

Cuttlefish dynamic camouflage: responses to substrate choice and integration of multiple visual cues

Justine J. Allen^{1,*}, Lydia M. Mäthger¹, Alexandra Barbosa^{1,2,†},
Kendra C. Buresch¹, Emilia Sogin¹, Jillian Schwartz¹,
Charles Chubb^{3,4} and Roger T. Hanlon¹

¹Marine Biological Laboratory, 7 MBL Street, Woods Hole, MA 02543, USA

²ICBAS—Institute of Biomedical Sciences Abel Salazar, University of Porto, Porto, Portugal

³Department of Cognitive Sciences and ⁴Institute for Mathematical Behavioral Sciences, University of California, Irvine, CA 92697, USA

Prey camouflage is an evolutionary response to predation pressure. Cephalopods have extensive camouflage capabilities and studying them can offer insight into effective camouflage design. Here, we examine whether cuttlefish, *Sepia officinalis*, show substrate or camouflage pattern preferences. In the first two experiments, cuttlefish were presented with a choice between different artificial substrates or between different natural substrates. First, the ability of cuttlefish to show substrate preference on artificial and natural substrates was established. Next, cuttlefish were offered substrates known to evoke three main camouflage body pattern types these animals show: Uniform or Mottle (function by background matching); or Disruptive. In a third experiment, cuttlefish were presented with conflicting visual cues on their left and right sides to assess their camouflage response. Given a choice between substrates they might encounter in nature, we found no strong substrate preference except when cuttlefish could bury themselves. Additionally, cuttlefish responded to conflicting visual cues with mixed body patterns in both the substrate preference and split substrate experiments. These results suggest that differences in energy costs for different camouflage body patterns may be minor and that pattern mixing and symmetry may play important roles in camouflage.

Keywords: behaviour; *Sepia officinalis*; body pattern; habitat preference; visual perception

1. INTRODUCTION

This study is the first to address whether cuttlefish show any preference to settle on a particular substrate type. In cephalopods, colour and pattern changes are under fast neural control (e.g. Holmes 1940; Packard 1972; Hanlon 2007; Hanlon *et al.* 2009) and many cephalopod species, particularly shallow-water octopus and cuttlefish, are able to successfully camouflage themselves on most natural substrates. We therefore did not expect cuttlefish (*Sepia officinalis*) to show substrate preference but could not rule it out for two reasons. First, a substrate preference may indicate that one particular body pattern may be more effective at fooling a predator's eye than the other patterns, and animals may consequently choose to rest, and camouflage themselves, on one specific substrate rather than another. Second, a substrate preference may indicate that one particular body pattern may be less costly energetically.

Many animals show habitat and substrate choice for a variety of reasons. For example, in some fish (e.g. sticklebacks and pupfish; Webster & Hart 2004; Horstkotte & Plath 2008), spiders (Morse 2006) and birds (e.g. curlew; Finn *et al.* 2008), substrate choice is related to

foraging. Egg laying and nesting behaviour of insects, reptiles and birds are also often associated with particular substrate choices (damselflies, Matushkina & Gorb 2007; lizards, Doody *et al.* 2006; black-throated blue warblers, Steele 1993). Substrate choice can be related to communication and copulation success (e.g. wolf spiders; Hebets *et al.* 2008). Animals make substrate choices as part of antipredator behaviours such as hiding and shoaling (e.g. English whiting, Atkinson *et al.* 2004; mollies, Bradner & McRobert 2001; roach and perch, Christensen & Persson 1993). Camouflage is also a primary reason for substrate choice in many animals that have fixed, or slowly changing, body patterns across the animal kingdom (Cott 1940), from insects to arachnids and crustaceans to vertebrates (e.g. mayfly larvae, Tikkanen *et al.* 2000; spiders, Heiling *et al.* 2005; tiger prawns, Kenyon *et al.* 2003; sculpin, Brown 1991; weedy seadragons, Sanchez-Camara *et al.* 2006; flatfish, Moles & Norcross 1995; Stoner & Ottmar 2003; Stoner & Titgen 2003; Ryer *et al.* 2004, 2008). Moving to the wrong substrate may render an animal with a fixed body pattern conspicuous (Merilaita *et al.* 1999).

Cuttlefish (*S. officinalis*) camouflage is impressive not only because of the speed at which these animals can change patterns and colours but also because their camouflage is apparently very effective at deceiving the visual capabilities of their varied predators (e.g. Hanlon &

* Author for correspondence (jallen@mbl.edu).

† Present address: National Center of Scientific Research, National Museum of Natural History, UMR 7179, Brunoy, France.

Messenger 1988, 1996; Hanlon *et al.* 2009). Cuttlefish employ three major body pattern types for camouflage: Uniform and Mottle patterns that function by generally matching the background, and Disruptive patterns that primarily act to break up the animal's recognizable outline (Cott 1940; Hanlon 2007; Hanlon *et al.* 2009). Background matching has recently been defined as a form of camouflage where 'the appearance generally matches the colour, lightness and pattern of one (specialist) or several (compromise) background types' (Stevens & Merilaita 2009a, p. 424) or 'there is a general resemblance but not exact pattern match to the immediate background' (Hanlon *et al.* 2009, p. 432). Disruptive camouflage has recently been defined as: 'a set of markings that creates the appearance of false edges and boundaries and hinders the detection or recognition of an object's, or part of an object's, true outline and shape' (Stevens & Merilaita 2009b, p. 484).

In the laboratory, we and others have successfully evoked background matching (Uniform and Mottle body patterns) and Disruptive body patterns in cuttlefish. Uniform patterns are generally evoked on uniform substrates, such as uniform artificial computer printouts or fine sand with little or no contrast (e.g. Mäthger *et al.* 2007; Allen *et al.* 2009). Mottle patterns are evoked on small-scale substrates with moderate-to-high contrast, such as small black and white checkerboards or natural substrates with small particles (check or particle size 3–12% of the animal's white square component; see Barbosa *et al.* 2007, 2008b and Chiao *et al.* in press for details). Disruptive patterns are evoked on large-scale substrates with high contrast and defined edges, such as large black and white checkerboards or natural rocks (white check or white rock size 40–120% of the animal's white square; Mäthger *et al.* 2006, 2007; Barbosa *et al.* 2007; Kelman *et al.* 2007; Shohet *et al.* 2007; Allen *et al.* 2009; Zylinski *et al.* 2009). Cuttlefish can show these three body pattern types with variations; for example, cuttlefish may show a Mottle pattern with a few disruptive components, such as the white square or white head bar (e.g. Mäthger *et al.* 2006; Barbosa *et al.* 2007). Cuttlefish camouflage is robust; these animals will camouflage on any substrate, natural or artificial (e.g. Chiao *et al.* 2005; Allen *et al.* 2009; Zylinski *et al.* 2009), regardless of the presence of a predator in the tank (J. J. Allen, L. M. Mäthger, K. C. Buresch, R. T. Hanlon *et al.* 2009, unpublished data).

