Juvenile plaice (*Pleuronectes platessa*) produce camouflage by flexibly combining two separate patterns

Emma J. Kelman, Palap Tiptus and Daniel Osorio*

School of Life Sciences, University of Sussex, Brighton BN1 9QG, UK

*Author for correspondence (e-mail: d.osorio@sussex.ac.uk)

Summary

Plaice (*Pleuronectes platessa*) is a flatfish well-known for the ability to vary its body pattern, probably for camouflage. This study investigates the repertoire of patterns used by juvenile plaice, by describing how they respond to shifts between three artificial backgrounds. Two basic patterns are under active control, fine 'spots' and coarser 'blotches'. These patterns are superimposed on a fairly uniform ground. For the six plaice studied, the levels of expression of the spot and blotch patterns varied continuously and independently according to the visual background, and in a manner consistent with their being cryptic. The repertoire of plaice appears to be intermediate between the tropical flatfish *Bothus ocellatus*, which has three separate basic patterns, and two temperate species *Paralichthys lethostigma* and *Pseudopleuronectes americanus*, which have one each. It is interesting to consider how mixing a small number of coloration patterns is effective for camouflage, and why the demands of this task may lead to differences between species.

Key words: flatfish, camouflage, coloration, vision, *Pleuronectes platessa*.

Introduction

Bottom dwelling flatfish (Pleuronectiformes) and cephalopod molluscs are known for being able to produce a range of body patterns, which are thought to be for camouflage, and – at least in cephalopods – for communication. In some respects these patterns resemble expressions of emotion by humans and other mammals. Both involve co-ordinated actions of many muscles, which in the fish and cephalopods regulate the visible area of pigmented chromatophore cells (Burton, 1999). Just as facial expressions combine a small number of basic forms – associated with emotional states (Darwin, 1872; Leyhausen, 1956), there is evidence that fish and cephalopods have a number of basic patterns. About 13 basic body patterns are recognised in cuttlefish *Sepia officinalis* [Cephalopoda (Hanlon and Messenger, 1988; Crook et al., 2002)], whereas the flatfish *Bothus ocellatus* reportedly has three (Ramachandran et al., 1996). Our interest is in how juvenile plaice (*Pleuronectes platessa*; Pleuronectiformes) generate their repertoire of coloration patterns. Although in the absence of an experimental study it is hard to prove (e.g. Cuthill et al., 2005; Ruxton et al., 2004), it is a reasonable assumption that cryptic camouflage is the primary concern (Sumner, 1911; Hewer, 1931). Plaice are cryptic to human observers, but there is no suggestion that they are aposematic, that body patterns serve interspecific communication, or that plaice change their appearance with sufficient speed to confuse predators (Hanlon et al., 1999). Our observations of responses to artificial backgrounds are consistent with pattern changes enhancing crypsis, but it is not the purpose of this study to establish the effectiveness of the changes to natural predators.

Natural textures and camouflage design

The difficulty with proving that a pattern is cryptic and understanding visual camouflage in general is that for humans [and *a fortiori* for other species (Majerus et al., 2000; Heiling et al., 2003)], there is no systematic method for predicting whether any two visual textures will match (Julesz et al., 1978; Julesz, 1981; Victor et al., 2005). For humans, colour and the spatial frequency spectrum are relevant, but do not completely specify the appearance of visual texture (Victor et al., 2005). Thus camouflage design for military purposes is as much an art as a science. Similar problems apply to understanding biological camouflage (Ruxton et al., 2004) (but see Kiltie et al., 1995).

It is therefore worthwhile to test the efficacy of cryptic patterns experimentally, as did, Cuthill and co-workers (Cuthill et al., 2005) who asked how 'wing patterns' of moth-like baits affected the rate of avian predation. Species that vary their body patterns allow an alternative experimental approach. One can investigate the range of patterns used, how they are generated and how the animal selects a particular pattern.

