



**This electronic thesis or dissertation has been  
downloaded from Explore Bristol Research,  
<http://research-information.bristol.ac.uk>**

*Author:*

**Vincent Venables, Sian**

*Title:*

**A new function for polarization vision**

*mitigation of visual noise from dynamic illumination*

**General rights**

Access to the thesis is subject to the Creative Commons Attribution - NonCommercial-No Derivatives 4.0 International Public License. A copy of this may be found at <https://creativecommons.org/licenses/by-nc-nd/4.0/legalcode>. This license sets out your rights and the restrictions that apply to your access to the thesis so it is important you read this before proceeding.

**Take down policy**

Some pages of this thesis may have been removed for copyright restrictions prior to having it been deposited in Explore Bristol Research. However, if you have discovered material within the thesis that you consider to be unlawful e.g. breaches of copyright (either yours or that of a third party) or any other law, including but not limited to those relating to patent, trademark, confidentiality, data protection, obscenity, defamation, libel, then please contact [collections-metadata@bristol.ac.uk](mailto:collections-metadata@bristol.ac.uk) and include the following information in your message:

- Your contact details
- Bibliographic details for the item, including a URL
- An outline nature of the complaint

Your claim will be investigated and, where appropriate, the item in question will be removed from public view as soon as possible.



**This electronic thesis or dissertation has been  
downloaded from Explore Bristol Research,  
<http://research-information.bristol.ac.uk>**

*Author:*

**Vincent Venables, Sian**

*Title:*

**A new function for polarization vision: mitigation of visual noise from dynamic illumination.**

**General rights**

Access to the thesis is subject to the Creative Commons Attribution - NonCommercial-No Derivatives 4.0 International Public License. A copy of this may be found at <https://creativecommons.org/licenses/by-nc-nd/4.0/legalcode> This license sets out your rights and the restrictions that apply to your access to the thesis so it is important you read this before proceeding.

**Take down policy**

Some pages of this thesis may have been removed for copyright restrictions prior to having it been deposited in Explore Bristol Research. However, if you have discovered material within the thesis that you consider to be unlawful e.g. breaches of copyright (either yours or that of a third party) or any other law, including but not limited to those relating to patent, trademark, confidentiality, data protection, obscenity, defamation, libel, then please contact [collections-metadata@bristol.ac.uk](mailto:collections-metadata@bristol.ac.uk) and include the following information in your message:

- Your contact details
- Bibliographic details for the item, including a URL
- An outline nature of the complaint

Your claim will be investigated and, where appropriate, the item in question will be removed from public view as soon as possible.

---

# **A new function for polarization vision: mitigation of visual noise from dynamic illumination**

---

Siân Vincent Venables

1640150

School of Biological Sciences

University of Bristol

A dissertation submitted to the University of Bristol in accordance with the requirements for award of the degree of Master of Science by Research in the Faculty of Life Sciences.

December 2021

Word count: 9910



## **Abstract**

Caustics are a visual hindrance for animals in shallow underwater environments. Downwelling light is refracted from surface waves onto the seafloor, creating bands of light that fluctuate in both time and space. This dynamic illumination creates a flickering environment that could cause problems for vision, for instance; preventing detection of prey, conspecifics or approaching predators. Maximov (2000) suggested that colour vision may have evolved to mitigate the impact of this unfavourable environmental condition. He suggests that the presence of multiple types of colour receptors and the antagonistic relationship between them could enable discrimination between an object and its background (based on spectral qualities), while ignoring the achromatic variations in illumination caused by caustics. If colour sensitivity was a result of living in habitats subject to water caustics, then polarization sensitivity may have evolved for a similar function. To explore this possibility, crabs (*Carcinus maenas*) and cuttlefish (*Sepia officinalis*) were presented with predator-like stimuli (an expanding disc on a screen) overlaid with video of either static or dynamic caustics. Dynamic caustics prevented stimulus detection and greatly reduced response probability, whereas static caustics did not. However, when the stimulus was presented with only a contrast in polarization (instead of in intensity), the ability to detect the expanding disc in dynamic caustics was greatly enhanced, resulting in a similar response probability in both caustic treatments. This study is the first to demonstrate that polarization vision reduces the negative impact of dynamic illumination underwater for visual tasks such as predator-detection.



## **Dedication**

I want to dedicate this thesis to my family and friends, for their continuous support and interest in my research; and especially to those who poked fun at me for putting crabs through their paces on treadmills.

## **Acknowledgements**

A huge thank you to Martin How for the constant support, guidance and positivity throughout my research, and for making my masters so enjoyable. Thank you also to Christian Drerup whom I collaborated with, and to the students and staff I met at the Marine Biological Association for making my stay in Plymouth so welcome and productive (especially Emily Sullivan, Emma Stuart, Kevin Atkins and Jasmine Somerville, for their assistance with cuttlefish care and experiments).

## **Collaboration**

Collaboration with Christian Drerup involved discussing the conceptual aspects and experimental design of my project. Christian ran separate and different experiments, using the same caustic playbacks and animals, however the experiments in this study were independently completed and analysed by myself.

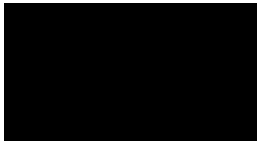




## **Author's Declaration**

I declare that the work in this dissertation was carried out in accordance with the requirements of the University's Regulations and Code of Practice for Research Degree Programmes and that it has not been submitted for any other academic award. Except where indicated by specific reference in the text, the work is the candidate's own work. Work done in collaboration with, or with the assistance of, others, is indicated as such. Any views expressed in the dissertation are those of the author.

SIGNED:



DATE: 22/12/2021



## **Table of Contents**

Abstract .....	2
Dedication and Acknowledgments .....	4
Collaboration.....	4
Author’s Declaration .....	6
Table of contents.....	8
List of Figures and Tables.....	9
<b>1. Introduction Chapter .....</b>	<b>11</b>
1.1 Introduction.....	11
1.2 Background.....	11
1.2.1 What is the polarization of light?.....	11
1.2.2 Where can the polarization of light be found and how do animals use it?.....	11
1.2.3 What are water caustics?.....	13
1.3 How do caustics affect the visual ecology of animals?.....	13
1.4 Visual sensitivity of crabs and cuttlefish.....	14
1.5 What do we know about the wavelength, intensity and polarization of caustics?.....	15
1.6 Could polarization vision help reduce the problem of caustics?.....	16
1.7 Aims of the current study and outline of the thesis structure.....	17
<b>2. Data Chapter.....</b>	<b>20</b>
2.1 Introduction.....	20
2.2 Methods.....	20
2.2.1 Animals.....	20
2.2.1.1 <i>Carcinus maenas</i> .....	20
2.2.1.2 <i>Sepia officinalis</i> .....	21
2.2.2 Experimental apparatus.....	21
2.2.2.1 <i>Carcinus maenas</i> .....	21
2.2.2.2 <i>Sepia officinalis</i> .....	23
2.2.3 Determining stimulus contrasts and the properties of caustics.....	25
2.2.4 Experimental protocol.....	26
2.2.4.1 <i>Carcinus maenas</i> .....	26
2.2.4.1.1 Experiment 1.....	26

2.2.4.1.2	Experiment 2.....	27
2.2.4.2	<i>Sepia officinalis</i> .....	27
2.2.4.2.1	Experiment 3.....	27
2.2.4.2.2	Experiment 4.....	28
2.2.5	Categorizing the response.....	28
2.2.6	Analysis.....	30
2.3	Results.....	30
<b>3.</b>	<b>Discussion Chapter.....</b>	<b>34</b>
3.1	Discussion.....	34
3.2	Summary.....	37
	<b>Bibliography.....</b>	<b>39</b>
	<b>Appendices.....</b>	<b>45</b>

**Figures and Tables**

<b>Figure 1.</b>	Screenshot images of both natural and artificial water caustics.....	16
<b>Figure 2.</b>	Photographs of the experimental set up.....	25
<b>Figure 3.</b>	Response curves illustrating the threshold of detection of a stimulus against either static or dynamic caustics.....	32
<b>Table 1.</b>	A description of each crab and cuttlefish behaviour counted as a positive response.....	29
<b>Table 2.</b>	The 50% sigmoid values and their corresponding 95% confidence intervals for the intensity and polarization stimuli.....	31



## **INTRODUCTION CHAPTER (1)**

### **1.1 Introduction**

Caustic flicker is a widespread form of visual noise within underwater habitats, that impedes visual tasks such as object-detection. The angle at which sunlight refracts through the water depends on the ever-changing shape and size of the surface wave, resulting in patterns of light falling on the sea floor that vary in both time and space. This fluctuation in illumination can prevent effective predator and prey detection, but this study aims to demonstrate how animals can mitigate this impact by exploiting an alternative modality of light: polarization. Despite caustics creating a visually-complex scene in terms of light intensity, approaching predators may still be detectable when viewed against a differently-polarized background. Determining this new function of polarization sensitivity may have a wider significance throughout the animal kingdom, and may even explain the evolution and origin of this type of vision.

### **1.2 Background**

#### **1.2.1 What is the polarization of light?**

Light is an electromagnetic wave, in which photons oscillate with both a magnetic and electric field perpendicular to the direction of travel (Foster et al. 2018). Polarization is a fundamental property of light, and refers to the orientation at which the electric field propagates as it moves through space. A beam of light from the sun consists of many waves propagating in different orientations, and this light has the lowest possible degree of polarization (DoP = 0). The degree of polarization is highest (DoP = 1) when all the photons oscillate on one plain with the same angle of polarization (AoP) or electric vector axis (e.g., a horizontal or vertical e-vector). Occasionally the e-vector of a photon can rotate either clockwise or anticlockwise around the axis of travel, and this light is circularly polarized (Foster et al. 2018).

#### **1.2.2 Where can the polarization of light be found and how do animals use it?**

Depending on the sun's position, a pattern of polarization can be seen in the sky. Atmospheric particles smaller than the wavelength of light (as well as fluctuations in air pressure) will scatter photons from the sun, so that under certain conditions a proportion of them propagate on the same axis (Rayleigh scattering, Young, 1982). As the position of the sun changes, the band of strongest polarization (90° from the sun) will move, allowing animals to use the sky and horizon for navigation and body-axis orientation purposes, even when the sun is obscured by cloud-cover (Foster et al. 2018). Many invertebrates have specialised ommatidia in the dorsal rim area (DRA) of their

compound eyes, comprising photoreceptors sensitive to the polarization of light (Labhart & Meyer 1999). The desert ant, *Cataglyphis fortis*, uses the celestial polarization pattern as a compass sense for path integration when foraging (Müller & Wehner, 2007). Moonlight is scattered in the same way as sunlight, with a higher DoP when a higher proportion of the moon's surface is reflecting light (Foster et al. 2019). Nocturnal species such as the South African dung beetle, *Escarabaeus lamarcki*, exploit this cue (along with other celestial cues) to navigate at night and roll dung in a straight line back to their burrow (El Jundi et al. 2015). However, these cues can be masked from anthropogenic light pollution, preventing animal navigation at night (Kyba et al. 2011).