We first tested whether cuttlefish can show a substrate preference at all (§2b). Next, we tested substrate choice using both artificial and natural substrates selected to evoke each of three camouflage body patterns (Uniform, Mottle and Disruptive; §2c). Three variations of natural substrates were tested to examine whether substrate depth influences cuttlefish substrate choice. 'Glued' natural substrates removed the depth variable (because the substrate could not be moved by the animal), the 'loose' substrates allowed some substrate depth while partially revealing each cuttlefish's body pattern and the 'deep' substrates allowed the cuttlefish to completely bury themselves. In a third experiment, we tested cuttlefish camouflage when each eye was presented with conflicting visual cues to investigate whether the cuttlefish brain may prioritize a particular body pattern over another (§2d). Since cuttlefish are driven to camouflage on any substrate on which they are placed, these experiments, by design,

also tested whether cuttlefish have a preference to show one of the three camouflage body pattern types.

2. MATERIAL AND METHODS

(a) *Animals*

European cuttlefish, *S. officinalis*, were hatched and maintained at the Marine Resources Center of the Marine Biological Laboratory in Woods Hole, MA. Twenty animals (mantle length: 5.2–7.9 cm) were tested on the binary substrates: 10 on the artificial substrates and 10 on the natural substrates. Forty animals (mantle length: 3–5 cm, dorsoventral height: approx. 1.5 cm) were used in the substrate preference experiment, 10 in each variation. Ten animals (mantle length: 2.9–3.6 cm) were tested in the split substrate experiment. In all, this paper reports data from 70 cuttlefish.

(b) *Experiment 1: binary substrates*

(i) *Artificial substrate*

Preliminary observations suggested that cuttlefish dislike being placed on white or very light substrates, both artificial (laminated white sheets of paper) and natural (white or bleached sand). To determine if cuttlefish prefer alternative substrates to white backgrounds, 10 animals were tested on a laminated, artificial substrate that was half white, half uniform grey. The walls of the arena (described below in §2b(iii)) were divided to correspond to the adjacent substrate.

(ii) *Natural substrate*

Previous laboratory and field observations suggest that cuttlefish often bury themselves in soft substrates (e.g. Boletzky 1983; Hanlon & Messenger 1988; Poirier *et al.* 2004). Ten cuttlefish were tested on a natural sand substrate. Half of the floor of the experimental arena was made up of substrate glued to a rigid piece of plastic that prevented the cuttlefish from burying their bodies. Superficially level with the glued half, the other half of the substrate was made up of the same sand (approx. 4 cm deep; referred to as deep). The walls of the arena were uniform grey.

(iii) *Experimental setup and procedure*

In both the artificial and the natural versions of the binary substrate experiment, 10 cuttlefish were placed, one at a time, in a rigid plastic circular arena that was 25.5 cm in diameter and approximately 10 cm tall. Each animal was allowed to acclimate to the experimental tank for a minimum of 15 min. Once each animal had settled, its position was recorded and a digital still image was taken with a Nikon Coolpix 5400 camera 0, 4 and 8 min after settling. Data were analysed using a one-tailed permutation test because we expected the animals to prefer the grey background (artificial substrate) and the deep substrate (natural substrate); the probability of choosing grey or deep was $p = 0.5$.

(c) *Experiment 2: substrate preference*

To test whether cuttlefish have a preference for settling on a substrate known to evoke a particular body pattern, cuttlefish were presented with artificial or natural substrates known to evoke the three main body patterns (Uniform, Mottle and Disruptive). Each animal's settling location was recorded and analysed.

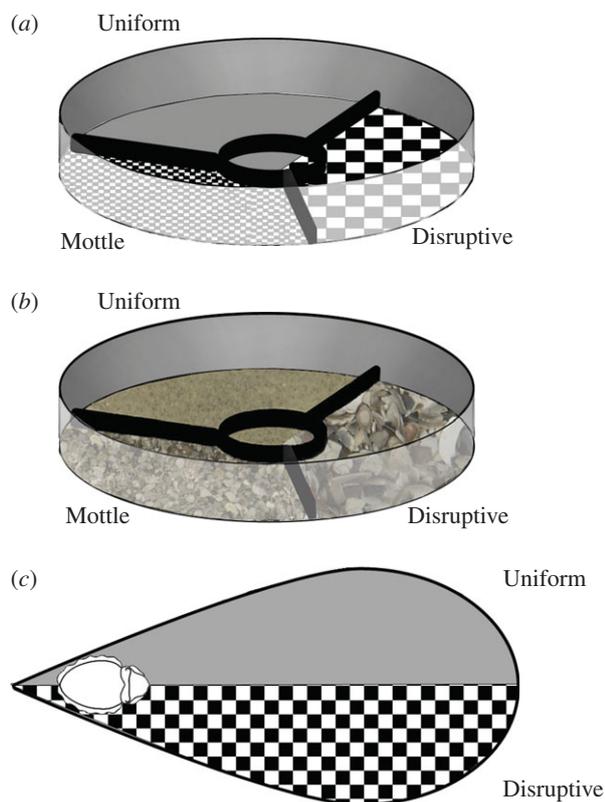


Figure 1. Illustration of set-up for substrate preference experiment on (a) artificial and (b) natural substrates showing arena, substrates and dividers. (c) Illustration of split substrates experimental set-up (top view). Note the position of cuttlefish (drawings are not to scale).

(i) Artificial substrates

Ten animals were given the option to settle on one of the three different laminated artificial substrates or a central area where these three substrates intersected (figure 1a): (i) uniform grey was used to evoke a Uniform pattern; (ii) a high-contrast black and white checkerboard with a check size of about 4 per cent of the animal's white square area (rectangular-shaped chromatic component located in the centre of the animal's mantle; Hanlon & Messenger 1988) was used to evoke a Mottle pattern; and (iii) a high-contrast black and white checkerboard with a check size of about 40 per cent of the animal's white square area was used to evoke a Disruptive pattern (Barbosa *et al.* 2007).

