

The response of squid and fish to changes in the angular distribution of light

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This paper describes the responses of a squid (*Alloteuthis subulata*) and a fish (*Trachurus trachurus*) to changes in the angular distribution of light.

An apparatus was made that simulated the angular distribution of daylight in the sea. The apparatus enabled the direction of the brightest light to be changed and the positions of the animals in response to these changes were observed. Squid viewed head-on were observed to roll by a maximum of 20° when the incident light source was at angles between 20° and 90° (where 0° is vertically downwards). When viewed laterally, i.e. in the pitch plane, the squid were observed to position themselves more closely with respect to the angle of the light source, they swam in a near vertical plane when the incident light source was at an angle of 90°. Swimming movements in the roll and pitch plane became more horizontal with positions of the light source between 90 and 180°. Horse mackerel, in contrast, inclined their dorsal surfaces to almost perfectly match the angle of the incident light source, even swimming upside-down when light came from below. These experiments also revealed that squid display a counter-shading chromatophore pattern ('Flexible Countershading') in response to light coming from the sides, which involves darkening the side of the body facing the brightest light. The use of chromatophores in this way may explain why the dorsal light reflex in squid is so weak compared to that of fish.

INTRODUCTION

Alloteuthis subulata are small squid (mantle length approximately 15 cm), which are common in the English Channel. They are fascinating animals, which show the sophisticated colour changes that are commonly employed by cephalopods for camouflage and communication. These colour changes are under nervous control and they are brought about by the action of hundreds of chromatophores (pigment containing organs) and iridophores (light reflecting cells) in the skin (Hanlon, 1982, 1988; Cornwell et al., 1997; Hanlon et al., 1999, Mäthger & Denton, 2001).

Horse mackerel (*Trachurus trachurus*) are typical representatives of silvery fish that camouflage themselves by countershading (Cott, 1940; Denton & Nicol, 1965a,b). Denton & Nicol (1966) and Rowe & Denton (1997) found that the light reflectors on the scales of *T. trachurus* are oriented towards the vertical, so that the intensity of the reflected light equals the intensity of the background for most angles of view. However, certain movements of the fish can cause some parts of the body to give off bright reflections and it appears likely that these reflections can be used by neighbouring fish to aid orientation in schools (Denton & Rowe, 1994; Rowe & Denton, 1997). Both *A. subulata* and *T. trachurus* are schooling animals, for which communication must play an important role in controlling the movements of individuals.

Light in the sea plays a key role in camouflage and signalling of marine animals and their responses to changes in the external light field may offer important insights into the behaviour of these animals. Work on the

squid *Lolliguncula brevis* has shown that if presented with light coming from the side (at 90°) the squid roll their dorsal sides towards the light by no more than 10 to 20° (Preuss & Budelmann, 1995). This is very unlike some fish, which incline their backs dramatically in response to light coming from the side, some even swimming upside-down if light comes from beneath (Holst, 1935, 1950). Turning the dorsal surface towards the brightest light is known as the 'dorsal light reflex'. It was first described, for insects and crabs, by Buddenbrock (1915).

The properties of daylight in the sea differ in a number of ways from those found on land. On a sunny day, radiance near the sea surface is greatest in the direction to which sunlight is refracted on penetrating into the sea. With increasing depth the influence of the sun becomes less important and the angular distribution of daylight approximates to an asymptotic condition, for which the radiance is greatest in a directly downwards direction and falls with an increase in angle to a value up to more than one hundred times less for light travelling directly upwards. When a cloud obscures the sun the greatest values of radiance are found in a directly downwards direction at all depths (Preisendorfer, 1959; Tyler, 1960, 1963; Jerlov & Fukuda, 1960; Jerlov, 1976).

