7 cycles per degree (Fritsch et al. 2017a). *P. flavescens* is a benthopelagic species and has single and double cones with peak sensitivity at 456, 531 and 553 nm (Utne-Palm and Bowmaker 2006). Visual acuity for gobies can be estimated with 2.36 cycles per degree (Pierotti et al. 2020). The two observers probably differ in their ability to distinguish chromatic signals in the long wavelengths, as *P. flavescens'* long-wavelength cone sensitivity is peaking at a longer wavelength compared to *T. delaisi*.

Objectives

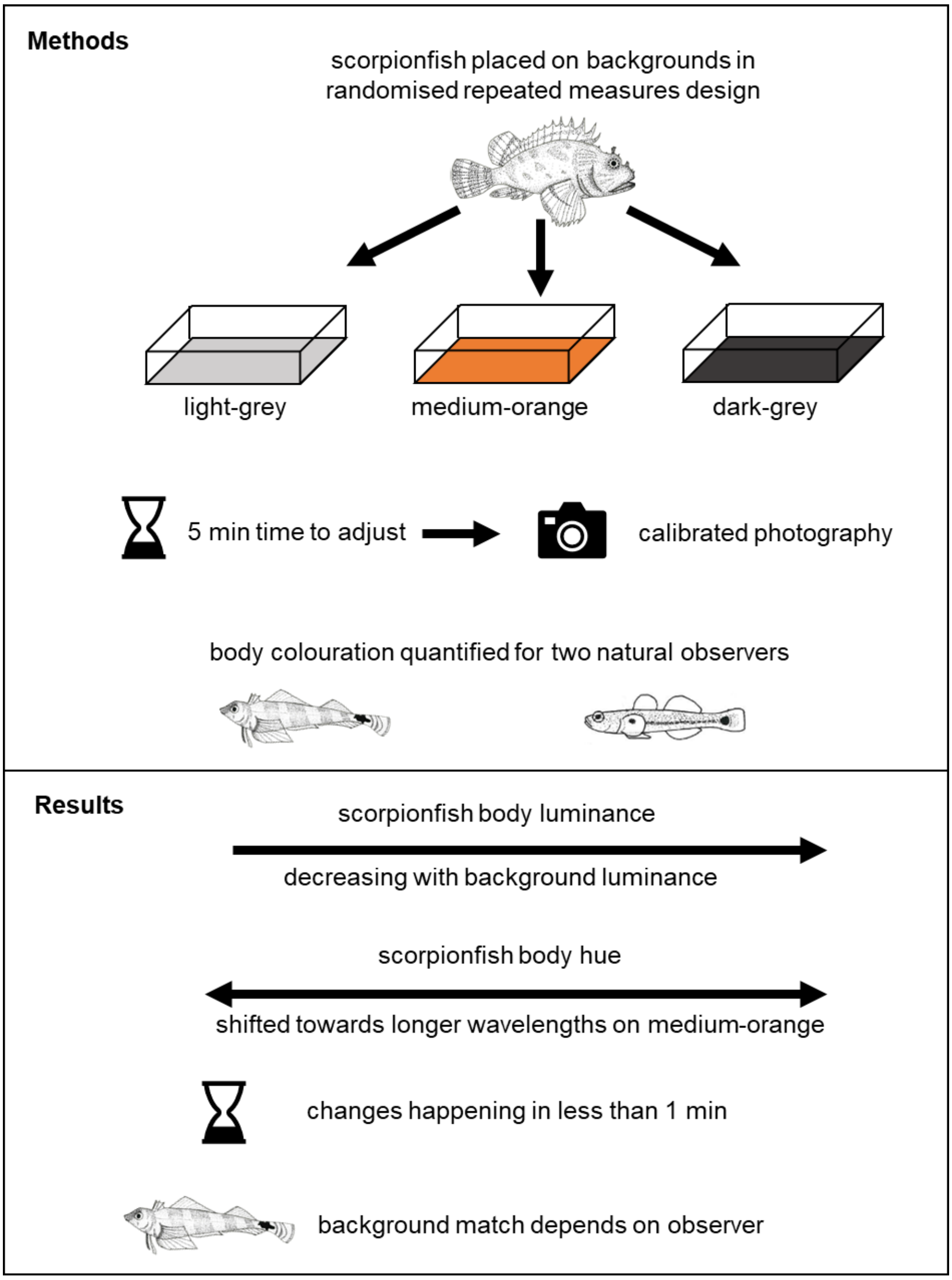
My research aims to empirically test potential camouflage strategies in scorpionfish, specifically in the two species *Scorpaena maderensis* and *Scorpaena porcus*. Here, I focussed on the question how scorpionfish can camouflage in a heterogeneous environment. By quantifying the physiological response of the scorpionfish on different backgrounds, I tested whether scorpionfish can dynamically change their body colouration (chapter 1), including body pattern (chapter 2). In a behavioural experiment, I tested whether scorpionfish show active background choice that facilitates camouflage (chapter 3). Additionally, I quantified how well scorpionfish body colouration matches their natural substrates to understand whether they have a generalist body colouration (chapter 3). Whenever assessing colouration or camouflage of scorpionfish, I used visual modelling to account for prey fish vision. The objective of these studies was to allow a first assessment of camouflage strategies we can find evidence for in these fishes. This work can thus hopefully provide a basis for further camouflage research on scorpionfish, including the assessment of established and the finding of novel camouflage strategies. This is important for two main reasons: camouflage research in predators is rare compared to research in prey, and scorpionfish represent an excellent group to study camouflage in predators. Moreover, scorpionfish ecology and behaviour are understudied to date, even though these fishes are an important part of marine benthic communities.

Chapter 1 – Scorpionfish rapidly change colour in response to their background

Related publication

John, L., Santon, M., & Michiels, N. K. (2023). Scorpionfish rapidly change colour in response to their background. *Frontiers in Zoology*, *20*, 10.

Visual summary



Summary

Here, we aimed to understand whether the two scorpionfishes *Scorpaena maderensis* and *S. porcus* change their body colouration to adjust to different backgrounds. In a repeated measures design, we placed scorpionfish on three artificial backgrounds and quantified changes in their body luminance and hue as perceived by two potential prey fishes, the triplefin *Tripterygion delaisi* and the goby *Pomatoschistus flavescens*. Since both scorpionfish species are also red fluorescent, we additionally quantified their display of red fluorescence on the different backgrounds. The three artificial backgrounds all differed in perceived luminance, where the darkest and lightest backgrounds were grey, but the intermediate background was orange. We proposed that scorpionfish would change their body colouration and become darker or lighter corresponding to luminance of the background, and shift their hue towards longer wavelengths on the orange background compared to the grey backgrounds, as this would facilitate background matching. We proposed that the display of red fluorescence should be linked to hue, meaning more red fluorescence should be displayed on the medium/orange background. Scorpionfish were placed on all three backgrounds in a randomised repeated measures design. We took standardised images with a calibrated camera in top view after one and five minutes that scorpionfish were placed on a background to document changes in body colouration. These images were then processed using the MICA toolbox (Troscianko and Stevens 2015; van den Berg et al. 2020) to extract scorpionfish luminance and hue as perceived by their prey. We measured display of red fluorescence from additional fluorescence photos that were taken after five minutes. We then analysed statistically whether these variables differed depending on what background scorpionfish were placed on. Moreover, we calculated achromatic and chromatic contrast of scorpionfish body colouration to their background to assess background matching. Because scorpionfish changed quicker than initially expected, we additionally measured luminance change at a higher temporal resolution in a second experiment.

Results and discussion

For both scorpionfish species, body luminance and hue as perceived by their prey differed depending on the background (Figure 9). Luminance was adjusted on all three backgrounds. About 50 % of the total luminance change observed after one minute was achieved very rapidly, in five to ten seconds. Hue was shifted towards longer wavelengths on the medium/orange background, compared to both grey backgrounds. These results indicate that scorpionfish adjusted their body colouration to improve their camouflage and reduce contrast to the background. Looking at achromatic and chromatic contrast of scorpionfish to the backgrounds, however, revealed a poor background match in all cases. This shows how the exposure to artificial backgrounds can induce a physiological response while it probably does not allow the camouflage effect that scorpionfish would achieve in nature. Especially the high achromatic contrast to the dark/grey and light/grey backgrounds suggests that in their natural environment, scorpionfish should avoid such backgrounds when pursuing optimal camouflage (Stevens and Ruxton 2019), or show a camouflage strategy other than background matching (Cuthill et al. 2005; Stevens and Merilaita 2009b). We explored this idea further in chapter 3. Moreover, changes in luminance were stronger than changes in hue (as indicated by a higher effect size for luminance change). This finding suggests that scorpionfish are either better at dynamically changing luminance than (the tested) hue or that achromatic cues are more important than chromatic cues while adjusting to backgrounds.

Chromatic contrast differed considerably depending on which prey fish vision was modelled. While colour of scorpionfish and background were similar from triplefin visual perspective, they were clearly distinguishable from goby visual perspective. These results illustrate the need to consider the visual system properties of naturally relevant observers and show that camouflage will oftentimes not work equally well for all observers (Fennell et al. 2019).

While scorpionfish hue was more long-wavelength-shifted on the medium/orange background compared to the grey backgrounds, their luminance and their red fluorescence display was highest on the light/grey background and intermediate on the medium/orange background. These patterns suggest that luminance and hue are regulated by two different types of chromatophores (Nilsson Sköld et al. 2013, 2016) and that red fluorescence display is related to scorpionfish luminance (Wucherer and Michiels 2014). This contradicts our hypothesis that red fluorescence is related to hue regulation of the fish, but does not exclude the possibility that red fluorescence could be important for the scorpionfish’s camouflage.

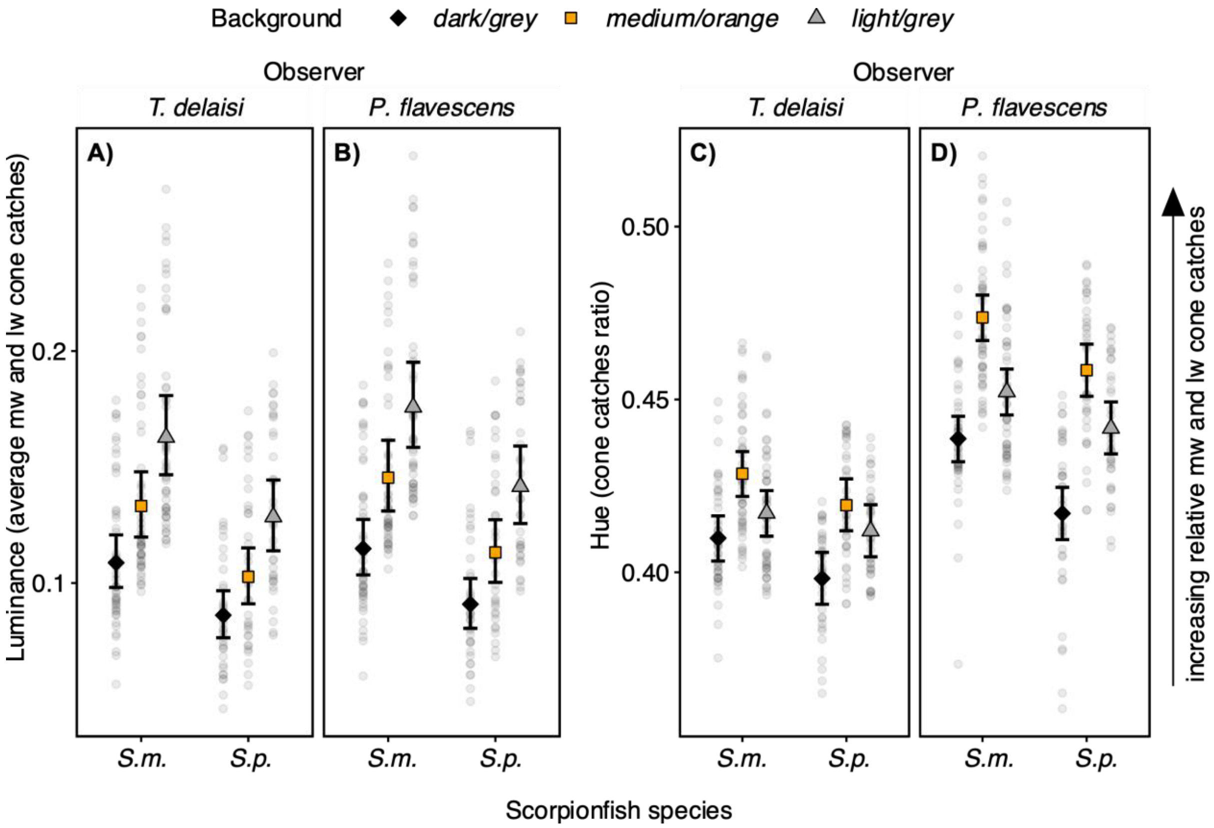


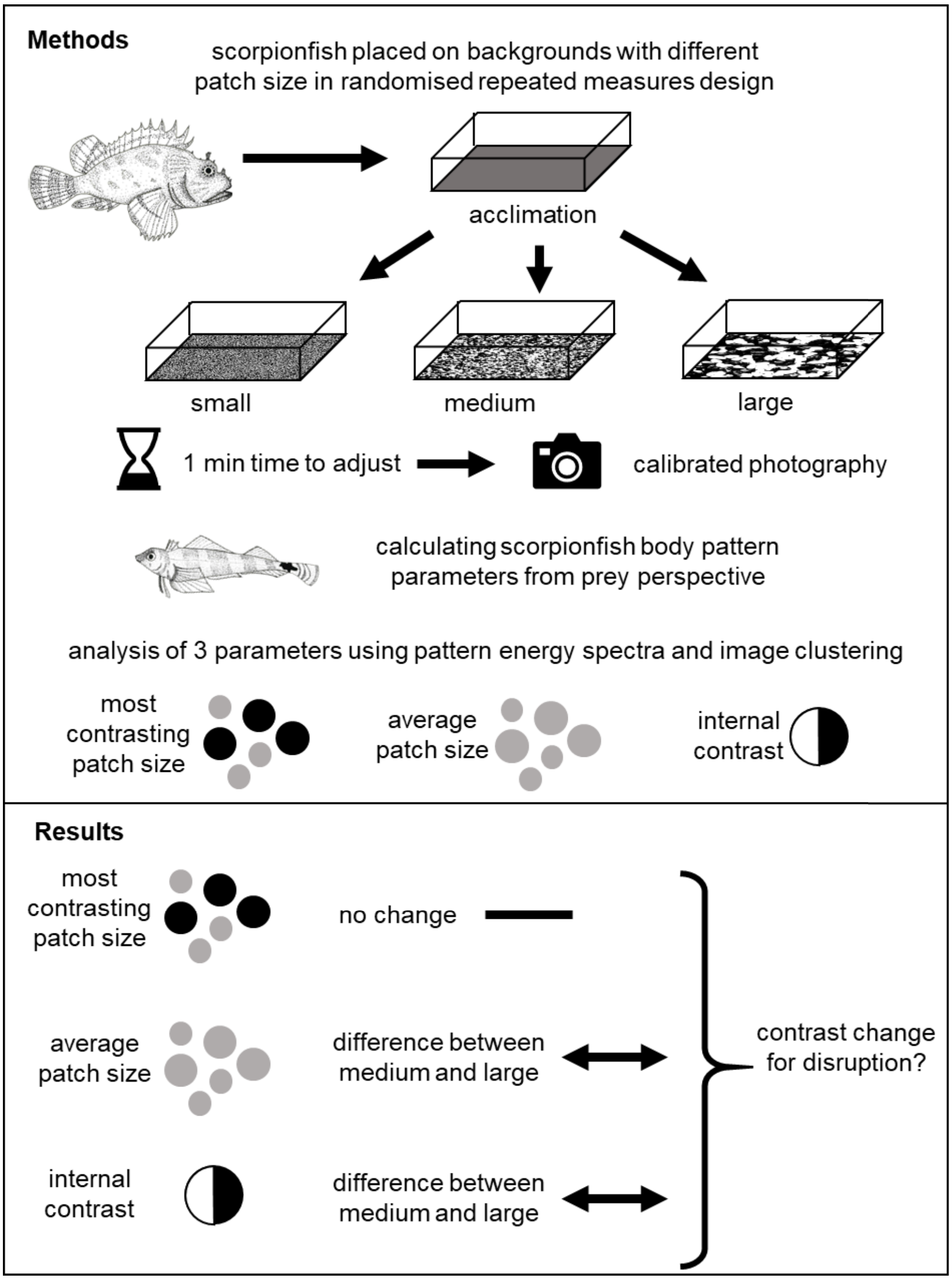
Figure 9: Scorpionfish change luminance and hue change across backgrounds. Scorpionfish luminance (A, B) and hue (C, D) from *Tripterygion delaisi* (A, C) and *Pomatoschistus flavescens* (B, D) visual perspective. *S. m.* = *Scorpaena maderensis*, *S. p.* = *S. porcus*. Each point represents a measurement for each individual scorpionfish (N = 24 *S. maderensis*, N = 18 *S. porcus*) averaged over the two time points (after one and five minutes adaptation time to the backgrounds). Markers with vertical bars represent predicted mean and 95% compatibility intervals (CIs) based on generalised linear mixed models (see John et al. 2023, Appendix A for details). The strength of the difference between two groups increases with decreasing degree of overlap of their 95% CIs.

Chapter 2 - Scorpionfish adjust skin pattern contrast on different backgrounds

Related manuscript

John, L., Santon, M., & Michiels, N. K. (2024). Scorpionfish adjust skin pattern contrast on different backgrounds. *Ecology and Evolution*, 14:e11124.

Visual summary



Summary

Here, we tested whether the two scorpionfishes *Scorpaena maderensis* and *S. porcus* change their body pattern in response to background pattern. After an acclimation on a uniform grey background, in a repeated measures design, we placed scorpionfish on three experimental black-and-white backgrounds that differed in their pattern patch size. All backgrounds had the same average luminance and contrast as perceived by the scorpionfish. The background with intermediate patch size was designed to have average patch size of scorpionfish pattern as measured in a previous study (John et al. 2023). We predicted that scorpionfish would decrease their own patch size on the background with smaller patch size and increase their own patch size on the background with larger patch size. Comparing scorpionfish body colouration between the acclimation and the black-and-white backgrounds, we predicted that fish would increase their internal pattern contrast. This could mediate disruptive colouration (Stevens and Merilaita 2009b), a beneficial strategy on complex backgrounds (Price et al. 2019) such as our high contrasting experimental backgrounds. We took standardised images with a calibrated camera in top view after one minute that scorpionfish were placed on a background to document changes in body pattern. These images were then processed using the MICA toolbox (Troscianko and Stevens 2015; van den Berg et al. 2020) to extract scorpionfish pattern as perceived by their prey fish *Tripterygion delaisi*. To analyse scorpionfish pattern, we used two approaches: the pattern energy analysis (Stoddard and Stevens 2010) and the Quantitative Colour Pattern Analysis (QCPA) (van den Berg et al. 2020). These allowed us to extract the dominant (i.e. most contrasting) patch size, the average patch size and internal pattern contrast as three parameters describing scorpionfish pattern. We then analysed statistically whether these parameters differed depending on which experimental background scorpionfish were placed on, and whether internal pattern contrast differed between the acclimation and the first experimental background.

Results and discussion

Scorpionfish did not change size of their most contrasting pattern components (Figure 10, shape of curve does not differ between backgrounds) but showed changes in average patch size, mainly between the medium and large patch size backgrounds. Moreover, their internal pattern contrast was increased on the background with medium compared to large patch size. An increase of internal pattern contrast was

also evident when comparing the acclimation to the first experimental background that an individual was placed on, regardless of patch size of that background (Figure 10, lower mean pattern energy on acclimation for all pattern size bins).

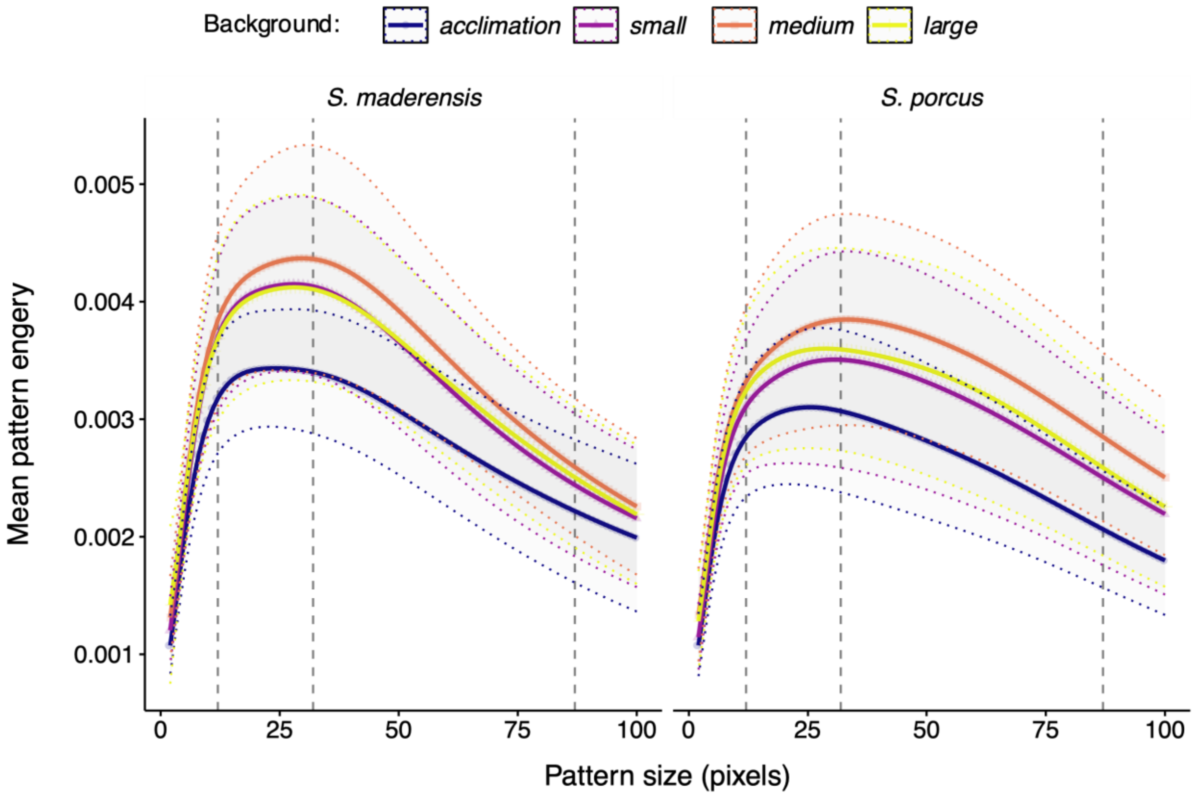


Figure 10: Scorpionfish pattern on different backgrounds. Pattern energy spectra (pattern energy for each patch size) of *Scorpaena maderensis* and *S. porcus* body pattern on the acclimation (uniform) and the three experimental backgrounds. Dashed vertical lines indicate the most contrasting patch size of the experimental background pattern (small: x = 12, medium: x = 32, large: x = 87). The solid lines indicate mean pattern energy over all individuals with standard deviation outlined as the dotted lines. Pattern energy is defined as the standard deviation of the luminance channel's cone catches of the filtered pixels and gives an indication of contrast. The x-axis describes pattern size in pixels and refers to pattern size bins that were used to filter the images. The shape of the curve shows which patch size is most contrasting (peak of curve), the area under the curve indicates how contrasting the fish are overall.

Concerning the most contrasting patch size, scorpionfish seem to have a fixed pattern. Therefore, they do not seem to follow the strategy of pattern matching to adjust to differently patterned backgrounds. Instead, the upregulation of internal contrast on the experimental backgrounds suggests that scorpionfish rather employ colour change to increase disruptive colouration on highly contrasting and complex backgrounds. Average patch size and internal contrast are closely related because an increase in contrast can create new boundaries within the fish pattern and thereby define new, smaller patches. It is therefore likely that the changes observed in average patch size are a by-product of contrast adjustment. We propose that this is the case rather than vice versa because average patch size probably contributes less to camouflage performance, as pattern is mainly defined by the most contrasting and thus most salient patch size (Stoddard and Stevens 2010). Instead, contrast regulation contributes to camouflage by mediating disruptive colouration (Stevens and Merilaita 2009b) and intensifying most contrasting patch size, and is therefore likely regulated as a response to the backgrounds.