(ii) Natural substrates

Thirty cuttlefish were tested on three natural substrates: (i) sand to evoke Uniform, (ii) small shells (Kona Coast Aragonite, Tidal Marine Substrates by Seachem; mean particle diameter: 0.26 cm) to evoke Mottle, and (iii) large shells (African Cichlid mix, CaribSea, Inc.; mean particle diameter: 1.15 cm) to evoke Disruptive body pattern. In the first version of this experiment, 10 animals were tested on natural substrates that had been glued in place. This ensured that each animal experienced the substrates in the same way. In the second version, 10 different animals were tested after approximately 1 cm of loose substrate (referred to as loose) was placed on top of the glued substrates; this allowed the animals to feel the substrate and toss substrate particles on top of their mantle and fins without completely obscuring the body pattern. In a third version, 10 different animals

were tested after approximately 4 cm of loose substrate (referred to as deep) was spread on top of the glued substrates; this gave the animals the option of completely burying themselves into the substrate (e.g. Boletzky 1983; Hanlon & Messenger 1988; Poirier *et al.* 2004).

(iii) Experimental setup and procedure

A circular arena was divided into three regions of equal area (each 543.57 cm²), each covered with one of the three substrates. A 2.5 cm tall grey wall separated the three regions. This wall blocked other substrates from view while the cuttlefish was settled on one of the three substrates but allowed the animal to easily swim from one substrate to another. A smaller central area (201.6 cm²) was used to hold animals before trials began (figure 1a,b). The experimental tank was supplied with running sea water, lit evenly and shielded from outside stimuli using a black plastic curtain (Boal & Ni 1996).

For each trial, a cuttlefish was placed in a confined glass container inside the central area and allowed to view all substrate options for 5 min. Then, the divider system was placed in the experimental tank and the glass container was removed, leaving the animal in the small central area (figure 1a,b). For the loose and deep natural substrates, each cuttlefish was released into the centre portion of the divider system; the walls stayed in place to support the substrate in each section. In all versions, the animal was then allowed to swim about and select a substrate on which to settle. After the animal was sitting calmly, the trial began. A still image was taken with a Sony HDR-HC1 HDV video camera 12 min after acclimation. To control for possible differences in water flow or light field that may influence the animals' choice, the substrate was rotated three times. That is, each substrate (evoking Uniform, Mottle or Disruptive) was oriented towards the water source for a series of trials. Each animal was tested three times, once on each of three substrate rotations.

(iv) Analysis

To see whether our data gave any significant indication of preference, we assumed that responses are jointly independent across all trials and tested the null hypothesis that all of our subjects were equally likely to select each of the four options in each trial. Under this null hypothesis, the statistic below has a chi-square distribution with three degrees of freedom (e.g. Zar 1984), where o_k (the observed value) equals the total number of trials in which a cuttlefish settled on substrate k ($k = 1, 2, 3$ and 4, representing Centre, Uniform, Mottle and Disruptive, respectively) and 7.5 (the expected value: 10 animals times 3 rotations = 30 trials, divided by 4 possible substrates = 7.5) is the expected number of times a cuttlefish would sit on substrate k if substrate choice were due to chance alone.

$$X = \sum_{k=1}^4 \frac{(o_k - 7.5)^2}{7.5}$$

For the artificial and glued natural substrates, each image was analysed using an automated grading system to verify the expected cuttlefish body pattern (granularity statistics). Body patterns are distinguished by the scale of light and dark patches that make up the pattern. Large-scale patches are Disruptive, moderate-scale are Mottle and small-scale are Uniform. The method is further described in Barbosa *et al.* (2008b). The images from the loose substrate preference

experiment were not analysed with this system because the cuttlefish often tossed substrate particles on their mantles in an attempt to bury themselves. Instead, body patterns were confirmed visually. Images from the deep substrate experiment did not undergo this image analysis system for the same reason; animals frequently buried into the substrate, occluding most or all of their body patterns.

(d) *Experiment 3: split substrate*

In this experiment, we tested whether the cuttlefish brain prioritizes visual cues from one particular substrate over another. We predicted cuttlefish would show a body pattern in response to the more important cue if a hierarchy of cues exists. The eyes of a cuttlefish are situated on opposite sides of the head, taking in visual information monocularly from the left and right hemispheres. Here, we designed an experimental chamber in which we presented each cuttlefish eye with visual stimuli known to evoke different camouflage body patterns.

(i) *Substrates*

Six laminated artificial substrates were used: three control substrates and three experimental substrates. Uniform grey was used to evoke Uniform coloration, and two high-contrast black and white checkerboards to evoke Mottle and Disruptive coloration. The area of the checks used on these substrates was 5 per cent and 55 per cent, respectively, of the area of the white square component (e.g. Barbosa *et al.* 2007). The control substrates covered the entire bottom of the experimental chamber and were paired with a matching wall. The experimental substrates were presented on half of the floor and adjacent wall of the arena (figure 1c). Stimuli for the following combinations of body patterns were used: (i) Uniform/Disruptive, (ii) Mottle/Disruptive, and (iii) Mottle/Uniform. Artificial, rather than natural, substrates were used to carefully control visual cues while maintaining the same substrate texture.

(ii) *Experimental set-up and procedure*

A teardrop-shaped arena was placed inside an experimental tank supplied with circulating sea water. For each trial, a cuttlefish was placed in the arena and allowed to acclimate. A trial began when the cuttlefish had settled with the posterior end of its mantle in the point of the teardrop and its head facing the wide side of the arena, a position that most animals assumed readily (figure 1c). Cuttlefish were not tethered or otherwise restricted from movement during these experiments; animals proved to remain stationary for the duration of the trials. Digital photographs were taken at 4, 8 and 12 min after acclimation using a Nikon Coolpix 5400 camera. Cuttlefish were tested randomly until all 10 had been tested on all six substrates.

(iii) *Analysis*

Granularity statistics were gathered using an automated image analysis algorithm (see Barbosa *et al.* 2008b for method). The statistics from each of the three images per trial were averaged, so each trial contributed one data point to the distribution. The mean of all 10 animals on each substrate was then plotted and the shape of the resulting line was compared with that of all other substrates tested.

3. RESULTS

(a) *Experiment 1: binary substrates*

(i) *Artificial substrate*

The response to the half white, half uniform grey substrate was robust: all 10 animals settled on the grey half of the substrate in all three images taken for each trial. As expected, all animals showed a Uniform body pattern. Under the null hypothesis that our subjects have no preference for the grey over the white background, the probability of the obtained result (that all 10 subjects chose the grey background) is $p = (0.5)^{10}$, where 0.5 is the probability of choosing the grey substrate and 10 is the number of animals that actually chose the grey substrate. Because this is a one-tailed test, we reject the null hypothesis with a p -value of <0.001 .