Many surfaces, such as glossy leaves and still water will also cause the polarization of reflected light. The butterfly species, *Papilio aegus*, identifies horizontally polarized surfaces on foliage to use for their oviposition sites (Kelber et al. 2001, Blake et al. 2019). Many insects will use light reflected from bodies of water, as a cue for locating their aquatic habitat (Sharkey et al. 2015). Water skaters (*Gerridae sp.*) will use their polarization sensitivity to provide a better view down into the water column, by cutting out the reflected light from water's surface (Leggett, 1976). Man-made materials such as glass windows, buildings and roads will also produce polarized reflections, creating a sensory trap (Robertson & Horváth, 2019).

Underwater, there is a strong band of polarization perpendicular to the downwelling light entering and refracting through 'Snell's window' (Lynch, 2015). The water flea, *Daphnia pulex*, uses the surrounding level of polarization to gauge depth and migrate towards deeper water, to evade predators nearer the surface or nearer the shore (Schwind, 1999). The sediment load in the water column causes the same light-scattering effect that occurs in the atmosphere, and so to any species with the ability to perceive this, objects will appear as a contrasting unpolarized silhouette against the evenly-polarized background space-light (Marshall et al. 2019). To compensate for this conspicuousness, many fish will use photonic structures to manipulate the AoP and DoP they reflect. The Atlantic herring, *Clupea harengus*, uses the optical properties of their skin to reflect polarized light, hiding their body outline against the polarized backdrop (Jordan et al. 2012). A terrestrial example of this would be butterflies with polarized wing patterns living in an open and highly-polarized habitat, while butterflies living in a cluttered environment such as a forest, will have a low polarization profile to avoid standing out from the background (Douglas et al. 2007). These polarized patterns might therefore be of preference, as an indication of quality, and used in mate choice (Sweeney et al. 2003). The exceptional ability to perceive such small contrasts in polarization, such as in the cuttlefish *Sepia plangon*, could also be a consequence of an 'arms race' between species able to use polarized reflections for camouflage and species with polarization sensitivity (Temple et al. 2012).

Most marine invertebrates are sensitive to the polarization of light; their long visual pigment molecules are often aligned in parallel by being constrained within adjacent microvillus membrane structures, rendering the photoreceptor cell sensitive to light polarized in that particular orientation (Labhart, 2016). Very little is known about how polarization visual systems are affected by dynamic lighting, such as water caustics. Animals living in environments subject to visual noise and fluctuations in irradiance may well share an advantage from having polarization sensitivity.

### **1.2.3 What are water caustics?**

In shallow waters (<5m), downwelling light can be refracted from surface ripples to create a mesh of moving waves of illumination across the sea floor, known as water caustics (McFarland & Loew, 1983). These are generated when concave and convex parts of water's surface refract and focus rays of light, creating areas of both converging and diverging incident light (Lock & Andrews, 1992, Schechner & Karpel, 2004). This spatio-temporal variation of illumination is also described as caustic flicker, caustic networks or wave lensing. Underwater caustics are most visible when the incident light projects orthogonally onto the sea floor. The dynamic pattern of illumination consists of a mosaic of elliptical cells, which become more elongated when the surface they reflect off is less than 90° from the sun (Loew & McFarland, 1990). The spatial frequency of the pattern (the level of detail) is highest at around five times the wave's crest-to-crest distance below the surface, and will lessen with increasing depth, turbidity and diffuse light (Lynch & Livingstone, 2001, Schechner & Karpel, 2004). Short and steep waves of higher amplitude, will therefore focus light closer to the surface and accentuate these fluctuations in irradiance (Schenk, 1957).

### **1.3 How do caustics affect the visual ecology of animals?**

Water caustics are prevalent in coral reefs and numerous other types of shallow water habitat (<5m deep, as reported by McFarland & Loew, 1983). They create a continuously moving light environment that conceals moving individuals, allowing prey to evade their predators (Matchette et al. 2018). By associating with visually-noisy environments, individuals can exploit the reduced visibility and take refuge within it, in turn influencing predator-prey interactions (Matchette et al. 2019, 2020). Seeking out habitats with dynamic visual noise can reduce an individual's signal-to-noise ratio (SNR), whereby the caustic flicker interferes with their true identification signal and their predator's search-images (Galloway et al. 2020). However, sedentary organisms, or 'sit-and-wait' predators, potentially stand out and have a higher risk of being spotted when stationary against this moving background. Because of this, some species may have to avoid such habitats altogether (Attwell et al. 2020). To counteract this conspicuousness, some will use 'motion masquerade' in



dynamic environments, and only alter their behaviours at times of high visual noise (Evans et al. 2018). For example, Hanlon and Messenger (2018) propose that octopuses match the speed of flickering water caustics as they move. A terrestrial example is the neotropical vine snake, *Oxybelis aeneus*, which shows rhythmic body movements to mimic wind-blown leaves (Fleishman, 1985). Both visual and tactile cues (and even artificial wind alone) elicited the same response. Dynamic visual noise can also hide an individual's communication signal; the Jacky dragon lizard (*Amphibolurus muricatus*) relies on aggressive visual displays towards their rivals when defending a territory, and will extend the duration of their tail-flicks in response to increased movement from wind-blown vegetation (Peters et al. 2007). The lengthened signal can therefore be clearly differentiated from their surroundings.

Prey species subject to a visually-noisy environment may have evolved colouration mimicking the wave-induced caustic flicker, such as vertical barring and vermiculation (markings resembling the track of a worm) (Cuthill et al. 2019). The undulant colouration of dwarf and minke whales (*Balaenoptera bonaerensis*) is also thought to be an instance of background matching in water caustics or dappled light (Merilaita & Stevens, 2011). Other strategies could include silver or iridescent scales of fish to create a dynamic flickering as they swim, which predators might struggle to discriminate from the surrounding flickering light (Cuthill et al. 2019). This strategy is likely to be highly advantageous in rivers, allowing individuals to hide amongst the fast directed caustics from waterflow. Cephalopods such as the Humboldt squid, *Dosidicus gigas*, have evolved a dynamic 'shimmer' pattern, to deceive the receiver of their signal (How et al. 2017). Bands or spots of colour will move across the skin in a random non-synchronous fashion, creating waves of complex patterns (Rosen et al. 2015). In doing this, *D. gigas* may be masking their body outline whenever they venture into shallow waters, by imitating the caustics that surround them (dynamic crypsis) (Gilly et al. 2012).

#### **1.4 Visual sensitivity of crabs and cuttlefish**

Animals negotiating areas subject to varying illumination, such as near the water surface, show a maximal response to the dominant frequencies found in this fluctuating light (McFarland & Loew, 1983). Crabs and cuttlefish will be exposed to caustics in their natural environment, and may too match their visual sensitivity to the frequencies dominant in the caustics that surround them. To understand how crabs and cuttlefish are perceiving caustics, as well as the simulated caustics presented within in this study, their critical fusion frequency (CFF) needs to be established. The CFF is the frequency at which animals perceive flickering light as constant illumination due to repeated stimuli fusing together (McFarland & Loew, 1983). In brighter environmental conditions with more

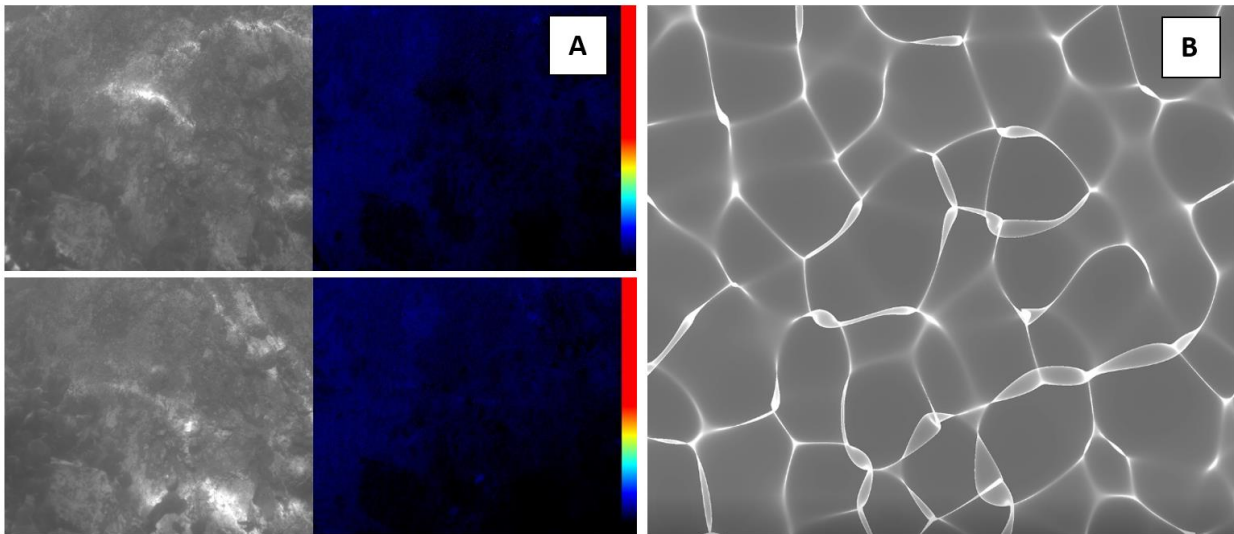
available visual information, animals can better detect moving objects without any blurring, hence a higher CFF value (as demonstrated in diurnal/nocturnal species of anoline lizard, Jenssen & Swenson, 1974).

*S. officinalis* has been shown to perceive a CFF of 24Hz (Nelson, 2003), while the CFF value for *C. maenas* has not yet been documented. *C. maenas* is a nocturnal species, and therefore may have a CFF value comparable to that of the nocturnal swimmer crab, *Portunus xantusii* (25.5Hz, Grober, 1990). Crabs were also observed under relatively low-light conditions within this study, meaning their expected CFF value is likely to be lower than the peak values reported in the literature. For example, the diurnal fiddler crab *Uca pugilator*, has a CFF of 50Hz which drops down to 32Hz when 'dark-adapted' (Layne et al. 1997). Although the experiments in this study were not dealing with flashing stimuli per se, establishing these CFF values enabled suitable refresh-rates (monitor update frequencies) to be chosen for the caustic video used. Having a frame-rate higher than the animal CFF value meant that the motion of the caustics appeared smooth and regular, as indeed natural water caustics would (as opposed to jerky stop-start motion profiles Hough, 2014).