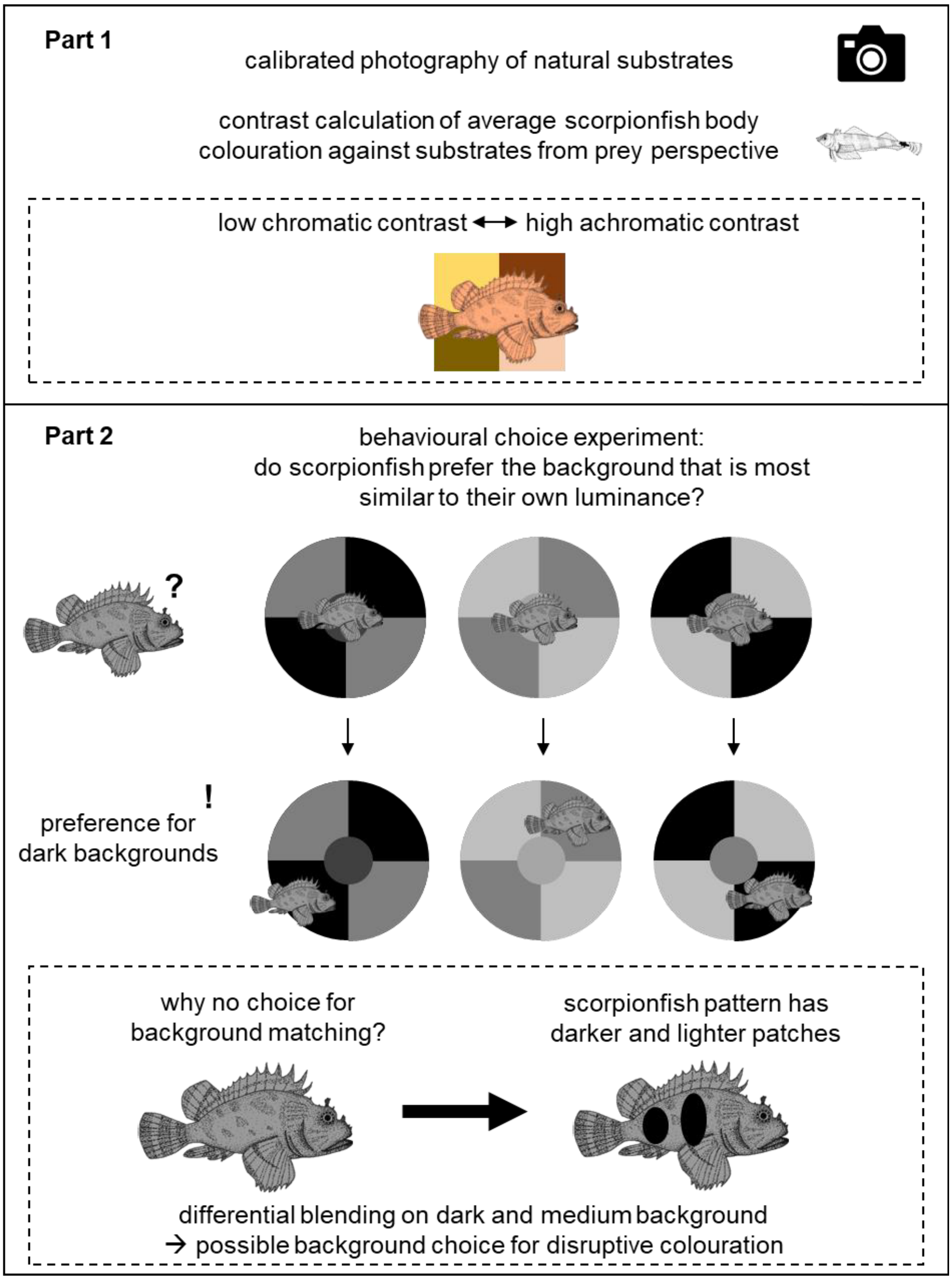
An additional observation in this dataset was that while average values of pattern patch size between species were similar, the variation in the raw data differed. Higher interindividual variation in patch size in *S. porcus* compared to *S. maderensis* indicates higher phenotypic variation in this species and suggests the possibility of differential use of camouflage strategies between species. However, such effects could also stem from differential genetic variation between the species or the local populations. In general, scorpionfish pattern is heterogeneous, meaning fish display not only patches of a given size but of different size over their whole body (Figure 10, spectra have a plateau from ~20 to 40 pixels). This could function as a generalist body pattern that aids camouflage on multiple backgrounds, reducing the need to modulate body pattern (Merilaita et al. 1999; Briolat et al. 2021), a strategy known from animals found on highly complex and heterogeneous backgrounds (Hughes et al. 2019; Nokelainen et al. 2019).

Chapter 3 - Generalist camouflage and background choice in scorpionfish

Related manuscript

John, L., Santon, M., & Michiels, N. K. Generalist camouflage and background choice in scorpionfish. In preparation.

Visual summary



calibrated photography of natural substrates

contrast calculation of average scorpionfish body colouration against substrates from prey perspective

low chromatic contrast ↔ high achromatic contrast

behavioural choice experiment: do scorpionfish prefer the background that is most similar to their own luminance?

preference for dark backgrounds

why no choice for background matching?

scorpionfish pattern has darker and lighter patches

differential blending on dark and medium background
→ possible background choice for disruptive colouration

Summary

Here, we aimed to understand whether the two scorpionfishes *Scorpaena maderensis* and *S. porcus* have a generalist body colouration on their natural backgrounds and whether they show an active background choice to facilitate camouflage. We took standardised photographs of natural substrates in the scorpionfish's habitat and calculated chromatic and achromatic contrast of average scorpionfish body colouration (data generated in chapter 2) against the substrates, from the visual perspective of the two prey fishes *Tripterygion delaisi* and *Pomatoschistus flavescens*. In a behavioural experiment, we tested scorpionfish preferences for backgrounds differing in perceived luminance. For this purpose, scorpionfish were placed in arenas with two different backgrounds that they could choose between, and were video-recorded for 10 minutes. We predicted that scorpionfish would prefer to settle on the background that would be most similar to their own average body colouration, a strategy to facilitate background matching (Stevens and Ruxton 2019). We also used calibrated photography to document scorpionfish body colouration after the experiment, to relate body colouration parameters to their choice. In the same experimental setup, we additionally tested scorpionfish preferences for backgrounds differing in their degree of complexity. We predicted that scorpionfish would prefer to settle on the more complex background to enhance camouflage (Kjernsmo and Merilaita 2012).

Results and discussion

Scorpionfish had low chromatic contrast on a range of natural substrates, but high achromatic contrast that would reveal them to their prey (Table 1). However, we calculated these contrasts with body colouration data of scorpionfish adjusted to a background of intermediate perceived luminance. Scorpionfish can become more dark or light (John et al. 2023) and it is likely that they would have adjusted to the natural substrates accordingly, possibly lowering achromatic contrast. The low chromatic contrast instead shows that even without adjustment, scorpionfish have a reasonable chromatic background match to natural substrates that could reduce their need to choose backgrounds based on chromatic cues.

Table 1: Scorpionfish have low chromatic contrast on natural substrates. Chromatic and achromatic contrast of average scorpionfish body colouration against different natural substrates in Just Noticeable Differences, as perceived by their prey fish *Tripterygion delaisi*. Contrasts are based on model estimates. Highlighted are values with compatibility intervals that include one JND (dark grey) and three JND (light grey), where one JND is the detection threshold under optimal viewing conditions and three JND is a more conservative detection threshold that can be assumed for natural conditions (Siddiqi et al. 2004). See John et al. in prep., Appendix C, Table 3 for extended table.

	Chromatic contrast		Achromatic contrast	
	S. maderensis	*S. porcus*	*S. maderensis*	*S. porcus*
Rubble and sand	1.09	0.93	10.42	9.07
Seagrass leaves	1.17	1.02	12.76	11.40
Turf algae	2.19	1.98	10.49	11.00
Red sponge	3.91	3.68	17.84	14.23
Yellow algae	3.85	3.62	9.44	6.85
Seagrass stems	7.03	6.78	17.75	14.54

Therefore, we decided to use only achromatic backgrounds in the background choice experiments. Against our prediction, scorpionfish did not show a preference for the background that was most similar to their own average body luminance. Instead, they preferred the darkest background, accepting a high achromatic contrast to the background as a consequence. One explanation for this behaviour could be that scorpionfish, like many other fishes (Bradner and McRobert 2001; Kjernsmo and Merilaita 2012; Smithers et al. 2018), have a preference for dark backgrounds, potentially because they indicate shelter and scorpionfish were attempting to hide when placed in the new environment. On the other hand, we need to consider that scorpionfish body colouration and its function for camouflage might be more complex. We offered scorpionfish a background that was close to their average body luminance, based on the assumption that scorpionfish would choose according to what background would offer the best background match. However, scorpionfish have a complex pattern with contrasting patches of different luminance. When calculating achromatic contrast to the preferred background using luminance of single patches within the body pattern instead of using average body luminance, we can see that the lowest contrast is found between dark patches and the dark background (Figure 11). Scorpionfish adjusted the luminance or their dark patches in relation to the chosen background, potentially improving differential blending and increasing internal pattern contrast (see John et al. in prep., Appendix C, Figure 4), both factors that enhance disruptive colouration (Stevens and Merilaita 2009b).

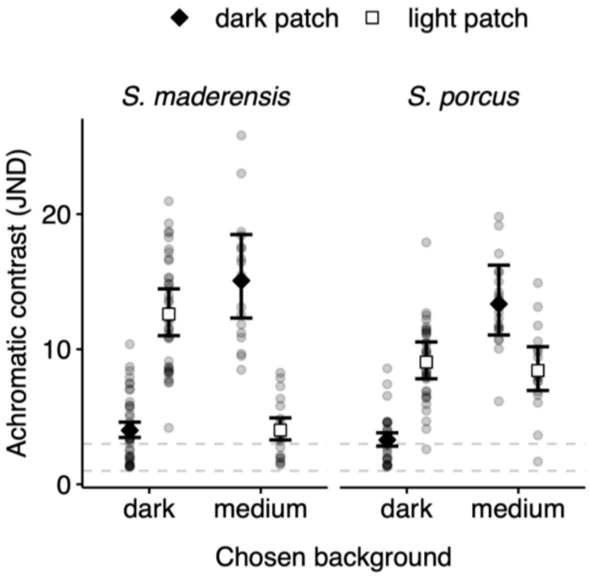


Figure 11: Distinct patches of scorpionfish body colouration match different backgrounds. Achromatic contrast as Just Noticeable Differences (JND) of two distinct patches of scorpionfish body colouration against their preferred background in the luminance experiment as perceived by *Tripterygion delaisi*. The dashed lines indicate one and three JND, where one JND is the detection threshold under optimal viewing conditions and three JND is a more conservative detection threshold that can be assumed for natural conditions (Siddiqi et al. 2004). Colour and shape of the markers indicate the body colouration patch. Grey points represent contrast per individual (n = 23 *Scorpaena maderensis*, n = 22 *S. porcus* tested in the luminance experiment in three choice treatments, cases where the light background was preferred were excluded here because of the low number of individuals that showed this preference). Markers with vertical bars represent predicted medians and 95% compatibility intervals (CIs) based on a generalised linear mixed model (see John et al. in prep., Appendix C for details). The strength of the difference between two groups increases with decreasing degree of overlap of their 95% CIs.

When confronted with backgrounds of varying degrees of complexity, fish chose randomly. The lack of a preference for a more complex background could either indicate that fish do not follow this camouflage strategy, or that they did not respond to our artificial backgrounds as we expected, potentially because we did not create complexity in a way that it is found in nature (Dimitrova and Merilaita 2010; Rowe et al. 2021).

Final remarks and limitations

The combined results from my studies on *Scorpaena maderensis* and *Scorpaena porcus* draw a conclusive picture of how these fishes camouflage in heterogeneous environments. By assessing body colouration and camouflage from the prey's visual perspective, I can show that the strategies employed might be beneficial for the scorpionfish to hide from their prey. Both species perform colour and pattern change, background choice, and have a generalist body colouration. This demonstrates how we need to consider that well camouflaged animals will usually employ multiple strategies to maintain camouflage in their oftentimes heterogeneous environments.

How is dynamic camouflage regulated?

There are indications that achromatic cues in the background are more strongly driving scorpionfish adjustment to the backgrounds than chromatic cues (John et al. 2023, John et al. in prep.). Moreover, changes in scorpionfish body luminance relate to changes in their internal pattern contrast, which emphasises the importance of luminance change to regulate camouflage strategies in these animals (John et al. 2024; John et al. in prep.). Achromatic cues are also driving background choice in the tested species (John et al. in prep.). Low variation in perceived colour of natural substrates but high variation in perceived luminance could explain the role of background luminance as a pivotal factor regulating dynamic camouflage strategies in scorpionfish. This idea is supported by the finding that scorpionfish seem to have low chromatic contrast on many natural backgrounds, potentially mitigating the need to assess chromatic cues to perform dynamic camouflage strategies (John et al. in prep.). However, teasing apart achromatic and chromatic vision is controversial (Caves et al. 2019). Research on different animals suggests that achromatic and chromatic vision are mediated by different pathways, as different cones are responsible for either (Olsson et al. 2018). Yet, there is evidence for the crossing and interaction of these two pathways, especially when we assess colour perception behaviourally (Caves et al. 2019). It is therefore critical to emphasise here that in their natural environment, scorpionfish likely will respond to an interaction of achromatic and chromatic cues. Notably, this argumentation is entirely based on the assumption that scorpionfish use only vision to assess their environment and respond accordingly.

However, recent evidence suggests that colour change in fishes could also be driven by light-sensitive skin cells (Kingston and Cronin 2016; Schweikert et al. 2023). Such dermal photoreceptors could offer an assessment of the background independently of vision (Mäthger et al. 2010; Kingston et al. 2015). The mechanism described by Schweikert et al. (2023) moreover indicates that dermal photoreceptors can respond to the state of chromatophores, which could be integrated as a feedback system to improve background matching. Such a feedback system could allow fine-tuned adjustment to backgrounds of different perceived luminance. A mechanism like this could explain the gradual luminance changes observed in scorpionfish (John et al. 2023). It is possible that both vision and dermal photoreception are involved in scorpionfish colour change. A contribution of dermal photoreception could help explain the importance of achromatic cues mediating change, as such a system would not provide colour discrimination (Schweikert et al. 2023).

Disruptive colouration

While understanding the importance of disruptive colouration for scorpionfish was not initially a focus of my research, I repeatedly found indications that this is one of their essential camouflage strategies. Maximum disruptive contrast and differential blending are the two core principles in disruptive colouration (Stevens and Merilaita 2009b). Colour change in the two species therefore directly influences disruptive colouration by altering internal pattern contrast (John et al. in prep.). Scorpionfish moreover preferred backgrounds that offered lowest contrast to individual patches within their pattern instead of their average colouration (John et al. in prep.). This indicates that differential blending and thus disruptive colouration is preferred over background matching under certain conditions, showing how scorpionfish can flexibly switch between camouflage strategies. These findings highlight the conclusion that the two scorpionfish species are generalists that can camouflage on a variety of backgrounds (Price et al. 2019).

Natural observers

Visual modelling is an essential part of understanding camouflage in the correct ecological context. While both scorpionfish species prey on small fishes and invertebrates, I only modelled prey fish vision. Many small marine invertebrates such as cleaner shrimp have monochromatic vision and lower visual acuity than the two prey fishes (Caves et al. 2016). However, my studies involved either understanding how chromatic vision impacts camouflage (chapter 1 and 3) or how pattern is perceived, which needs a certain level of visual acuity (chapter 2), and I therefore neglected monochromatic, low acuity vision. However, it is possible that such different visual system properties reveal new functions of scorpionfish colouration and could therefore be incorporated into future studies. Eventually, testing predation success would be necessary to fully understand the function of camouflage strategies in regard to the natural observers.

Comparing two species

All empirical tests were done with two closely related species of scorpionfish, which allowed understanding whether the observed strategies were species-specific. Finding differences between these species would be highly interesting because it could link to differences in their ecologies. The two species are found in the same habitat, but it is uncertain whether they show the same microhabitat use. Personal observations during the specimen collection in my studies allow a first suggestion that *S. maderensis* might have a more limited microhabitat use than *S. porcus*. This was however not reflected in differences in their camouflage, as the species' overall responses were similar and led to the same conclusions for both species. This confirms that colour and pattern change, as well as a preference to settle on dark backgrounds are camouflage strategies that did not evolve on species level. Indeed, it is likely that many more scorpionfish species show these strategies, as indicated by several personal observations of various scorpionfishes in the field.

Outlook

There are many points touched upon in my thesis that remain uncertain to date and that I want to highlight as possible future research directions.

Directly related to my study species, one camouflage strategy is of particular interest. The observation that one species might have a higher intraspecific diversity in body pattern than the other (John et al. 2024) should be followed up on in more detail. The concept of phenotypic variation for camouflage is known, but often studied only theoretically by using computer animations (Bond and Kamil 2002; Troscianko et al. 2021). Here, a negative frequency dependent selection of common phenotypes can be confirmed. However, studying this phenomenon in a natural population to understand its effect for camouflage needs to involve multiple populations or even species that have a different degree of phenotypic variation as a control group. *S. maderensis* and *S. porcus* potentially show such a difference but are known to otherwise employ similar camouflage strategies (John et al. 2023; John et al. 2024; John et al. in prep.). Therefore, they could be an interesting system to study phenotypic variation and its effect on predation success in nature. Moreover, finding differences between how closely related species camouflage can be important to understand how these differences relate to differences in ecology and microhabitat use. For instance, populations or species with higher phenotypic variation could have a broader range of prey because prey that can form a search image can better avoid individuals from populations or species with low phenotypic variation.

Aside from this specific example, scorpionfishes are generally a well-suited group to better understand how camouflage strategies evolved in relation to ecological factors. From observations alone, it seems as if there are camouflage strategies that are present in most scorpionfish species, but also strategies that single species display, or that seem to have evolved several times independently. An intriguing example of this would be the evolution of masquerade. Examples of specialized masquerade can be found in species of different genera within the scorpionfishes (e.g. *Taenianotus triacanthus* and *Pteroidichthys amboinensis*) (Smith et al. 2018). This could allow using phylogenetic studies in combination with assessment of ecology to understand what factors promote the evolution of such a specific camouflage strategy.

Such studies could also offer a better understanding of how species rely on specific ecological factors or microhabitats for their camouflage to work. In the light of current benthic habitat degradation in marine environments (Harris 2020), it is important to know what microhabitats are vital for certain species that rely on camouflage for survival. Moreover, comparing microhabitat use in relation to camouflage could reveal which species will be more or less affected by changing microhabitats, and how this might affect species composition. Changes in species composition in benthic communities can have extensive consequences for the whole ecosystem (Depczynski and Bellwood 2003; Bellwood et al. 2006; Brandl et al. 2019).

Scorpionfishes are a family of predatory fishes that are all well camouflaged and therefore would be an excellent group to study the relevance of their predatory lifestyle on the evolution of camouflage. While camouflage is a research topic traditionally focussing on camouflaged prey, it is evident that studying camouflaged predators can help understand how camouflage is related to lifestyle and specifically predation strategy (Pembury Smith and Ruxton 2020). For instance, the evolution of mechanisms to enhance complete motionlessness, for which we can find evidence in the scorpionfishes, would be especially beneficial for sit-and-wait predators.

Eventually, by empirically testing camouflage strategies of the two scorpionfish species *S. maderensis* and *S. porcus*, this work creates a basis for further research in this direction and contributes to a better understanding of the ecology of these fishes, an important part of the Mediterranean marine benthic community.

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Appendix

Appendix A

Related publication to chapter 1

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RESEARCH **Open Access**

Scorpionfish rapidly change colour in response to their background

Leonie John[1*], Matteo Santon[1,2] and Nico K. Michiels[1]

Abstract

Background To facilitate background matching in heterogenous environments, some animals rapidly change body colouration. Marine predatory fishes might use this ability to hide from predators and prey. Here, we focus on scorpionfishes (Scorpaenidae), well-camouflaged, bottom-dwelling sit-and-wait predators. We tested whether *Scorpaena maderensis* and *Scorpaena porcus* adjust body luminance and hue in response to three artificial backgrounds and thereby achieve background matching. Both scorpionfish species are also red fluorescent, which could contribute to background matching at depth. Therefore, we tested whether red fluorescence is also regulated in response to different backgrounds. The darkest and the lightest backgrounds were grey, while the third background was orange of intermediate luminance. Scorpionfish were placed on all three backgrounds in a randomised repeated measures design. We documented changes in scorpionfish luminance and hue with image analysis and calculated contrast to the backgrounds. Changes were quantified from the visual perspective of two potential prey fishes, the triplefin *Tripterygion delaisi* and the goby *Pomatoschistus flavescens*. Additionally, we measured changes in the area of scorpionfish red fluorescence. Because scorpionfish changed quicker than initially expected, we measured luminance change at a higher temporal resolution in a second experiment.

Results Both scorpionfish species rapidly adjusted luminance and hue in response to a change of background. From prey visual perspective, scorpionfishes' body achromatic and chromatic contrasts against the background were high, indicating imperfect background matching. Chromatic contrasts differed considerably between the two observer species, highlighting the importance of choosing natural observers with care when studying camouflage. Scorpionfish displayed larger areas of red fluorescence with increasing luminance of the background. With the second experiment, we showed that about 50% of the total luminance change observed after one minute is achieved very rapidly, in five to ten seconds.

Conclusion Both scorpionfish species change body luminance and hue in response to different backgrounds within seconds. While the achieved background matching was suboptimal for the artificial backgrounds, we propose that the observed changes were intended to reduce detectability, and are an essential strategy to camouflage in the natural environment.

Keywords Background matching, Calibrated image analysis, Camouflage, Colour change, Predator–prey interactions, Scorpionfish, Visual modelling, Biofluorescence

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Background

Background matching, where body colouration and pattern of an animal are similar to the background, is one of the most common strategies to hide from predators or prey [1, 2]. To match the background in a heterogenous environment, animals may have a fixed colour and pattern that performs sub-optimally with a wide range of backgrounds, actively choose matching substrates by relocating, or adjust their appearance in response to backgrounds by changing colour and pattern [3, 4]. Depending on the underlying mechanism, this colour change can happen rapidly, over seconds to a few minutes, or slowly, over hours or days or even months [3, 5, 6]. Rapid colour change is mediated by chromatophores containing pigment organelles that can be aggregated or dispersed within the cell [5]. Depending on the pigment, chromatophores can be divided into different types. While melanophores are the type that typically regulates luminance change, others allow changes in hue and/or saturation [5]. This physiological, rapid colour change for camouflage has been documented in reptiles [7, 8], and in marine animals such as cephalopods (e.g. [9, 10]). Only a few fish species have been studied in this context, for example flatfish [11, 12] and rock pool gobies [13–15]. Studies that empirically measure rapid colour change for camouflage from the visual perspective of natural observers are scarce [7, 13–15].

Red (long wavelength) fluorescence is a widespread component of body colouration in fishes, and is particularly common among gobies (*Bryaninops*, *Eviota*), triplefins (*Enneapterygius*, *Tripterygion*), dragonets (*Synchiropus*) and small wrasses (*Cirrhilabrus, Paracheilinus*), but also larger cryptic predatory fishes [16, 17]. With increasing depth in marine environments, longer wavelengths are absorbed faster than shorter wavelengths, resulting in a blue-green shifted light environment below ten meters [18]. Hence, red reflective objects appear dull grey at such depth, whereas red fluorescent structures can still show subtle grades of redness because they absorb short (blue-green) light and re-emit the energy at longer (red) wavelengths. Many marine substrates are red fluorescent, particularly when dominated by calcareous algae and other sedentary organisms such as corals [19]. For cryptic and benthic fishes, such as the scorpionfishes, it has therefore been suggested that red fluorescence contributes to background matching at depth as a subtle but possibly important colour component [16, 20].

The scorpionfishes (Scorpaenidae) are a family of benthic predators that rely on camouflage for hunting. As sit-and-wait predators, they remain motionless until prey comes close enough to be caught rapidly via suction feeding. Such ambush predators therefore face strong pressure to evolve particularly good camouflage [21]. Background matching can help to decrease detectability by prey [1] and could therefore increase foraging success. Colour change has the potential to allow for background matching on various substrates, generating a broader range of suitable microhabitats for hunting [21]. Given their wide distribution, high species diversity, benthic sit-and-wait predation tactic and diverse camouflage strategies, scorpionfish are an ideal system for experimental studies of fish camouflage. Yet, research on this topic is rare [22].

In this study, we explored colour change in scorpionfishes. We chose to test two species, *Scorpaena maderensis* and *Scorpaena porcus* (Fig. 1), to understand whether colour change would be species-specific. We tested whether (1) scorpionfish rapidly change their body luminance and hue when placed on different backgrounds, and (2) how well they match their background by doing so. Such results may depend on the visual system of the observer, which is highly variable in marine animals [23, 24]. We therefore assessed the objectives from the visual perspective of two prey fish species as ecologically relevant observers with differing spectral sensitivity, the triplefin *Tripterygion delaisi* and the goby *Pomatoschistus flavescens*. To test objective (1), we placed individual scorpionfish of the two species on three artificial backgrounds: (a) low luminance, achromatic *dark/grey*, (b) medium luminance, chromatic *medium/orange*, and (c) high luminance, achromatic *light/grey*. We expected both scorpionfish species to change luminance and show the

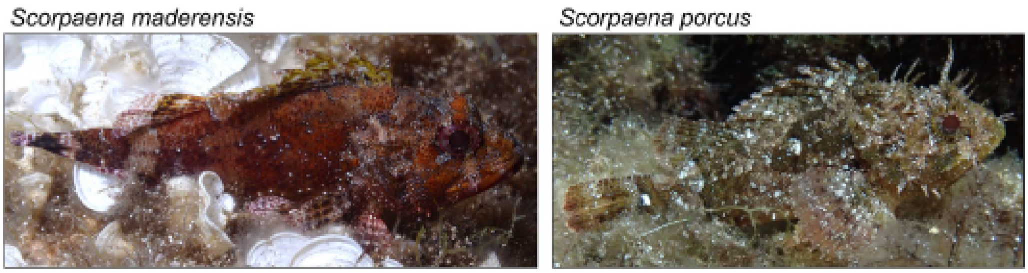

Fig. 1 *Scorpaena maderensis* (left) and *S. porcus* (right) in their natural environment. Photos by LJ

lowest luminance on the *dark/grey* background, medium luminance on the *medium/orange* background and highest luminance on the *light/grey* background. As for the hue, we expected scorpionfish to show a similar hue on the *dark/grey* and the *light/grey* backgrounds, but hue to be shifted to longer wavelengths on the *medium/orange* background. We quantified scorpionfish body luminance and hue based on cone catches for the two observers at one and five minutes after relocation to a new background. To test objective (2), we assessed the degree of background matching by calculating achromatic and chromatic contrast of scorpionfish body against the background from the visual perspective of the same two observers. We expected that scorpionfish display a similar luminance and hue to the background and therefore show a low contrast on all backgrounds. We expected both scorpionfish species to show a similar degree of background matching. We also tested whether (3) red fluorescence is part of the expected hue change mechanism. We therefore measured the total area of scorpionfish body showing red fluorescence when placed on the different backgrounds. We expected fish to show more fluorescence on the *medium/orange* background compared to the other backgrounds, analogous to the expected hue change. Because both scorpionfish species occur in shallow water but can also be found at depths of 30–40 m [25, 26], regulating red fluorescence together with red reflectance could enhance background matching at depths were long-wavelength light is scarce. In this first experiment, we observed that luminance and hue changes were happening faster than initially expected, i.e. in less than a minute. To (4) quantify how rapid this change was, we conducted a second experiment where we documented body luminance of scorpionfish every five seconds for 30 s, after relocation from a black to a white background.