(ii) *Natural substrate*

The response to the half deep sand, half glued sand substrate was also robust: all 10 animals settled on the deep half of the substrate in all three images taken for each trial. Eight of the 10 animals tested buried into the deep substrate. The animals that did not bury showed the expected Uniform body pattern. Therefore, under the null hypothesis that our subjects have no preference for the deep sand over the glued sand, the probability of the obtained result is $p = (0.5)^{10}$, where 0.5 is the probability of choosing the deep substrate and 10 is the number of animals that actually chose the deep substrate. Because this is a one-tailed test, we reject the null hypothesis with a p -value of <0.001 .

(b) *Experiment 2: substrate preference*

Image analysis confirmed that body patterns were consistent with expected results on the grey, small check and large check substrates, as well as sand, small shells and large shells substrates, i.e. Uniform, Mottle and Disruptive patterns were shown, respectively. Animals that had settled in the centre region (see figure 1a,b) for both artificial and natural substrates showed mixed body patterns. Image analysis data are not shown, but see images in figure 2a–c. None of the animals settled in one particular spot in the arena, implying they were not influenced by water flow or variation in the light field.

(i) *Artificial substrates*

Our data do not support the hypothesis that cuttlefish have a strong preference for a particular substrate when given the choice between four areas of artificial substrates (uniform grey, small check, large check and centre; $X = 3.87$, $p = 0.276$; figure 2a). One out of ten animals settled on the same substrate in all three substrate rotations while three animals sat on a different substrate for each rotation.

(ii) *Natural substrates*

Glued substrates. Our data do not support the hypothesis that cuttlefish have a strong preference for a particular substrate when given the choice between four glued natural substrates (sand, small shells, large shells and centre; $X = 6.53$, $p = 0.09$; figure 2b). Cuttlefish were slightly more likely to settle on the small shells (37%) than any other substrate. None of the cuttlefish settled on the same substrate for all three rotations; six out of ten animals settled on a different substrate for each rotation.

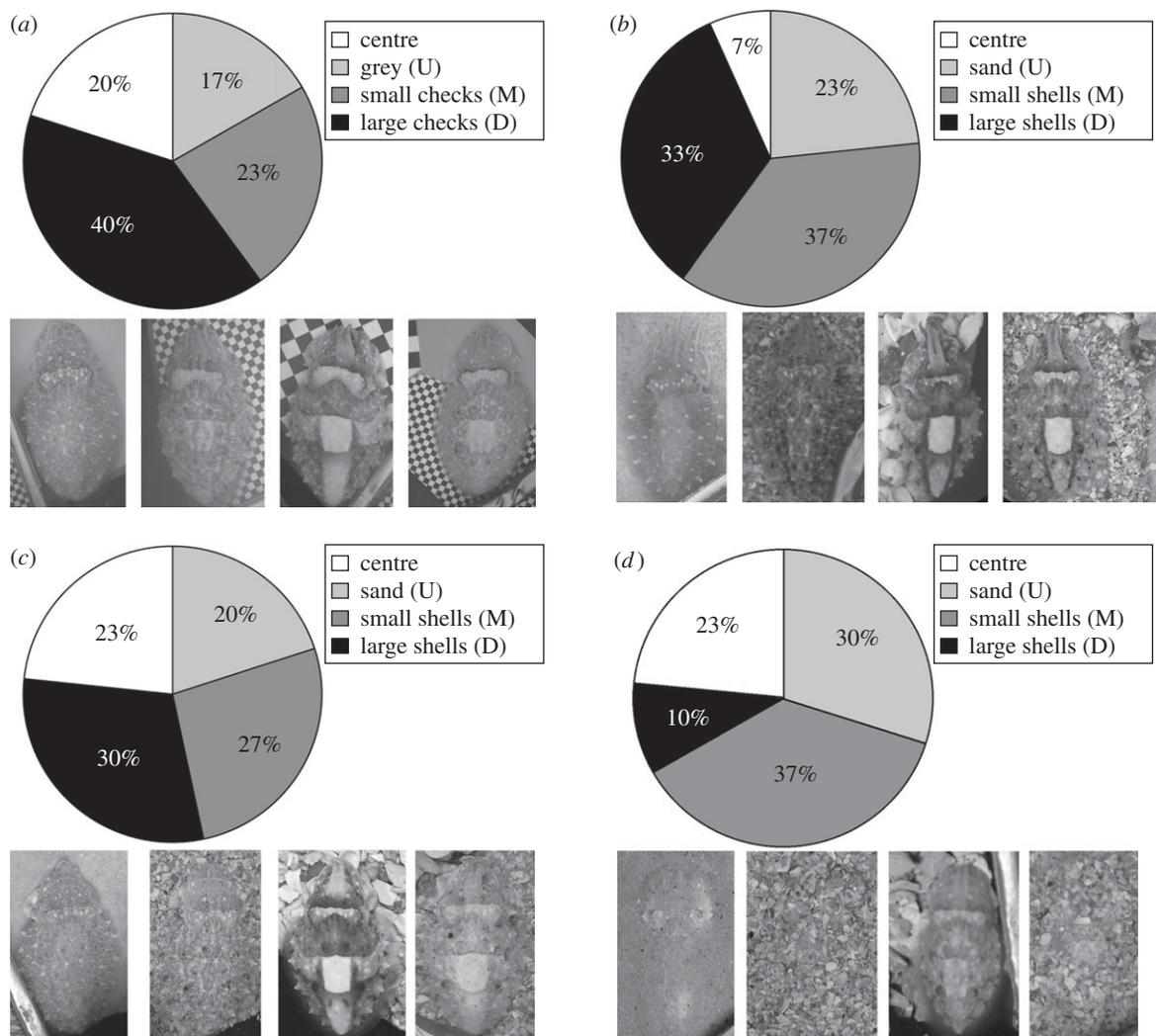


Figure 2. Substrate preference experiment: results with representative images; pie graphs show percentages of trials in which cuttlefish sat on each substrate. (a) Artificial substrates; images left to right: on grey (U), small checks (M), large checks and centre. (b) Natural, glued substrates. (c) Natural, loose substrates (depth: 1 cm of same substrates). (d) Natural, deep substrates (depth: 4 cm of same substrates). For b, c and d, images left to right: sand (U), small shells (M), large shells (D) and centre. Abbreviations: U, Uniform; M, Mottle and D, Disruptive.

Loose substrates. Our data do not support the hypothesis that cuttlefish have a strong preference for a particular substrate when given the choice between four loose natural substrates (same substrates as glued substrates experiment), 1 cm deep to allow partial burying behaviour while exposing most of the body pattern ($X = 0.67$, $p = 0.88$; figure 2c). None of the cuttlefish sat on the same substrate for all three rotations; four out of ten animals settled on a different substrate for each rotation.