### **1.5 What do we know about the wavelength, intensity and polarization of caustics?**

It has been previously established that water caustics are largely achromatic (Maximov, 2000), and research, including work by Schenck (1957), has also focused on the intensity fluctuations within caustics. Depending on the shape and size of the surface-wave, the intensity and the distance from the surface in which the intensity peaks can vary drastically. In short steep waves, the intensity of the downwelling incident light is highest nearer the surface, while long and flat waves produce caustics with highest intensity at lower depths. It's theorised that the peak intensity of wave-induced caustics can potentially reach six times the average intensity level (Schenck, 1957). This huge variation in intensity explains the impedance animals experience from living in an environment subject to dynamic lighting.

Despite the extensive knowledge on the intensity of caustics, little work has been done to quantify the DoP they exhibit. In this study, the DoP of natural caustic flicker was measured at Oyster Cove, Devon, UK (GPS: 50.41785, -3.55599). A polarization camera (Triton machine vision camera, Thinklucid.com, Lucid Vision Labs, Canada) was used to capture the properties of water caustics and helped determine how the visual noise is being perceived by marine animals. The video camera footage illustrated the high modulation of intensity within water caustics, but showed low-level noise in terms of the DoP (Fig. 1A).



**Fig. 1. Images of both natural and artificial water caustics (A)** Two screenshots of natural water caustics taken from polarization camera footage at Oyster Cove, Devon, UK (50.41785, -3.55599). The water in which the footage was taken was approximately 1m deep, and the caustics were captured against a sheet of black PVA plastic at a depth of approximately 30cm and at an angle of about 45°. The weather conditions were sunny with intermittent cloud and a gentle breeze. The two screenshots are subsequent frames (15 frames per second) and each screenshot shows an image of caustics in intensity (left) and in DoP (right). The DoP images have a colour scale bar ranging from 0% DoP (black) to 100% (red). **(B)** A screenshot of the caustic video that was played on loop during the experiments; the pattern was either static or dynamic (generated on [www.dualheights.se/caustics/](http://www.dualheights.se/caustics/)).

### **1.6 Could polarization vision help reduce the problem of caustics?**

Another way to overcome the challenge of visual noise could be to use an alternative modality of light for object detection purposes; in the form of polarization vision. Many invertebrates have evolved polarization vision for multiple purposes; enabling better navigation, habitat selection, conspecific communication and predator/prey detection (Yadav & Shein-Idelson, 2021). Polarization sensitivity is known to increase salience in visual tasks by improving object-background contrast, which can even help with spotting transparent prey items (Johnsen et al. 2011, Shashar et al. 1998). Prey that are using wavelength- or intensity-based camouflage may also be exposed in polarization, such as fish with silvery scales that mirror the surrounding light, or with countershading colouration (Shashar et al. 2000).

Polarization vision has been shown to be highly beneficial in visual noise such as from turbid water (Liu et al. 2018). In a study on two cuttlefish species, *Sepia prashadi* and *Sepia pharaonis*, a computer-generated looming stimulus (an expanding black circle to imitate an approaching predator) was presented either with only a polarization or intensity component, through varying levels of turbidity (Cartron et al. 2013). The cuttlefish showed stronger responses to the polarized stimuli, clearly demonstrating the advantage that polarization sensitivity and contrast vision

provides in turbid water. More recently it has been shown that captive cuttlefish exposed to turbid water from a young age actually experience improved and accelerated development of their polarization sensitivity (Georger et al. 2021).

In parallel to this, polarization vision may also potentially increase contrast and improve object-detection in environments subject to water caustics. Water caustics are clearly a visual intensity problem for animals (Fig. 1A), so perhaps the polarization of light is used to overcome this issue. It has been suggested that caustics played a salient role in the evolution of colour vision, as removing the impedance of the visual noise was only made possible from the interaction between two spectrally distinct types of photoreceptors (Maximov, 2000). With a lack of colour vision, cuttlefish (*Sepia* spp.) and other cephalopods have evolved an enhanced sensitivity to the polarization of light (Temple et al. 2012), and so polarization vision may well have had a similar evolutionary origin.

### **1.7 Aims of the current study and outline of the thesis structure**

When there are strong fluctuations in illumination underwater from caustic flicker, some species will avoid this environment as the visual noise disrupts their predator and prey detection (Matchette et al. 2019). However, many species can successfully tolerate such conditions, maybe as a result of having polarization vision. Previous research has explored the benefits of polarization vision in visually-noisy environments such as turbid water (Cartron et al. 2013), but no studies have looked into the potential benefits in an environment with high levels of water caustics. To investigate this possibility, two species with polarization sensitivity from UK coastal waters were chosen; the common cuttlefish, *Sepia officinalis*, and one of their prey species, the European shore crab, *Carcinus maenas*. Having experiments with both *S. officinalis* and *C. maenas* gives a wider picture of how the responses of linked predator and prey species compare and interact. By understanding the way in which the prey crabs are perceiving the world around them, may give insight into why cuttlefish have also evolved such successful polarization vision.

Despite it being well known that water caustics inhibit the detection of prey (Matchette et al. 2018, 2019, 2020), very little research has been performed on the impact of caustics on detection of predators and antipredator behaviour. Therefore, similar to the work of Cartron et al (2013) and Smithers et al (2019), the animals in this study were presented with computer-generated looming stimuli (an expanding disc on a screen) to imitate an approaching predator. The stimuli were displayed in either polarization or intensity contrasts, and instead of varying the levels of water turbidity, the stimuli were viewed against video of either static or moving caustics. It was predicted that, consistent with Maximov's (2000) theory, the test animals would perform better when detecting polarized cues compared to those that were unpolarized. Their adapted visual system

would enable them to block out the visual noise just as they do in turbid water, and perceive an increased contrast of the looming stimulus against its background. The aim of this study was therefore to observe whether there is an advantage to having polarization sensitivity in environments with water caustics, allowing individuals to exploit a complex moving environment not accessible to other species. This thesis will include a second chapter consisting of methodology and data analysis, and a third discussion chapter.



## **DATA CHAPTER (2)**

### **2.1 Introduction**

Few studies have delved into the impact of spatio-temporal variation of light on the visual ecology of marine animals, and in turn their resulting behavioural and phenotypic adaptations. Polarization sensitivity may well be an adaptation to coping with such visual noise. To support this theory, four experiments in this study were carried out to determine whether polarization vision enables improved predator detection in environments subject to water caustics. Crabs and cuttlefish were presented with predator-like stimuli (an expanding disc on a screen) overlaid with video of water caustics. The stimuli were either presented in only intensity or polarization, - in order to compare the difference in response probability. These experiments will therefore explore a new purpose and function for polarization vision, bringing improved understanding of underwater visual ecology; a world we know little about.

### **2.2 Methods**

#### **2.2.1 Animals**

##### **2.2.1.1 *Carcinus maenas***

*C. maenas* of mixed sex were collected from Clevedon beach, UK (GPS: 51.43707, -2.86637), where they live within upper intertidal zone rockpools. The crab carapaces ranged from approximately 20-50mm. They were collected in batches of 25 individuals, driven back to the University of Bristol and housed within individual Tupperware boxes. The boxes all stood upright within a shallow salt water aquarium which was cleaned and had the water replaced between each experiment. The boxes had small 5mm holes punched into the sides allowing for ~2cm of each box to be immersed in and filled with water. Each box contained a few rocks or shells for the crabs to take refuge under or to stand above the water's surface. Crabs were fed twice a week, with defrosted mussels, cockles or prawns.

Each crab was used only once per experiment, and they were kept in the lab for no longer than a week. Once the 25 individuals were finished with, they were returned to the location they were collected from, after collecting another batch. 50 crabs were used per experiment and so 100 crabs were used in total for the two crab experiments. Only small and young crabs were collected (big adult crabs were less responsive) and were used only a few days after collection to prevent fatigue, stress or long-term habituation to lab conditions from affecting their response. Measurements were mostly carried out in the morning between 9am and midday, as the crabs also exhibited an overall

lessened response later in the day (perhaps related to tidal rhythms associated with the collection site).

The water was maintained at a salinity ranging between 25-35 ppt using Premium reef salt (Tropical Marine Centre, Bristol, UK). Water was maintained at a constant room temperature (~20°C) and a naturally occurring light cycle was maintained for the duration of the experiment (i.e., 12hr:12hr light to dark alternation). Experimental protocols were carried out in accordance with ethical regulations and guidelines (School of Biological Sciences, University of Bristol – UIN/21/061).

### **2.2.1.2 *Sepia officinalis***

*S. officinalis* of mixed sex were raised from eggs in an aquarium within the Sea Water Hall research lab in the Marine Biological Association, Plymouth, UK. There were 35 individuals, ranging between around 8-12cm in length and they were fed a live shrimp 3 times a day; morning around 9am, midday, and late afternoon around 4pm. The shrimp were either ordered from LiveFoods.co.uk or collected from Mount Batton beach, Plymouth. On weekends, the cuttlefish were only fed one or two shrimp a day at midday. On one day of the week at midday, the cuttlefish were fed a single crab (ranging between ~1.5-4cm), also collected from Mount Batton beach.

*S. officinalis* were housed individually in fibreglass tanks (30-by-65-by-40cm), which were scrubbed and siphoned twice a week. The tanks were equipped with plastic plants and a few rocks to provide refuge. All tanks had a circulation of water from a large flow-through system (supplied by fresh seawater from Plymouth sound) providing exceptionally stable water quality throughout the experiment. Water temperature remained stable at around 17°C, with slight variation depending on changes in weather. It was ensured that a naturally occurring light cycle was maintained for the duration of the experiment (i.e., 12hr:12hr light to dark alternation). Experimental protocols were carried out in accordance with the 'Animal ethics policy' (Animal Ethical Review Committee (ERC), Marine Biological Association, 2002).

## **2.2.2 Experimental apparatus**

### **2.2.2.1 *Carcinus maenas***

To determine the effect of visual noise on the threshold of predator detection of *C. maenas*, a dual-projector system was used to present carefully calibrated predator-like stimuli in varying contrasts, against static or dynamic caustic flicker backgrounds. To clearly observe the behavioural responses to these visual stimuli, crabs were tethered above a spherical treadmill consisting of a Styrofoam ball (10cm in diameter) suspended in a flow of air (supplied from a compressed air tap) entering through a tube into the bottom of a hemispherical cup (Fig. 2A). *C. maenas* were secured



on-top of the ball using 'hook-and-loop' Velcro; a small square of 'loop' Velcro was attached to the middle of the dorsal carapace using 2 small dots of cyanoacrylate glue. This was completed with all individuals before starting the experiment. The individual was tethered to a square of 'hook' Velcro on a metal rod in a clamp apparatus, holding the crab above the ball and preventing the individual from rotating, so that their field of view was fixed forwards. The rotation of the ball allowed the crab to walk freely in any direction.