Near the sea surface the direction of maximum radiance depends on the angle of the sun and the cloudiness of the sky, whereas at greater depths the external light field is more constant. Nevertheless, the brightest light almost always comes from above, while the dimmest light comes from beneath. Submarine daylight must therefore furnish animals with important information about their spatial orientation. Gravity also provides animals with information

about their spatial orientation. Cuttlefish (*Sepia officinalis*), for example, have a sophisticated 'Countershading Reflex' (CSR), in which the body part facing upwards is darkened by expansion of the chromatophores. The CSR appears to be primarily based upon gravity perception (Ferguson & Messenger, 1991; Ferguson et al., 1994).

The experiments described here were carried out to study the responses of the squid *A. subulata* to changes in the angular distribution of light. As the reflective system of these squid takes advantage of the properties of daylight in the sea (Mäthger & Denton, 2001), squid were expected to have a fine perception of small changes in the external light field. The objective was to compare the responses of *A. subulata* with those of *T. trachurus* and investigate why the responses to changes in the external light field apparently differ so substantially between squid and fish.

MATERIALS AND METHODS

Animals

Squid *Alloteuthis subulata* (Lamarck) (body length: 10–15 cm) and young horse mackerel *Trachurus trachurus*

(Linnaeus) (body length: 8–12 cm) were trawled off the Plymouth coast and kept in the laboratory's closed circulating seawater system at approximately 16°C.

Equipment and definitions of angles

The apparatus used to investigate the responses of animals to changes in the angular distribution of light is shown in Figure 1A. A plastic tube (constructed from two black PVC dustbins) (length: 112 cm, diameter: 40 cm) was submerged in a glass tank (153×91×91 cm), which was connected to the circulating seawater system. A window (approximately 20 cm wide) was cut along the length of the tube and covered with a piece of white opal Perspex, to which a waterproof white light source (8–12 V) could be attached. The inside of the tube was lined with a plastic sheet with graded shading, from white, at the side of the opal Perspex, to black on the bottom. The tube was attached to the sides of the tank, allowing it to be rotated to any angle around its long axis. The front end of the tube was cut open and replaced with a lid of clear Perspex, so that the animals could be observed and video recorded. Squid were video recorded using a Panasonic camcorder.