Deep substrates. Our data do not support the hypothesis that cuttlefish have a strong preference for a particular substrate when given the choice between four deep natural substrates (same substrates as glued substrates experiment), 4 cm deep to allow complete burying behaviour ($X = 4.67$, $p = 0.20$; figure 2d). However, 67 per cent of the time cuttlefish chose substrates made up of small particles in which they could bury themselves (sand and small shells; the large shells were too large for the cuttlefish to shift around to bury themselves). The animals that chose to settle in the centre buried themselves in either the sand or small shells subdivision of

the central area; none settled on the large shells in the centre. Including these trials, the animals chose substrates where they could bury themselves in 90 per cent of the trials ($23 + 30 + 37\%$; figure 2d). Five of the ten animals settled on the same substrate for all three rotations; none settled on a different substrate in each rotation.

(c) Experiment 3: split substrate experiment

(i) Control substrates

The mean granularity curves (which are quantitative representations of each body pattern) for the control substrates were shaped as expected (figure 3a); that is, cuttlefish showed the appropriate body patterns, i.e. Uniform, Mottle and Disruptive (figure 3b(i–iii)). Cuttlefish showing Uniform had low relative energy across all six granularity bands, meaning the body patterns were generally uniform. Cuttlefish showing Mottle had more energy in the second and third granularity bands with a generally gradual slope, meaning the body patterns were made up of mostly medium-sized light and dark patches with few large or very tiny patches. Cuttlefish showing Disruptive

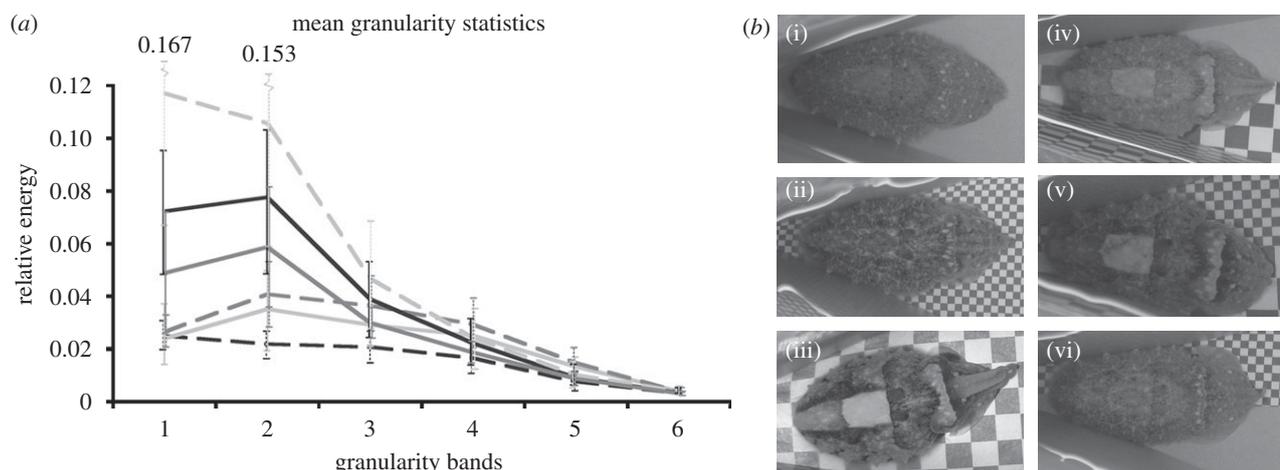


Figure 3. (a) Split substrates experiment: results of image analysis for images of cuttlefish on six different substrates. Control substrates (Uniform, black; Mottle, dark grey; Disruptive, light grey) are shown with broken lines; experimental split substrates (Uniform/Disruptive, dark grey; Mottle/Disruptive, black; Mottle/Uniform, light grey) are shown with solid lines. See text and Barbosa *et al.* (2008b) for details of image analysis. Error bars are standard deviation. (b) Representative images of cuttlefish sitting on control substrates: (i) Uniform, (ii) Mottle and (iii) Disruptive; and experimental split substrates: (iv) Uniform/Disruptive, (v) Mottle/Disruptive and (vi) Mottle/Uniform.

had the most energy in the first and second granularity bands with a steep slope, meaning the body patterns were made up of mostly large light and dark patches with few moderate or tiny patches (figure 3a).

(ii) Experimental split substrates

For each experimental split substrate, the granularity curve fell *between* the curves for the control version of each substrate (figure 3a), indicating the cuttlefish were responding to cues from each half of the split substrate with components from multiple body patterns.

Grey/large checks (Uniform/Disruptive) split substrate. On the grey/large checks split substrate (Uniform/Disruptive), the granularity curve (solid, medium grey line in figure 3a) was similar in shape to the large checks (Disruptive) control (dashed, light grey line in figure 3a) but was lower in amplitude (figure 3a). That is, the images show cuttlefish with a generally Uniform pattern plus a few Disruptive components (see Hanlon & Messenger 1988 for details of different disruptive components): white square, white head bar, dark anterior head bar; a few animals showed some lightening in the white mantle bar region and some weak expression of median mantle stripes (figure 3b(iv)).

Small checks/large checks (Mottle/Disruptive) split substrate. The granularity statistics for images of cuttlefish on the small checks/large checks (Mottle/Disruptive) produced a curve (solid, dark grey line in figure 3a) that was similar in shape to the control large checks but lower in amplitude, resulting in a line that fell *between* the small checks (Mottle) control and the large checks (Disruptive) control (figure 3a). Images from the small checks/large checks substrate showed cuttlefish with body patterns that were generally Mottle, especially around the lateral edges of the animals, with a few Disruptive components: white square component, white head bar, dark anterior head bar, median mantle stripes, anterior transverse mantle line and white posterior triangle (figure 3b(v)).

Small checks/grey (Mottle/Uniform) split substrate. The granularity statistics from images of cuttlefish sitting on

the small checks/grey (Mottle/Uniform) split substrate produced a curve (solid, light grey line in figure 3a) that fell between the small checks (Mottle) control and the grey (Uniform) control (figure 3a). Image data from the small checks/grey split substrate showed cuttlefish with a generally Uniform body pattern with a few larger scale patches distinctive of Mottle body patterns (figure 3b(vi)).

4. DISCUSSION

Although cuttlefish have the ability to show a preference for sitting on a particular substrate (e.g. the grey half of the artificial substrate in the Binary substrates experiment), our results suggested they do not have a preference for a particular substrate type they might encounter in nature (sand, small shells and large shells). Our results also suggested cuttlefish do not have a preference for showing a particular body pattern. Instead, these animals take visual cues from their surroundings and respond with an appropriate camouflage pattern regardless of substrate type.