The treadmill sat in the centre of a 40-by-40-by-40cm photography cube tent (PhotoSel Limited, UK) made from white fabric. The front side of the tent was kept open and a hole was cut into the top surface for a video camera (Panasonic HC-X800M) to record through, which was positioned above the tent using a tripod (Fig. 2B). Directly in front of the open side of the experimental area, was a custom-made screen that the stimuli could be projected onto, made from a sheet of 0.5 film diffuser (Lee Filters, Andover, UK). The screen was positioned 20cm from the centre of the Styrofoam ball. To avoid the crab getting distracted by other stimuli in the room, a curtain attached to the cube tent covered the edges of the screen (Fig. 2B). The absolute irradiance of the testing area was recorded using a calibrated spectrometer (Ocean Optics USB 2000) and a cosine corrector (Ocean Optics) attached to a 400micron diameter optic fibre. A Gershan tube (Ocean Optics) attached to an optic fibre measured the relative radiance of light emitted from the display screen (Appendix 1). Visual stimuli were presented to the animals using two overlaid screens. Using MATLAB (R2021a, MathWorks, Natick, USA), an expanding black disc (full size 10.5cm) against a plain grey background was plotted on one screen (with a frame rate of 30Hz) to simulate an approaching predator. Caustic flicker video was generated using Caustics Generator Pro software (Fig. 1B, [www.dualheights.se/caustics/](http://www.dualheights.se/caustics/)). The caustic flicker video was played on loop on the second screen and the caustics pattern was either static or dynamic (moving). The static caustic flicker video had a frame-rate of 30 frames per second (allowing for a reduced file size for the non-moving stimulus video), and the dynamic caustic flicker had a frame-rate of 60 frames per second. This refresh-rate of the moving caustic video was higher than the expected CFF of the two chosen species (see 'Visual sensitivity of crabs and cuttlefish' section), so that the animals would perceive smooth motion profiles of animated caustics and objects (Hough, 2014). The chosen spatial frequency of the pattern was determined using the 5m depth value on Caustics Generator Pro, and the speed of the dynamic caustics used was modified using MATLAB. The optimal speed and spatial frequency of the dynamic caustic videos was determined in a preliminary experiment (see 'Determining stimulus contrasts and the properties of caustics' section).

As a result of this experimental set-up, the animals would in theory be perceiving a screen in front of them, showing the looming predator-like stimuli against a grey background, with caustic

lighting playing across it. This would recreate a very simplified version of what an animal would perceive in their natural environment; caustics reflecting off both the approaching predator and surrounding substrate (such as sand, rock or coral). However, realistically, caustics would also be cast over and around the subject animal itself, and so a subsequent study could project caustics from above in order to recreate their natural environment in an alternative way.

Two systems were used for presenting this stimulus. For the first experiment, two overlaid intensity-only images (the expanding disc and the caustic video) were presented on the screen, by having one projector sitting on-top of another (positioned ~1m from the screen so that the projector windows were ~30-by-38cm). For the second experiment, one projector presented an intensity-only image of the caustic video, overlaid onto a polarization-only image of the expanding disc presented on a custom-made intensity/polarization screen. The screen was constructed by extracting the LCD panel from a Dell 1905fp vertical alignment-type computer monitor (Dell House, Bracknell, UK) and removing the front-most polarization filter. A sheet of 0.5 diffuser (Lee Filters, Andover, UK) was spray-glued to the innermost surface of the LCD panel, against which the intensity image of the digital projector was cast. This resulted in a combined intensity/polarization display, on which moving images could be produced varying in polarization or intensity (see Smithers et al. 2019 for further details). Polarized sunglasses were used throughout the experiment to check that the expanding disc was in position.

MATLAB was programmed to produce 4 audio beeps during a trial: 1) the start of the trial; 2) the disc appeared at its smallest size; 3) the point at which the disc had stopped expanding (End size = 30% of the height of the projected image, loom time = 3 seconds for crabs, 1 second for cuttlefish); 4) the disc disappeared (3 seconds after maximum expansion). The audio beeps were recorded on the video camera via an audio cable from the computer to the camera's microphone port, enabling clear observations when scoring the data. Audio beeps were muted from external speakers so that the animals could not hear and respond to the sound. The real-time view from the digital camera was also delivered to an LCD display via an HDMI cable, allowing remote viewing of the experimental animal. The computer and viewing monitors were kept outside of the experimental area, so that the responses could be observed without disturbing the individual being tested.

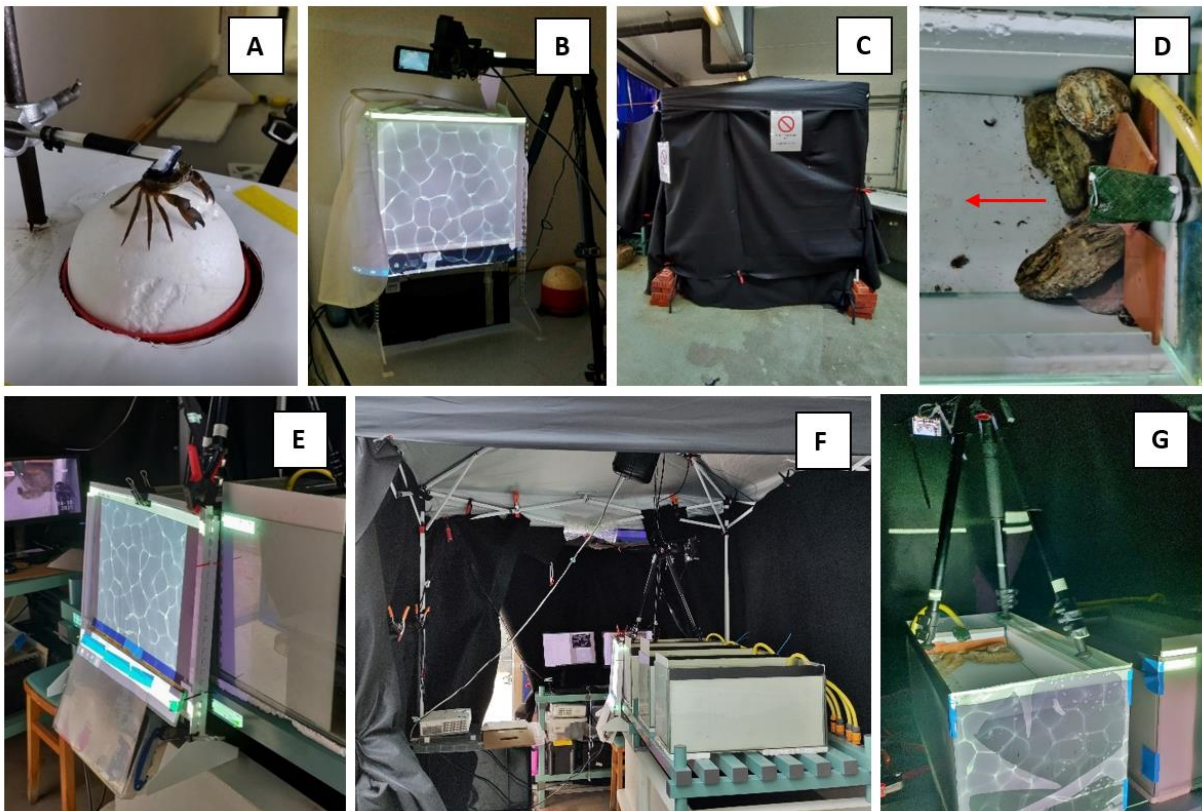
#### **2.2.2.2 *Sepia officinalis***

The experimental apparatus used to study cuttlefish responses was similar to that used for crabs, with a few notable exceptions relating to the differences in natural history. A rectangular aquarium tank (60-by-30-by-31cm) was positioned on a surface ~1m from the ground and enclosed in a gazebo covered in black cloth to create a shaded environment (Fig. 2C). The tank was filled with

approximately 45L of circulating seawater, with the same salinity and temperature as the water in the original tanks that the cuttlefish were housed in. Rocks were positioned in a V-shape at the back of the tank, which encouraged the cuttlefish to sit in a consistent position 35-50cm from the front of the tank and facing towards the stimulus screen (Fig. 2D).

With the exception of the front-most face of the aquarium, the inner surfaces were lined with white plastic glued to the glass, to prevent the cuttlefish from getting distracted by their own reflection and to eliminate intensity artefacts caused by the internal reflection of polarized stimuli (Foster et al. 2019). For the first cuttlefish experiment, a sheet of diffuser film (0.5, Lee Filters) was taped to the front surface of the tank, to act as a projection screen for the visual stimuli. For the second experiment, the polarization screen was clipped onto the front surface of the tank (Fig. 2E) instead of the stimulus being projected onto the diffuser originally used.

In the two cuttlefish experiments, the projector and MATLAB systems for presenting the caustics and stimuli (and the method for measuring the relative radiance/absolute irradiance) were the same as used in the two crab experiments (see 'Experimental apparatus, *Carcinus maenas*' section above). The projectors sat ~1m from the front of the tank and were aligned so that both projector windows were ~30 by 38cm, covering the whole of the front of the tank. Four sets of aquarium tanks were constructed side-by-side to enable a higher throughput of data collection (Fig. 2F). The projectors sat on a large box on top of a wheeled platform, enabling them to be rolled to an adjacent tank once a cuttlefish had been tested. A digital video camera was positioned above the tanks using a tripod, so that the cuttlefish could be filmed from above (Fig. 2G).



**Fig 2. Photographs of the experimental apparatus for both *Carcinus maenas* and *Sepia officinalis*.** (A) The spherical treadmill (consisting of a Styrofoam ball suspended in a flow of air) for clear observations of *C. maenas* responses to the presented stimulus. (B) The video camera and tripod positioned above the experimental area (photography tent) for recording responses of *C. maenas*. (C) The gazebo covered in black felt to enclose *S. officinalis* in darkness. (D) Rocks positioned in a V-shape to encourage *S. officinalis* to sit facing forwards towards the stimulus (in direction of arrow). (E) The polarization screen clipped onto the front surface of the tank for presenting the stimulus in polarized light. (F) The four sets of aquarium tanks constructed side-by-side to enable a higher throughput of data collection. (G) The video camera positioned on top of the tanks so that *S. officinalis* could be filmed from above.

### 2.2.3 Determining stimulus contrasts and the properties of caustics

A scoping study was firstly carried out to investigate the range of intensity contrast values (measured as Weber contrast) that the crabs and cuttlefish were behaviourally sensitive to in the experimental apparatus. Most individuals responded to the expanding disc with a Weber contrast value of around -0.054 (calculated from the measured relative radiance). The contrast needed to be slightly above the threshold of detection so as not to overwhelm the background caustic stimuli or cause the animal significant stress. As a result, a range of stimulus intensity contrasts was picked between 0 and -0.08 to fall either side of this threshold.