This study investigates responses to artificial backgrounds, and aims to identify the strategy used to produce the repertoire

or gamut of patterns that is expressed. One option is to use a relatively large set of different patterns, and to express them one at a time. This strategy is used by human military camouflage. An alternative is to combine flexibly a small number of patterns to match a range of backgrounds. These patterns would, in effect, be a basis-set for generating a range of visual textures (or images). Other possibilities can easily be imagined. If the patterns are used for camouflage the relative merits of different strategies may depend on how natural backgrounds vary. For example, whether there are a number of distinct kinds, or continuous gradation between a few basic types of visual texture (Julesz, 1981; Kiltie et al., 1995; Cuthill et al., 2005).

We note that an interpretation in terms of the relationship between the repertoire of body patterns and visual texture of backgrounds as an adaptation for cryptic camouflage, although reasonable, is not essential to the validity of our observations. They could for instance be related to the information content of a communication system (Leyhausen, 1956; Crook et al., 2002).

Flatfish achieve short-term colour variation by means of melanophores that are under neuronal control (Burton, 2002), and which expand to produce dark patches and/or mask the iridophores [i.e. white structural reflectors (Lanzing, 1977); see Fig. 1A]. In a study of how visual backgrounds affect coloration in two species of flatfish, southern flounder (*Paralichthys lethostigma*) and winter flounder (*Pseudopleuronectes americanus*), Saidel (Saidel, 1988) concluded that both the mean reflectance and the contrast of the background have an influence. Maximal contrast in pattern of *Paralichthys* ranged from 14% to 70%, according to the background. These two species did not match further aspects of visual texture, for example whether the background was fine or coarse. In particular, for *Paralicthys* "*all features of skin texture were present for all adaptive changes*" [(Saidel, 1988), p. 495]. This limitation is not faced by the tropical flounder *Bothus ocellatus* (Ramachandran et al., 1996), where a principal components analysis of their body patterns suggested that they have three basic patterns. In *Bothus* the authors observed continuous variation, but did not quantify the levels of expression. For juvenile plaice used here coloration turned out to be sufficiently simple for us to analyse their body patterns by visual inspection (Fig. 1A).

Materials and methods

Six wild-caught juvenile plaice (*Pleuroncetes platessa* L.; 64–110 mm in length and 35–72 mm width) were used. They were held in a circular tank (1.2 m in diameter, 0.2 m depth with natural seawater pumped from offshore) at between 14°C and 16°C; maintained under a 12 h:12 h L:D lighting regime; and fed daily *ad libitum* with frozen krill (*Euphausia superba*).

Experiments were performed in a glass tank (0.9 m×0.9 m) on artificial backgrounds (Fig. 1A) made of substrate fixed to 0.5 m×0.5 m Perspex sheets with aquarium sealant (Geocel Ltd, UK). In preliminary tests from a range of backgrounds we chose three that caused the plaice to produce clearly distinct patterns (Fig. 1A). These were as follows: (1) the glass aquarium raised above a wooden table, giving a uniform pattern; (2) black and white gravel with the average diameter of three millimetres; and (3) natural gravel (with an average diameter of five millimetres) with blue circles of 60 mm in diameter painted randomly on the gravel. Hereafter these are called backgrounds 1, 2 and 3. In experiments, the backgrounds were paired, to provide the six possible transitions: i.e. 1→2, 1→3, 2→1, 2→3, and 3→1, 3→2.

At the start of an experiment a plaice was placed directly on the first substrate. Preliminary observations showed that plaice completed any changes to their body pattern within 15 min, so the fish was left for this time to adjust, after which a digital photograph was taken (Olympus C-300 Zoom, Olympus America Inc, 3.0 Megapixel and 2.8× optical zoom). The fish was then transferred to the second background, and a further image taken after 15 min. Each of the six plaice was tested once for each of the six background transitions.

Scoring and analysis of responses

The procedure described above produced 72 images (2 images for each plaice ×6 combinations of backgrounds ×6 plaice). A grading scheme was used to specify the how strongly the two body patterns were expressed. Grading was done by two people blind to the background, by removing the latter in Adobe Photoshop. On the rare occasions where the two scores differed they were averaged. Each body pattern was assigned a grade on a four-point scale (Fig. 1C) ranging from 0 (not expressed) to 3 (strongly expressed). The spot and the blotch were scored separately, i.e. the scorers graded the spots first, and then blotches. Also, care was taken to identify the positions of all the variable melanophore clusters, as these vary slightly between individual fish.