Results

Changes in luminance and hue

Both scorpionfish species changed luminance according to the background (Fig. 2A, B). Scorpionfish body luminance differed for all background comparisons, for both scorpionfish species and regardless of observer (see Table 1A, median differences and 95% CIs deviate from

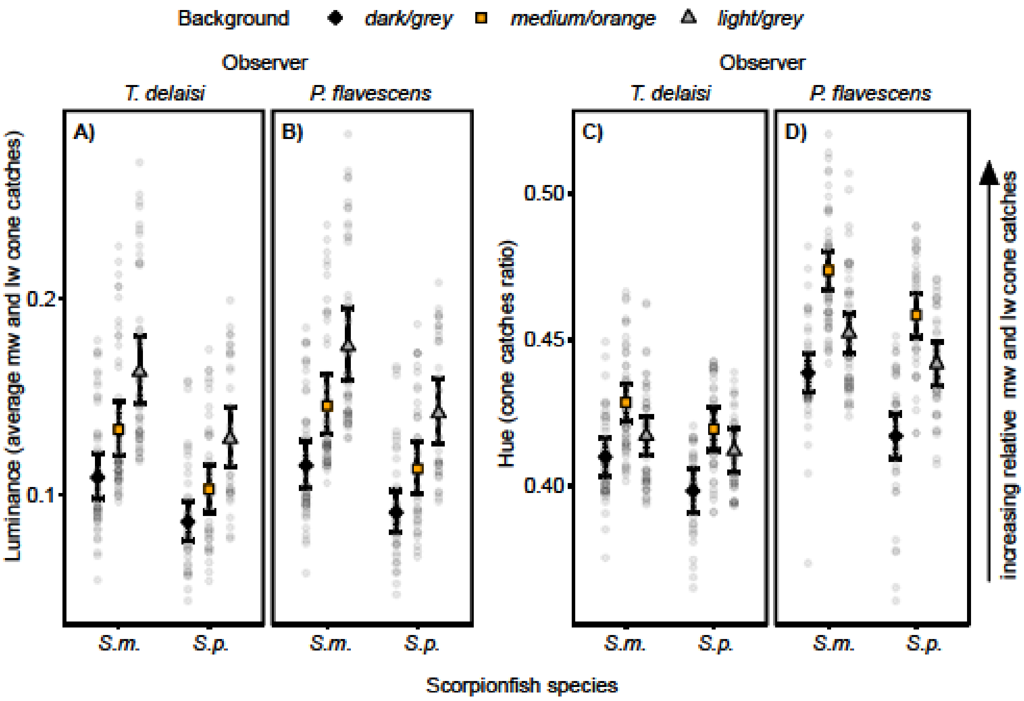

Fig. 2 Scorpionfish luminance and hue change across backgrounds. Scorpionfish luminance (average of the medium (mw) and long wavelength (lw) cone catches) from **A** *Tripterygion delaisi* and **B** *Pomatoschistus flavescens* visual perspective. Scorpionfish hue (ratio of short compared to medium and long wavelength cone catches, where higher values indicate a shift towards longer wavelengths, see Methods) from **C** *T. delaisi* and **D** *P. flavescens* visual perspective. *S. m.* = *Scorpaena maderensis*, *S. p.* = *S. porcus*. All panels show model estimates and raw data for all combinations of *background*, *species* and *observer*. Each point represents a measurement for each individual fish (N = 24 *S. maderensis*, N = 18 *S. porcus*) averaged over the two time points (after one and five minutes adaptation time to the backgrounds, see Methods). Markers with vertical bars represent predicted mean and 95% compatibility intervals (CIs) derived from 10,000 simulations of the posterior distribution of model parameters. The strength of the difference between two groups increases with decreasing degree of overlap of their 95% CIs

Table 1 Median differences in luminance and hue between all background combinations

	Scorpaena maderensis			*Scorpaena porcus*		
Background	**Median**	**Lower CIs**	**Upper CIs**	**Median**	**Lower CIs**	**Upper CIs**
(A) Luminance. $R^2_{marg}=0.387$, $R^2_{cond}=0.937$						
Observer = *T. delaisi*						
Medium/orange–dark/grey	0.024	0.020	0.030	0.017	0.013	0.021
Medium/orange–light/grey	– 0.030	– 0.036	– 0.024	– 0.026	– 0.032	– 0.021
Light/grey–dark/grey	0.054	0.047	0.062	0.042	0.036	0.049
Observer = *P. flavescens*						
Medium/orange–dark/grey	0.030	0.025	0.036	0.022	0.018	0.027
Medium/orange–light/grey	– 0.030	– 0.037	– 0.025	– 0.028	– 0.034	-0.023
Light/grey–dark/grey	0.061	0.054	0.069	0.051	0.044	0.059
(B) Hue. $R^2_{marg}=0.597$, $R^2_{cond}=0.881$						
Observer = *T. delaisi*						
Medium/orange–dark/grey	0.019	0.015	0.023	0.021	0.017	0.026
Medium/orange–light/grey	0.011	0.008	0.015	0.007	0.003	0.012
Light/grey–dark/grey	0.007	0.003	0.011	0.014	0.009	0.018
Observer = *P. flavescens*						
Medium/orange–dark/grey	0.035	0.031	0.039	0.042	0.037	0.046
Medium/orange–light/grey	0.022	0.018	0.025	0.017	0.012	0.021
Light/grey–dark/grey	0.014	0.010	0.017	0.025	0.020	0.029

Median differences of A) luminance and B) hue between all combinations of *background*, *species* and *observer*. Estimated effect sizes are reported as the median difference and its 95% compatibility intervals (CIs), calculated from 10,000 simulations of the posterior distribution of model parameters. N = 24 for *S. maderensis* and N = 18 for *S. porcus*. Effect size strength increases with increasing deviation of median differences from zero, and the robustness of the result increases with decreasing degree of overlap of the 95% compatibility intervals (CIs) with zero

zero for any given comparison). As expected, mean luminance of both scorpionfishes was lowest on the *dark/grey* background, intermediate on the *medium/orange*, and highest on the *light/grey* background (Fig. 2A, B, Table 1A), showing that the observed body luminance change follows the direction of luminance change of the background. Luminance of *Scorpaena maderensis* was overall higher than that of *S. porcus* (median difference of luminance averaged over *background* and *observer*: 0.029, 95% CI 0.011 to 0.049). Comparing the two observers, results for luminance change were similar (Table 1B, compare median differences for the same scorpionfish species and background comparison between the section "Observer = *T. delaisi*" and section "Observer = *P. flavescens*", 95% CIs overlap). Scorpionfish had on average a slightly higher luminance from *P. flavescens* visual perspective (Fig. 2B) than from *T. delaisi* visual perspective (Fig. 2A) (median difference of scorpionfish luminance averaged over *background* and *species:* 0.010, 95% CI 0.008 to 0.012).

Both scorpionfishes also changed hue in response to the background (Fig. 2C, D). Scorpionfish body hue differed for all background comparisons for both scorpionfishes and regardless of the observer (see Table 1B, median differences and 95% CIs deviate from zero for any given comparison). As expected, mean hue was shifted towards longer wavelengths (i.e. a higher hue value) for both scorpionfishes on the *medium/orange* background compared to the *dark/grey* and *light/grey* background (Fig. 2C, D, Table 1B). Hue also differed on the *light/grey* and *dark/grey* backgrounds (Table 1B, see *light/grey–dark/ grey* comparisons), being shifted towards longer wavelengths on the *light/grey* background (Fig. 2C, D). In general, hue of *S. maderensis* was more long-wavelength shifted compared to *S. porcus* (median difference of hue averaged over *background* and *observer*: 0.012, 95% CI 0.003 to 0.021). Hue perception was different depending on the observer, hue changes were stronger from *P. flavescens* compared to *T. delaisi* visual perspective (Table 1B, compare median differences for the same scorpionfish species and background comparison between the section "Observer = *T. delaisi*" and section "Observer = *P. flavescens*", 95% CIs mostly do not overlap). Scorpionfish had on average a more long-wavelength shifted hue from *P. flavescens* (Fig. 2D) compared to *T. delaisi* visual perspective (Fig. 2C) (median difference of scorpionfish hue averaged over *background* and *species:* 0.033, 95% CI 0.032 to 0.035).

Background matching

Mean achromatic contrast of scorpionfish body against the background was above the detection threshold on all backgrounds, regardless of scorpionfish or observer species (Fig. 3A, B, all predicted means and their 95% CIs are above one JND). Both scorpionfish species showed the lowest mean achromatic contrast on the *medium/orange* background (Fig. 3A, B, 95% CIs of predicted means do not overlap with *dark/grey* or *light/grey*). Achromatic contrast was similar from both visual perspectives (Fig. 3A, B) (median difference of scorpionfish body achromatic contrast against the background averaged over *background* and *species:* 0.23, 95% CI −0.04 to 0.49).

Mean chromatic contrast of scorpionfish body against the background was above detection threshold on all backgrounds, regardless of scorpionfish or observer species (Fig. 3C, D, all predicted means and their 95% CIs are above one JND). On which background scorpionfish had the lowest and highest mean chromatic contrast was depending on scorpionfish and observer species (Fig. 3C, D, see Additional file 1: Table S1B for all comparisons between chromatic contrast on all backgrounds). Chromatic contrast was clearly higher when calculated from *P. flavescens* visual perspective (Fig. 3D) compared to *T. delaisi* visual perspective (Fig. 3C) (median difference of scorpionfish body chromatic contrast against the background averaged over *background* and *species*: 3.11, 95% CI 3.03 to 3.20).

Changes in fluorescence

The fluorescent area varied between all backgrounds for both scorpionfish species (see Table 2, median differences and CIs deviate from zero for any given comparison). Against our expectations, mean fluorescent area was not largest on the *medium/orange* background, but increased with increasing background luminance in both species (Fig. 4, Table 2, see *medium/orange–dark/grey* and *medium/orange–light/grey* comparisons, median differences and CIs deviate from zero, where fluorescent area is larger on the *light/grey* than on the *medium/orange* background). Across all backgrounds, *S. maderensis* showed a larger fluorescent area than *S. porcus* (median difference of *fluorescent area* between *species*, averaged over *background*: 1930.71 pixels, 95% CI 533.76 to 3137.43).

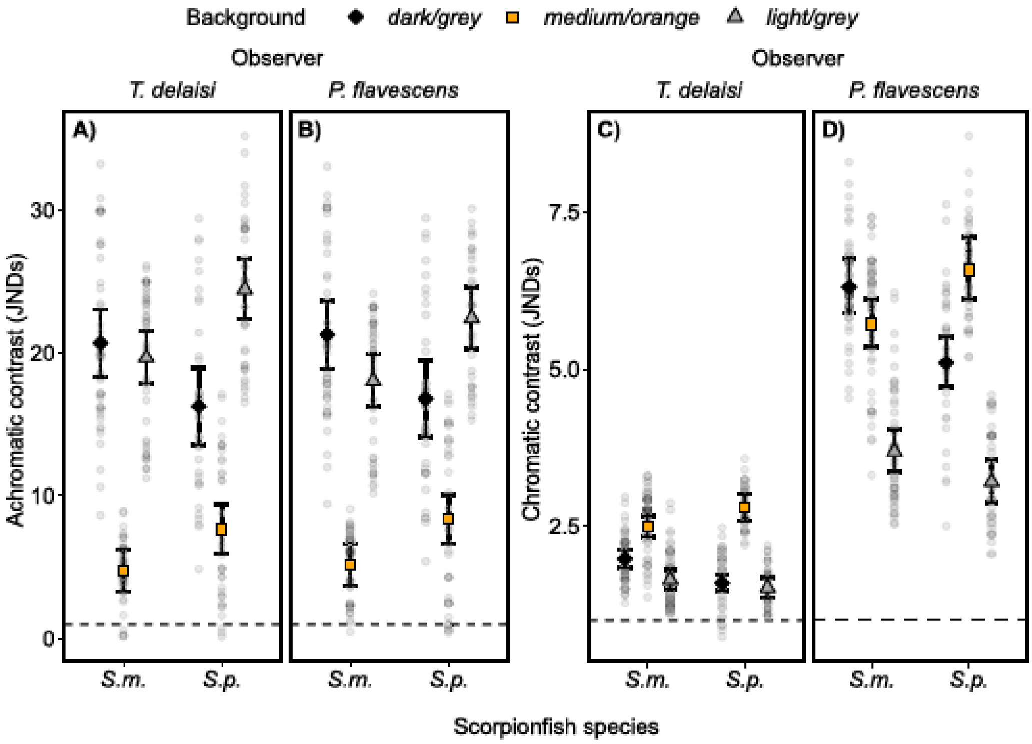

Fig. 3 Achromatic and chromatic contrasts of scorpionfish body against the background are above detection threshold. Achromatic contrast from **A** *Tripterygion delaisi* and **B** *Pomatoschistus flavescens* visual perspective in Just Noticeable Differences (JNDs). Chromatic contrasts from **C** *T. delaisi* and **D** *P. flavescens* visual perspective in JNDs. Dashed line = detection threshold of one JND. *S. m.* = *Scorpaena maderensis*, *S. p.* = *S. porcus*. All panels show model estimates and raw data for all combinations of *background*, *species* and *observer*. Each point represents a measurement for each individual fish (N = 24 *S. maderensis*, N = 18 *S. porcus*) averaged over the two time points (after one and five minutes adaptation time to the backgrounds, see Methods). Markers with vertical bars represent predicted mean and 95% compatibility intervals (CIs) derived from 10,000 simulations of the posterior distribution of model parameters. The strength of the difference between two groups increases with decreasing degree of overlap of their 95% CIs

Table 2 Median differences in fluorescent area of scorpionfish body across backgrounds for both scorpionfish species

	Scorpaena maderensis			***Scorpaena porcus***		
Background	**Median**	**Lower CIs**	**Upper CIs**	**Median**	**Lower CIs**	**Upper CIs**
Difference in fluorescent area (absolute pixel count). $R^2_{marg} = 0.373$, $R^2_{cond} = 0.804$						
Medium/orange–dark/grey	2244.95	1399.03	3278.23	1364.06	737.62	2350.44
Medium/orange–light/grey	– 2213.41	– 3711.56	– 897.32	– 924.62	– 2048.1	– 55.90
Light/grey–dark/grey	4457.48	3191.07	5957.11	2307.90	1357.15	3674.72

Estimated effect sizes are reported as the median difference and its 95% compatibility intervals (CIs), calculated from 10,000 simulations of the posterior distribution of model parameters. N = 21 for *Scorpaena maderensis* and N = 16 for *S. porcus*. Effect size strength increases with increasing deviation of median differences from zero, and the robustness of the result increases with decreasing degree of overlap of the 95% compatibility intervals (CIs) with zero

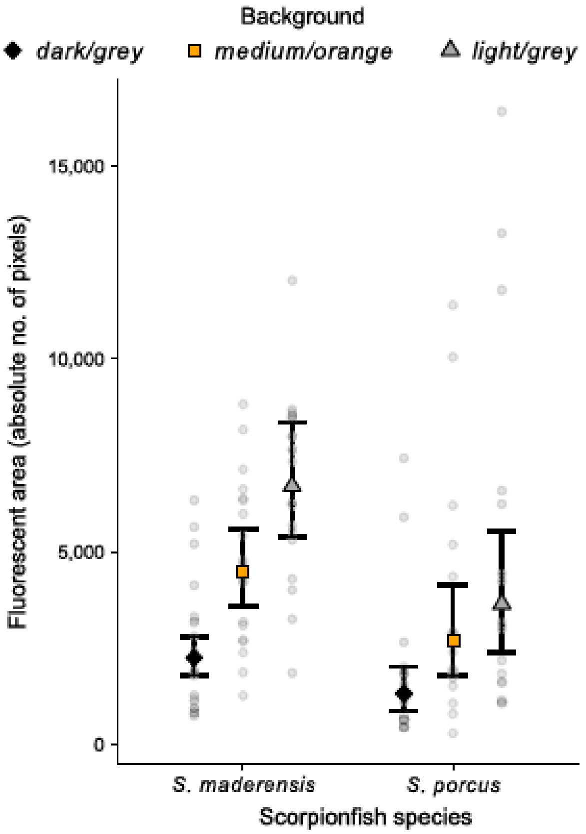

Fig. 4 Fluorescent area of scorpionfish body increases with background luminance. The figure shows model estimates and raw data for each background and scorpionfish species. Each point represents a measurement for each individual fish (N = 21 *Scorpaena maderensis*, N = 16 *S. porcus*). Fluorescent area is given in absolute pixel count. Markers with vertical bars represent predicted mean and 95% compatibility intervals (CIs) derived from 10,000 simulations of the posterior distribution of model parameters. The strength of the difference between two groups increases with decreasing degree of overlap of their 95% CIs

Rate of luminance change

S. maderensis individuals took on average about 10 s to achieve 50% and 23 s to achieve 80% of the body luminance change measured over the observation time of 60 s (Fig. 5). For *S. porcus*, more than 50% of the change was already achieved after 5 s, and 80% after 20 s (Fig. 5).

Discussion

Changes in luminance and hue

As expected, both species of scorpionfish changed their body luminance according to the luminance of the background. The lowest body luminance was observed on the *dark/grey* background, intermediate luminance on the *medium/orange* and the highest luminance on the *light/grey*. Scorpionfish also changed their body hue to longer wavelengths when placed on the *medium/orange* background compared to the other two backgrounds. Luminance and hue change were also connected, as shown by the shift in hue between the *dark/grey* and the *light/grey* background. The two grey backgrounds had a chromatic contrast below detection threshold from scorpionfish visual perspective (see Methods), and we therefore expected scorpionfish to display a similar hue on both backgrounds. However, in natural environments, changes of background luminance and hue usually come together, especially for carotenoid-based colours [27]. This dependence of luminance and hue occurrence and perception might explain the observed shift in scorpionfish body hue on the lighter background. Another reason for the observed shift in hue between the grey backgrounds could be a passive hue change as the scorpionfish changed luminance. Although the proximate mechanisms of colour change have not been investigated in scorpionfish, the observed colour change is probably due to the aggregation or dispersion of pigment organelles in chromatophores, a common mechanism present in many fish species [28]. Luminance changes are probably mediated by melanophores [5], and pigment organelle aggregation in the melanophores might have affected the hue of the scorpionfish as well, e.g. by exposing underlying structures in the fish skin [29]. However, the stronger change in body hue towards longer wavelengths on the *medium/orange* background compared to both grey

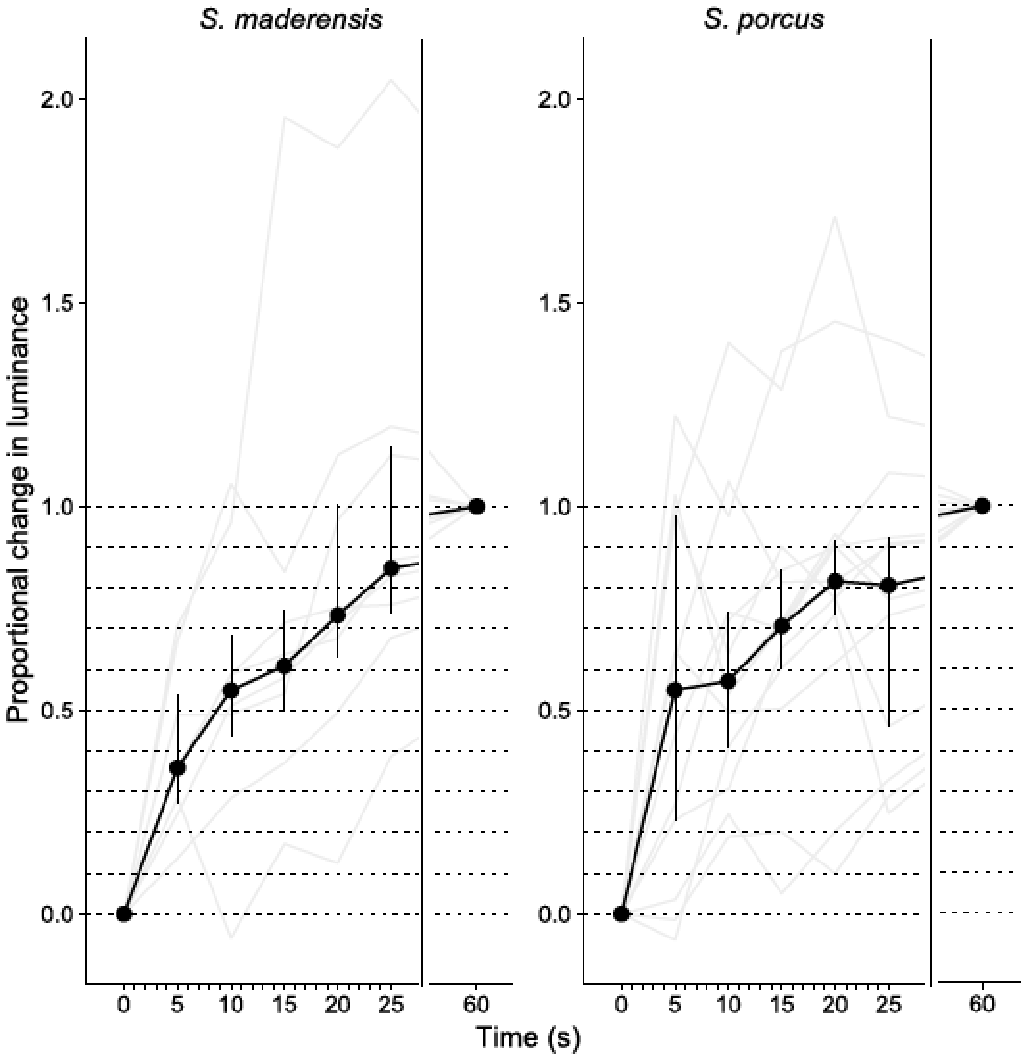

Fig. 5 Time needed for *Scorpaena maderensis* and *S. porcus* to change body luminance. Figure shows median (points) and interquartile range (vertical bars) of the proportional change in body luminance every five seconds for 25 s, relative to initial ($y = 0$) and final luminance ($y = 1$) measured for each individual (see Methods). The black curve connects medians for each time point. The grey lines connect each data point per individual fish. The dotted horizontal lines indicate every 10% step from 0 to 100%, the dashed vertical line represents is a gap with no data between 25 and 60 s. N = 9 for *S. maderensis* and N = 13 for *S. porcus*

backgrounds indicates that hue can be regulated actively, by an additional type of chromatophores. If luminance and hue change were mediated only by the same type of chromatophores, the long wavelength shift in body hue should have shown a similar pattern to luminance change across all three backgrounds. Such fine regulation of luminance and hue by different chromatophore types could allow scorpionfish to camouflage on different backgrounds [5, 13]. Both scorpionfish species tested showed similar results, suggesting that such colour change mechanisms may be present across the family Scorpaenidae, which are all benthic ambush predators. Still, *S. maderensis* appeared redder and lighter on each background compared to *S. porcus*. These species-specific differences might be related to differences in the species' ecology, or to different camouflage strategies [30]. Possible defining factors, e.g. microhabitat use and related background preferences, are however unknown for these two species or any other scorpionfish. Our study shows to what extent these species can adjust body luminance and hue, which is valuable information for further studies investigating their camouflage on natural backgrounds.

Background matching

Contrary to our expectations, scorpionfish did not match the artificial backgrounds very well. Achromatic contrasts of fish body against the backgrounds were clearly above detection threshold, especially on the two grey backgrounds. Yet, the fish did show a strong luminance change in the predicted direction, which likely reduced the contrast to background luminance. Moreover, on backgrounds that are difficult to match, fish may rely on other camouflage strategies such as disruptive colouration [31], which we did not quantify here. It is possible that fish changed colour to increase disruption, e.g. by changing certain patches in their pattern to increase pattern contrast or facilitate differential blending [32]. The poor achromatic match we observed may be explained by our use of artificial backgrounds of extremely low and high luminance, which might differ to the luminance range of natural backgrounds. Similarly, the orange hue we used might have been too artificial for the scorpionfishes, which may explain that even though fish adjusted body hue towards longer wavelengths on the *medium/orange* background, they still had high contrast to this background. Another explanation for this could be the scorpionfish's limited ability to discriminate long wavelengths given their spectral sensitivity [33]. We cannot exclude that longer adaptation time would have allowed for further improvement of background matching [7, 34, 35]. Even though neither scorpionfish species matched the backgrounds well when considering both achromatic and chromatic contrast, the response into the predicted directions suggests the luminance and hue changes were meant to improve background matching. Further studies are needed to test how well scorpionfish can match the background of natural substrates and which further camouflage strategies are deployed.

While changes in body luminance and achromatic contrasts against the backgrounds were comparable for both modelled observers, this was different for body hue and chromatic contrasts. Chromatic contrast of scorpionfish body against the backgrounds was higher from *P. flavescens* than from *T. delaisi* visual perspective, where it was below three JNDs on all backgrounds. While we do not have behavioural data on actual detection thresholds in *T. delaisi*, a conservative approach of three JNDs as detection threshold has been used for many animals including fishes [13, 35, 36], indicating that the chromatic contrast would be difficult to perceive at least from *T. delaisi* visual perspective. *P. flavescens* is a trichromat with a spectral sensitivity shifted to longer wavelengths compared to *T. delaisi*, which explains the better colour discrimination in the long wavelengths. These results highlight the importance to consider different observers when investigating animal colour change. In cases where scorpionfish match background luminance well, chromatic contrast might still reveal them to certain observers.

Changes in fluorescence

We predicted that red fluorescence would be upregulated on the *medium/orange* background. Even though our experiment was carried out under surface light conditions, we expected fluorescence to be increased on a red reflective background, since we did not expect the fish to have a physiological colour change mechanism that would be regulated differently depending on a specific light environment. Contrary to our prediction, the area of scorpionfish body showing fluorescence was not largest on the orange background, but on the lightest background. This suggests that display of red fluorescence depends on background luminance. This may be a consequence of melanosome aggregation on light backgrounds, an effect also known from other fishes [29]. How strong the contribution of red fluorescence is relative to reflectance in this experiment, or at depth, where red reflectance is much lower [16, 20], cannot be assessed with our data.

Rate of luminance change

Comparing the measurements taken after one and five minutes, luminance did not change much anymore, indicating that changes took place within one minute, before the first photo in experiment 1 was taken. A separate assessment of the rate of luminance change in experiment 2 showed that about 80% of the change achieved after one minute happened already within the first 20 to 25 s. More than 50% of the change was achieved after 10 s in *Scorpaena maderensis*, but already after 5 s in *S. porcus*. Such rapid colour change for camouflage is also known from tropical flounders [11].