Secondly, another preliminary experiment helped determine the optimal properties of the simulated water caustics to be used (Appendix 2). Caustic backgrounds of varying speeds (static,

slow, fast) and levels of spatial frequency (low, moderate, high) were projected over the top of an expanding disc with the previously chosen Weber contrast value of -0.054. The treatments also included a greyscale plain background instead of a caustic pattern, used as a control to rule out the possibility of individuals responding to the caustic pattern alone. It was found that individuals could respond to the expanding disc when the caustics were static and slow or out of focus/blurry (low spatial frequency). Faster and more defined caustics however, prevented stimulus detection, demonstrating the impedance that visually-noisy environments have on predator detection. From this, the static and fast caustics of high spatial frequency were chosen for the 'static' and 'dynamic' treatments within the following main experiments. Establishing which properties of caustics did and did not prevent stimulus detection helped to then observe any differences in response probability when using a polarized stimulus. This would therefore highlight the predicted advantage in having polarization vision in these visually-noisy environments.

## **2.2.4 Experimental Protocol**

### **2.2.4.1 *Carcinus maenas***

#### **2.2.4.1.1 Experiment 1:**

In order to determine whether water caustics influence the threshold of predator detection, crabs were presented with a series of expanding discs varying in contrast and viewed against two different caustic backgrounds; static and dynamic. Each crab was left to acclimatise on the treadmill for 3 minutes, before being shown seven expanding disc stimuli, with 1-minute intervals (each crab spent approximately 20 minutes on the treadmill in total). The stimulus consisted of an expanding disc varying with the background in the following Weber contrasts: -0.074, -0.0685, -0.063, -0.054, -0.045, -0.0225 and 0. These contrasts correspond to the following RGB values on the loom generator within MATLAB: 20, 25, 30, 35, 40, 45 and 50, on a background of 50 (with the same value assigned to each of the three colour channels). Discs were expanded using a geometric profile that approximately simulated the approach of a sphere (full size 10.5cm) from a distance of 5m. The discs were overlaid with a video of one of the caustic light treatments, which played continuously on loop.

Crabs were used in pairs, for example, once the seven expanding disc stimuli had been presented to one crab with the first caustic treatment, the second crab was put on the treadmill and presented with the same seven stimuli, but this time using the second caustic treatment, before returning to the first crab with the second caustic treatment (and so on). This allowed each crab to have a 20-minute break between caustic treatments, to rest and rehydrate. The contrast values were fully randomised for each presentation and the caustic treatment order was alternated

between pairs of crabs to avoid confounds related to order effects such as of habituation, fatigue or daily activity cycles.

#### 2.2.4.1.2 Experiment 2:

The second crab experiment aimed to determine whether having polarization vision can overcome the problem of visual noise when detecting predators in dynamic caustic environments. The exact same procedure was carried out, but with the expanding disc presented in polarization contrasts. The expanding disc varied in the percent of horizontally-oriented polarization as follows: 38%, 40%, 42%, 44%, 46%, 48%, 50%. These were all presented against a 50% polarized background, so that each stimulus varied in contrast from the background in 2% increments. These contrast values correspond to the following RGB values on the loom generator within MATLAB: 176, 173, 171, 168, 165, 162 and 159, on a background of 159 (see Smithers et al 2019 for more details on the polarization screen measurements).

#### **2.2.4.2 *Sepia officinalis***

##### 2.2.4.2.1 Experiment 3:

Again, this experiment set out to demonstrate the influence of water caustics on the threshold of predator detection, but with cuttlefish subjects (*S. officinalis*) rather than crabs (*C. maenas*). At 9am on each day of experimentation, the projectors, camera and computer were turned on and put in position for the first tank to be used. Light from the projectors was blocked, the front wall of the aquarium was covered with opaque white card and the side doors of the gazebo were kept open, to avoid a large and sudden change in illumination when transferring the cuttlefish. Four cuttlefish were transferred to the four experimental tanks using a net and bucket and were fed a live shrimp to help them settle. They were left to acclimatise to their new tanks for 4 hours, and within this time their original housing tanks were cleaned.

At 1pm, the white card was removed from the front surface of the first tank, so that only the first cuttlefish was exposed to light from the stimulus screen. The projector was slowly uncovered, and the side doors of the gazebo were closed to block out the majority of light from outside. This procedure again created a gradual change in illumination. The video camera was positioned and focused on the test cuttlefish after the changed lighting for accurate behavioural observations. The first cuttlefish was left to acclimatise to the new light conditions for 10 minutes, before being shown 5 stimuli, with 5-minute intervals. Following this, they had 10 minutes to acclimatise to the second

caustic treatment, before seeing the same 5 stimuli again. The stimulus was an expanding disc (10.5cm full size) in a range of 5 different intensity contrast values: -0.078, -0.074, -0.063, -0.045 and 0 (Weber contrast). These contrast values correspond to the following RGB values on the loom generator within MATLAB: 10, 20, 30, 40 and 50, on a background of 50. The contrast values were randomised to avoid order effects, and the caustic treatment order was alternated between cuttlefish, to control for any effects of habituation and fatigue. Each cuttlefish took approximately an hour in total and so the experiment ran until around 5pm, after which they were returned to their original tanks. Four cuttlefish were used per day, and due to time constraints, only 15 individuals could be used for each of the two experiments.

#### 2.2.4.2.2 Experiment 4:

The second cuttlefish experiment (the fourth and last experiment in total) again aimed to determine whether polarization vision aids predator detection in dynamic caustics. The exact same procedure was carried out as experiment 3, but with the expanding disc in contrasts of polarization only. The polarization properties of the expanding disc were 20%, 29%, 36%, 43%, and 50% viewed against a 50% polarized background, representing increments in contrast of 7-9%. These contrast values correspond to the following RGB values on the loom generator within MATLAB: 199, 189, 179, 169 and 159, on a background of 159.

### 2.2.5 Categorizing the response

Trials were started when a crab was walking on the treadmill and when a cuttlefish was still and facing within 180° of the stimulus monitor, to ensure the individuals were seeing the stimulus and to clearly observe their response. Behaviours were only counted as a definite response to the stimuli when they occurred after the second audio beep, which was when the expanding disc appeared (see 'Experimental apparatus, *Carcinus maenas*' section). For example, if the crabs froze directly on the second beep, or more than 3 seconds after the last beep, the behaviour was not counted as a positive response. For data analysis, the responses were recorded as binary data, with 1 being a positive response, and 0 being no response.

A positive response to the stimulus with *C. maenas* included one or a combination of these behaviours: freezing, slowing to a stop, a clear increase or decrease in speed, tucking the legs and/or claws towards the body for protection, and lastly, extending the claws in an aggressive posture (Table 1). A positive response to the stimulus with *S. officinalis* included one or a combination of these behaviours: a very quick flash of colour across the whole or part of the body (including very

subtle changes in colour), a sudden contraction of the body (jerk movement), or a sudden movement of the eyes towards the screen (Table 1).

**Table 1.** A description of each cuttlefish (*S. officinalis*) and crab (*C. maenas*) behaviour that was counted as a positive response to the presented stimulus (for all four experiments).

<b>Study Species</b>	<b>Behaviour</b>	<b>Description of Behaviour</b>
<b><i>Carcinus maenas</i></b>	Freezing	The crab will stop walking abruptly and its posture will freeze.
	Slowing to a stop	The crab will be walking at a more-or-less consistent walking speed, before decreasing in speed until it stops walking altogether.
	Clear increase or decrease in speed	The crab will be walking at a more-or-less consistent walking speed, before increasing/decreasing the pace until it is walking at a noticeably different speed than it was at the start of the trial.
	Tucking in legs and/or claws	The crab will pull in the claws and legs towards the body to protect itself, or the crab may continue to walk whilst only pulling the claws in against the body.
	Extending claws	The claws extend outwards away from the body in an aggressive posture.
<b><i>Sepia officinalis</i></b>	Rapid colour change	A part of (or the majority of) the body shows a rapid change in colour; the skin flashes either a darker or brighter shade, and is often very subtle and fast, lasting under a second before returning to the original colour.
	Contraction of body (jerk movement)	A rapid compression and decompression of the mantle tissue, like a pulsation. This may be a result of filling the mantle with water (hyperinflation), which King & Adamo (2006) suggest might help the cuttlefish escape using jet propulsion.
	Eye movement towards stimulus screen	Cuttlefish will be sitting still with their eyes fixed in one position, before showing an optokinetic response; reflexive eye movement induced by motion in the visual field (Collewyn, 1970). The external eye muscles can be observed to move towards the stimulus.



## 2.2.6 Analysis

Videos of the animals were manually scored blind to produce binary response data for each of the four experiments. Similar to How et al (2012), the data were analysed using a linear mixed-model with one between-subjects factor, “caustic treatment” (static, dynamic), and one within-subjects factor, “stimulus contrast value” (5-7 Weber or polarization contrasts). The glmer function within the lme4 package was used in R 4.0.3 (64 bit), to compare and observe both the effect of contrast and of caustic treatment on the data (crab and cuttlefish response). The ‘drop1’ function was used to perform an analysis of deviance, to compare models with and without contrast, treatment, or the interaction term (contrast\*treatment). Crab and cuttlefish identity was set as a random factor, to account for biases such as sex and body size of individuals.

## 2.3 Results

In all experiments, stimulus contrast had a significant effect on response probability (Fig. 3A; Comparison of GLMM models with and without contrast:  $\chi^2 = 258.53$ ,  $df = 1$ ,  $p < 0.001$ ) (Fig. 3B,  $\chi^2 = 260.75$ ,  $df = 1$ ,  $p < 0.001$ ) (Fig. 3C,  $\chi^2 = 59.8$ ,  $df = 1$ ,  $p < 0.001$ ) (Fig. 3D,  $\chi^2 = 62.08$ ,  $df = 1$ ,  $p < 0.001$ ). As the stimulus increased in contrast from its background, the response probability would also increase. The majority of crabs responded by freezing or slowing to a stop. Bigger crabs would occasionally extend their claws away from the body in an aggressive posture, whereas the smaller crabs tucked in the legs and claws as a more frequent response. Cuttlefish predominately responded to the stimulus by showing a quick and subtle flash of colour across the whole of the body, combined with a body contraction/jerk movement (Table 1). Cuttlefish of different sizes however, did not noticeably vary in their type of response.