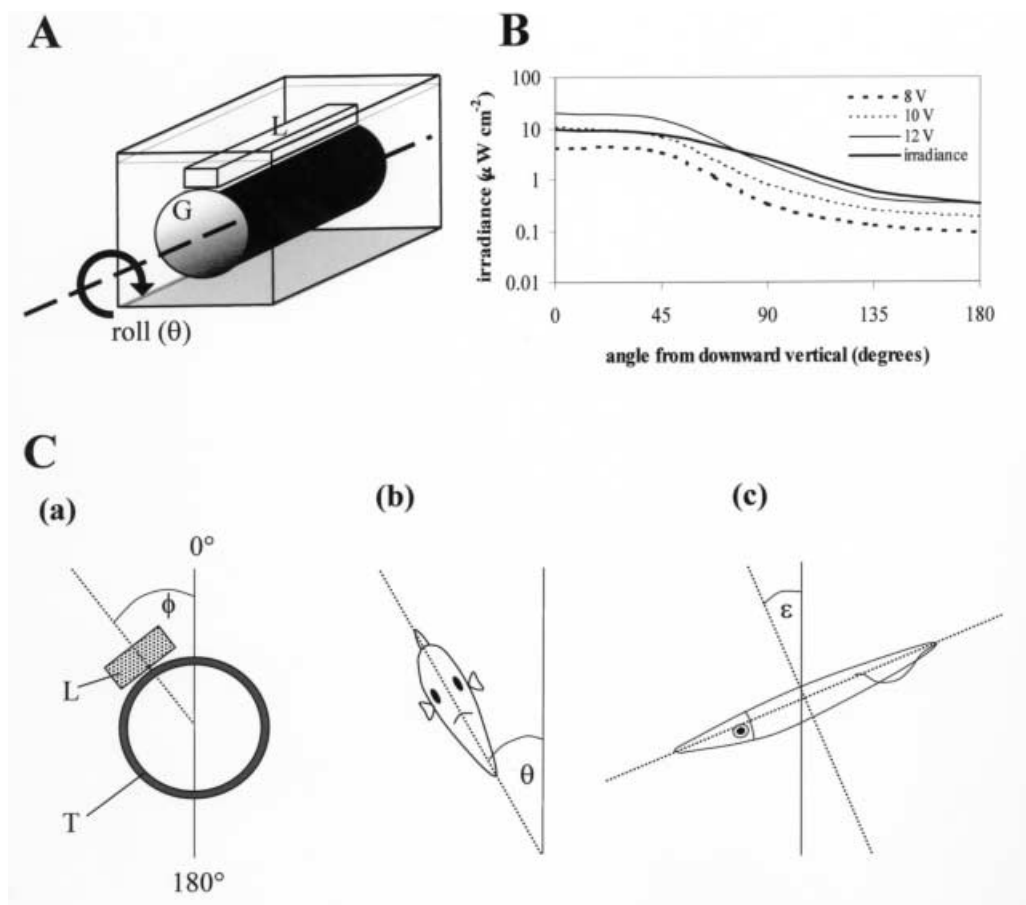


Figure 1. (A) Apparatus used to simulate the angular distribution of daylight in the sea. A tube (40 cm diameter, 112 cm length) was fixed inside a glass tank. The tube was equipped with a light source and could be rotated around its long axis, as shown by arrow. L, light source; T, black tube; G, plastic sheet with graded shading; (B) irradiance measurements of light inside the apparatus shown in (A). The values are given for three voltages of the light source used during experiments. Plotted also is irradiance of daylight in the sea (as arbitrary values), obtained by integration of the radiance values given by Tyler (1963) over the entire hemisphere. The angles on the abscissa represent angles from vertical, i.e. 0 degrees: upwards (facing light); 180 degrees: downwards (facing away from light); (C) diagram defining the angles used in the text. (a) The angle of tilt (ϕ) of the light source, L, light source, T, black tube; (b) the angle of roll (θ) of *Alloteuthis subulata* and *Trachurus trachurus*, viewed anteriorly or posteriorly; (c) the angle of pitch (ϵ) of an animal viewed from the side.

Some still photographs were taken on a Nikon camera. Between five and ten animals were used per experiment. Figure 1B shows measurements of irradiance inside the apparatus for three different voltages of the light source that were used. These measurements were made using an Advantest Optical Powermeter (TQ 8210). For comparison the figure also shows irradiance of daylight in the sea, obtained by integrating the radiance values obtained from the equation given by Tyler (1963) over the whole hemisphere. It can be seen that the irradiance inside the apparatus closely resembles the irradiance of daylight in the sea.

These measurements should, however, only be used as a rough guideline. Irradiance does not convey information about small differences in light intensity with changes in the angle of viewing. Animals in the sea most probably have the ability to detect small differences in light intensity, so that radiance measurements would have been more useful. The main limitation of these measurements is that the irradiance values of upwelling light in the apparatus are greater than the radiance values of upwelling light in the midwaters of the sea. Although the irradiance measurements shown here are sufficient to characterize the light field in the apparatus, in future studies it should be attempted to make more precise radiance measurements.

The angles that describe the orientation of the light source and the animals are shown in Figure 1C. The vertical is defined as 0° , the angles given in the text are those between the vertical and a line indicating the orientation of the light source or an animal relative to the vertical. The orientation of the light source is given by drawing a line perpendicular to the light source and measuring the angle (ϕ) between that line and the vertical (Figure 1Ca). The orientation of a squid or fish in the roll plane, i.e. when observed head-on or tail-on, is given by drawing a line through the dorso-ventral midline of the animal and measuring the angle (θ) between that line and the vertical (Figure 1Cb). There was usually no appreciable difference between the orientation of the head and the mantle of a squid in the roll plane. The orientation of animals in the pitch plane, when observed laterally, is measured by drawing a line perpendicular to the antero-

posterior line of the animal and measuring the angle (ε) between that line and the vertical (Figure 1Cc). These angles were measured by 'grabbing' still images from the video recordings obtained during the experiments. Significance tests of measured angles were carried out using a 1-sample *t*-test.