Conclusions

This is the first study investigating whether scorpionfish adjust body luminance and hue to a given background. While fish were unable to match the extreme, artificial backgrounds below detection threshold, we show that both species rapidly change colour in the expected direction. As sit-and-wait predators, scorpionfish are an ideal group to study camouflage of predators from prey visual perspective. While this study focussed on two species of scorpionfish and tested background matching only, there are more species and types of camouflage worth exploring in this family.

Methods

Study species

The first experiment was carried out in the Station de Recherches Sous-marines et Océanographiques (STARESO), Corsica, France in June and July 2021. The second experiment was carried out in the same location

Fig. 6 Setup of experiment 1. **A** Overview of the setup, **B** trays with the three backgrounds used in the first experiment (from left to right: *dark/grey*, *medium/orange*, *light/grey*)

in July 2022. Madeira rockfish *Scorpaena maderensis* and the black scorpionfish *Scorpaena porcus* (Fig. 1) were caught with hand nets while SCUBA diving under the station's general sampling permit. All fish were kept in flow-through tanks (125 × 55 × 58 cm/400 L). Both species are ambush predators that sit motionless between rocks or algae and sedentary animals on natural hard substrates [25]. Scorpionfish are generalists that feed on a variety of small fishes and invertebrates. Both species mainly occur above 30–40 m [25, 26]. Fish sampled for our study were collected in 2–10 m depth. Observations under natural light conditions in the field indicate that both species can change colour, and that they are red fluorescent (personal observations).

Experiment 1

Experimental setup

To elicit changes in body colouration, fish were alternately placed in three white polyethylene trays (40 × 30 × 9 cm), each with a different uniformly coloured bottom (Fig. 6B). The walls of all trays were kept white. The three backgrounds were an achromatic, low luminance background (*dark/grey*), a chromatic, medium luminance background (*medium/orange*), and an achromatic, high luminance background (*light/grey*). We expected fish to show changes in luminance across all three backgrounds. Changes in hue on the orange background, but not on the grey backgrounds, would instead show that scorpionfish adjust body hue independently of luminance (see expectations in Introduction). We chose an orange reflective background to elicit hue changes in long wavelength body reflectance and fluorescence. If red fluorescence is part of dynamic background matching on long wavelength backgrounds, we expected to see a modulation of red fluorescence on the *medium/orange* background only. We did not test fish on fluorescent backgrounds or under deep-water light conditions since we did not expect the fish to distinguish between red fluorescence or reflectance, nor to have a physiological colour change mechanism that depends on the current light environment. We expected fish to simply regulate red fluorescence depending on the amount of red in the background, regardless of its origin. The *dark/grey* and *light/grey* backgrounds were plastic sheets spray-painted with black or light-grey spray paint (black: Marabou do it Colourspray black satin matt, Germany; light-grey: Maison Déco Relook Tout galet satin matt, France), and glued onto the bottom of the trays. The *medium/orange* background consisted of filter paper (LEE filter no. 204, Full C.T. Orange, Hampshire, UK) placed on the white bottom of the tray, and covered by a transparent plastic sheet. We chose to use filter paper for this background because all commercial orange spray paints we tried were fluorescent, which interfered with fish fluorescence photography (see below).

We quantified background appearance using a spectroradiometer (SpectraScan PR-740, Photo Research, New York, USA, with MS-75 standard lens) positioned on a tripod looking down at a 20° angle at the tray from a distance of ~ 100 cm to measure background reflectance relative to a diffuse white reflectance standard measured in the same way (SRS-99–010, Labsphere, NH, USA) (reflectance spectra in Additional file 1: Figure S2). To assess how scorpionfish would perceive the backgrounds, we calculated achromatic and chromatic contrasts between the backgrounds from a scorpionfish visual perspective by implementing their spectral sensitivities and cone ratio in the Receptor Noise Limited model [37] using the pavo R-package [38] in R (version 4.1.1) [39] (Table 3). *S. porcus* vision is characterized by short-wavelength single cones with average sensitivity peaking at 455 nm and medium-wavelength double cones with average sensitivity peaking at 530 nm ([33], as cited in [24]). The single to double cone ratio is 1:1 [40]. We assume similar visual properties for *S. maderensis*, for which there is no published record.

Each tray contained two centrally placed PTFE diffuse grey standards (12% and 72% grey, Berghof Fluoroplastic

Table 3 Achromatic and chromatic contrasts between backgrounds from scorpionfish visual perspective

Backgrounds compared	Achromatic contrast (JND)	Chromatic contrast (JND)
Light/grey–dark/grey	20.95	0.99
Medium/orange–light/grey	9.29	5.38
Medium/orange–dark/grey	11.67	6.37

Contrasts are expressed in Just Noticeable Differences (JNDs) for each background comparison, as perceived by scorpionfish. Contrasts below one JND are not distinguishable, and increasing values indicate an increasing probability of detection [36]. All backgrounds differ in luminance (achromatic contrast). The medium luminance background (*medium/orange*) has a comparable achromatic distance to both the high and the low luminance background. Difference in colour (chromatic contrast) between the two grey backgrounds is not distinguishable, whereas the *medium/orange* background shows contrasts above detection threshold to both grey backgrounds

Technology GmbH, Eningen unter Achalm, Germany) and a scale bar (Fig. 6B). Trays were filled with fresh sea water before each trial. Trials took place outside in a shaded area under the open blue sky. Photos to document change in luminance and hue were taken with a calibrated Nikon D4 DLSR camera (NIKON CORPORATION, Tokyo, Japan, Micro-Nikkor 60 mm lens, RAW format, ISO and aperture fixed) positioned in the same way as the spectroradiometer (Fig. 6A).

Since reflectance and fluorescence both contribute to body colouration under daylight, we estimated changes in red fluorescence separately by using a 3D-printed, cylindrical photo-chamber that was placed over the scorpionfish on its current background (Additional file 1: Figure S3). The top-lid of the chamber included a ring-light source and camera-holder for an Olympus Tough TG-6 (Olympus Europa SE & Co. KG, Hamburg, Germany, RAW format, ISO and aperture fixed). The ring-light (WEEFINE ringlight 3000, WEEFINE Technology, China) was set to "blue" and covered with an additional cyan filter (LEE filter no. 172, Lagoon Blue, Hampshire, UK) to block wavelengths above 540 nm. The camera was instead equipped with a double red filter (LEE filter no. 106, Primary Red, Hampshire, UK) to block light below 580 nm. This combination of light and filters assured that only cyan excitation light reached the fish, and that only red fluorescent emission reached the camera.

Experimental procedure

We tested 24 *S. maderensis* and 18 *S. porcus*. Mean standard length of both species was similar on average (*S. maderensis*: 7.04 ± 1.03 cm (mean ± SD), *S. porcus*: 7.03 ± 1.84 cm), and *S. porcus* had a slightly larger body area than *S. maderensis* on average when photographed from the top (*S. maderensis*: 7.58 ± 2.09 cm^2, *S. porcus*: 8.41 ± 4.14 cm^2). Each individual was tested on each background. At the start of the experiment, a fish was transferred into a medium luminance grey acclimation-box filled with fresh sea water, where it stayed for ten minutes. This acclimation period ensured initial short-term adaptation of each fish to the same background. Each fish was subsequently placed on the first of the three experimental backgrounds. It was photographed as soon as it settled (within a minute). A second photo was taken after five minutes adaptation time (Fig. 7A, B). Immediately after this, we placed the cylindrical photo-chamber on the fish, added a non-fluorescent red diffuse reflectance standard (SCS-RD-010, Labsphere, NH, USA) next to it, closed the chamber (details above), turned on the light source and took a photo (Fig. 7C). Taking a fluorescence photo took about 30 s. Subsequently, the fish was placed in the next tray and the procedure was repeated for the other two backgrounds. Exposing a fish to all backgrounds required around 20 min. The acclimation period was not repeated between backgrounds. In which order the fish were exposed to the three backgrounds was balanced across all individuals of a species to account for a potential effect of background order. After a completed trial, fish were either immediately brought back to the field or returned to a temporary housing tank. Each individual was used only once.

Image analysis

To quantify changes of luminance and hue between backgrounds, we used the Multispectral Image Analysis and Calibration (MICA) Toolbox plugin [41] for ImageJ

Fig. 7 Scorpionfish can adjust body luminance, and display red fluorescence. Exemplary photos of the same *S. maderensis* individual **A** on the *dark/grey* and **B** on the *light/grey* background and **C** of a fluorescence photo of a different *S. maderensis* individual (adapted to the *dark/grey* background)

(version 1.53o) [42]. Images were normalized with the 12% and 72% grey standards present in each tray, and converted into 32-bit multispectral images. For every image, we selected two regions of interests (ROI): (a) the 'body' of the fish, excluding the fins since they were transparent, and (b) a ca. 1 cm^2 sample of the 'background' (for more detail on the ROI selection, see Additional file 1: Figure S4). We also measured standard length of each fish relative to the size standard and extracted the area of the fish body in cm^2. All images were then batch-processed using a custom-written routine for MICA in ImageJ. First, reflectance images were converted to a cone-catch model, which included the spectral sensitivity of the camera and a modelled observer, and the spectra of photography and model illuminant, which were both a D65 spectrum. We chose D65 as the model illuminant since this was the light spectrum under which the experiment was run and under which the scorpionfish adjusted to the backgrounds. We modelled the vision of the yellow black-faced blenny *Tripterygion delaisi*, a common species and prey of scorpionfish. *T. delaisi* has single cones with average peak sensitivity at 468 nm, and double cones with average sensitivity peaking at 517 and 530 nm [43]. Since we were focusing on hue change in the long-wavelength part of the visible spectrum, we also modelled a natural observer with a better ability to perceive long wavelength changes, the two-spotted goby *Pomatoschistus flavescens*, which also occurs in the natural range of the scorpionfish. This fish has single and double cones with peak sensitivity at 456, 531 and 553 nm [44]. We assumed a Weber fraction of 0.05 for the most abundant cones and for the luminance channel for both species [45, 46], and a cone ratio (from shortest to longest wavelength photoreceptor) of 0.25:1:1 for *T. delaisi* [47] and 0.72:1:0.6 for *P. flavescens* [44, 48]. We defined the luminance channel as the average cone catches of the two longer wavelength sensitive cones, as fish likely perceive achromatic (luminance) contrasts through these photoreceptors [49]. The routine further processed the images to adjust for *T. delaisi* foveal spatial acuity of 7 cycles per degree [47] and 2.36 cycles per degree for goby vision [50] for a viewing distance of 30 cm by using the Gaussian Acuity Control and the Receptor Noise Limited (RNL) Ranked Filter functions of the MICA toolbox [51]. We then measured cone catches for the ROI 'body' and 'background' for both observers. To assess scorpionfish changes in luminance, we compared luminance channel cone catches measured for 'body' [35]. To assess changes in hue, we instead calculated the ratio of the difference between the cone catches of the short wavelength receptor and the sum of the two longer wavelength sensitive receptors and the total cone catches (*T. delaisi*: $\text{hue} = ((\lambda_{max}530 + \lambda_{max}517) - \lambda_{max}468) / (\lambda_{max}530 + \lambda_{max}517 + \lambda_{max}468)$, *P. flavescens*: $\text{hue} = ((\lambda_{max}553 + \lambda_{max}531) - \lambda_{max}456) / (\lambda_{max}553 + \lambda_{max}531 + \lambda_{max}456)$), following previous studies [13, 14]. Finally, we calculated the contrast of fish against the background as perceived by the observers, to see how well scorpionfish were matching the backgrounds by comparing the ROI 'body' to the ROI 'background' for each image. Achromatic and chromatic contrasts were calculated implementing the Receptor Noise Limited model [37] informed with the cone catches of the three chromatic channels, and the luminance channel cone catches using the pavo R-package [38] in R, where we set weber fraction and cone ratios for each observer as described above [13, 14, 35, 36]. Contrasts are reported as Just Noticeable Differences (JNDs), where values below one JND indicate an indistinguishable contrast and higher values indicate an increased probability of detection [36, 37, 46].

Fluorescence photos were corrected for differences in shutter speed by adjusting exposure to the same speed for each photo of one individual in the program Olympus Workspace (version 1.5, OM Digital Solutions Corporation), and subsequently exported as TIF. Images were imported in ImageJ, and only the red channel was selected. To filter out noise, we removed all pixels with a brightness threshold below 100 (RGB scale), which was defined beforehand by manually testing different thresholds and identifying the most conservative threshold where background pixels (i.e. noise) were removed, but not pixels of the fish for any given background used. We counted the remaining pixels with 'Analyse Particles' to quantify changes fluorescent area within the fish body.

Experiment 2

Experimental setup

To measure the rate of luminance change more precisely, we tested fish in a different setup. A white shallow plastic tray (40 × 60 × 9 cm) was divided into two compartments (40 × 30 × 9 cm each) by a removeable plastic wall. One compartment was kept white, while the other side was covered in black plastic. We chose to use black and white backgrounds instead of the same backgrounds as in experiment 1 since we wanted to record the fastest possible luminance change and we expected fish to change most rapidly if they would be moved between extremes. A moveable transparent plastic cylinder of 15 cm diameter and 8 cm height was placed in the tray. It had a small plastic edge at the bottom (2 × 1 cm) which served as a scale bar and on which two PTFE diffuse grey standards (12% and 72% grey, Berghof Fluoroplastic Technology GmbH, Eningen unter Achalm, Germany) were attached. To move the cylinder from the outside, it had a transparent handle reaching out of the tray. A Nikon D4 DLSR camera (NIKON CORPORATION, Tokyo, Japan, Micro-Nikkor 60 mm lens, RAW format, ISO and aperture fixed) was positioned on a tripod looking down at a 10° angle at the tray from a distance of ~ 120 cm.

Experimental procedure

To quantify the rate of luminance change, we tested 9 *S. maderensis* and 14 *S. porcus* in the setup for experiment 2. An individual was placed in the cylinder in the black compartment of the tray for one minute acclimation time. Then, the separating wall was pulled out and the fish was moved into the white compartment. We then took a photo every second for 30 s, and a last photo after 60 s. We assumed that the final luminance for short-term adaptation was achieved after this one minute since we observed in the first experiment that fish changed very little between one minute and five minutes adaptation time (Additional file 1: Figure S5). Fish were returned back into the field after the experiment.

Image analysis

Photos taken to measure the rate of luminance change were analysed with the same MICA toolbox routine used for experiment 1. We selected photos of the fish when first settled on the new background (second 0), and from second 5, 10, 15, 20, 25 and 60. For each fish, we only selected and measured a specific patch (Fig. 5A, dark dorsal patch behind the head framed by the gill covers), because this patch was easy to locate and select as an ROI in every individual regardless of its position. We then converted the images to *T. delaisi* vision as described above, and extracted luminance channel cone catches to test hypothesis 5). We chose to only present the data from *T. delaisi* vision as luminance perception of both observers is comparable (see Fig. 1A, B).

Statistical analysis

Experiment 1

We implemented generalized linear mixed models with the glmmTMB R-package [52] following a custom-written guided linear modelling R-routine [53]. Model assessment followed the guidance of Santon et al. [53]. We computed randomized quantile residuals with the R-package DHARMa [54], and inspected their distribution within and among factor predictor levels that are included or not in the models, and performed posterior predictive checks to assess model dispersion and overall model fit. Models were initially implemented using the most appropriate family distribution based on the nature of the response variable. The family was sometimes adjusted after model assessment to better capture the observed data.

Data from the first experiment originated from 42 individuals (24 *S. maderensis* and 18 *S. porcus*) that were used to test objectives 1 and 2 (see Introduction). Observations at the two time points (minute 1 and 5) were averaged since there was little variation between these two observations (Additional file 1: Figure S5). To assess changes in scorpionfish body luminance and hue (1), we implemented a generalised linear mixed model using a Gamma distribution (link = log) for the response variable *luminance*, and one using a Gaussian distribution for *hue*. Both models included the fixed effects *background (dark/grey, medium/orange, light/grey)*, *scorpionfish species* (*S. maderensis*, *S. porcus*) and *observer* (*T. delaisi*, *P. flavescens*), and their interaction. *Fish ID* was used as a random intercept to account for the repeated measurements of each fish [55]. We further included a random slope over *background* in the *luminance* model, to account for differences in the predictor-response relationship between individual fish [56]. To assess how well scorpionfish matched their backgrounds (2), we implemented a generalised linear mixed model using a Gaussian distribution for the response variable *achromatic contrast*, and one using a Tweedie distribution (link = log) for *chromatic contrast*. The fixed effects and random intercept were identical as described above. We further included a random slope over *background* in the *chromatic contrast* model. For each model, random slopes were added when the differences in group means of interest varied among the random predictors' levels.

We did not obtain fluorescence photos for 5 of the 42 individuals because of temporary technical difficulties with the photo-box and therefore used data from only 37 individuals (21 *S. maderensis* and 16 *S. porcus*) to test objective 3 (see Introduction) and assess changes in the response variable *fluorescent area* (i.e. the area of scorpionfish body showing fluorescence). For this model, we used a negative binomial distribution (link = log). Since this variable was not based on visual modelling, we here only included the fixed effects *background*, *species*, and their interaction. *Fish ID* was also included as random intercept.

We report R^2-values as a measure of fit for each model and report both the marginal R^2 (variance explained by fixed effects only) and the conditional R^2 (variance explained by entire model) [57] (Table 1, 2 and Additional file 1: S1), using the *r2* function of the performance package [58]. For graphical displays of the results, our figures present model predicted means and their 95% compatibility (i.e. credible) intervals calculated from the posterior distributions of fitted values obtained from 10,000 sets of model parameters [52]. The same posterior distribution of fitted values was used to compute and report median differences between factor levels and their 95% compatibility intervals for all combinations of factor predictors of interest (Tables 1,2 and Additional file 1: S1). Effect size strength increases with increasing deviation of differences from zero, and the robustness of the result increases with decreasing degree of overlap of the 95% compatibility intervals (CIs) with zero. We refrain from reporting associated *p*-values because they offer limited information about the biological relevance of the observed effects [59, 60].

Experiment 2

We visualised data from 9 *S. maderensis* and 14 *S. porcus* to evaluate how fast scorpionfish adjust body luminance to the background (objective 4, see Introduction). One *S. porcus* was excluded from the graphs since it showed little change of luminance within one minute and did not seem to adjust to the background (absolute difference between t_0 and $t_{60} < 0.001$ luminance channel cone catches). We calculated the proportional change in luminance at each time point (second 5, 10, 15, 20, 25), scaled for the total luminance change of every individual fish from initial to final luminance. We used the luminance channel cone catches of second 0 (t_0) as the initial value for luminance and of second 60 (t_{60}) as the final value for luminance, and calculated proportional change at time t_x as follows: proportional change t_x = (luminance t_x − luminance t_0)/(luminance t_{60} − luminance t_0). We then plotted the medians and interquartile range of these proportional change values over time to display how much time was needed to complete a certain percentage of the overall achieved luminance change.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12983-023-00488-x.

Additional file 1. Table S1: Median differences in achromatic and chromatic contrasts. **Figure S2**: Reflectance spectra of the three backgrounds and the acclimation box. **Figure S3**: Photo-chamber for fluorescence photos. Supplementary Methods: ROI selection; **Figure S4**: Example of ROI selection. **Figure S5**: Luminance of scorpionfish body between time points.

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Author contributions

LJ, MS and NKM conceived the study. LJ collected the data. MS provided custom-written template scripts for image analysis and statistical analysis. LJ analysed the data and drafted the manuscript. All authors edited the manuscript and approved the final version.

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Availability of data and materials

The datasets generated and analysed during the current study are available on Figshare: https://doi.org/10.6084/m9.figshare.22059092. The script used for statistical analysis can be found in Santon et al. [53].

Declarations

Ethics approval and consent to participate

We comply to the EU animal welfare legislation (Directive 2010/63/EU) in that our research was not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. It is therefore no subject to approval as an animal experiment.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

Appendix B

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RESEARCH ARTICLE

Scorpionfish adjust skin pattern contrast on different backgrounds

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Abstract

The two scorpionfish species *Scorpaena maderensis* and *S. porcus* are well camouflaged ambush predators that rapidly change body colouration to adjust to background colour in less than 1 min. We tested whether individuals of both species also adjust body pattern to that of the background. We placed fish on backgrounds of different pattern granularity and quantified the change in fish body pattern over 1 min. We used calibrated image analysis to analyse the patterns from the visual perspective of a prey fish species using a granularity (pattern energy) analysis and an image clustering approach. In our experiment, fish did not change their most contrasting pattern components as defined by the dominant marking size, but changed their average marking size. Moreover, fish responded with a change in pattern in contrast to the different experimental backgrounds, especially when compared to the acclimation phase. These results indicate that scorpionfish have one main pattern that can be adjusted by modulating its internal contrast. A reduction in pattern contrast could thereby improve background matching, while an increase could promote camouflage via disruptive colouration.

KEYWORDS
background matching, camouflage, colour change, disruptive colouration, pattern energy analysis, QCPA

TAXONOMY CLASSIFICATION
Behavioural ecology, Sensory ecology, Zoology

1 | INTRODUCTION

Many animals use camouflage to hide from predators or prey, which can be achieved with different strategies (Stevens & Merilaita, 2009a). A body colouration and pattern very similar to that of the background could allow camouflage through background matching (Stevens & Merilaita, 2009a). Such a specific phenotype can, however, bring the disadvantage that camouflage is restricted to a specific background, often a background that is homogeneous in colouration and pattern (Briolat et al., 2021; Price et al., 2019). Disruptive colouration, on the other hand, can work on more heterogeneous backgrounds (Cuthill et al., 2005; Price et al., 2019; Robledo-Ospina et al., 2017). Here, contrasting markings can create false edges and disrupt the body outline and shape, which makes it more difficult to be detected or recognised as such (Stevens & Merilaita, 2009b). Some animals dynamically change their body colouration and pattern, which can allow them to camouflage on multiple backgrounds, and to switch between camouflage strategies (Duarte et al., 2017).

Cuttlefish are renowned for their ability to change pattern in response to different backgrounds (Barbosa et al., 2008; Hanlon & Messenger, 1988; How & Santon, 2022; Mäthger et al., 2007; Osorio et al., 2022). Highly variable patterns can be produced by the high-dimensional control and flexible grouping of chromatophores (Woo et al., 2023). While cephalopods are unrivalled, fishes show remarkable pattern change too. Some flatfishes can switch between two to three different body patterns (Kelman et al., 2006; Ramachandran et al., 1996; Tyrie et al., 2015), and express up to six pattern components (Ramachandran et al., 1996). Nassau groupers and slender filefish display up to three body patterns in response to different natural substrates (Allen et al., 2015; Watson et al., 2014). The rock pool goby *Gobius paganellus* changes its pattern depending on the substrate granularity by modulating the contrast of certain bars within its pattern (Smithers et al., 2017). Many more fish species could potentially adjust body pattern for camouflage, as the ability to rapidly change colour is widespread in fishes (Nilsson Sköld et al., 2013).

Scorpionfishes are sit-and-wait predators that show various camouflage strategies (John et al., 2023; Santon et al., 2018). Studying their ability to dynamically change body colouration can help to understand how they improve camouflage and therefore potentially increase predation success. A previous study has shown that the two species *Scorpaena maderensis* and *Scorpaena porcus* can rapidly adapt to background colour (John et al., 2023). Field observations indicate that *S. porcus* individuals vary in their skin pattern (Figure 1, personal observations by LJ). While those might be individual differences only, scorpionfishes' ability to change body colour raises the possibility that they can also adapt their pattern in response to specific background features.

Therefore, we tested whether both scorpionfish species adjust their body pattern in response to background pattern granularity (i.e. the patch size within the pattern) to increase background pattern matching. After an acclimation phase on a uniform grey background, we placed individuals on three experimental backgrounds of different granularity but similar average luminance and contrast estimated using the spectral sensitivities of scorpionfish (Govardovskii & Zueva, 1988; John et al., 2023; Schweikert et al., 2018). The *medium granularity* background was designed based on the average scorpionfish body patch size observed in a previous study (John et al., 2023), the *fine granularity* and *coarse granularity* backgrounds instead had a smaller and larger patch size. We documented scorpionfish body pattern after 1 min on each background using calibrated image analysis and compared whether their pattern differed depending on the background granularity. We expected that fish would change their pattern granularity depending on background granularity. In particular, we expected fish to show smaller patch size on the fine granularity background and larger patch size on the coarse granularity background when compared to their patch size on the medium granularity background. We decided to include fish pattern contrast into our analysis because we also suspected that scorpionfish could increase pattern contrast on the high-contrasting experimental backgrounds, regardless of background granularity, when compared to the uniform acclimation background. We used different image analysis approaches to compare scorpionfish pattern metrics calculated from the visual perspective of a potential prey fish, the triplefin *Tripterygion delaisi*.

2 | MATERIALS AND METHODS

2.1 | Experimental animals

Experiments were carried out in the Station de Recherches Sous-marines et Océanographiques (STARESO), Corsica, France in June and July 2022 and followed the general procedure and setup used by John et al. (2023). Madeira rockfish *Scorpaena maderensis* and the black scorpionfish *Scorpaena porcus* were caught with hand nets while SCUBA diving under the station's general sampling permit. We followed the EU animal welfare legislation's directive (Directive 2010/63/EU) to ensure that our research was not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. Fish were kept in shaded outside flow-through seawater tanks (210 × 120 × 50 cm/1200 L) exposed to the natural light cycle. Both species are ambush predators that sit motionless on various natural hard substrates and feed on small fish and invertebrates (Louisy, 2002). Both species can adjust body colouration to the background in less than a minute (John et al., 2023). Observations in the field show a high pattern variability between individuals, yet it remains unclear whether scorpionfish can adjust skin pattern to that of the background (Figure 1).