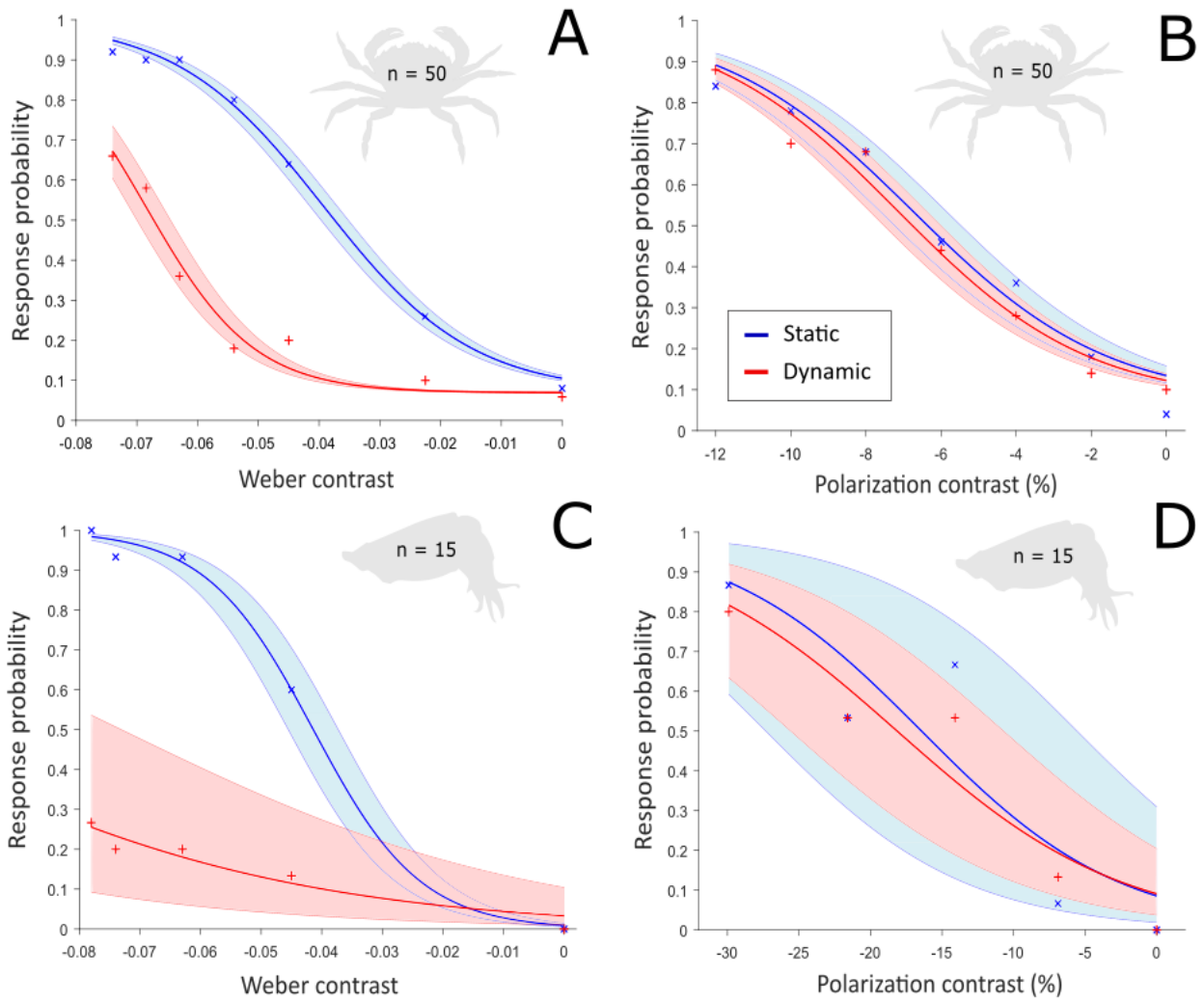
Crabs and cuttlefish had a lower threshold of detection (an overall higher proportion of positive responses across 7 Weber contrasts) in static caustics than in dynamic. They were far less effective at detecting intensity-based predator-like stimuli against dynamic caustic backgrounds than static (Fig. 3AC; Comparison of GLMM models with and without treatment: for crabs;  $\chi^2 = 121.7$ ,  $df = 1$ ,  $p < 0.001$ ; and for cuttlefish:  $\chi^2 = 67.1$ ,  $df = 1$ ,  $p < 0.001$ ), but this impact was completely mitigated when stimuli were presented in polarization, so that the type of caustics had no significant effect on response and a similar response probability was seen for both caustic treatments (Fig. 3BD; for crabs:  $\chi^2 = 0.34$ ,  $df = 1$ ,  $p = 0.56$ , and for cuttlefish:  $\chi^2 = 0.18$ ,  $df = 1$ ,  $p = 0.67$ ). For intensity-only stimuli, there was a clear interdependence of the effect of treatment and contrast on response probability (Comparison of GLMM models with and without the contrast\*treatment interaction term for crabs:  $\chi^2 = 4.48$ ,  $df = 1$ ,  $p = 0.034$ , and for cuttlefish:  $\chi^2 = 5.48$ ,  $df = 1$ ,  $p = 0.019$ ), but this was not

the case for the polarization-based stimuli (for crabs:  $\chi^2 = 0.015$ ,  $df = 1$ ,  $p = 0.90$ , and for cuttlefish:  $\chi^2 = 0.20$ ,  $df = 1$ ,  $p = 0.65$ ).

The fitting function 'sigm\_fit.m' (Pavao, 2016) was used to find the best sigmoid curve to fit the response data, starting at the level of false positives (mean response probability for zero contrast stimuli) and ending at 1. This also calculates a 95% confidence interval for the 50% point on the sigmoid (Table 2), which was used to plot dotted sigmoid lines at the upper and lower bounds (Fig. 3).

**Table 2.** The 50% sigmoid values and their corresponding 95% confidence intervals, in both static and dynamic caustic treatments for the intensity and polarization stimuli. The values were calculated using the 'sigm\_fit.m' sigmoid fitting function (Pavao, 2016).

Species	Intensity Stimuli (Weber Contrast)		Polarization Stimuli (% Polarization Contrast)	
	Static Caustics	Dynamic Caustics	Static Caustics	Dynamic Caustics
Crab	-0.039	-0.067	-6.75	-7.14
( <i>Carcinus maenas</i> )	(95% CI 0.0022)	(95% CI 0.0031)	(95% CI 0.88)	(95% CI 0.75)
Cuttlefish	-0.041	-0.11	-16.44	-18.14
( <i>Sepia officinalis</i> )	(95% CI 0.0041)	(95% CI 0.041)	(95% CI 10.08)	(95% CI 7.43)



**Fig. 3.** Response curves of *Carcinus maenas* and *Sepia officinalis*, illustrating their threshold of detection of a stimulus (an expanding disc on a grey background) against either static caustics (shown in blue) or dynamic caustics (shown in red). (A) *C. maenas* response probability to the stimulus presented in a range of Weber contrasts. (B) *C. maenas* response probability to the stimulus presented in a range of polarization contrasts. (C-D) As above but for *S. officinalis*. The shaded areas represent the 95% confidence intervals for each sigmoid curve.



## **DISCUSSION CHAPTER (3)**

### **3.1 Discussion**

Among its many uses, such as navigation, communication and object-detection, polarization vision has been proven to also provide a way of reducing visual noise, for example from turbid murky water (Cartron et al. 2013, Sharkey et al. 2015). The experiments in this study have uncovered a new function for polarization sensitivity; the reduction of visual noise caused by fast flickering water caustics. When the predator-like stimuli were intensity-based, both crabs and cuttlefish were less able to detect the expanding disc in dynamic caustics than in stationary caustics (Fig. 3). However, when the stimulus was presented in polarized light only, the type of caustics had no effect on response. Dynamic caustics no longer had an impact on stimulus detection; a similar proportion of responses was observed for both caustic treatments. This demonstrated how having polarization sensitivity increased object-detection by mitigating the visual-impedance from water caustics.

The crabs and cuttlefish had a number of different ways in which they responded to the stimulus (Table 1). Most crabs would freeze or slow to a stop in response to the expanding disc, or even tuck in their legs and claws. The larger crabs however, would extend their claws out from the body in an aggressive posture. This variation may have been down to differences in previous encounters; larger crabs are likely to be older with more experience of defending themselves against threats or rival individuals. Whereas as a smaller crab, tucking appendages in for protection is potentially more advantageous. Cuttlefish also exhibited multiple forms of behaviour in response to the stimulus, although a correlation with size was not noticeably apparent. Further research could investigate the effect of age, size, sex and species, to better understand some of these behavioural differences between individuals.

Varying levels of illumination are prevalent amongst different aquatic environments. Directed water flow, wind, cloud movement and convective heating of air will all result in surface ripples and waves, which refract downwelling light onto substrate that is close to the surface (McFarland & Loew, 1983). Caustics are therefore most common in shallow water environments (<5m, McFarland & Loew, 1983), such as coral reefs, mangroves and estuaries, as well as freshwater habitats such as rivers, streams, ponds and lakes. Dappled light from overhead foliage can create a similar problem for terrestrial or in-shore animals (Matchette et al. 2019). Previous researchers have theorised that those organisms living in such dynamically lit environments may use light modalities other than intensity to mitigate the effects of the visual noise. For instance, Maximov (2000) suggested that colour vision originally evolved to improve object-detection in visual noise such as caustic flicker. Even prior the evolution of vertebrate colour vision, horizontal cells (interneurons) within the retina provided an antagonistic interaction between adjacent photoreceptors, but with the presence of

multiple types of cone cell, it is believed to enable even more efficient edge enhancement and contrast discrimination in the visual field. By being able to discriminate between multiple wavelengths, features in the visual field can be bound and perceived as single or separate objects. The varying waves of illumination from caustics are achromatic, and so having colour vision to discriminate between an object and its background could become highly important in visually-complex environments with dynamic visual noise. Monochromatic species such as the cuttlefish, may have therefore evolved an alternative strategy to reduce the impact of dynamic lighting. Their polarization vision is the most acute amongst the animal kingdom (Temple et al. 2012), and so the evolution of this type of visual system may well be a result of both a lack of colour sensitivity and the presence of flickering caustics that hide prey and approaching predators. This theory has been suggested previously in the striped pyjama squid, *Sepioloidea lineolata* (Talbot & Marshall, 2010), but this study presents the first evidence that polarization vision improves visual performance in water caustics, thus providing strong evidence to support this hypothesis.

Different parts of the visual system are frequently used for different visual tasks (Viviani & Aymoz, 2001). Looking into the neural pathways involved in polarization vision may provide further understanding of how and why polarization sensitivity enhances object detection in visually-noisy environments. Both crustaceans and cephalopods have a dipolar (2 channel) polarization vision system, where half of their polarization-sensitive photoreceptors have microvilli (containing the visual pigments) aligned perpendicular to the microvilli of the other half, creating two channels of sensitivity to horizontally and vertically polarized light respectively (Snyder, 1973). In the fiddler crab, *Afruca tangeri*, the signal output from these photoreceptors combine as they synapse with an interneuron within the external layers of the lamina. This interneuron synapses with three other types of neuron. One conveys intensity-only information, acquired by sharing synaptic connections with the seven main photoreceptor cells in the ommatidium (R1-7). The other two carry separate streams of information from the horizontal and vertical channels of polarization (Bernard & Wehner, 1977). The three channels terminate in the medulla, and it's theorised that prior to this, signals from the two polarization channels project into a pathway independent from the intensity information, creating a parallel system for independently processing intensity and polarization of light (Smithers et al. 2019). By being processed in parallel, it potentially enables access to a broader range of contrast information and enhances the ability to discriminate between objects and their background. Analogous to intensity and colour perception in humans, neurons relaying the two sources of information don't interfere or inhibit each other, but combine later during visual processing and decision making (Smithers et al. 2019). For example, if intensity information is hard to acquire, i.e., the predator is hard to spot from a particular viewing angle due to camouflage

colouration or undesirable lighting conditions, then the polarization of light can provide an alternative way to expose the threat. Similarly, this independent processing of polarization and intensity information might be beneficial in water caustics; flickering illumination will prevent the binding of features in the environment, but an object will still look silhouetted against the polarized backdrop despite unreliable intensity information.