To investigate the relationship between the visual background and coloration we first looked at the level of expression of the two patterns once animals had settled for at least 20 min (Fig. 1B). χ^2 tests were used to establish the significance of differences observed (Table 1). In addition to describing these settled patterns we scored the pattern change associated with the transition to a new background (Fig. 1C). For each of the six possible transitions, changes were scored +1 for an increase, 0 for no change and –1 for a decrease in the level of expression. To distinguish effects of the background from the (non-directional) effects of a change we combined data for opposite pairs of transitions (e.g. 1→2 and 2→1, etc.; Fig. 1C). Thus an increase in the expression of a feature associated with the transition 1→2 is equivalent to a decrease for the transition 2→1 etc. This gave a set of 12 separate measurements for each pair of backgrounds; one forward and one reverse for each of the six fish. As the tests were done at different times they are independent. A sign test was applied to non-zero scores to determine the effect of background on the change in body pattern.
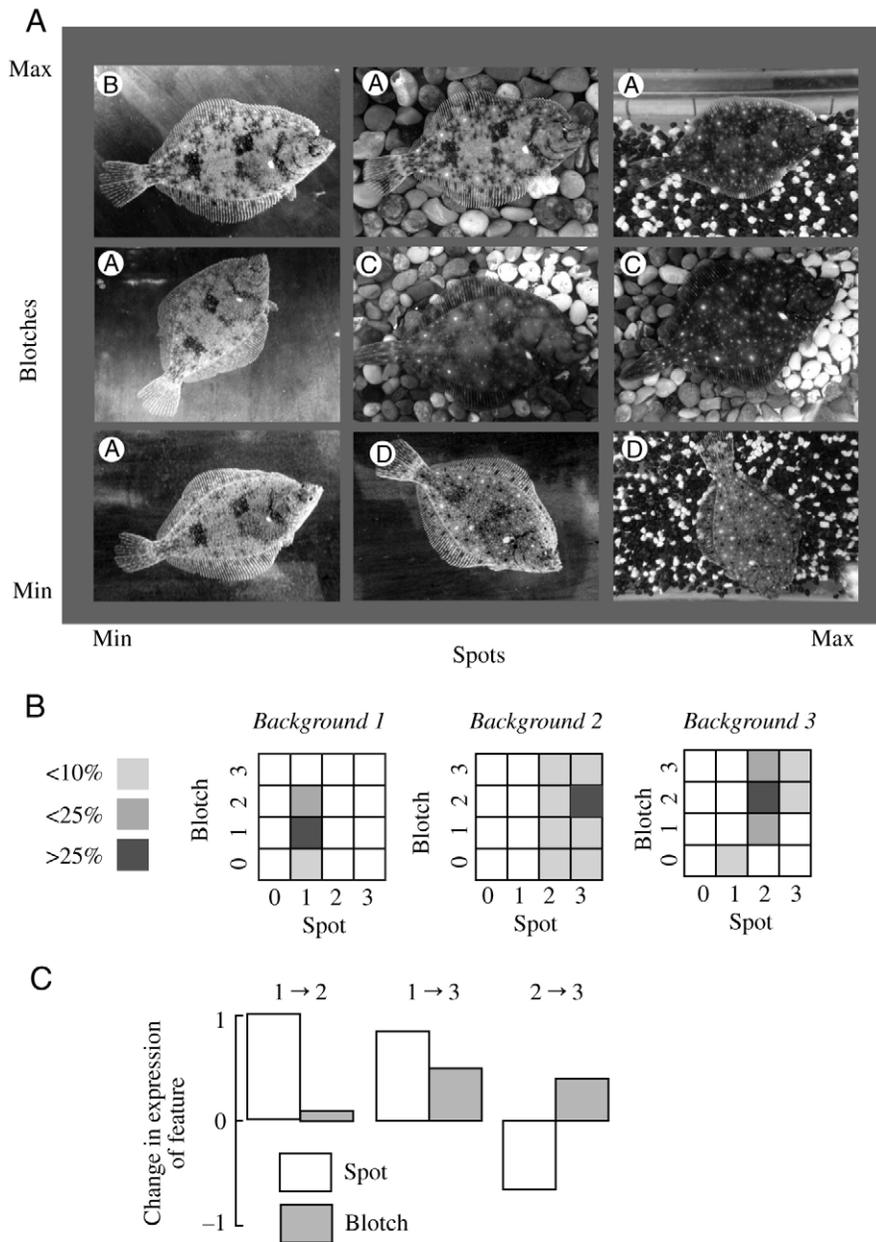
Fig. 1. (A) Photographs showing varying levels of expression of spots and blotches. Letters A–D identify four individual plaice out of the six used in the study. The modifiable features can be seen by comparing the images at the four corners of the figure. Spots are readily identified, the blotches are confusable with fixed dark patches, three of which are clearly seen on fish A. Images were scored blind (see Materials and methods). The three different backgrounds are illustrated. Background 1 in the left column, background 2 at the lower right and background 2 at the upper middle. It is apparent that the fish can regulate the expression of spots and blotches continuously and independently. On background 1, spots and blotches are expressed at a low level. On background 2, spots are dominant, with the white spots giving an impression of transparency, which is a very effective camouflage technique. On background 3, both spots and blotches are clearly expressed. (B) Responses of plaice to the three different backgrounds. Data are for six individuals each recorded twice on the three backgrounds, after at least 15 min acclimatisation. Plots show the levels of expression of spots and blotches in each of the twelve images for each background. The greyscale indicates the proportion of occasions on which a given combination of spots and blotches were seen. Table 1. The background has significant effect on coloration pattern (see Table 1). (C) Changes in level of expression of spots and blotches associated with the transition between each of the three pairs of backgrounds. The values plotted are based on scores of the sign but not the magnitude of the change in expression associated with a transition between a pair of backgrounds. See main text for details. Changes in levels of expression all differ significantly from zero ($P<0.05$), with the exception of blotches in transition 1→2 (see Results).