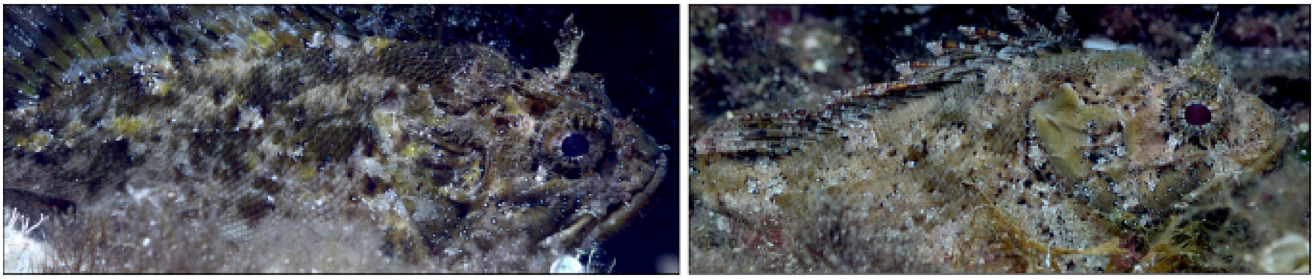

FIGURE 1 Two *Scorpaena porcus* individuals with different skin patterns. Photo by M S.

2.2 | Experimental setup

To elicit changes in body pattern, fish were alternately placed in three polyethylene trays (40 × 30 × 9 cm), each with a background of different pattern granularity (Figure 2). An identical tray was used for initial acclimation but had a uniform grey background. Backgrounds were printed on underwater paper (no. 3487; Avery Zweckform GmbH, Germany) with a laser printer (Kyocera ECOSYS P7240cdn KX, Kyocera, Japan) and then laminated with matte laminating pouches (125 micron, no. S-PP525-22, PRT GmbH, Switzerland). The three experimental backgrounds were black-and-white patterns of different granularity (fine, medium, coarse). Patterned backgrounds were created by taking photos of sand, gravel and small pebbles of different sizes. We used ImageJ (version 1.53o; Schneider et al., 2012) to convert the photos into masks showing 50% black and 50% white, to keep the contrast and average luminance perceived by the fish similar. The medium granularity roughly matched the average stripe size of scorpionfish estimated from a previous study (average grain size = 0.4 cm^2; John et al., 2023). The other two granularities were distinctly smaller (average grain size = 0.1 cm^2) and larger (average grain size = 1 cm^2). To create the uniform acclimation background, we took standardised photos of the three experimental and acclimation backgrounds of different grey levels in the setup, and calculated their average luminance using scorpionfish spectral sensitivity (Govardovskii & Zueva, 1988; John et al., 2023; Schweikert et al., 2018). The acclimation background's grey level chosen was the one closest to the average luminance of the experimental backgrounds.

Trials took place outside the station, in a shaded area under the open sky. A small transparent plastic frame of 24 × 18 cm and 2 cm height was placed in the centre of the tray to prevent fish from hiding in the corners or edges of the tray (Figure 2). Top view photos of fish were taken using a calibrated Nikon D4 DLSR camera (NIKON CORPORATION, Tokyo, Japan, Nikkor 60 mm macro lens, RAW format, ISO and aperture fixed) positioned on a tripod at a 20° angle, and a ~100 cm distance from the tray. Each tray contained two centrally placed PTFE diffuse grey standards (12% and 72% grey, Berghof Fluoroplastic Technology GmbH, Germany) and a scale bar. Each tray was also equipped with an Olympus Tough TG-6 camera (Olympus Europa SE & Co. KG, Hamburg, Germany, RAW format, ISO and aperture fixed) placed in a 3 cm wide compartment on the side of the tray (Figure 2). The camera was completely hidden during the trials, and only a small window for the lens was opened at the end of each trial to take a side view photo of the fish. We used a picture of a Mini ColourChecker Card (X-Rite Inc., Grand Rapids, MI, USA) to calibrate the Olympus camera in the experimental setup under the same light conditions as in the experiments (Troscianko & Stevens, 2015; van den Berg et al., 2020).

2.3 | Experimental procedure

We tested 21 *S. maderensis* and 30 *S. porcus*. Body size of the two species was similar on average (body area in top view for *S. maderensis*: 6.3 ± 2.0 cm^2 (mean ± SD), and for *S. porcus*: 6.4 ± 2.3 cm^2). Each individual was alternately placed on each experimental background. Trays were filled with fresh seawater before each trial. At the start of the experiment, each fish was first placed in the acclimation tray, and then on the three experimental backgrounds. We chose the uniform background as acclimation to obtain a reference image for each individual on a non-patterned (uniform) background, and to acclimate the fish to the luminance of the experimental backgrounds before starting the trials. A fish was photographed from the top 1 min after being transferred in the tray (minute 1) and after 5 min (minute 5). Then, the transparent frame was removed and a piece of PVC tube with 12% and 72% grey standards oriented sideways was inserted in the tray, opposite to the side with the Olympus camera compartment. The fish was gently moved until it settled next to the standards. Then, the small window in the camera compartment was opened to take a side view photo. The fish was then placed in the next tray and the procedure was repeated for the other backgrounds. For transferring fish between backgrounds, we used hand nets. The six possible background orders (for the three experimental backgrounds) were randomised and fully balanced across all individuals of each species. All individuals were used only once and then returned to the field.

2.4 | Image analysis

2.4.1 | Granularity analysis

To analyse fish body pattern, we used the multispectral image calibration and analysis (MICA) Toolbox plugin (version 2.2.2; Troscianko & Stevens, 2015) for ImageJ (version 1.54d). Images were normalised with the 12% and 72% grey standards and converted into 32-bit multispectral images. For each image, we selected a region of interest (ROI) on the body of the fish. We always excluded the fins and paid attention to only select the part of the body that was illuminated at the same angle as the grey standards used to normalise the images. All photos were then batch-processed using a custom-written routine for MICA in ImageJ (John et al., 2023). First, body area for each fish was extracted as the number of pixels contained in the ROI 'body', to later calculate body area in cm^2 using the scale in the photos. To exclude potential effects that fish body orientation in the tray could have on the pattern analysis, all top view photos were rotated in such a way that all fish were oriented in the same way. Then, images were converted to cone catches using a cone-catch model that was computed using the spectral sensitivity of the camera and of a modelled observer, and the D65 spectrum as illuminant. We used D65 as illuminant because fish adjusted to backgrounds under this spectrum. We modelled the vision of the yellow black-faced blenny *Tripterygion delaisi*, a common prey species of scorpionfish in the Mediterranean Sea (Santon et al., 2021), following previous studies (Bitton et al., 2017; John et al., 2023; Santon et al., 2020). *T. delaisi* has single cones with average peak sensitivity at 468 nm, and double cones with average sensitivity peaking at 517 and 530 nm (Bitton

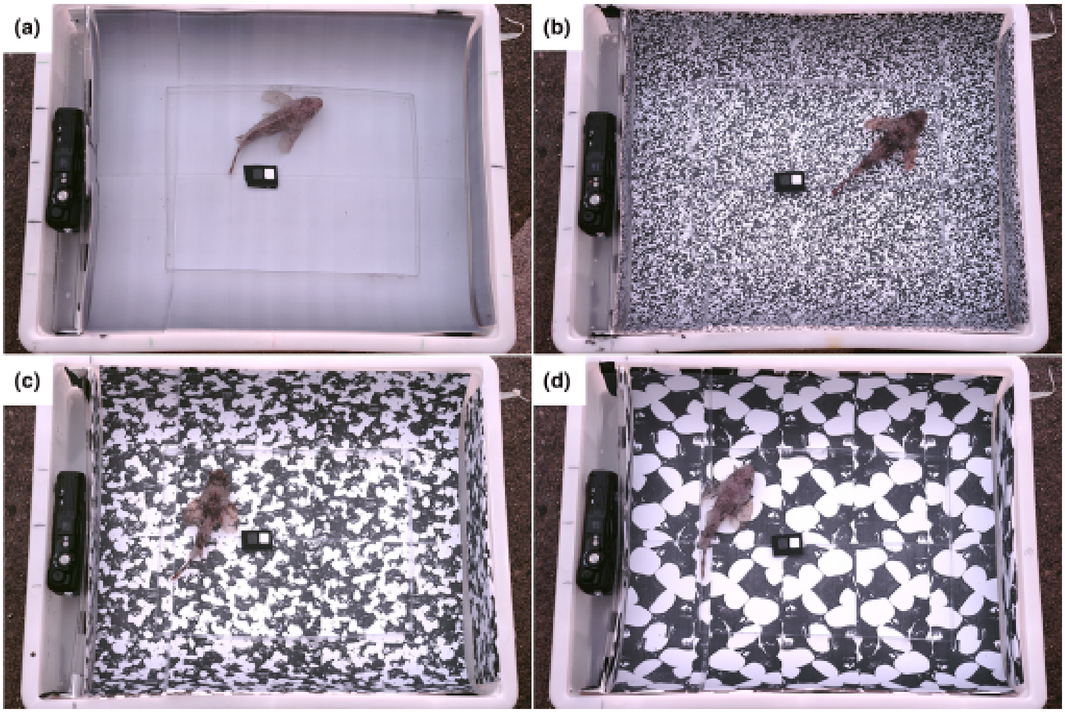

FIGURE 2 Exemplary top view photos in the experimental setup with the same *Scorpaena porcus* individual (a) at the acclimation background and the three experimental backgrounds with (b) fine, (c) medium and (d) coarse granularity. Body area of this individual was 6.5 cm^2. The left side of each tray has a small compartment with a camera for side view photos. Fish are kept in the centre of the tray with a transparent plastic frame (best visible in a).

et al., 2017). We assumed a Weber fraction of 0.05 for the most abundant cones (Champ et al., 2016; Olsson et al., 2018), and estimated it to 0.1 for the short wavelength cones based on cone abundance ratios (from shortest to longest wavelength photoreceptor) of 1:4:4 (Fritsch et al., 2017). We defined the luminance channel as the average cone catches of the two longer wavelength-sensitive cones, as fish likely perceive achromatic (luminance) contrasts through this channel (Lythgoe, 1979). The routine further processed the images to adjust for *T. delaisi* foveal spatial acuity of 7 cycles per degree (Fritsch et al., 2017; Santon et al., 2019), for a viewing distance of 20 cm (a relevant viewing distance in nature (Santon et al., 2021)), by using the Gaussian Acuity Control and the Receptor Noise Limited (RNL) Ranked Filter functions of the MICA toolbox (van den Berg et al., 2020). We then ran a granularity (pattern energy) analysis on the fish body using the 'Pattern Colour & Luminance Measurements' function of the toolbox (Troscianko & Stevens, 2015). This function uses fast Fourier transformation to produce images on different spatial scales and measures their pattern energy, defined as the standard deviation of the luminance channel's cone catches of the filtered pixels. By comparing pattern energy at different spatial scales (granularity bands), a dominant (highest energy, i.e. most contrasting) marking size can be determined (Barbosa et al., 2008; Stoddard & Stevens, 2010) (Figure 3, granularity spectrum for the four backgrounds). We analysed 99 granularity bands ranging from 2 to 100 pixels in size (i.e. using 1-pixel steps) for the top view photos and 30 granularity bands ranging from 2 to 150 pixels (i.e. using 5-pixel steps) for the side view photos. Analyses were stopped after 100 and 150 pixels because wider bin sizes exceeded the maximum fish and background patch size. Granularity bands differed between top and side view photos because the two cameras used had a different resolution, so analyses of top and side view photos can also not directly be compared (resolution of RNL rank filtered images: top view = 83 pixels per cm, side view = 80 pixels per cm). We visually inspected the granularity spectra derived from the side view photos and did not see any difference in fish pattern depending on the background (Figure A1). Because this was similar to the results derived from the top view photos, we focused on the top view for further analyses. To get the granularity spectra of the experimental backgrounds, we randomly chose eight top view photos per background type from our dataset and selected a 10 × 20 cm background patch in each image as an ROI 'background'. This large sampling area was to ensure that we would capture the granularity of each background type. The photos were processed as described above. We analysed 15 granularity bands ranging from 2 to 150 pixels (i.e. using 10-pixel steps) for the background samples. We reduced the number of granularity bands for the backgrounds to reduce computation time for the large samples and because the 15 bands seem to give a high enough resolution.

2.4.2 | QCPA analysis

The granularity analysis is widely used to assess dominant marking size in animal pattern research (Pérez-Rodríguez et al., 2017).

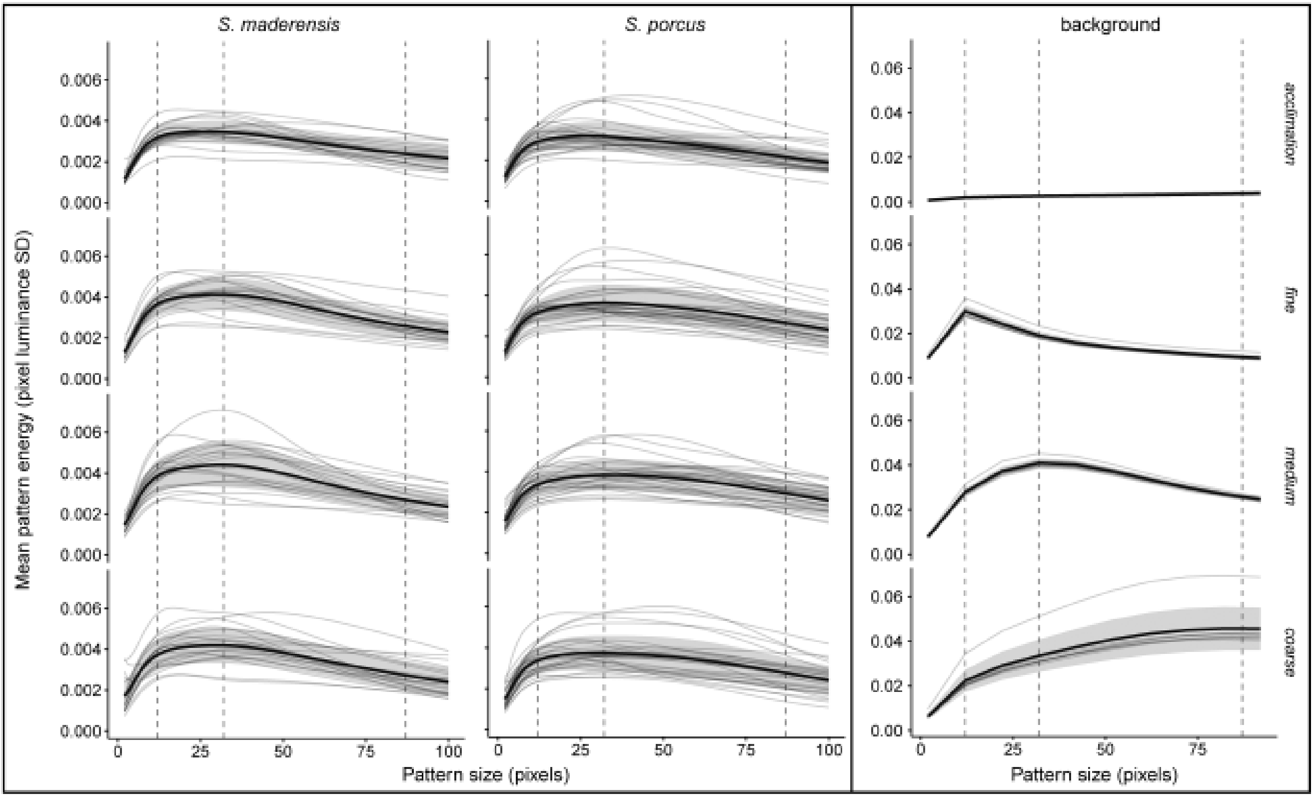

FIGURE 3 Pattern energy spectra (pattern energy for each pattern size bin) of the acclimation (uniform) and the three experimental backgrounds (right block), and of fish pattern for *Scorpaena maderensis* and *S. porcus* on each background (left block). Dashed vertical lines indicate the dominant marking size of the experimental background pattern (fine: x = 12, medium: x = 32, coarse: x = 87). The grey lines are spectra for each individual. The black lines indicate mean pattern energy over all individuals with standard deviation as the grey shaded area. Note the difference in pattern energy (range of y-axis) between fish and backgrounds. Pattern energy is defined as the standard deviation of the luminance channel's tone patches of the filtered pixels (see Section 2).

However, we decided to consider an additional approach to investigate fish pattern in more detail. We used RNL Clustering on the RNL rank-filtered images to apply the colour adjacency analysis (CAA) from the Quantitative Colour Pattern Analysis (QCPA) (van den Berg et al., 2020). CAA creates clusters of pixels of the same colour and luminance within a pattern, based on a given perception threshold. We used the average size of these clusters as an additional measure of pattern granularity. While from the granularity analysis, we can extract the size of the most contrasting patches (dominant marking size), the CAA gives average patch size regardless of contrast (all contrasts above our given perception threshold). The comparison of these two metrics therefore allows us to understand whether fish change patch size overall (CAA) or specifically the dominant marking size (granularity analysis). We further used the local edge intensity analysis (LEIA) on the RNL rank-filtered images to compare the mean luminance contrast value across edges within the fish body to test whether pattern contrast changed, irrespective of patch size (van den Berg et al., 2020). Chromatic contrasts were not analysed because, from *T. delaisi* perspective, there were almost no perceivable chromatic contrasts within the fish body pattern. For both analyses, we used a perception threshold of one just noticeable difference (JND) for *T. delaisi* vision. We ran the same analysis on the images with the background samples (see above).

2.5 | Statistical analysis

We implemented generalised linear mixed models with the glmmTMB R-package (Brooks et al., 2017) following a custom-written guided linear modelling R-routine (Santon et al., 2023). Model assessment followed the guidance of Santon et al. (2023), focusing on the inspection of the distribution of randomised quantile residuals, computed with the R-package DHARMa (Hartig, 2022), within and among factor predictor levels that were included or not in the models, and performed posterior predictive checks to assess model dispersion and overall model fit. Models were initially implemented using the most appropriate family distribution for the nature of the response variable.

Data analysed originated from 51 individuals (21 *S. maderensis* and 30 *S. porcus*). We only analysed observations after 1 min of exposure to the backgrounds because our previous study showed that scorpionfish change colour in less than 1 min (John et al., 2023), and because the granularity analysis spectra comparing measurements after 1 and 5 min were similar (Figure A2). To compare fish patterns on the different experimental backgrounds, we implemented generalised linear mixed models using a Gamma distribution (link = log) for the response variables *dominant marking size* (granularity analysis), *patch size* (CAA) and *pattern contrast* (LEIA), and specified *background* (fine, medium, coarse), scorpionfish *species* (*S. maderensis*, *S.*

porcus), and their interaction as fixed effects in each model. *Fish ID* was used as a random intercept to account for the repeated measurements of each fish. We added random slopes over a specific predictor when effect sizes' direction substantially varied among fish (Korner-Nievergelt et al., 2015). We therefore only included a random slope over *background* in the *dominant marking size* model.

To further investigate whether fish pattern contrast changed between the acclimation and the first experimental background, we created a subset of the data that only included observations for the acclimation and the first experimental background each fish was tested on. We implemented a generalised linear mixed model using a Gamma distribution (link = log) for the response variable *pattern contrast* (LEIA), with *event* (acclimation, first experimental background) and *first background type* (fine, medium, coarse) as main fixed effects. *First background type* was a variable created to group observations of the acclimation with the first experimental background type and included to compare whether a change in contrast differed between the experimental backgrounds. *Fish ID* was included as a random intercept.

We report R^2-values as a measure of fit for each model and report both the marginal R^2 (variance explained by fixed effects only) and the conditional R^2 (variance explained by entire model) (Nakagawa & Schielzeth, 2013) (Tables 1, 2), using the *r2* function of the performance package (Lüdecke et al., 2021). For graphical displays of the results, our figures present model predicted means and their 95%

TABLE 1 Pairwise contrasts of fish pattern (A) dominant marking size, (B) average patch size and (C) luminance contrast expressed as the response ratio between all combinations of background for both scorpionfish species.

	Scorpaena maderensis			*Scorpaena porcus*		
	Response ratio	Lower CIs	Upper CIs	Response ratio	Lower CIs	Upper CIs
(A) Dominant marking size (R^2_{cond} = .637, R^2_{marg} = .050)						
Fine – medium	1.07	0.90	1.27	1.04	0.90	1.20
Fine – coarse	1.00	0.82	1.22	1.10	0.94	1.30
Medium – coarse	0.94	0.81	1.09	1.06	0.94	1.20
(B) Average patch size (R^2_{cond} = .511, R^2_{marg} = .066)						
Fine – medium	**1.11**	**1.01**	**1.22**	1.04	0.97	1.13
Fine – coarse	1.00	0.91	1.10	**0.91**	**0.84**	**0.98**
Medium – coarse	**0.90**	**0.82**	**0.99**	**0.87**	**0.80**	**0.94**
(C) Luminance contrast (R^2_{cond} = .813 R^2_{marg} = .045)						
Fine – medium	**0.94**	**0.89**	**0.99**	0.96	0.91	1.01
Fine – coarse	1.06	1.00	1.13	**1.06**	**1.01**	**1.11**
Medium – coarse	**1.13**	**1.06**	**1.20**	**1.10**	**1.05**	**1.16**

Note: Effect size is proportional to the deviation of ratios from one, and the robustness of the result increases with decreasing degree of overlap of the 95% compatibility intervals (CIs) with one. Response ratios with CIs excluding one are highlighted in bold. $N = 21$ for *S. maderensis* and $N = 30$ for *S. porcus*.

TABLE 2 Pairwise contrasts of fish pattern luminance contrast (mean contrast of edges in LEIA) expressed as the response ratio between the acclimation and the first experimental background for both scorpionfish species, either pooling all measurements in the first experimental background regardless of background type, or split by background.

	Response ratio	Lower CIs	Upper CIs
Scorpaena maderensis			
Acclimation – first experimental background (pooled)	**0.80**	**0.75**	**0.85**
Acclimation – fine	**0.77**	**0.69**	**0.85**
Acclimation – medium	**0.83**	**0.74**	**0.92**
Acclimation – coarse	**0.81**	**0.72**	**0.91**
Scorpaena porcus			
Acclimation – first experimental background (pooled)	**0.87**	**0.83**	**0.92**
Acclimation – fine	**0.87**	**0.80**	**0.95**
Acclimation – medium	**0.83**	**0.76**	**0.91**
Acclimation – coarse	**0.91**	**0.83**	**0.99**

Note: Effect size is proportional to the deviation of ratios from one, and the robustness of the result increases with decreasing degree of overlap of the 95% compatibility intervals (CIs) with one. Response ratios with CIs excluding one are highlighted in bold. $N = 21$ for *S. maderensis* and $N = 30$ for *S. porcus*. R^2_{cond} = .820, R^2_{marg} = .254.

compatibility intervals calculated from the posterior distributions of fitted values obtained from 10,000 sets of model parameters (Brooks et al., 2017). We further used the emmeans package (Lenth, 2023) to compute pairwise contrasts expressed as ratios between factor levels and their 95% compatibility intervals for all combinations of factor predictors of interest (Tables, 1, 2). Effect size strength increases with increasing deviation of differences from one, and the robustness of the result increases with decreasing degree of overlap of the 95% compatibility intervals (CIs) with one.

3 | RESULTS

3.1 | Change in pattern granularity

3.1.1 | Dominant marking size (granularity analysis)

From inspecting the granularity spectra, we cannot see changes in dominant marking size when fish were placed on backgrounds of different granularity (Figures 3 and 4a; Table 1A). This becomes particularly evident when looking at how the spectra instead differed between backgrounds (Figure 3). On average, fish show a constant dominant marking size similar to that of the medium granularity background (Figure 4a). However, fish have a relatively heterogenous pattern granularity. While the mean curves peak at around 32 pixels, pattern energy remains high between ~20 and 40 pixels (Figure 3). For very regular patterns, a steeper peak around dominant marking size would be expected. Dominant marking size of *S. maderensis* is similar to that of *S. porcus* (dominant marking size between species ratio averaged over *background*: 0.87, 95% CI 0.75–1.01). Variance of dominant marking size was higher for *S. porcus* ($\sigma^2 = 97.98$) than for *S. maderensis* ($\sigma^2 = 77.45$), while their body sizes were comparable (see Section 2).

3.1.2 | Average patch size (CAA)

Both species show differences in average patch size depending on experimental backgrounds. *S. maderensis* shows a smaller average patch size on the medium, compared to the fine and coarse background (Figure 4b, Table 1B). *S. porcus* shows a larger average patch size on the coarse, compared to the medium and fine background (Figure 4b, Table 1B). Patch size of *S. maderensis* is similar to that of *S. porcus* (patch size between species ratio averaged over *background*: 1.02, 95% CI 0.93 to 1.13).

3.2 | Change in pattern luminance contrast (LEIA)

3.2.1 | Comparison between experimental backgrounds

Both species show a lower pattern luminance contrast on the coarse, compared to the medium granularity background (Figure 4c, Table 1C). Pattern contrast of *S. maderensis* is similar to that of *S. porcus* (pattern contrast between species ratio averaged over *background*: 1.03, 95% CI 0.92 to 1.16).

3.2.2 | Comparison between acclimation and first experimental background

Fish increased the contrast of their pattern when moved from the acclimation to the first experimental background, regardless of its granularity (Figure 5, Table 2).

4 | DISCUSSION

We investigated whether the two scorpionfish species *Scorpaena maderensis* and *S. porcus* change their pattern depending on the granularity of their visual background. Fish changed their average patch size and pattern contrast. However, dominant marking size, the most contrasting component of the pattern (Barbosa et al., 2008; Stoddard & Stevens, 2010), was not modulated on different granularity backgrounds. This was in contrast to other camouflaged benthic fishes such as different species of flounder, which can adapt their body pattern dominant marking size flexibly to different substrate granularities (Akkaynak et al., 2017; Ramachandran et al., 1996). Possibly, scorpionfish did not change in our experiment because the right cues to induce pattern change, such as specific pattern components, or even tactile or olfactory cues, were missing (Stevens & Ruxton, 2019). However, there might well be morphological or physiological restraints that prevent scorpionfish from modulating pattern elements, such as the inability to regulate the chromatophores of different skin patches independently. Similar to other fishes such as the rock pool goby *Gobius paganellus* (Smithers et al., 2017), or the flatfishes *Paralichthys lethostigma* and *Pseudopleuronectes americanus* (Saidel, 1977), scorpionfish seem to have one dominant body pattern with a given patch size, which can be modulated by adjusting the contrast between patches.