A predator against a substrate with caustic lighting playing across it is a visually-noisy scene in terms of intensity, but has low-level noise in polarization. This assumption was supported by quantifying the intensity and polarization of natural water caustics using a polarization camera (Fig. 1A). Conversely, Sabbah and Shasher (2006) discovered that light refracted from surface waves in open water with a featureless backdrop has a higher variability in polarization than in irradiance, and may serve to enhance the detection of objects (rather than obscure them) in the following way: With no surface to reflect from, caustics are not so visible in intensity, leaving only fluctuations in polarization as a visible attribute. The AoP and DoP of light entering through Snell's window is distorted and altered from the curvature of the surface waves, and animals inhabiting this domain will exploit this by matching their spectral sensitivity to the dominant frequencies of the fluctuating light (Mcfarland & Loew, 1983). The higher fluctuations in polarization than irradiance therefore suggest that polarization sensitivity may not only enhance object-detection in shallow water, but in the featureless water column too. A potential study to follow could therefore investigate the effects of deep-water (>5m) light fluctuations on the visual ecology of marine life. This could be implemented by instead presenting the caustic flicker pattern in polarization contrasts rather than altering the properties of the expanding disc. A further experiment instead using video of natural water caustics would also validate the use of computer-generated caustics.

Additional questions could be asked to explore the benefit of polarization vision for object-detection in other types of visual noise, such as blooms of bioluminescent algae, or turbidity from microplastics. Perhaps even anthropogenic light pollution from harbours or ink secretions from escaping prey could be considered as visual noise. For example, in an evolutionary 'arms race', predators of cephalopods may have utilized polarization vision to prevent an ink decoy from obscuring their view. However, Bush & Robison (2007) suggest that the squid, *Galiteuthis phyllura*, fill their transparent body cavity with ink to potentially prevent polarized reflections from the mantle tissue, implying ink may have properties that impede object detection by polarization vision. Alternatively, the impact of water caustics on cognitive tasks other than object detection could be considered, such as on group cohesion. For example, in a paper by Matchette & Herbert-Read (2021), water caustics have been found to promote social behaviour in fish, such as shoaling. The reduced visibility from increased visual noise resulted in fish increasing the time spent in closer

proximity to others. The fish were thought to be substituting individually-acquired environmental cues for the acquisition of social cues, enabling faster detection of threats in a visually-noisy environment. Therefore, there may be multiple potential benefits from having polarization vision when caustics are prevalent, for example, whereby the need to shoal may be reduced.

### **3.2 Summary**

Water caustics are dynamic patterns of illumination found widespread within shallow underwater habitats. Downwelling light is refracted from the convex and concave areas of surface waves, creating flickering bands of light on the sea-floor. This visual noise can mask signals and cues within an animal's environment, making predator and prey detection considerably more challenging. For many species, polarization vision is known to help increase the contrast of an object against its background in the visual field, therefore increasing visibility in visual noise such as from turbid water (Cartron et al. 2013). This study therefore set out to determine whether polarization-sensitive animals share this same advantage in water caustics. An expanding disc on a screen was used to simulate an approaching predator, and was overlaid with video of water caustics. Crabs and cuttlefish both exhibited an overall lower response probability to this intensity-based stimulus when the caustics were dynamic rather than static. However, when the stimulus was presented with only a polarization component, there was no effect of the caustic treatment on the response probability. The ability to perceive the polarization of light enabled the crabs and cuttlefish to detect the stimulus irrespective of whether the caustics were static or dynamic; effectively cancelling out the visual noise. This study therefore uncovered a new function for polarization vision; mitigating the impact of water caustics. This research demonstrated how species can adapt their visual system to adhere to challenging and hindering environmental conditions, allowing them to exploit a niche that other species are unable to access.





## **Bibliography**

- Attwell, J.R., Ioannou, C.C., Reid, C.R. and Herbert-Read, J.E., 2020. Fish avoid visually noisy environments that reduce their perceptual abilities. *bioRxiv*.
- Bernard, G.D. and Wehner, R., 1977. Functional similarities between polarization vision and colour vision. *Vision research*, 17(9), pp.1019-1028.
- Blake, A.J., Go, M.C., Hahn, G.S., Grey, H., Couture, S. and Gries, G., 2019. Polarization of foliar reflectance: novel host plant cue for insect herbivores. *Proceedings of the Royal Society B*, 286(1915), p.20192198.
- Bush, S.L. and Robison, B.H., 2007. Ink utilization by mesopelagic squid. *Marine Biology*, 152(3), pp.485-494.
- Cartron, L., Josef, N., Lerner, A., McCusker, S.D., Darmaillacq, A.S., Dickel, L. and Shashar, N., 2013. Polarization vision can improve object detection in turbid waters by cuttlefish. *Journal of experimental marine biology and ecology*, 447, pp.80-85.
- Collewijn, H., 1970. Oculomotor reactions in the cuttlefish, *Sepia officinalis*. *Journal of Experimental Biology*, 52(2), pp.369-384.
- Cuthill, I.C., Matchette, S.R. and Scott-Samuel, N.E., 2019. Camouflage in a dynamic world. *Current Opinion in Behavioural Sciences*, 30, pp.109-115.
- Douglas, J.M., Cronin, T.W., Chiou, T.H. and Dominy, N.J., 2007. Light habitats and the role of polarized iridescence in the sensory ecology of neotropical nymphalid butterflies (*Lepidoptera: Nymphalidae*). *Journal of Experimental Biology*, 210(5), pp.788-799.
- El Jundi, B., Foster, J.J., Byrne, M.J., Baird, E. and Dacke, M., 2015. Spectral information as an orientation cue in dung beetles. *Biology letters*, 11(11), p.20150656.
- Evans, J.C., Dall, S.R. and Kight, C.R., 2018. Effects of ambient noise on zebra finch vigilance and foraging efficiency. *PloS one*, 13(12), p.e0209471.
- Foster, J.J., Kirwan, J.D., El Jundi, B., Smolka, J., Khaldy, L., Baird, E., Byrne, M.J., Nilsson, D.E., Johnsen, S. and Dacke, M., 2019. Orienting to polarized light at night—matching lunar skylight to performance in a nocturnal beetle. *Journal of Experimental Biology*, 222(2).
- Foster, J.J., Temple, S.E., How, M.J., Daly, I.M., Sharkey, C.R., Wilby, D. and Roberts, N.W., 2018. Polarization vision: overcoming challenges of working with a property of light we barely see. *The Science of Nature*, 105(3-4), p.27.

Fleishman, L.J., 1985. Cryptic movement in the vine snake *Oxybelis aeneus*. *Copeia*, 1985(1), pp.242-245.

Galloway, J.A., Green, S.D., Stevens, M. and Kelley, L.A., 2020. Finding a signal hidden among noise: how can predators overcome camouflage strategies? *Philosophical Transactions of the Royal Society B*, 375(1802), p.20190478.

Georger, A., Darmaillacq, A.S., Shashar, N. and Dickel, L., 2021. Early exposure to water turbidity affects visual capacities in cuttlefish (*Sepia officinalis*). *Frontiers in Physiology*, 12, p.88.

Gilly, W.F., Zeidberg, L.D., Booth, J.A.T., Stewart, J.S., Marshall, G., Abernathy, K. and Bell, L.E., 2012. Locomotion and behaviour of Humboldt squid, *Dosidicus gigas*, in relation to natural hypoxia in the Gulf of California, Mexico. *Journal of Experimental Biology*, 215(18), pp.3175-3190.

Grober, M.S., 1990. Luminescent flash avoidance in the nocturnal crab *Portunus xantusii*: II. Cardiac and visual responses to variations in simulated luminescent flashes. *Journal of Experimental Biology*, 148(1), pp.427-448.

Hanlon, R.T. and Messenger, J.B., 2018. Cephalopod behaviour. *Cambridge University Press*.

Hough, A.R., 2014. Automation of discrimination training for cuttlefish (Mollusca: Cephalopoda). *Keystone Journal of Undergraduate Research*, 2, pp.15-21.

How, M.J. and Marshall, N.J., 2014. Polarization distance: a framework for modelling object detection by polarization vision systems. *Proceedings of the Royal Society B: Biological Sciences*, 281(1776), p.20131632.

How, M.J., Norman, M.D., Finn, J., Chung, W.S. and Marshall, N.J., 2017. Dynamic skin patterns in cephalopods. *Frontiers in physiology*, 8, p.393.

How, M.J., Pignatelli, V., Temple, S.E., Marshall, N.J. and Hemmi, J.M., 2012. High e-vector acuity in the polarization vision system of the fiddler crab *Uca vomeris*. *Journal of Experimental Biology*, 215(12), pp.2128-2134.

Jenssen, T.A. and Swenson, B., 1974. An ecological correlate of critical flicker-fusion frequencies for some *Anolis* lizards. *Vision research*, 14(10), pp.965-970.

Johnsen, S., Marshall, N.J. and Widder, E.A., 2011. Polarization sensitivity as a contrast enhancer in pelagic predators: lessons from in situ polarization imaging of transparent zooplankton. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 366(1565), pp.655-670.

- Jordan, T.M., Partridge, J.C. and Roberts, N.W., 2012. Non-polarizing broadband multilayer reflectors in fish. *Nature photonics*, 6(11), pp.759-763.
- Kelber, A., Thunell, C. and Arikawa, K., 2001. Polarization-dependent colour vision in *Papilio* butterflies. *Journal of Experimental Biology*, 204(14), pp.2469-2480.
- King, A.J. and Adamo, S.A., 2006. The ventilatory, cardiac and behavioural responses of resting cuttlefish (*Sepia officinalis*) to sudden visual stimuli. *Journal of Experimental Biology*, 209(6), pp.1101-1111.
- Kyba, C.C., Ruutz, T., Fischer, J. and Hölker, F., 2011. Lunar skylight polarization signal polluted by urban lighting. *Journal of Geophysical Research: Atmospheres*, 116(D24).
- Labhart, T., 2016. Can invertebrates see the e-vector of polarization as a separate modality of light? *Journal of Experimental Biology*, 219(24), pp.3844-3856.
- Labhart, T. and Meyer, E.P., 1999. Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microscopy research and technique*, 47(6), pp.368-379.
- Layne, J.E., Wicklein, M., Dodge, F.A. and Barlow, R.B., 1997. Prediction of maximum allowable retinal slip speed in the fiddler crab, *Uca pugilator*. *The Biological Bulletin*, 193(2), pp.202-203.
- Leggett, L.M.W., 1976. Polarized light-sensitive interneurons in a swimming crab. *Nature*, 262(5570), pp.709-711.
- Liu, F., Han, P., Wei, Y., Yang, K., Huang, S., Li, X., Zhang, G., Bai, L. and Shao, X., 2018. Deeply seeing through highly turbid water by active polarization imaging. *Optics letters*, 43(20), pp.4903-4906.
- Lock, J.A. and Andrews, J.H., 1992. Optical caustics in natural phenomena. *American journal of physics*, 60(5), pp.397-407.
- Loew E.R, and McFarland W.N, 1990. The underwater visual environment. *The visual system of fish* (eds Douglas R, Djamgoz M), pp. 1-40.
- Lynch, D.K., 2015. Snell's window in wavy water. *Applied optics*, 54(4), pp. B8-B11.
- Lynch, D.K., Livingston, W.C., 2001. Colour and light in nature. *Cambridge university press*.