Results and discussion

From preliminary studies using a wide range of backgrounds, the three experimental backgrounds that were used for systematic study were those that gave the widest range of patterns (Fig. 1A). We found, by visual inspection, that patterns could be completely characterised in terms of the level of expression of two types of feature, namely small discrete 'spots', and larger 'blotches'. There was also no sign that the intensity of any spot was varied independently of the other spots, and the same applied to blotches. We can therefore conclude that spots and blotches represent two basic patterns. The expression of each of these patterns varied continuously, and independently of the other (Fig. 1A,B; Table 1). We note that whereas it was not difficult to analyse the plaice patterns (non-parametrically) by visual inspection, this method would

be much more difficult with higher dimensional systems such as that of the flatfish *Bothus* or of *Sepia* and other cephalopods where more powerful statistical image processing may be required (Ramachandran et al., 1996; Crook et al., 2002; Anderson et al., 2003).

There are approximately equal numbers of dark and light spots, which lie roughly symmetrically about the midline running from the head to the tail (when the fish is viewed from above). The spots are less prominent close to this midline. Blotches are dark, and they too are most prominent near the sides of the body, but in fact lie in six transverse bands, which form regular but 'blurred' stripes running across the animal. Lanzing (Lanzing, 1977) also noted on the approximate symmetry of plaice body patterns. Given that dorsal and ventral parts of fish are not normally mirror images this distribution is

Table 1. *Expression of spots and blotches by plaice settled for 15 min on the three backgrounds*

	Level of expression			
	0	1	2	3
Spots				
Background	0	1	2	3
1	0	12	0	0
2	0	0	5	7
3	0	1	9	2
Blotches				
Background	0	1	2	3
1	1	9	2	0
2	2	1	6	3
3	1	3	5	3

Each of the six fish was tested twice on each of the three backgrounds; values are the scores for each individual test. Given the balanced design we treated samples as independent using two-sided χ^2, with Fisher's exact test where appropriate.