The Colour Adjacency Analysis revealed small changes in average patch size depending on background granularity. This indicates that fish changed their pattern in response to the background, but without modulating their dominant, most contrasting marking size. *S. maderensis* have the smallest average patch size on the medium granularity background, and *S. porcus* have a smaller patch size on both fine and medium granularity backgrounds. However, the average patch size remains substantially larger than that of the medium granularity background. Therefore, it is unclear how these small observed changes may affect the fishes' camouflage. It is indeed more plausible that changes in average patch size might only be a by-product of the changes in pattern contrast that we observed. As fish modulate contrast within their pattern, perceived size of some patches may vary due to some edges blending in or becoming more apparent. Pattern contrast of both species

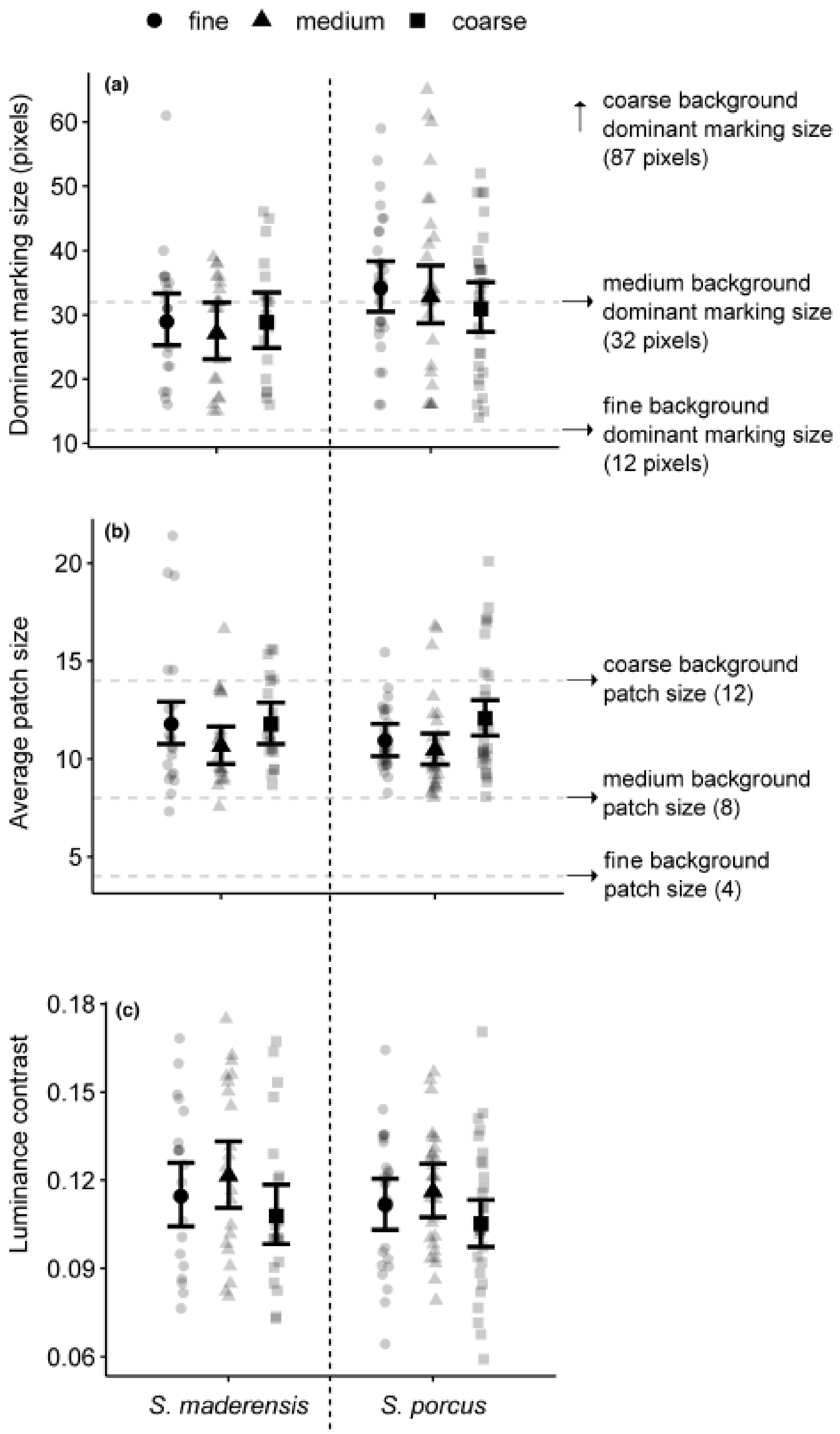

FIGURE 4 Fish pattern metrics depending on background granularity. (a) Dominant marking size is the spatial scale (measured in pixels) showing the highest contrast per individual fish, based on the granularity analysis. Dashed horizontal lines indicate the dominant marking size of the experimental backgrounds (fine: $y = 12$, medium: $y = 32$, coarse: $y = 87$). (b) Average patch size is the average size of clusters derived from the RNL clustered image of the fish body in the QCPA. Dashed horizontal lines indicate the average patch size of the experimental background pattern (fine: $y = 4$, medium: $y = 8$, coarse: $y = 14$). (c) Luminance contrast is given as the mean contrast value of edges based on the LEIA. Points represent observations of each individual fish ($N = 21$ *Scorpaena maderensis*, $N = 30$ *S. porcus*). Markers with vertical bars represent predicted medians and 95% compatibility intervals (CIs) derived from 10,000 simulations of the posterior distribution of model parameters. The strength of the difference between two groups increases with decreasing degree of overlap of their 95% CIs.

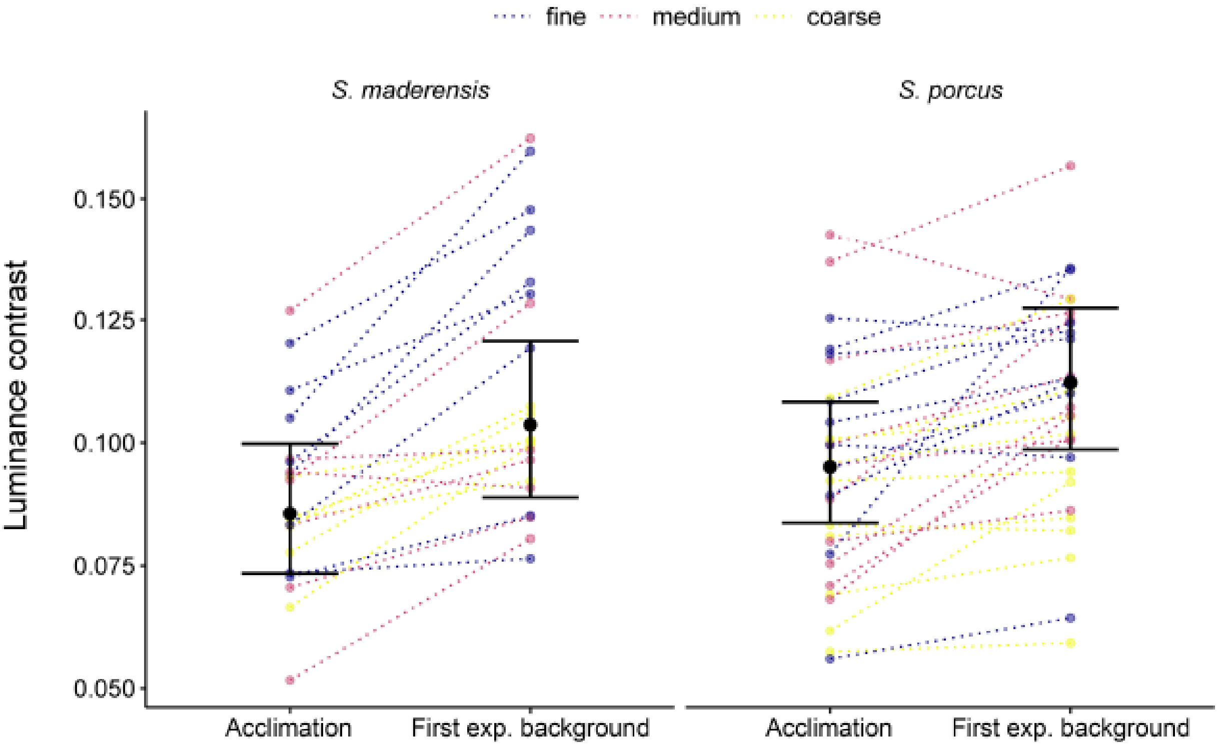

FIGURE 5 Fish change their pattern contrast between acclimation and the first experimental background. Contrast is given as the mean contrast value of edges based on the LEIA. The horizontal lines connect data points of an individual, colours indicate the background type used in the first experimental background. Points represent observations of each individual fish ($N = 21$ *Scorpaena maderensis*, $N = 30$ *S. porcus*). Markers with vertical bars represent predicted medians and 95% compatibility intervals (CIs) derived from 10,000 simulations of the posterior distribution of model parameters. The strength of the difference between two groups increases with decreasing degree of overlap of their 95% CIs.

in the Local Edge Intensity Analysis was highest on the medium granularity background. Possibly, fish increased pattern contrast especially on the medium granularity background because this is closest to their own dominant marking size. Increasing pattern contrast on a similar background could improve background pattern matching by intensifying the pattern through the increasing contrast. Furthermore, all fish had a substantially lower pattern contrast in the acclimation uniform background compared to the fine, medium and coarse experimental ones. While this could be a result of differences in pattern granularity, it is likely that the difference in contrast induced this change, as the acclimation was the only background without contrast. On backgrounds with high-contrast patterns, fish might increase their pattern contrast regardless of background granularity to improve disruption by displaying maximum disruptive contrast (Stevens & Merilaita, 2009b). We suggest that both contrast and granularity may impact the pattern regulation of the fish. It is known that cuttlefish use both pattern size and pattern contrast as cues to adjust their body pattern and that backgrounds with higher contrast elicit body patterns with higher contrast (Barbosa et al., 2008).

An individual fish pattern is relatively heterogenous in terms of dominant marking size and average patch size, meaning fish have patches of different sizes and not a very regular pattern. This could function as a generalist body pattern that works well on multiple backgrounds, reducing the need to modulate body pattern (Briolat et al., 2021; Merilaita et al., 1999), a strategy known from animals found on highly complex and heterogenous backgrounds (Hughes et al., 2019; Nokelainen et al., 2019). Moreover, scorpionfish could show further adaptations that improve their camouflage and reduce the need to adjust pattern to different backgrounds, such as an active background selection (Stevens & Ruxton, 2019). While we overall observed similar results for both scorpionfish species, there seems to be a higher individual variation of dominant marking size in *S. porcus* compared to *S. maderensis*, and this cannot be explained by a systematic variation in body size. High individual variation in pattern could benefit camouflage by disrupting the search image of prey or preventing search image formation (Bond & Kamil, 2002; Stevens et al., 2014; Surmacki et al., 2013). Individual pattern variation can also be favoured by living in a very heterogeneous habitat (Merilaita et al., 1999), and it is possible that the species differ in their microhabitat use with *S. porcus* living in more complex microhabitats or having a more generalist habitat use. An assessment of scorpionfish colouration and behaviour in their natural environment could help to understand the importance of skin pattern for their camouflage and consequently, prey capture success.

AUTHOR CONTRIBUTIONS

Leonie John: Conceptualization (equal); data curation (lead); formal analysis (lead); investigation (lead); methodology (lead); visualization (lead); writing – original draft (lead); writing – review and editing (equal). **Matteo Santon**: Conceptualization (equal); methodology (supporting); writing – review and editing (equal). **Nico K. Michiels**: Conceptualization (equal); writing – review and editing (equal).

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT

All data collected and analysed in the study will be available on Figshare, https://doi.org/10.6084/m9.figshare.24560323.

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APPENDIX

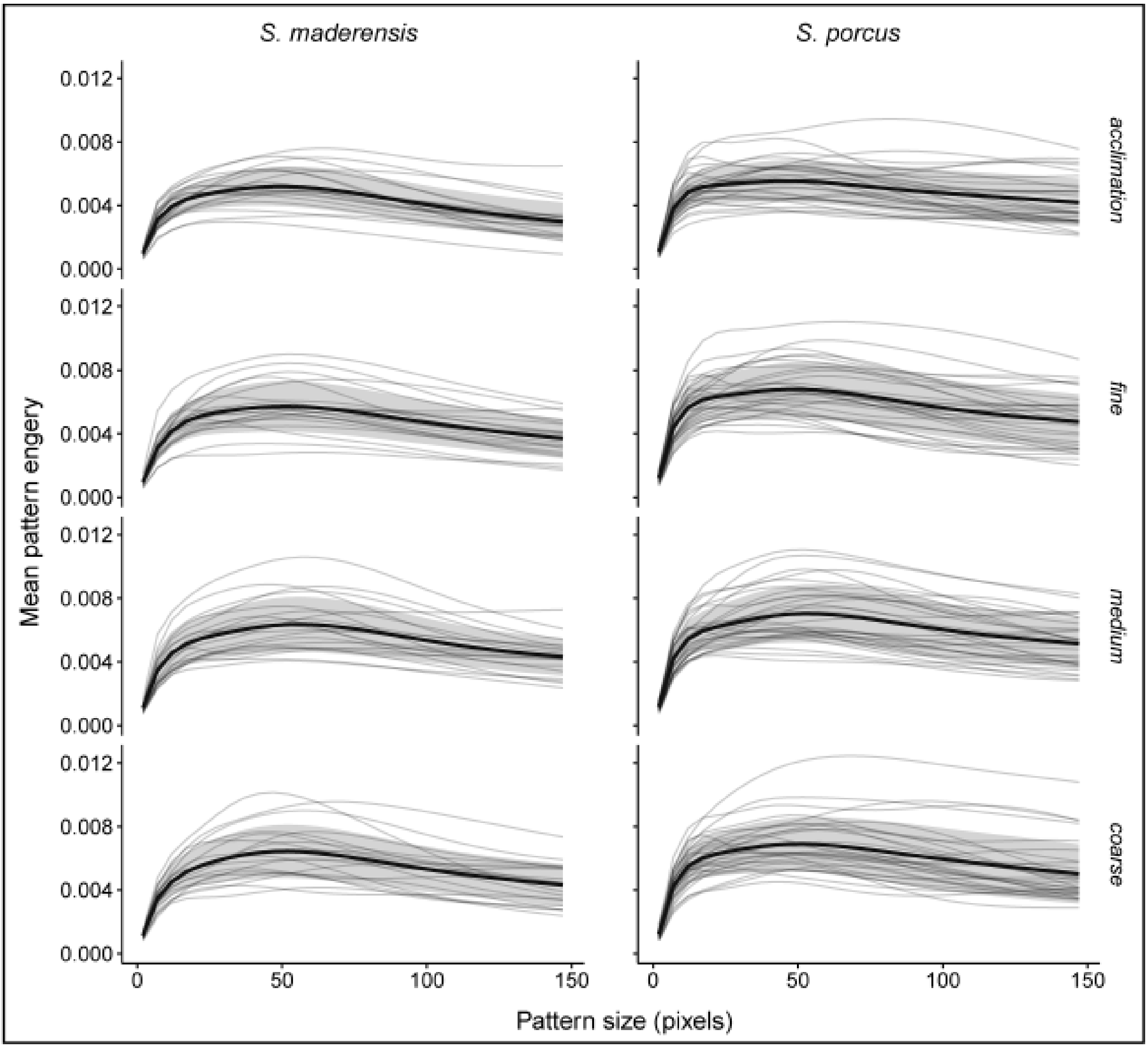

FIGURE A1 Pattern energy spectra (pattern energy for each pattern size bin) of *Scorpaena maderensis* (*n* = 20) and *S. porcus* (*n* = 30) on the acclimation (uniform) and the three experimental backgrounds based on the side view photos. The grey lines are spectra for each individual. The black line indicates mean pattern energy over all individuals with standard deviation as the grey shaded area. Pattern energy is defined as the standard deviation of the luminance channel's one matches of the filtered pixels (see Section 2).

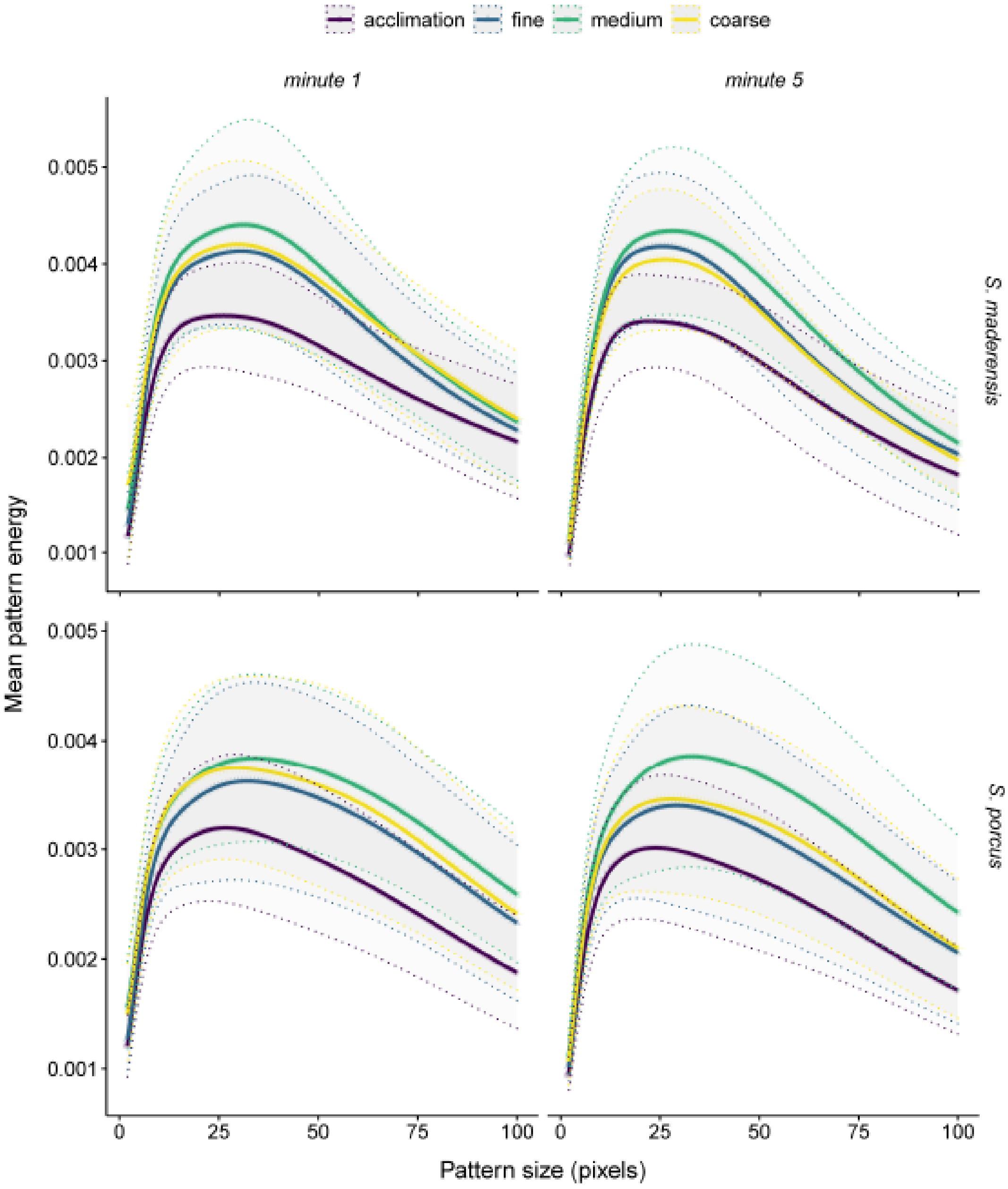

FIGURE A2 Pattern energy spectra (pattern energy for each pattern size bin) of *Scorpaena maderensis* ($n=21$) and *S. porcus* ($n=30$) on the acclimation (uniform) and the three experimental backgrounds, split by the timepoint at which the measurement was taken (after 1 or 5 min). The thick lines indicate mean pattern energy, the dotted lines indicate its standard deviation. Pattern energy is defined as the standard deviation of the luminance channel's zone patches of the filtered pixels (see Section 2).

Appendix C

Related publication to chapter 3

John, L., Santon, M., & Michiels, N. K. (2024). **Generalist camouflage and background choice in scorpionfish**. In preparation.

Generalist camouflage and background choice in scorpionfish

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Abstract

For a successful hunt, marine ambush predators such as scorpionfish need to be well camouflaged in the eyes of their prey. We explored two strategies that the two Mediterranean scorpionfish species *Scorpaena maderensis* and *S. porcus* might employ to maintain their camouflage in a heterogeneous environment. A generalist body colouration allows an imperfect but reasonable background match on many natural substrates. We calculated chromatic and achromatic contrast of scorpionfish against a range of natural substrate samples using calibrated photography and visual modelling of three natural observers. Scorpionfish matched many substrates with relatively low chromatic contrasts for all observers, but had high achromatic contrast. As an alternative strategy, fish might choose to settle on backgrounds on which they are most cryptic and avoid others. We tested scorpionfish in a behavioural experiment where they were able to choose between A) backgrounds that were similar or contrasting to their own average luminance and B) backgrounds that were more or less complex. Scorpionfish did not prefer backgrounds of luminance similar to their own average luminance but settled more often on darker backgrounds. Scorpionfish chose backgrounds randomly when they differed in complexity only. We propose that the preference for dark backgrounds can enhance disruptive colouration by showing that certain patches within the fish pattern matched best the dark background. Considering their ability to change colour to intensify disruptive colouration, and the chromatic background match on multiple natural substrates, we conclude that scorpionfish are generalists that can camouflage in a range of microhabitats using different strategies.

Keywords

background choice, background matching, habitat complexity, generalist camouflage, heterogeneous habitat, visual modelling

Introduction

Animals show different strategies to camouflage in heterogeneous or changing environments (Hughes *et al.*, 2019). They can flexibly change colour (Duarte *et al.*, 2017), have a generalist body colouration that allows camouflage on several backgrounds (Briolat *et al.*, 2021; Nokelainen *et al.*, 2019), or actively choose backgrounds that maximise their crypsis (Stevens & Ruxton, 2019). In the marine environment, some fishes preferentially settle on backgrounds that match their own body colouration (Smithers *et al.*, 2018; Tyrie *et al.*, 2015). An alternative, yet not exclusive, strategy to improve crypsis is to choose more complex backgrounds. 'Visual clutter' in the background is characterised by variation in colour, shapes and edge orientations, with more variable backgrounds being more complex (Dimitrova & Merilaita, 2010; Rowe *et al.*, 2021). Complex backgrounds overload observers with visual information and may complicate detection of prey or predators (Dimitrova & Merilaita, 2010, 2012; Merilaita, 2003; Xiao & Cuthill, 2016), even compensating for suboptimal background matching (Rowe *et al.*, 2021). However, investigations of choice in the context of background complexity are rare and remain inconclusive (Kjernsmo & Merilaita, 2012; Perkovic & Mettke-Hofmann, 2018).

Scorpionfishes (family Scorpaenidae) are well-camouflaged sit-and-wait marine benthic predators that attack approaching prey by sudden suction feeding. The Mediterranean species *Scorpaena maderensis* and *Scorpaena porcus* are found on a variety of substrates that differ in colour, luminance, pattern and complexity (personal observations). Both species are known to rapidly change their colour and pattern depending on background features, with the strongest changes observed being changes in luminance (John *et al.*, 2023). These changes also regulate internal contrast and average patch size of the skin pattern (John *et al., accepted*). *S. porcus* is a dichromat with cones with maximum peak absorbance at short (455 nm) and medium (530 nm) wavelengths (Govardovskii & Zueva, 1988; Schweikert *et al.*, 2018). We do not have data for the visual system of *S. maderensis* but assume similar properties. As dichromats, scorpionfish should have a limited ability to distinguish between differently coloured substrates. If changes in body colouration are controlled by vision (Duarte *et al.*, 2017; Stevens, 2016), it is therefore possible that scorpionfish adjustment to different backgrounds is not primarily driven by chromatic but achromatic vision. Moreover, they could benefit greatly from a generalist body colouration that allows settling on substrates without assessing their colouration (Hughes *et al.*, 2019; Merilaita *et al.*, 1999).

We therefore investigated 1) whether scorpionfish can distinguish colours in their natural habitat and how well their body colouration matches different substrates. We used calibrated photography of natural substrates and visual modelling to quantify chromatic differences between substrate types from scorpionfish visual perspective. Additionally, we quantified scorpionfish background match against those substrates from the visual perspective of conspecifics and two prey fish. We expected that scorpionfish would show a low chromatic contrast on most of the natural substrates where they usually settle. Given the naturally high variation in perceived luminance of backgrounds, we expected scorpionfish to have a higher and more variable achromatic contrast to the substrates.

We further investigated 2) whether scorpionfish use perceived luminance as a cue to actively choose the backgrounds to settle on. We therefore conducted a behavioural experiment where scorpionfish were allowed to choose between grey backgrounds of different luminance. We expected scorpionfish to prefer backgrounds that were closer to their average body luminance. An alternative strategy for background choice that would be less dependent on the own body colouration would be a preference depending on degree of background complexity. We tested this concept in the same experimental setup. We expected scorpionfish to choose the more complex background because increased background complexity could improve crypsis.

Because we found an unexpected preference of scorpionfish for the darker backgrounds, we investigated 3) the background matching of scorpionfish body colouration on their preferred backgrounds. We measured body colouration of distinct patches within the fish pattern and calculated contrast to the background and internal pattern contrast to understand their choice in the context of potential benefits for their camouflage.