Marshall, N.J., Powell, S.B., Cronin, T.W., Caldwell, R.L., Johnsen, S., Gruev, V., Chiou, T.H.S., Roberts, N.W. and How, M.J., 2019. Polarisation signals: a new currency for communication. *Journal of Experimental Biology*, 222(3), p.134213.

Matchette, S.R., Cuthill, I.C., Cheney, K.L., Marshall, N.J. and Scott-Samuel, N.E., 2020. Underwater caustics disrupt prey detection by a reef fish. *Proceedings of the Royal Society B*, 287(1924), p.20192453.

Matchette, S.R., Cuthill, I.C. and Scott-Samuel, N.E., 2018. Concealment in a dynamic world: dappled light and caustics mask movement. *Animal Behaviour*, 143, pp.51-57.

Matchette, S.R., Cuthill, I.C. and Scott-Samuel, N.E., 2019. Dappled light disrupts prey detection by masking movement. *Animal Behaviour*, 155, pp.89-95.

Matchette, S.R. and Herbert-Read, J.E., 2021. Dynamic visual noise promotes social attraction, but does not affect group size preference, in a shoaling fish. *Animal Behaviour*, 177, pp.39-48.

Maximov, V.V., 2000. Environmental factors which may have led to the appearance of colour vision. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, 355(1401), pp.1239-1242.

McFarland, W.N. and Loew, E.R., 1983. Wave produced changes in underwater light and their relations to vision. *Predators and prey in fishes* (pp. 11-22). Springer, Dordrecht.

Merilaita, S. and Stevens, M., 2011. Crypsis through background matching. *Animal camouflage: mechanisms and function*, pp.17-33.

Müller, M. and Wehner, R., 2007. Wind and sky as compass cues in desert ant navigation. *Nature*, 94(7), pp.589-594.

Nelson, L., 2003. An investigation of the phototransduction cascade and temporal characteristics of the retina of the cuttlefish, *Sepia officinalis*. <http://hdl.handle.net/10026.1/2520>. Retrieved March 14, 2022.

Pavao, R., (2016). `sigm_fit` ([https://www.mathworks.com/matlabcentral/fileexchange/42641-sigm\\_fit](https://www.mathworks.com/matlabcentral/fileexchange/42641-sigm_fit)), MATLAB Central File Exchange. Retrieved January 19, 2021.

Peters, R.A., Hemmi, J.M. and Zeil, J., 2007. Signalling against the wind: modifying motion-signal structure in response to increased noise. *Current Biology*, 17(14), pp.1231-1234.

Robertson, B.A. and Horváth, G., 2019. Colour polarization vision mediates the strength of an evolutionary trap. *Evolutionary Applications*, 12(2), pp.175-186.

Rosen, H., Gilly, W., Bell, L., Abernathy, K. and Marshall, G., 2015. Chromogenic behaviours of the Humboldt squid (*Dosidicus gigas*) studied in situ with an animal-borne video package. *Journal of Experimental Biology*, 218(2), pp.265-275.

Sabbah, S. and Shashar, N., 2006. Underwater light polarization and radiance fluctuations induced by surface waves. *Applied optics*, 45(19), pp.4726-4739.

Schechner, Y.Y. and Karpel, N., 2004, November. Attenuating natural flicker patterns. *Oceans' 04 MTS/IEEE Techno-Ocean'04* (IEEE Cat. No. 04CH37600) (Vol. 3, pp. 1262-1268). IEEE.

Schenk, H., 1957. On the focusing of sunlight by ocean waves. *JOSA*, 47(7), pp.653-657.

Schwind, R., 1999. *Daphnia pulex* swims towards the most strongly polarized light—a response that leads to 'shore flight'. *Journal of Experimental Biology*, 202(24), pp.3631-3635.

Sharkey, C.R., Partridge, J.C. and Roberts, N.W., 2015. Polarization sensitivity as a visual contrast enhancer in the Emperor dragonfly larva, *Anax imperator*. *Journal of Experimental Biology*, 218(21), pp.3399-3405.

Shashar, N., Hagan, R., Boal, J.G. and Hanlon, R.T., 2000. Cuttlefish use polarization sensitivity in predation on silvery fish. *Vision research*, 40(1), pp.71-75.

Shashar, N., Hanlon, R.T. and Petz, A.D., 1998. Polarization vision helps detect transparent prey. *Nature*, 393(6682), pp.222-223.

Smithers, S.P., Roberts, N.W. and How, M.J., 2019. Parallel processing of polarization and intensity information in fiddler crab vision. *Science advances*, 5(8), p.eaax3572.

Snyder, A.W., 1973. Polarization sensitivity of individual retinula cells. *Journal of comparative physiology*, 83(4), pp.331-360.

Sweeney, A., Jiggins, C. and Johnsen, S., 2003. Polarized light as a butterfly mating signal. *Nature*, 423(6935), pp.31-32.

Talbot, C.M. and Marshall, J., 2010. Polarization sensitivity and retinal topography of the striped pyjama squid (*Sepioloidea lineolata*). *Journal of Experimental Biology*, 213(19), pp.3371-3377.

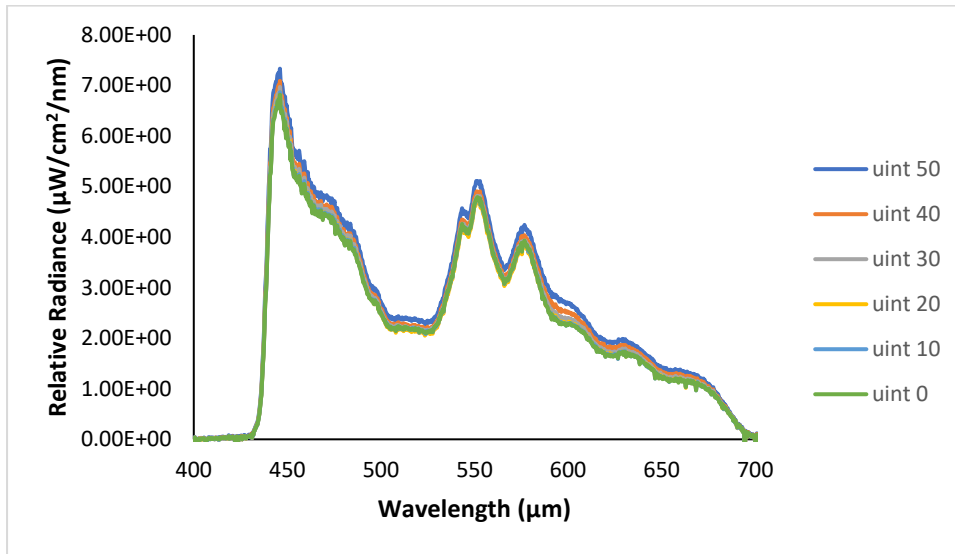
Temple, S.E., Pignatelli, V., Cook, T., How, M.J., Chiou, T.H., Roberts, N.W. and Marshall, N.J., 2012. High-resolution polarization vision in a cuttlefish. *Current Biology*, 22(4), pp.R121-R122.

Viviani, P. and Aymoz, C., 2001. Colour, form, and movement are not perceived simultaneously. *Vision Research*, 41(22), pp.2909-2918.

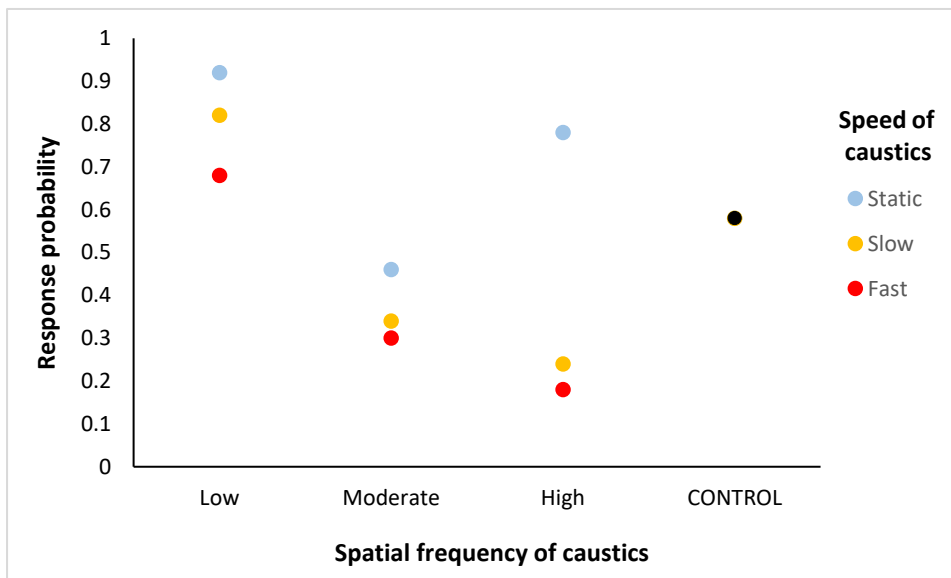
Yadav, P. and Shein-Idelson, M., 2021. Polarization Vision in Invertebrates: Beyond the Boundaries of Navigation. *Current Opinion in Insect Science*.

Young, A.T., 1982. Rayleigh scattering. *Physics Today*, 35(1), pp.42-48.

## Appendices



**Appendix 1. Calibrated relative radiance measurements for the expanding disc stimulus at 6 different intensity contrasts.** Measurements are taken from the light emitted from the display screen in experiment 1 (with *Carcinus maenas*) and experiment 3 (with *Sepia officinalis*), using a Gershan tube (Ocean Optics) attached to an optic fibre. The uint value is the shade of the expanding disc; where 0 = Black, and 50 = the same shade of grey as the background (control).



**Appendix 2. The response probability of *Carcinus maenas* (n = 50) to expanding disc stimuli overlaid with different types of caustic flicker pattern.** The treatments vary in speed and spatial frequency (level of detail), and the control treatment consists of a greyscale plain background instead of a caustic pattern.