The exception to this rule occurs when the substrate is fine, soft and deep enough to allow a cuttlefish to bury its body. When the only variable was substrate depth (Binary substrates experiment: natural substrate), all cuttlefish strongly preferred the deep half of the substrate. Although in the substrate preference experiment cuttlefish chose from the sections of deep natural substrates statistically equally, 90 per cent of the time they chose substrates in which they could completely bury their bodies. When *S. officinalis* have the opportunity to disappear into the finer substrates such as sand and small shells, they are likely to do so (figure 4).

The natural habitat of *S. officinalis* is visually and structurally diverse, and pressure from visual predators is probably the driving force behind the complex camouflage abilities of these animals. Because cuttlefish can change their pattern, colour, contrast intensity, physical texture and posture for camouflage in almost any

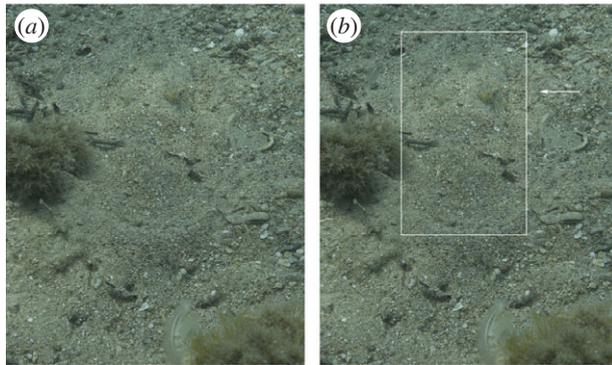


Figure 4. Cuttlefish *Sepia officinalis* showed no strong substrate preference except when they could bury themselves. This behaviour can be seen in the laboratory as well as in the field. (a) Field image of a cuttlefish buried in soft substrate. (b) Same field image; a box is drawn around the animal's body and an arrow points to the right eye to indicate cuttlefish location.

surrounding, they are not constrained to a particular visual microhabitat. Furthermore, having a substrate preference would be detrimental to cuttlefish survival; if cuttlefish were strongly attracted to rocks, for example, predators may learn to spend more time searching for cuttlefish on rocky substrates than on those with smaller particles. It is intuitive, therefore, and supported by the data presented in this paper as well as recent *in situ* observations, that cuttlefish do not have a preference when given a choice between substrates that evoke a Uniform, Mottle or Disruptive body pattern.

When presented with multiple cues on either artificial or natural substrates (i.e. when sitting in the centre section of the substrate preference experiment or when sitting on any of the experimental split substrates), cuttlefish responded with a mixed body pattern. This suggests that the animals integrate multiple visual cues from their environment and use skin components characteristic of different body patterns (Uniform, Mottle and Disruptive) to produce a mixed camouflage pattern. This illustrates the adaptability of the camouflage system in *S. officinalis*. Their camouflage body patterns lie along a continuum of possibilities with their landmark categories previously defined as Uniform, Mottle and Disruptive (e.g. Hanlon 2007; Hanlon *et al.* 2009).

It has been speculated that colour and pattern change for camouflage are energetically costly in cephalopods (Hanlon *et al.* 1999), as well as in chameleons (Stuart-Fox & Moussalli 2009). To the best of our knowledge, there are no data on the energy requirements for chromatophore expansion to produce each of the three types of camouflage body patterns in cuttlefish. However, this behavioural study suggests that any difference in the energy cost between Uniform, Mottle and Disruptive body patterns may be negligible because cuttlefish were statistically equally willing to sit on all substrates and show the appropriate body pattern. If chromatophore expansion was considerably energetically costly, cuttlefish may be more willing to sit on substrates on which fewer chromatophores need to be expanded (such as a light uniform substrate) than on substrates on which more chromatophores need to be expanded (such as rocks or large checks that evoke a Disruptive body pattern).

Furthermore, if chromatophore expansion required a great deal of energy, cuttlefish may choose to express the less costly of the two body patterns cued when presented with conflicting visual information (as in the split substrate experiment). Neither of these results was observed; this behavioural evidence therefore suggests that (i) the energy requirement for chromatophore expansion is comparable between the different camouflage body patterns shown by cuttlefish, or (ii) the differences in energetic costs are less important than the need to produce effective camouflage to avoid predation, regardless of the complexity or energetic costs of the resultant body pattern.

Cuttlefish have the ability to present chromatic components unilaterally (Hanlon & Messenger 1988; Langridge 2006; Barbosa *et al.* 2008a). When cuttlefish were presented with conflicting visual information into each eye, we found that they did not prioritize a particular substrate, nor were body pattern components presented unilaterally. Instead, cuttlefish integrated cues for multiple body patterns and generally showed symmetrical (when viewed from above), mixed camouflage patterns. This was shown by Langridge (2006), who reported a high degree of bilateral symmetry of camouflage body patterns in cuttlefish. Moreover, we have never noticed an octopus or cuttlefish using an asymmetrical (relative to anterior–posterior central body axis, viewed from above) body pattern for camouflage in our numerous underwater field images of benthic cephalopods in natural habitats. This is particularly interesting because there is some debate over whether body symmetry is advantageous (Troscianko *et al.* 2009) or disadvantageous (Cuthill *et al.* 2006a,b; Merilaita & Lind 2006) for camouflage. Our results, especially the split substrate experiment, suggest that for cuttlefish, overall body pattern symmetry is advantageous over asymmetry for effective camouflage or is important for another reason (e.g. a display of fitness), unrelated to camouflage.

In summary, cuttlefish (and it is most likely that this also applies to other benthic cephalopod species) probably do not have a preference for a particular substrate type on which to express a particular camouflage body pattern (although one species—*S. officinalis*—prefers substrates that allow the animals to bury themselves). This result is intuitive because these animals are under strong predation pressure and must be able to quickly and adaptively cope with diverse habitats.

Special thanks to MRC staff, interns and Liese Siemann for help and advice. This manuscript was improved by the helpful comments of two anonymous reviewers. A.B. was funded by POCI 2010 and Fundo Social Europeu through the FCT, Portugal (SFRH/BD/11303/2002). The Sholley Foundation is gratefully acknowledged.