Methods

1. Natural habitat sampling and background matching

The Madeira Rockfish *Scorpaena maderensis* and the Black Scorpionfish *Scorpaena porcus* are benthic ambush predators. Both species can change body colouration (John *et al.*, 2023) and pattern (John *et al., accepted*) in response to their background and likely more camouflage strategies (Santon *et al.*, 2018). We took standardised photos of natural substrates where scorpionfish were caught for the behavioural experiment, and where these species are known to occur from previous studies (John *et al.*, 2023, John et al., *accepted*). We focused on six common substrate types: (1) rubble covered with sand and epiphytes (*rubble and sand*), (2) *seagrass leaves*, which are partially covered by epiphytes, (3) *seagrass stems* just above the roots, (4) *turf algae*, (5) *yellow algae* (*Dictyota* cf. *fasciola*) and (6) a species of *red sponge* (cf. *Crambe crambe*). Raw photos were taken while SCUBA-diving at 6 m depth using a calibrated Nikon D4 DLSR camera (Nikkor 60 mm macro lens). This was an intermediate depth of the range where we typically caught scorpionfish (2-10 m). We used a pole with a dark grey standard (9 % grey) attached to its tip to place the standards in the images perpendicularly to the lens of the camera. The pole also served to standardise the distance between substrate and camera in each picture (~50 cm). To analyse substrate colour, we used the Multispectral Image Calibration and Analysis (MICA) Toolbox plugin (version 2.2.2) (Troscianko & Stevens, 2015) for ImageJ (version 1.54d). Images were normalised with the 9 % grey standard and converted into 32-bit multispectral images. For each image, we selected one or more regions of interest (ROIs), selecting only areas in the photo that were in the same plane as the grey standard and therefore had the same exposure. All images were then batch-processed using a custom-written routine for MICA in ImageJ (John *et al.*, 2023). Normalised images were converted to cone catches using the spectral sensitivity of the camera and of the modelled observer, and the spectra used during photography and as a model illuminant, which were measured in our sampling location at 6 m depth (Bitton *et al.*, 2017). We then measured cone catches for the ROIs in the image. The first two modelled observers were the prey fishes

Tripterygion delaisi (Santon *et al.*, 2020, 2021)(cone sensitivities peaking at 468, 517 and 530 nm (Bitton *et al.*, 2017) cone ratio 0.25:1:1 (Fritsch *et al.*, 2017)) and *Pomatoschistus flavescens* (cone sensitivities peaking at 456, 531 and 553 nm (Utne-Palm & Bowmaker, 2006), cone ratio 0.72:1:0.6 (Green *et al.*, 2019)), while the third was a conspecific scorpionfish *Scorpaena porcus* (cone sensitivities peaking at 455 and 530 nm (Govardovskii & Zueva, 1988; Schweikert *et al.*, 2018), cone ratio 1:1 (Lyall, 1957)). The spectral sensitivity of *S. maderensis* is not known, so we assumed it to be similar to the one of *S. porcus*. For all observers, we assumed a Weber fraction of 0.05 for the most abundant cones and for the luminance channel (defined as average cone catches of the two longer wavelength sensitive cones (John *et al.*, 2023; Lythgoe, 1979)) (Champ *et al.*, 2016; Olsson *et al.*, 2018). We used the Receptor Noise Limited model (Vorobyev & Osorio, 1998) in the R (version 4.1.1) (R Core Team, 2021) package pavo (Maia *et al.*, 2019) to calculate mean achromatic and chromatic contrasts between each substrate type using the cone catches measured from the images for each colour channel and the luminance channel, specifying the weber fraction and cone ratios of each observer as described above (John *et al.*, 2023). Contrasts are reported as Just Noticeable Differences (JND), where values below one JND indicate an indistinguishable contrast under optimal viewing conditions, and values above one indicate an increased probability of detection (Siddiqi *et al.*, 2004; Vorobyev & Osorio, 1998). Three JND are often considered as a more conservative approach to interpret results because a detection threshold of one JND is unlikely under natural viewing conditions (Abernathy *et al.*, 2017; Siddiqi *et al.*, 2004; da Silva *et al.*, 2020; Stevens *et al.*, 2014). Additionally, we used previously published data on scorpionfish colouration to calculate contrast between different individual scorpionfish and each of the natural substrates from the visual perspective of the three observers. Scorpionfish photos were taken in a previous study of *S. maderensis* and *S. porcus* on a grey experimental background (n = 20 *S. maderensis* and n = 30 *S. porcus*), and converted to cone-catch models using the same procedure as described above (John et al., *accepted*). These photos were taken under a D65 spectrum but converted using the light spectrum measured at 6m depth as model illuminant. Using the same procedure described above, we calculated contrast between the average scorpionfish body colouration and each sample of natural substrates (46 natural substrate photos in total).

2. Background choice

Experimental backgrounds

Luminance experiment

In the first choice experiment, we tested whether scorpionfish prefer a background with a perceived luminance that is more similar to their average body luminance, compared to a darker and lighter background. We restricted the testing to backgrounds varying in luminance because scorpionfish change luminance more than colour (John et al. 2023), and because we suspected that from their own perspective, they match colours in their natural habitat well (see methods section 1), which was confirmed by the outcome of this study (see results section 1). Scorpionfish were tested in three choice treatments, choosing between two backgrounds each, giving all combinations of three backgrounds: a light, medium and dark grey background (Figure 1A). To create the experimental backgrounds, we took standardised photos of a grey scale under experimental light conditions and calculated average luminance of the grey values from the perspective of the scorpionfish visual system (Govardovskii & Zueva, 1988; John *et al.*, 2023; John *et al., accepted*; Schweikert *et al.*, 2018). We then calculated the weber contrast of average scorpionfish body luminance when adjusted to a light, medium, and dark grey background (data from: John *et al.*, 2023; John *et al., accepted*) against each grey value of the scale. For the *medium* background, we chose the grey value that had the smallest weber contrast (0.03) to the medium-grey adapted scorpionfish. The *light* and *dark* backgrounds were instead created to be too light and dark to be matched by scorpionfish body luminance. Their grey values had the same high weber contrast (0.33) to the light- or dark-adapted scorpionfish. The central starting zone and the acclimation cylinder had an intermediate luminance between each combination of the two experimental backgrounds.

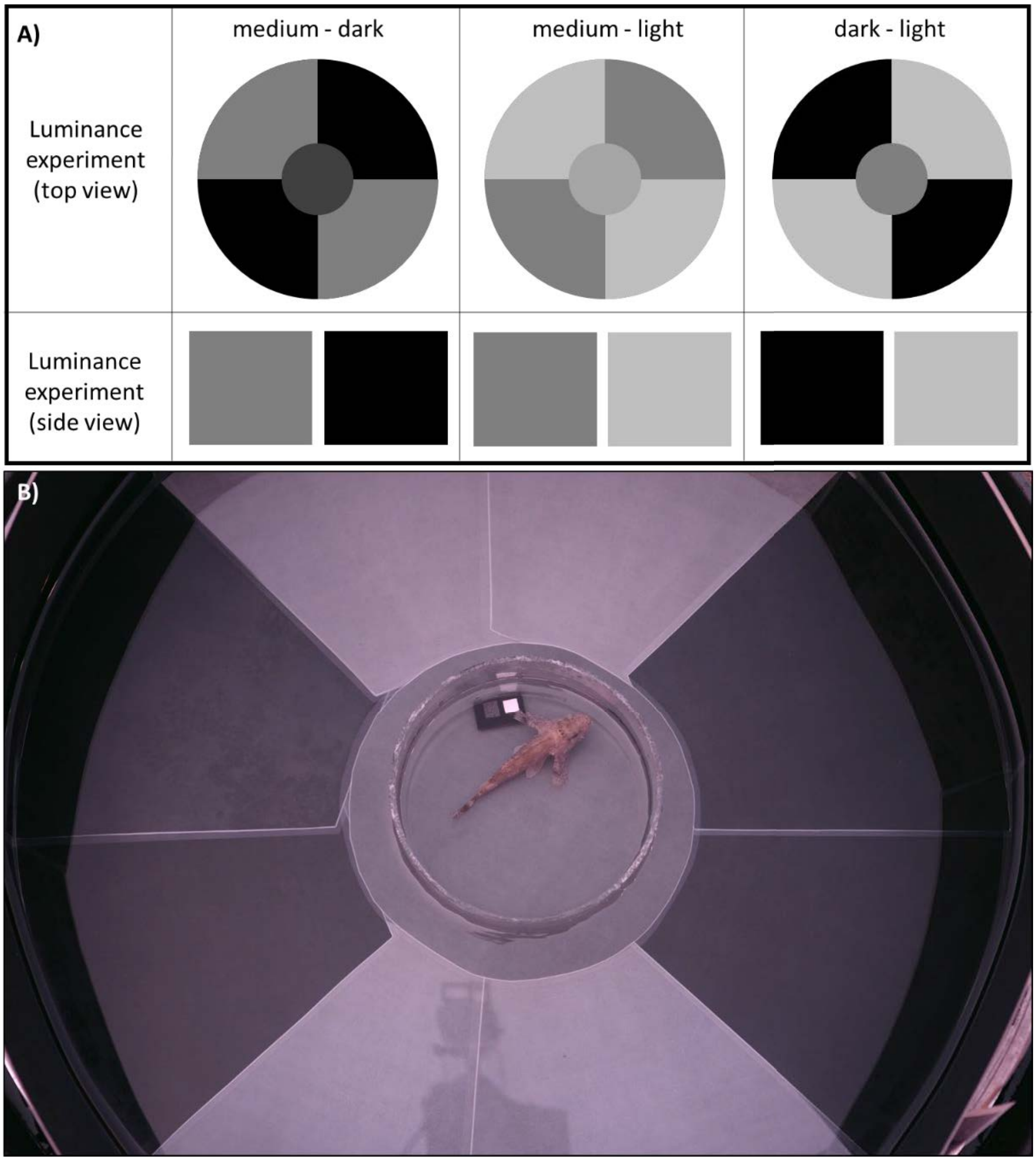

Figure 1: A) Schematic view of the three choice treatments in the luminance experiment. The circular area in the centre represents the starting zone. B) Exemplary photo of the setup, choice treatment ‘medium-dark’. The scorpionfish is in the acclimation cylinder without the opaque walls inserted and can watch the setup before being released.

Complexity experiment

Here, we tested whether scorpionfish prefer the more complex background when given a choice between two different levels of background pattern complexity. Background complexity can be defined by many different properties such as diversity of shapes, variability in edge orientation and luminance, or colour heterogeneity (Dimitrova & Merilaita, 2010; Rowe *et al.*, 2021). We designed all backgrounds to have the same average luminance, colour and contrast, but added a pattern including bars of different orientation. Scorpionfish have a body pattern with bars (John *et al., accepted*) and we used average width of these bars to design the rectangles used in our backgrounds (0.3 x 0.07 cm for *S. maderensis* and 0.3 x 0.09 cm for *S. porcus*, both bar sizes were equally frequent and randomly distributed on all backgrounds). All backgrounds had the same mean grey value equal to the medium grey in the luminance experiment, and the same number of bars per area in a lighter grey. All bars also had an identical (rectangular) shape, but their orientation differed, creating complex and simple backgrounds by altering edge orientation. We designed the complex background by having bars at randomly differing orientations, and the two simple backgrounds with bars equally oriented. Because scorpionfish might prefer backgrounds with a pattern that is similar to their own, one simple background featured vertical bars that would match the orientation of the bars in the body pattern of the scorpionfishes, while the other simple background had horizontal bars as a control. Scorpionfish tend to settle against vertical structures (the wall in the setup), and therefore would be viewed either from the side, matching the vertical bars of the wall, or from the top, matching the vertical bars of the bottom (Figure 2A). Scorpionfish were tested in three choice treatments, choosing between two backgrounds each, giving all combinations of three backgrounds: with random, vertical or horizontal bar orientation. We expected scorpionfish to preferentially settle on backgrounds with random bar orientation if they preferred complex over simple backgrounds. In case of a preference for pattern matching, we expected a preference for the backgrounds with vertical bar orientation instead.

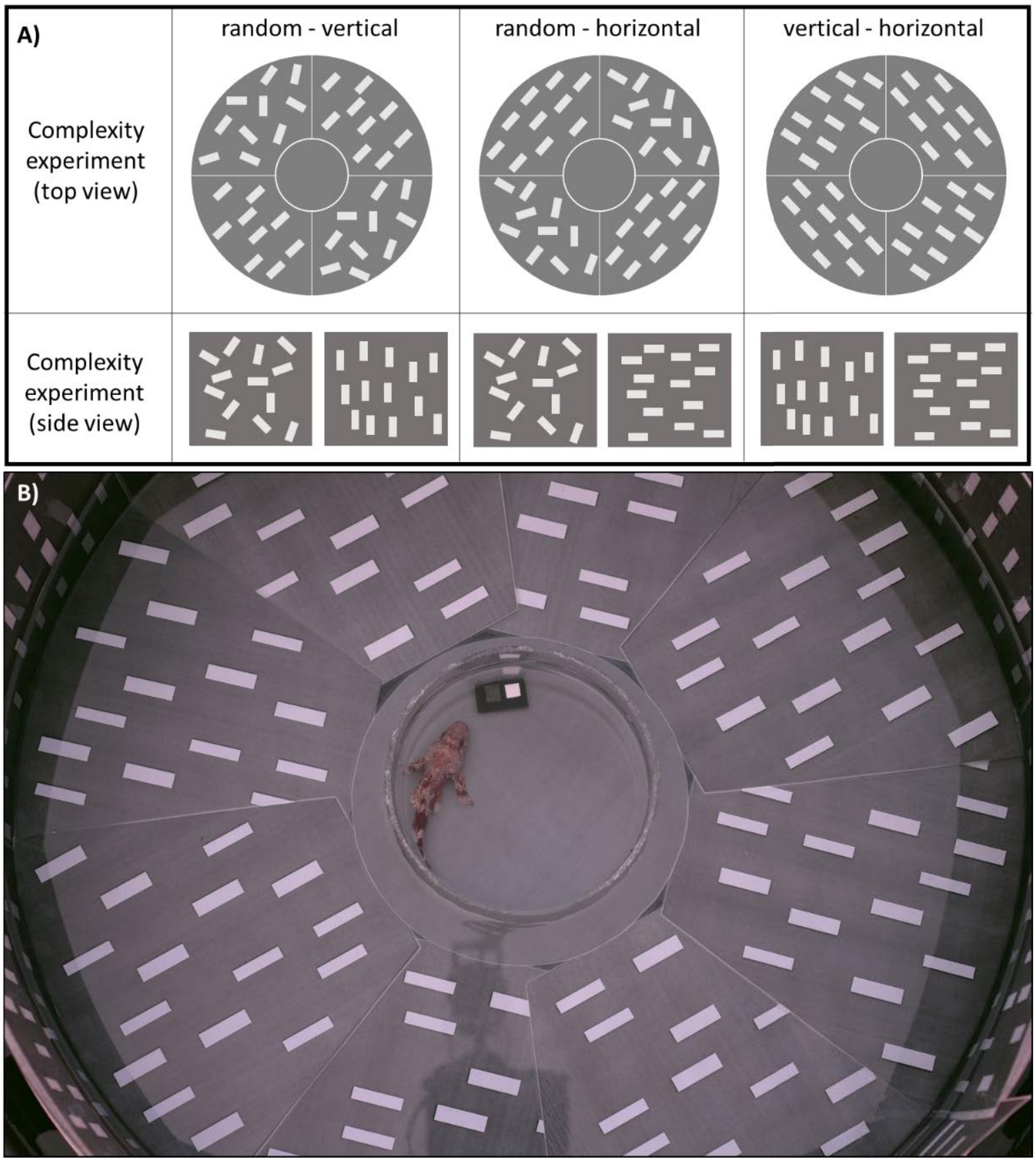

Figure 2: A) Schematic view of the three choice treatments in the complexity experiment. The circular area in the centre represents the starting zone. Note that the patterns are not to scale. B) Exemplary photo of the setup in the complexity experiment, choice treatment ‘vertical-horizontal’. The scorpionfish is in the acclimation cylinder without the opaque walls inserted and can watch the setup before being released.

Experimental setup and procedure

Experiments were carried out at the Station de Recherches Sous-marines et Océanographiques (STARESO), Corsica, France, in June and July 2023. Scorpionfish were caught under the station’s general sampling permit, using hand nets while SCUBA diving in depths of 2 to 10 m. We followed the EU animal welfare legislation’s directive (Directive

2010/63/EU) to ensure that our research was not likely to cause pain, suffering, distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle in accordance with good veterinary practice. Scorpionfish were kept in shaded outside flow-through seawater tanks (210 × 120 × 50 cm^3) exposed to natural light before and in between experiments. Each individual was tested in two independent experiments (details below) with at least one day between them. All individuals were eventually returned to the field. We tested 23 *S. maderensis* and 27 *S. porcus*.

We used three identical arenas made from plant pot saucers (60 cm diameter and 9 cm height, Primavera 70, Plastkon product s.r.o.) with inserted plastic cylinders (60 cm diameter and 21 cm height) as walls. The bottom and walls were covered with printed and laminated paper (matte laminating pouches 125 micron, no. S-PP525-22, PRT GmbH). For the luminance experiment, backgrounds were printed by WiesingerMedia GmbH (Tübingen). For the complexity experiment, backgrounds were printed on underwater paper (no. 3487, Avery Zweckform GmbH) with a laser printer (Kyocera ECOSYS P7240cdn KX). The setup was split into quadrants which were alternatingly covered with the two backgrounds (Figure 1). We chose this design to minimize possible side preference effects. The centre of the setup was a neutral starting zone (18 cm diameter). At the beginning of a trial, an acclimation cylinder (15 cm diameter, 10 cm height) of transparent plexiglass was placed in the starting zone. For the acclimation time, the cylinder had opaque inner walls of the same colour and luminance as the starting zone. Attached inside the cylinder were two grey standards (12 and 72 %) needed for calibrated photography (Figure 1B, 2B). Trials were filmed with a GoPro Hero 7, and RAW photographs were taken at the beginning and the end of trials with a calibrated Nikon D4 DLSR camera (Nikkor 60 mm macro lens). The camera was placed on a tripod in ~ 100 cm distance to the setup, looking down in a ~20 ° angle. The GoPro was mounted on top of the camera, looking down in approximately the same distance and angle. Setups were filled with fresh sea water before each trial (water level 9 cm). Starting a trial, a scorpionfish was caught from a housing tank using hand nets and transferred to the setup in a white bucket. After that, the

GoPro recording was started, and the scorpionfish gently transferred into the acclimation cylinder. Scorpionfish were allowed to acclimate for one minute. Then, the opaque walls of the acclimation cylinder were lifted, allowing the scorpionfish to see the rest of the setup for one minute. Photos of the fish were taken before and after this one minute acclimation. The acclimation cylinder was then lifted and the scorpionfish filmed for another 10 minutes. Then, the GoPro recording was stopped and fish were pulled towards the centre using the transparent acclimation cylinder. Fish were photographed immediately when positioned in the centre (back in the starting zone). Catching the fish with the cylinder, pulling it into the starting zone and taking a photo took less than five seconds, a time in which fish should not have been able to change colour again (John *et al.*, 2023). The scorpionfish were then placed back into the bucket with a hand net, either to be transferred to take the next choice treatment, or returned into the housing tank. The order of the two experiments and three choice treatments per experiment was balanced across individuals. Setup orientation was alternated and balanced across individuals for each choice treatment.

Behavioural data analysis

To analyse background preferences from the videos, we used the program BORIS (version 8.20.4) (Friard & Gamba, 2016). We measured how much time each individual settled on each background, only including events where scorpionfish did not move for at least five seconds (*duration settled*). If a scorpionfish was sitting on both backgrounds, we considered the background under its eyes as the one chosen. To understand whether there was a difference in the *duration settled* and the events where scorpionfish did not move for an extended period of time, we looked at an additional variable: we noted when an individual for the first time settled on a background and did not move for at least one minute (choice variable *first settled*). Because *duration settled* showed the same pattern as *first settled*, we display only results for *first settled* (Figure S1).

3. Background matching in luminance experiment

Because we found an unexpected preference of the scorpionfish for dark backgrounds, we decided to look at fish colouration related to choice in more detail. Therefore, we converted the images taken of the scorpionfish after a trial to cone catches for the observer *Tripterygion delaisi* (see procedure described above in Methods section 1). Our photography and model illuminant were a D65 spectrum because this was the spectrum under which the behavioural experiment took place. As region of interest (ROI), we selected specific parts of the scorpionfish pattern to analyse perceived luminance of both type of patches. Both scorpionfish species have a pattern of alternating dark and light bars and we selected two patches within those bars close to the tail fin that were typically well distinguishable. We also selected eight 1 cm^2 samples per background from randomly selected photos and calculated achromatic contrast of scorpionfish body colouration and the background that they chose per trial (for details on contrast calculation see Methods section 1). We moreover calculated achromatic contrast of both patches within one individual's pattern as an estimate of internal pattern contrast.

Statistical analysis

We used the glmmTMB package (Brooks *et al.*, 2017) to implement generalised linear mixed models for 1. achromatic and chromatic contrast of scorpionfish against natural substrates, 2. choice in both behavioural experiments and 3. scorpionfish body colouration parameters on the chosen background following a guided linear modelling routine for R (Santon *et al.*, 2023). The routine guides through model assessment inspecting the distribution of randomized quantile residuals, computed with the R-package DHARMa (Hartig, 2022), within and among factor predictor levels that were included or not in the models, and performing posterior predictive checks to assess model dispersion and overall model fit. We chose family distribution based on the nature of the response variable. We use the *r2* function of the performance package (Lüdecke *et al.*, 2021) to report marginal and conditional R^2 as a measure of fit for each model (Nakagawa & Schielzeth, 2013). We graphically report model predicted medians and their 95 % compatibility intervals (CIs) calculated from the posterior distributions of fitted values obtained from 10,000 sets of model parameters (Brooks *et al.*, 2017).

1. *Natural habitat sampling and background matching*

We implemented one model per scorpionfish species, both using a Gamma distribution (link = log) for the response variables *achromatic contrast* and *chromatic contrast*. We specified *substrate type* and *observer* and their interaction as fixed effects. We used *fish ID* and *photo ID* as random factors.

2. *Background choice*

From the initial 23 *S. maderensis* and 27 *S. porcus*, some individuals never settled on any experimental background. Therefore, we included only observations from 23 *S. maderensis* and 22 *S. porcus* individuals for the luminance experiment, and for 23 individuals for both species for the complexity experiment. To analyse a potential preference statistically, we transformed the variable *first settled* into a hypothesis-driven success/failure variable that indicated whether an individual first settled on the background we expected it to settle (1) or on the opposite background (0) (Table 1).

Table 1: Expectations of scorpionfish preference to settle on one of the backgrounds in each choice treatment.

	Choice treatment	**Success / Expected (1)**	**Failure / Unexpected (0)**
Luminance experiment	dark - light	dark	light
	medium - light	medium	light
	medium - dark	medium	dark
Complexity experiment	vertical - horizontal	vertical	horizontal
	complex - horizontal	complex	horizontal
	complex - vertical	complex	vertical

We implemented both models using a binomial distribution (link = logit) for the response variable *first settled (success/failure)* and had scorpionfish *species* and *choice treatment* and their interaction as fixed effects. We defined *fish ID* as random factor. A preference for one of two backgrounds can be assumed when the CIs are completely on that side of the probability scale, excluding the (random) probability of 0.5. To describe a preference or random choice better, we calculated median differences of choice to the random choice threshold 0.5. Differences and their 95% compatibility intervals (CIs) are calculated by subtracting the random choice threshold of 0.5 from estimates from 10,000 simulations of the posterior distribution of model parameters. CIs including zero indicate a random choice.

3. *Background matching in luminance experiment*

Because we wanted to understand how different patches in the scorpionfish body colouration contribute to camouflage, we analysed *luminance channel cone catches*, *achromatic contrast* against the backgrounds that fish chose, meaning the background they first settled on in each choice treatment, and *internal pattern contrast* of an individual on the chosen background. We excluded cases where individuals preferred the light background because of the low sample size. This left us with a total of 41 preferences for the dark background by *S. maderensis* and 34 by *S. porcus*, and 18 preferences for the medium background by *S. maderensis* and 20 by *S. porcus*. While inspecting the data we did not find an effect of choice treatment on scorpionfish body colouration and therefore did not include this factor into statistical analysis. We implemented models using a Gamma distribution (link = log) for the response variables *luminance channel cone catches*, *achromatic contrast* and *internal pattern contrast*. We specified scorpionfish *species*, *patch* and *chosen background* and their interaction as fixed effects. We used *fish ID* as random factor.

Results

1. Natural habitat sampling and background matching

From a scorpionfish's visual perspective, all substrates differed from each other with a chromatic contrast of more than one Just Noticeable Difference (JND) (Table 2). However, only the seagrass stems differed clearly from the other substrates with values above three JND.

Table 2: Chromatic contrast (JND) (mean ± SD) from the visual perspective of a scorpionfish, for all comparisons of natural substrates in the contrast analysis.

	Rubble and sand	Seagrass leaves	Turf algae	Red sponge	Yellow algae	Seagrass stems
	Scorpionfish view					
Rubble and sand	-					
Seagrass leaves	1.36 ± 1.42	-				
Turf algae	1.23 ± 1.33	1.90 ± 1.04	-			
Red sponge	2.05 ± 1.01	2.23 ± 0.85	2.28 ± 0.77	-		
Yellow algae	3.11 ± 1.35	3.91 ± 1.96	2.38 ± 1.08	3.29 ± 0.65	-	
Seagrass stems	8.22 ± 4.84	9.03 ± 5.12	7.50 ± 4.76	8.34 ± 4.70	5.37 ± 4.51	-

Both species showed low chromatic contrast below the detection threshold of one JND on rubble and sand from the scorpionfish and triplefin perspective, on seagrass leaves from triplefin perspective and on the red sponge from scorpionfish perspective (Table 3A). When considering a detection threshold of three JND, scorpionfish matched the colour of seagrass leaves and turf algae well (Table 3A). Achromatic contrast was above detection threshold of one and three JND for both species on all substrates for all observers (Table 3B).

Table 3: A) Chromatic and B) achromatic contrast of *Scorpaena maderensis* and *S. porcus* average colouration on different natural substrates as perceived by three natural observers. Estimates are based on individual calculations of Just Noticeable Difference (JND) of scorpionfish colouration (n = 20 *S. maderensis* and n = 30 *S. porcus* measured, adapted to a grey background) against each sample. Estimates are predicted medians and 95% compatibility intervals (CIs) derived from 10,000 simulations of the posterior distribution of model parameters. Values below one JND indicate optimal background matching. Values where the CIs include one or three JND are highlighted in bold and underlined respectively.