There are significant effects of the background. For spots comparing background 1 with combined data for backgrounds 2 and 3, at expression levels 0,1 *vs* 2,3: $P<0.001$. For spots comparing backgrounds 2 and 3 at expression levels 0–2 *vs* 3 $P=0.09$. For blotches comparing levels 1, 2, 3 separately and expression levels 0,1 *vs* 2, 3: $P=0.008$.

probably not due to developmental constraints, and perhaps implies that symmetry is not detrimental to camouflage (see also Osorio, 1996; Forsman and Merilaita, 2003; Langridge, 2006). In addition to the modifiable patterns flatfish have fixed markings (Fig. 1A, lower left panel) (Hewer, 1931). In our plaice these included large dark patches and a white spot near the opercular fin, which interestingly are rather asymmetrical.

Consistent with the colour changes being used for camouflage the six fish tested showed a significant relationship between the visual background and the patterns expressed (Fig. 1B, Table 1). Similarly, transitions between backgrounds gave systematic directional changes in body patterns (Fig. 1C). Combining data for a transfer from background 1 to background 2 (1→2) with those for the reverse shift, 2→1 (see Materials and methods), there was a significant increase in expression of spots associated with background 2 (χ^2 test, $P<0.001$) but no effect on the expression of blotches. Likewise between backgrounds 1 and 3, expression of both spots and blotches increased for background 3 (spots, $P<0.01$; blotches, $P<0.05$). Finally between backgrounds 2 and 3 the expression of spots decreased ($P<0.005$) and the expression of blotches increased ($P<0.05$) for background 3. Overall the selection of pattern according to background by the six plaice in this study was entirely consistent with the notion that the colour changes are for crypsis. Spots and blotches were faintest on the plain background (1), spots most strongly expressed on high-contrast gravel (2), and blotches predominant on pebbles (3). These observations of behaviour on artificial backgrounds indicate but do not, of course, prove that the animals tend to match the visual texture of the seafloor.

Comparison with other species

The plaice's system for producing body patterns can be compared with those of the other flatfish (Pleuronecticformes), namely southern flounder (*Paralichthys lethostigma*), winter flounder (*Pseudopleuronectes americanus*) (Saidel, 1988) and the eyed flounder *Bothus ocellatus* (Ramachandran et al., 1996). *Paralicthys* and *Pseudopleuronectes* have (at least) one such pattern, *Pleuronectes* two, and *Bothus* three. The three patterns used by *Bothus* were not explicitly identified (but defined in terms of their spatial frequency power spectra), and this may partly reflect the problem of separating the three components of a mixture by simple visual inspection. It is probable (but not certain) that *Bothus* like *Pleuronectes* can control the level of expression of the separate patterns continuously and independently (Ramachandran et al., 1996). Assuming that all systems are equally flexible the number of patterns that can be produced increases with the power of the number of degrees of freedom. Interestingly the timescales over which changes are effected appear to complement their versatility. *Bothus* takes less than 10 s to complete changes, whereas *Pleuronectes* takes about 10 min (unpublished observations). As the four species belong to three families (*Bothus*: Bothidae; *Paralicthys*: Paralicthyidae; and *Pleuronectes*, *Pseudopleuronectes*: Pleuronectidae) it is not possible to say whether the observed differences reflect visual ecology or phylogenetic constraints, but it is interesting to speculate that factors such as water turbidity and depth, the range of backgrounds in the habitat, and the animal's mobility could all affect the desirable repertoire and of speed change of body patterns. For example, *Bothus* lives in shallow waters of tropical coral reefs (Robins and Ray, 1986), where the clear viewing conditions may favour precise matching of background texture over a range of spatial frequencies in cryptic patterns. By comparison, the other species inhabit more turbid temperate seas where details of spatial patterns become blurred over comparatively short ranges, in which case a smaller repertoire of patterns may be adequate.

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