REFERENCES

- Allen, J. J., Mäthger, L. M., Barbosa, A. & Hanlon, R. T. 2009 Cuttlefish use visual cues to control three-dimensional skin papillae for camouflage. *J. Comp. Physiol. A* **195**, 547–555. (doi:10.1007/s00359-009-0430-y)
- Atkinson, C. J. L., Bergmann, M. & Kaiser, M. J. 2004 Habitat selection in whiting. *J. Fish Biol.* **64**, 788–793. (doi:10.1111/j.1095-8649.2004.00340.x)
- Barbosa, A., Mäthger, L. M., Chubb, C., Florio, C., Chiao, C.-C. & Hanlon, R. T. 2007 Disruptive coloration in

- cuttlefish: a visual perception mechanism that regulates ontogenetic adjustment of skin patterning. *J. Exp. Biol.* **210**, 1139–1147. (doi:10.1242/jeb.02741)
- Barbosa, A., Litman, L. & Hanlon, R. T. 2008a Changeable cuttlefish camouflage is influenced by horizontal and vertical aspects of the visual background. *J. Comp. Physiol. A* **194**, 405–413. (doi:10.1007/s00359-007-0311-1)
- Barbosa, A., Mäthger, L. M., Buresch, K. C., Kelly, J., Chubb, C., Chiao, C.-C. & Hanlon, R. T. 2008b Cuttlefish camouflage: the effects of substrate contrast and size in evoking uniform, mottle or disruptive body patterns. *Vis. Res.* **48**, 1242–1253. (doi:10.1016/j.visres.2008.02.011)
- Boal, J. G. & Ni, J. N. 1996 Ventilation rate of cuttlefish, *Sepia officinalis*, in response to visual stimuli. *Veliger* **39**, 342–347.
- Boletzky, S. V. 1983 *Sepia officinalis*. In *Cephalopod life cycles, vol. 1: species accounts* (ed. P. R. Boyle), pp. 31–52. New York, NY: Academic Press Inc.
- Bradner, J. & McRobert, S. P. 2001 Background colouration influences body colour segregation in mollies. *J. Fish Biol.* **59**, 673–681. (doi:10.1111/j.1095-8649.2001.tb02371.x)
- Brown, L. R. 1991 Differences in habitat choice and behavior among 3 species of sculpin (*Cottus*) in artificial stream channels. *Copeia* **1991**, 810–819. (doi:10.2307/1446408)
- Chiao, C.-C., Kelman, E. J. & Hanlon, R. T. 2005 Disruptive body patterning of cuttlefish (*Sepia officinalis*) requires visual information regarding edges and contrast of objects in natural substrate backgrounds. *Biol. Bull.* **208**, 7–11. (doi:10.2307/3593095)
- Chiao, C.-C., Chubb, C., Buresch, K. C., Barbosa, A., Allen, J. J., Mäthger, L. M. & Hanlon, R. T. In press. Mottle camouflage patterns in cuttlefish: quantitative characterization and visual background stimuli that evoke them. *J. Exp. Biol.*
- Christensen, B. & Persson, L. 1993 Species-specific anti-predatory behaviours: effects on prey choice in different habitats. *Behav. Ecol. Sociobiol.* **32**, 1–9. (doi:10.1007/BF00172217)
- Cott, H. B. 1940 *Adaptive coloration in animals*. London, UK: Methuen & Co. Ltd.
- Cuthill, I. C., Hiby, E. & Lloyd, E. 2006a The predation costs of symmetrical cryptic coloration. *Proc. R. Soc. B* **273**, 1267–1271. (doi:10.1098/rspb.2005.3438)
- Cuthill, I. C., Stevens, M., Windsor, A. M. M. & Walker, H. J. 2006b The effects of pattern symmetry on detection of disruptive and background-matching coloration. *Behav. Ecol.* **17**, 828–832. (doi:10.1093/beheco/arj015)
- Doody, J. S., Guarino, E., Georges, A. & Corey, B. 2006 Nest site choice compensates for climate effects on sex ratios in a lizard with environmental sex determination. *Evol. Ecol.* **20**, 307–330. (doi:10.1007/s10682-006-0003-2)
- Finn, P. G., Catterall, C. P. & Driscoll, P. V. 2008 Prey versus substrate as determinants of habitat choice in a feeding shorebird. *Estuar. Coast. Shelf Sci.* **80**, 381–390. (doi:10.1016/j.ecss.2008.09.001)
- Hanlon, R. T. 2007 Cephalopod dynamic camouflage. *Curr. Biol.* **17**, R400–R405. (doi:10.1016/j.cub.2007.03.034)
- Hanlon, R. T. & Messenger, J. B. 1988 Adaptive coloration in young cuttlefish (*Sepia officinalis* L.): the morphology and development of body patterns and their relation to behaviour. *Phil. Trans. R. Soc. Lond. B* **320**, 437–487. (doi:10.1098/rstb.1988.0087)
- Hanlon, R. T. & Messenger, J. B. 1996 *Cephalopod behaviour*. Cambridge, UK: Cambridge University Press.
- Hanlon, R. T., Forsythe, J. W. & Joneschild, D. E. 1999 Crypsis, conspicuousness, mimicry and polyphenism as antipredator defences of foraging octopuses on Indo-Pacific coral reefs, with a method of quantifying crypsis from video tapes. *Biol. J. Linn. Soc.* **66**, 1–22. (doi:10.1111/j.1095-8312.1999.tb01914.x)
- Hanlon, R. T., Chiao, C.-C., Mäthger, L. M., Barbosa, A., Buresch, K. C. & Chubb, C. 2009 Cephalopod dynamic camouflage: bridging the continuum between background matching and disruptive coloration. *Phil. Trans. R. Soc. B* **364**, 429–437. (doi:10.1098/rstb.2008.0270)
- Hebets, E. A., Elias, D. O., Mason, A. C. & Stratton, G. E. 2008 Substrate-dependent signalling success in the wolf spider *Schizocosa retrorsa*. *Anim. Behav.* **75**, 605–615. (doi:10.1016/j.anbehav.2007.06.021)
- Heiling, A. M., Chittka, L., Cheng, K. & Herberstein, M. E. 2005 Colouration in crab spiders: substrate choice and prey attraction. *J. Exp. Biol.* **208**, 1785–1792. (doi:10.1242/jeb.01585)
- Holmes, W. 1940 The colour changes and colour patterns of *Sepia officinalis* L. *Proc. Zool. Soc. Lond. A* **110**, 2–35.
- Horstkotte, J. & Plath, M. 2008 Divergent evolution of feeding substrate preferences in a phylogenetically young species flock of pupfish (*Cyprinodon* spp.). *Naturwissenschaften* **95**, 1175–1180. (doi:10.1007/s00114-008-0439-z)
- Kelman, E. J., Baddeley, R. J., Shohet, A. J. & Osorio, D. 2007 Perception of visual texture and the expression of disruptive camouflage by the cuttlefish *Sepia officinalis*. *Proc. R. Soc. B* **274**, 1369–1375. (doi:10.1098/rspb.2007.0240)
- Kenyon, R. A., Loneragan, N. R., Hughes, J. M. & Staples, D. J. 2003 Habitat type influences the microhabitat preference of juvenile tiger prawns (*Penaeus esculentus* Haswell and *Penaeus semisulcatus* de Haan). *Estuar. Coast. Shelf Sci.* **287**, 1–12.
- Langridge, K. V. 2006 Symmetrical crypsis and asymmetrical signalling in the cuttlefish *Sepia officinalis*. *Proc. R. Soc. B* **273**, 959–967. (doi:10.1098/rspb.2005.3395)
- Mäthger, L. M., Barbosa, A., Miner, S. & Hanlon, R. T. 2006 Color blindness and contrast perception in cuttlefish (*Sepia officinalis*) determined by a visual sensorimotor assay. *Vis. Res.* **46**, 1746–1753. (doi:10.1016/j.visres.2005.09.035)
- Mäthger, L. M., Chiao, C. C., Barbosa, A., Buresch, K. C., Kaye, S. & Hanlon, R. T. 2007 Disruptive coloration elicited on controlled natural substrates in cuttlefish, *Sepia officinalis*. *J. Exp. Biol.* **210**, 2657–2666. (doi:10.1242/jeb.004382)
- Matushkina, N. & Gorb, S. 2007 Mechanical properties of the endophytic ovipositor in damselflies (Zygoptera, Odonata) and their oviposition substrates. *Zoology* **110**, 167–175. (doi:10.1016/j.zool.2006.11.003)
- Merilaita, S. & Lind, J. 2006 Great tits (*Parus major*) searching for artificial prey: implications for cryptic coloration and symmetry. *Behav. Ecol.* **17**, 84–87. (doi:10.1093/beheco/arj007)
- Merilaita, S., Tuomi, J. & Jormalainen, V. 1999 Optimization of cryptic coloration in heterogeneous habitats. *Biol. J. Linn. Soc.* **67**, 151–161. (doi:10.1111/j.1095-8312.1999.tb01858.x)
- Moles, A. & Norcross, B. L. 1995 Sediment preference in juvenile pacific flatfishes. *Neth. J. Sea Res.* **34**, 177–182. (doi:10.1016/0077-7579(95)90025-X)
- Morse, D. H. 2006 Fine-scale substrate use by a small sit-and-wait predator. *Behav. Ecol.* **17**, 405–409. (doi:10.1093/beheco/arj053)
- Packard, A. 1972 Cephalopods and fish: the limits of convergence. *Biol. Rev.* **47**, 241–307. (doi:10.1111/j.1469-185X.1972.tb00975.x)
- Poirier, R., Chichery, R. & Dickel, L. 2004 Effects of rearing conditions on sand digging efficiency in juvenile cuttlefish. *Behav. Process.* **67**, 273–279. (doi:10.1016/j.beproc.2004.04.006)