A) Chromatic contrast

Substrate (*n* photos)	**Scorpionfish view**		**Triplefin view**		**Goby view**	
	Estimate	CIs	Estimate	CIs	Estimate	CIs
	Scorpaena maderensis (R^2_{cond} = 0.910, R^2_{marg} = 0.698)					
Rubble and sand (13)	**1.12**	**0.90, 1.38**	**1.09**	**0.88, 1.35**	1.58	1.27, 1.95
Seagrass leaves (9)	1.34	1.04, 1.73	**1.17**	**0.91, 1.51**	1.77	1.38, 2.29
Turf algae (9)	2.35	1.82, 3.04	2.19	1.70, 2.83	3.36	2.60, 4.36
Red sponge (3)	**1.50**	**0.97, 2.29**	3.91	2.53, 5.96	4.24	2.74, 6.47
Yellow algae (6)	4.96	3.64, 6.75	3.85	2.82, 5.22	6.66	4.87, 9.04
Seagrass stems (6)	9.40	6.95, 12.78	7.03	5.19, 9.54	12.10	8.91, 16.44
	Scorpaena porcus (R^2_{cond} = 0.902, R^2_{marg} = 0.710)					
Rubble and sand (13)	**0.96**	**0.77, 1.19**	**0.93**	**0.75, 1.15**	1.36	1.10, 1.68
Seagrass leaves (9)	**1.22**	**0.94, 1.58**	**1.02**	**0.79, 1.33**	1.58	1.22, 2.04
Turf algae (9)	2.10	1.63, 2.72	1.98	1.53, 2.55	3.03	2.34, 3.92
Red sponge (3)	**1.42**	**0.90, 2.19**	3.68	2.35, 5.67	4.00	2.55, 6.17
Yellow algae (6)	4.69	3.44, 6.41	3.62	2.65, 4.94	6.30	4.63, 8.60
Seagrass stems (6)	9.08	6.63, 12.47	6.78	4.95, 9.30	11.69	8.51, 16.01

B) Achromatic contrast

Substrate (*n* photos)	**Scorpionfish view**		**Triplefin view**		**Goby view**	
	Estimate	CIs	Estimate	CIs	Estimate	CIs
	Scorpaena maderensis (R^2_{cond} = 0.950, R^2_{marg} = 0.117)					
Rubble and sand (13)	10.24	7.05, 14.90	10.42	7.19, 15.23	10.01	6.87, 14.55
Seagrass leaves (9)	12.49	7.92, 19.76	12.76	8.11, 20.24	12.28	7.80, 19.49
Turf algae (9)	10.40	6.61, 16.21	10.49	6.66, 16.28	10.38	6.61, 16.12
Red sponge (3)	15.98	7.46, 34.35	17.84	8.35, 38.61	13.10	6.16, 28.33
Yellow algae (6)	8.89	5.13, 15.22	9.44	5.45, 16.13	7.91	4.55, 13.51
Seagrass stems (6)	26.11	15.08, 45.67	17.75	10.28, 30.81	15.81	9.16, 27.71
	Scorpaena porcus (R^2_{cond} = 0.882, R^2_{marg} = 0.135)					
Rubble and sand (13)	8.93	6.23, 12.75	9.07	6.35, 13.00	8.75	6.10, 12.49
Seagrass leaves (9)	11.15	7.20, 17.26	11.40	7.36, 17.71	10.98	7.10, 17.01
Turf algae (9)	10.91	7.09, 16.71	11.00	7.14, 16.79	10.88	7.06, 16.63
Red sponge (3)	12.92	6.19, 26.64	14.23	6.77, 29.28	11.91	5.68, 24.62
Yellow algae (6)	6.46	3.82, 10.80	6.85	4.04, 11.43	5.82	3.43, 9.75
Seagrass stems (6)	23.51	13.86, 40.00	14.54	8.58, 24.72	12.89	7.60, 21.90

2. Background choice

Both species preferred the dark over the light or medium background and the medium over the light background when they first settled (Figure 4A, Table 4A). Both species showed a random choice for backgrounds differing in complexity and edge orientation when they first settled for at least one minute (Figure 4B, Table 4B).

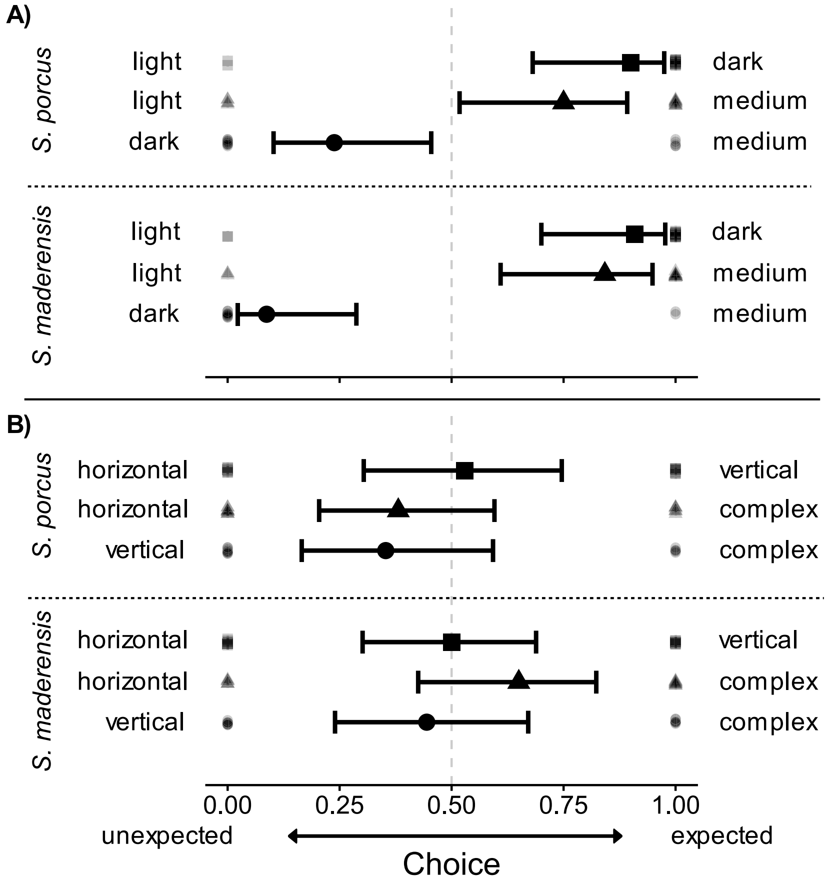

Figure 3: Choice of *Scorpaena maderensis* and *S. porcus* for all three background combinations in A) the luminance and B) the complexity experiment. Choice was defined by a scorpionfish settling for at least one minute for the first time. Points represent choice of each individual (n = 23 *S. maderensis*, n = 22 *S. porcus* for A) the luminance experiment and n = 23 *S. maderensis*, n = 22 *S. porcus* for B) the complexity experiment, note that not every individual settled in every choice treatment) and were scored with 1 for a choice that was expected by our hypothesis and with 0 for a choice against our hypothesis. Markers with horizontal bars represent predicted medians and 95% compatibility intervals (CIs) derived from 10,000 simulations of the posterior distribution of model parameters. The dashed line indicates the random choice threshold 0.5. CIs excluding 0.5 indicate a non-random choice.

Table 4: Median differences of choice to the random choice threshold 0.5 for A) the choice treatments in the luminance experiment (n = 23 *S. maderensis*, n = 22 *S. porcus*) and B) the choice treatments in

the complexity experiment (n = 23 *S. maderensis*, n = 23 *S. porcus*). Differences and their 95% compatibility intervals (CIs) are calculated by subtracting the random choice threshold of 0.5 from estimates from 10,000 simulations of the posterior distribution of model parameters. CIs excluding zero indicate a non-random choice and are highlighted in bold.

	S. maderensis		*S. porcus*	
Choice treatment	Estimate	CIs	Estimate	CIs
A) Luminance experiment (R^2_{cond} = 0.499)				
dark - light	**0.41**	**0.19, 0.48**	**0.40**	**0.18, 0.47**
medium - light	**0.34**	**0.10, 0.45**	**0.25**	**0.02, 0.39**
medium - dark	**-0.41**	**-0.48, -0.21**	**-0.26**	**-0.40, -0.04**
B) Complexity experiment (R^2_{cond} = 0.048)				
vertical - horizontal	0.00	-0.20, 0.19	0.03	-0.19, 0.24
complex - horizontal	0.15	-0.07, 0.32	-0.12	-0.30, 0.10
complex - vertical	-0.06	-0.26, 0.17	-0.15	-0.33, 0.09

3. Background matching in luminance experiment

S. maderensis decreased the luminance of both their dark and light patches when choosing the dark over the medium background (Figure 5A, Table 5A). *S. porcus* only decreased the luminance of their dark patch (Figure 5A, Table 5A). When calculating achromatic contrast of dark and light patches in the fish colouration to the background that fish preferred, lowest achromatic contrast was found for the dark patches on the dark background and for the light patches on the medium background (Figure 5B, Table 5B). *S. maderensis* had a similar internal contrast regardless of their preferred background while *S. porcus* reduced internal contrast on the medium compared to the dark background (Figure 5C, Table 5C).

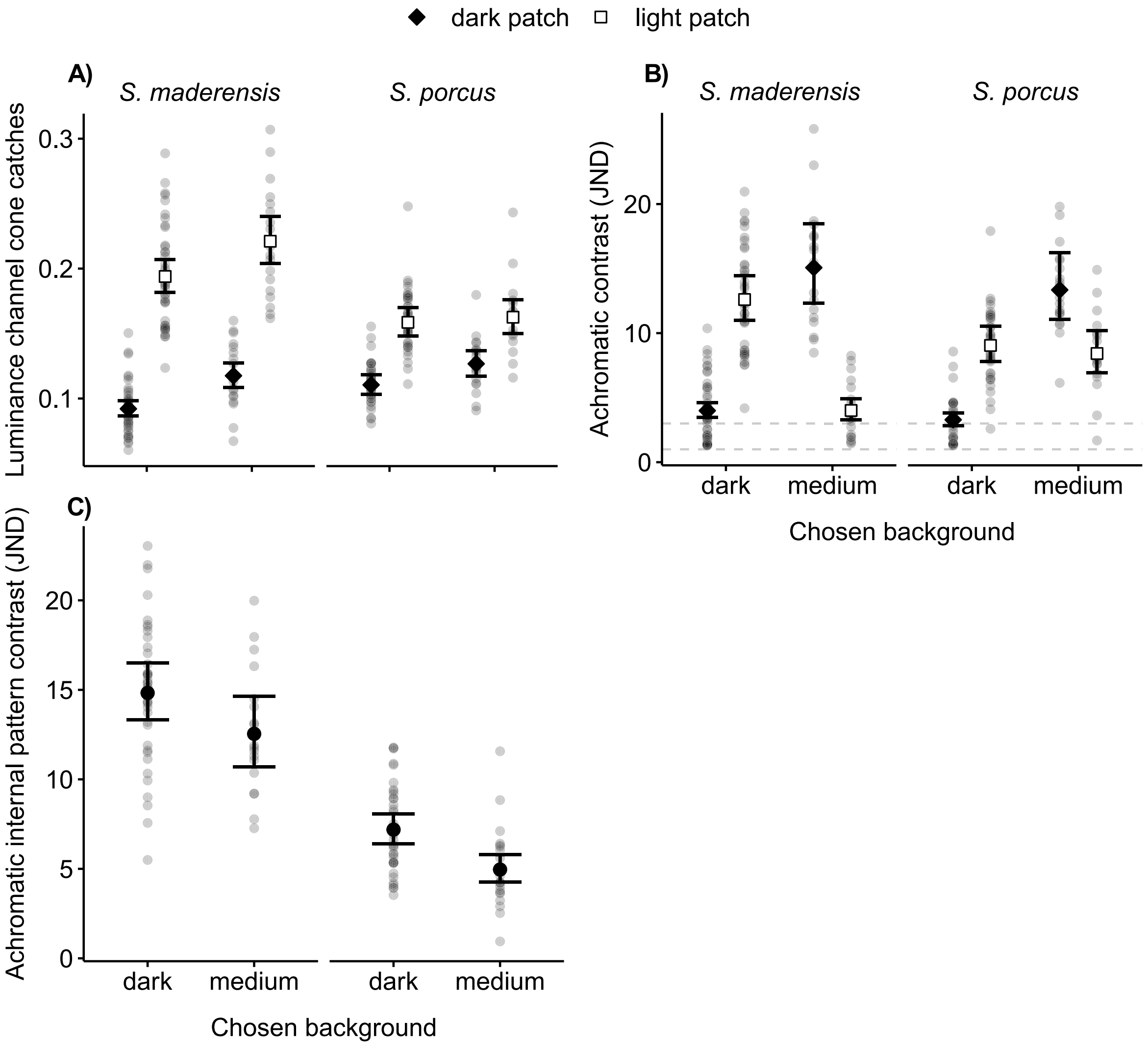

Figure 4: Body colouration of *Scorpaena maderensis* and *S. porcus* after choosing a background in the luminance experiment as perceived by *Tripterygion delaisi*. A) Luminance channel cone catches of the two selected patches of body colouration, and achromatic contrast as Just Noticeable Differences (JND) of the two patches B) against the preferred background and C) against each other. The dashed lines (B) indicate one and three JND. Colour and shape of the markers indicate the body colouration patch (A, B). Grey points represent individual cone catches (A) or JND (B, C). Markers with vertical bars represent predicted medians and 95% compatibility intervals (CIs) derived from 10,000 simulations of the posterior distribution of model parameters. Cases where the light background was preferred were excluded because of the low number of individuals that showed this preference (n = 23 *S. maderensis*, n = 22 *S. porcus* tested in the luminance experiment in three choice treatments).

Table 5: Pairwise contrasts for body colouration parameters of *Scorpaena maderensis* and *S. porcus* as perceived by *Tripterygion delaisi*, comparing parameters between the preferred backgrounds. Contrasts expressed as response ratios of A) luminance channel cone catches of the two selected patches of body colouration between the dark and medium background, and achromatic contrast as Just Noticeable Differences (JND) of the two patches B) against the preferred background and C) against each other, between the dark and medium background. Effect size is proportional to the deviation of ratios from one, and the robustness of the result increases with decreasing degree of overlap of the 95% compatibility intervals (CIs) with one. Response ratios with CIs excluding one are highlighted in bold. N = 23 for *S. maderensis* and N = 22 for *S. porcus*.

	S. maderensis		*S. porcus*	
	Estimate	CIs	Estimate	CIs
A) Luminance channel cone catches – dark vs. medium background preferred (R^2_{cond} = 0.847, R^2_{marg} = 0.722)				
dark patch	**0.78**	**0.73, 0.85**	**0.87**	**0.81, 0.94**
light patch	**0.88**	**0.81, 0.95**	0.98	0.90, 1.05
B) Achromatic contrast to background – dark vs. medium background preferred (R^2_{cond} = 0.647, R^2_{marg} = 0.632)				
dark patch	**0.26**	**0.21, 0.34**	**0.25**	**0.19, 0.31**
light patch	**3.15**	**2.48, 4.00**	1.07	0.85, 1.36
C) Achromatic internal pattern contrast – dark vs. medium background preferred (R^2_{cond} = 0.647, R^2_{marg} = 0.616)				
internal contrast	1.18	0.98, 1.42	**1.45**	**1.21, 1.74**

Discussion

Natural habitat sampling and background matching

Under perfect viewing conditions, scorpionfish should be able to differentiate between all substrate types as their chromatic contrast is above one Just Noticeable Difference (JND). However, under natural viewing conditions, it is unlikely that such low contrast values can be routinely detected. Therefore, other studies suggest to consider more conservative perception thresholds such as e.g. three JND to interpret results (Abernathy *et al.*, 2017; Siddiqi *et al.*, 2004; da Silva *et al.*, 2020; Stevens *et al.*, 2014). Considering such a conservative approach, four out of the six substrates had a similar colour from scorpionfish visual perspective. In an environment where common substrates vary but are similar relative to only a few substrates standing out strongly, a generalist body colouration might be promoted (Merilaita *et al.*, 1999).

Scorpionfish had a chromatic contrast below one JND only on few natural substrates and only from the visual perspective of the triplefin or conspecifics in the modelled depth at 6 m at mid-day light conditions. However, considering the conservative approach, scorpionfish matched several common substrate types, also from the perspective of the goby. Animals with a generalist body colouration typically match no or only few backgrounds perfectly, but show a sub-optimal match on many backgrounds (Hughes *et al.*, 2019). Our results support this idea for scorpionfish when looking at chromatic contrast only. However, looking at average achromatic contrast, scorpionfish did not match any of the substrates well. This was primarily caused by a high variation of perceived luminance of the natural substrates. Substrate luminance perception is influenced strongly by variable factors such as weather conditions, shading and texture of a substrate, which explains why achromatic contrast of scorpionfish against the substrates was high. Possibly it is also the reason why dynamic changes in luminance in these fishes are stronger than changes in hue (John *et al.*, 2023). We calculated scorpionfish contrast to the substrates with data of scorpionfish adjusted to a background of intermediate luminance (John et al., *accepted*). It is likely that they can achieve a lower achromatic contrast after adjustment to the background (John *et al.*, 2023).

Scorpionfish had a colour similar to rubble, sand and epiphytic growth on seagrass, all of which are common in the Mediterranean Sea. This could allow for reasonably good background matching on such substrates (Stevens & Merilaita, 2009a). But even on mismatching backgrounds scorpionfish could be camouflaged by means of masquerade, where animals appear like common, irrelevant objects to the viewer (Skelhorn, 2015). Scorpionfish have a complex skin texture including skin flaps and spines and typically sit motionlessly on the substrate. These features could enhance the masquerading effect and make the fish appear like an irrelevant object, for instance a rock overgrown with epiphytes.

Luminance experiment

In the choice experiment, scorpionfish preferentially settled on a darker background in all choice treatments. When choosing between a background of perceived luminance similar to their own body and a darker background, scorpionfish preferred the darker background and therefore potentially risked mismatch to the background. A preference for dark backgrounds was previously shown in several fish species (Bradner & McRobert, 2001; Kjernsmo & Merilaita, 2012; Smithers *et al.*, 2018) and might be linked to an escape response that scorpionfish showed in the setup. Individuals might have chosen the background that most resembled shelter in their natural habitat, i.e. dark cracks and crevices for hiding. We cannot exclude that in a foraging context under natural conditions, scorpionfish instead prefer to settle on backgrounds that are most similar to their own average body luminance. Nevertheless, we can conclude that scorpionfish do not settle randomly, but actively choose between backgrounds. Moreover, we used average scorpionfish colour to calculate background match, even though scorpionfish have a patchy and contrasting body pattern. Calculating background match of individual patches within the scorpionfish pattern revealed that their dark patches have the lowest achromatic contrast on the dark background. Therefore, scorpionfish may have preferred this background to improve camouflage through disruptive colouration. Here, the different patches could allow differential blending and in *S. porcus*, the increased internal contrast indicates aiming for maximum disruptive contrast (Stevens & Merilaita, 2009b).

Disruptive colouration is a more generalist camouflage strategy compared to background matching, because it offers crypsis on a wider range of backgrounds (Cuthill *et al.*, 2005; Phillips *et al.*, 2017; Price *et al.*, 2019; Robledo-Ospina *et al.*, 2017). Taking together the aspects of chromatic background matching of natural substrates, achromatic background choice, and disruptive colouration, scorpionfish seem to be generalists that can settle differently coloured substrates and use their colour change and possibly background choice to cope with luminance variation in their habitat.

Complexity experiment

Scorpionfish did not differentiate between backgrounds of different complexity when settling. While it is possible that scorpionfish do not exhibit a preference for more complex backgrounds, it is also likely that our experimental design failed to elicit a response. Our backgrounds only differed in the degree of edge orientation. It is possible that the difference in complexity was too weak or created by the wrong features, since visual complexity is normally defined by multiple factors (Dimitrova & Merilaita, 2010; Rowe *et al.*, 2021). Moreover, in the natural environment, substrate complexity can be assessed not only by visual cues but possibly also by tactile and olfactory cues. It is possible that scorpionfish indeed have a preference to settle on more complex backgrounds, but that their choice is guided by more than the visual sense (Stevens & Ruxton, 2019). Experiments in the natural environment could help to better understand whether there is a background preference in scorpionfish and what cues define it.

Species comparison

We tested two congeneric scorpionfish species to understand potential differences and similarities in their body colouration and behaviour. Since both species are competing for the same habitat and share the same lifestyle, differences in camouflage strategies could indicate relevant niche partitioning (Stevens & Ruxton, 2019). However, we find similar results for both species. For the background choice experiments, we cannot exclude that under more natural conditions, we would see differences between the species. *S. maderensis* is a species known to be common around eastern Atlantic islands and only in specific areas in the Mediterranean, predominantly the southern Mediterranean (La Mesa *et al.*, 2005). The species was rarely present in our sampling location in the northern Mediterranean several years ago, but is common now (personal observations by NKM and STARESO). Understanding their potential to outcompete congeneric species like *S. porcus* is therefore of great interest.

Author contributions

All authors conceived the study. LJ collected and analysed the data. MS supported statistical analysis. LJ drafted the manuscript. All authors edited the manuscript and approved the final version.

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Supplementary

Table S1: Chromatic contrast (JND) (mean ± SD) from the visual perspective of the triplefin and the goby, for all comparisons of natural substrates that were included in the contrast analysis (section 1). Values below one and three JND are bold and underlined respectively.

	Rubble and sand	Seagrass leaves	Turf algae	Red sponge	Yellow algae	Seagrass stems
	Triplefin view					
Rubble and sand	-					
Seagrass leaves	**0.72 ± 0.83**	-				
Turf algae	**0.73 ± 0.55**	1.14 ± 0.56	-			
Red sponge	2.47 ± 0.44	2.85 ± 0.72	1.99 ± 0.35	-		
Yellow algae	2.34 ± 0.64	2.77 ± 0.28	1.70 ± 0.40	1.63 ± 0.50	-	
Seagrass stems	5.60 ± 1.77	6.02 ± 1.86	4.96 ± 1.71	4.06 ± 1.73	3.31 ± 1.72	-
	Goby view					
Rubble and sand	-					
Seagrass leaves	1.70 ± 1.81	-				
Turf algae	1.56 ± 1.49	2.48 ± 1.03	-			
Red sponge	3.34 ± 1.16	3.96 ± 1.31	3.22 ± 0.89	-		
Yellow algae	4.16 ± 1.50	5.25 ± 1.63	3.14 ± 1.17	3.88 ± 0.82	-	
Seagrass stems	10.34 ± 5.60	11.41 ± 5.84	9.31 ± 5.53	9.50 ± 5.48	6.49 ± 5.26	-

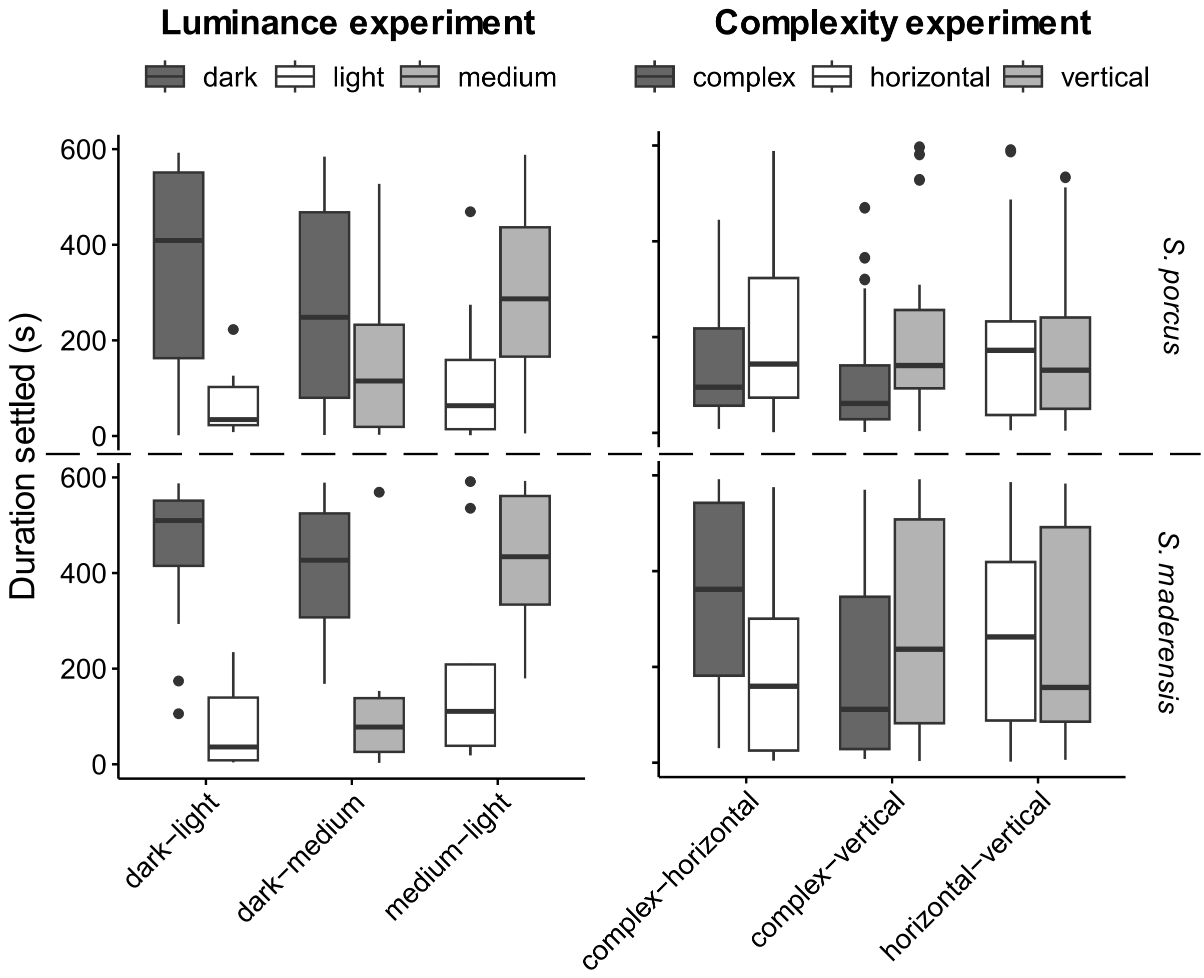

Figure S1: Absolute duration (seconds) that scorpionfish were settled on both backgrounds per background combination for the luminance experiment (left) and the complexity experiment (right), split by scorpionfish species (top row: *Scorpaena porcus*, bottom row: *S. maderensis*), background combination (x-axis) and background (colour).