- Ryer, C. H., Stoner, A. W. & Titgen, R. H. 2004 Behavioral mechanisms underlying the refuge value of benthic habitat structure for two flatfishes with differing anti-predator strategies. *Mar. Ecol. Prog. Ser.* **268**, 231–243. (doi:10.3354/meps268231)
- Ryer, C. H., Lemke, J. L., Boersma, K. & Levas, S. 2008 Adaptive coloration, behavior and predation vulnerability in three juvenile North Pacific flatfishes. *J. Exp. Mar. Biol. Ecol.* **359**, 62–66. (doi:10.1016/j.jembe.2008.02.017)
- Sanchez-Camara, J., Booth, D. J., Murdoch, J., Watts, D. & Turon, X. 2006 Density, habitat use and behaviour of the weedy seadragon *Phyllopteryx taeniolatus* (Teleostei: Syngnathidae) around Sydney, New South Wales, Australia. *Mar. Freshw. Res.* **57**, 737–745. (doi:10.1071/MF05220)
- Shohet, A. J., Baddeley, R. J., Anderson, J. C. & Osorio, D. 2007 Cuttlefish camouflage: a quantitative study of patterning. *Biol. J. Linn. Soc.* **92**, 335–345. (doi:10.1111/j.1095-8312.2007.00842.x)
- Steele, B. B. 1993 Selection of foraging and nesting sites by black-throated blue warblers. *Condor* **95**, 568–579. (doi:10.2307/1369601)
- Stevens, M. & Merilaita, S. 2009a Animal camouflage: current issues and new perspectives. *Phil. Trans. R. Soc. B* **364**, 423–427. (doi:10.1098/rstb.2008.0217)
- Stevens, M. & Merilaita, S. 2009b Defining disruptive coloration and distinguishing its functions. *Phil. Trans. R. Soc. B* **364**, 481–488. (doi:10.1098/rstb.2008.0216)
- Stoner, A. W. & Ottmar, M. L. 2003 Relationships between size-specific sediment preferences and burial capabilities in juveniles of two Alaska flatfishes. *J. Exp. Mar. Biol. Ecol.* **282**, 85–101. (doi:10.1016/S0022-0981(02)00447-1)
- Stoner, A. W. & Titgen, R. H. 2003 Biological structures and bottom type influence habitat choices made by Alaska flatfishes. *J. Exp. Mar. Biol. Ecol.* **292**, 43–59. (doi:10.1016/S0022-0981(03)00144-8)
- Stuart-Fox, D. & Moussalli, A. 2009 Camouflage, communication and thermoregulation: lessons from colour changing organisms. *Phil. Trans. R. Soc. B* **364**, 463–470. (doi:10.1098/rstb.2008.0254)
- Tikkanen, P., Huhta, A. & Muotka, T. 2000 Determinants of substrate selection in lotic mayfly larvae: is cryptic coloration important? *Arch. Hydrobiol.* **148**, 45–57.
- Troscianko, T. S., Benton, C. P., Lovell, P. G., Tolhurst, D. J. & Pizlo, Z. 2009 Camouflage and visual perception. *Phil. Trans. R. Soc. B* **364**, 449–461. (doi:10.1098/rstb.2008.0218)
- Webster, M. M. & Hart, P. J. B. 2004 Substrate discrimination and preference in foraging fish. *Anim. Behav.* **68**, 1071–1077. (doi:10.1016/j.anbehav.2004.04.003)
- Zar, J. H. 1984 *Biostatistical analysis*, 2nd edn. Englewood Cliffs, NJ: Prentice-Hall.
- Zylinski, S., Osorio, D. & Shohet, A. J. 2009 Perception of edges and visual texture in the camouflage of the common cuttlefish, *Sepia officinalis*. *Phil. Trans. R. Soc. B* **364**, 439–448. (doi:10.1098/rstb.2008.0264)