In order to study the chromatophore pattern produced by *A. subulata* in response to light from the side, each squid was placed in a glass tube (length: 13 cm, diameter: 5 cm) that was fixed inside a small glass tank ($30 \times 18 \times 18$ cm). Two white light sources were positioned on each side of the tank at angles of approximately 45° from the vertical. These were alternated in illuminating a squid from its left and right side. Squid were allowed approximately 5 min to settle before the start of experiments. The light intensities used varied between $39.5 \mu\text{W cm}^{-2}$ to $738 \mu\text{W cm}^{-2}$.

RESULTS

Orientation of body relative to light

Alloteuthis subulata

Squid viewed in the roll plane (i.e. head-on or tail-on), were observed to swim generally with little tilt, which appeared to be almost independent of the angle of the light source. If light came from directly above (0°) the dorsal surfaces of the squid were not inclined. With an increase in the angle of the light source the squid inclined their dorsal surfaces towards the light within a few seconds, although never exceeding an angle of 10 to 20° (Figure 2A,B). For example, with the light source at an angle of 20° the squid inclined their dorsal sides by 10 to 20° . They maintained this angle of tilt with the light source at angles between 20 and 90° . At angles of the light source greater than 90° the orientations of the squid became more and more horizontal and with the light source at an angle of 180° the squid did not incline their dorsal sides (Figure 3Aa). The orientations of squid were significantly different from the angles expected if the squid were to turn their dorsal sides towards the brightest light ($t=5.6$, $df=17$, $P<0.001$).

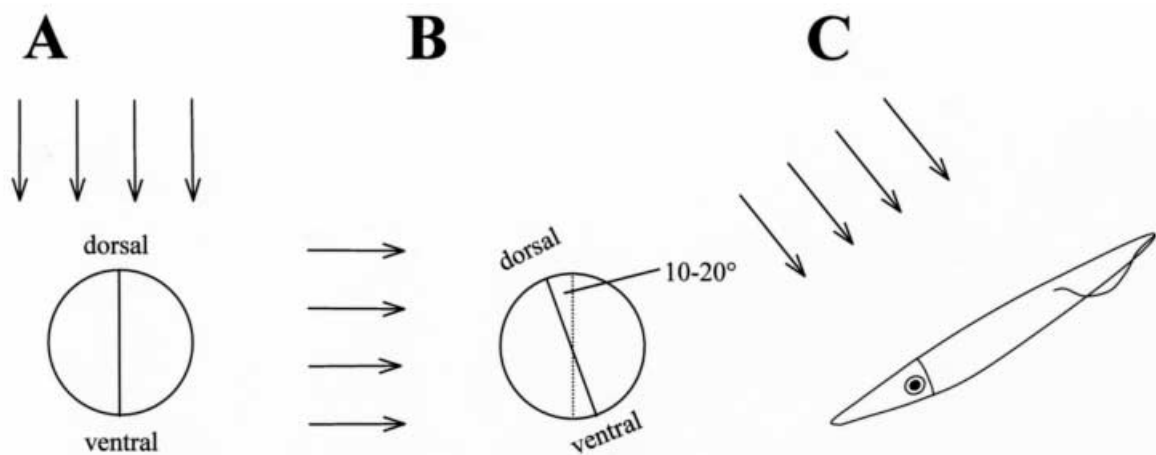


Figure 2. Diagram showing the orientation of the squid *Alloteuthis subulata*. (A) Orientation in the roll plane with light from directly above; (B) orientation in the roll plane with light coming from the side. The squid roll their dorsal sides towards the light by a maximum of 20 degrees; (C) orientation in the pitch plane. Squid turn their dorsal sides towards the light for angles of the light source up to approximately 70 degrees with the vertical. Direction of light source indicated by arrows.

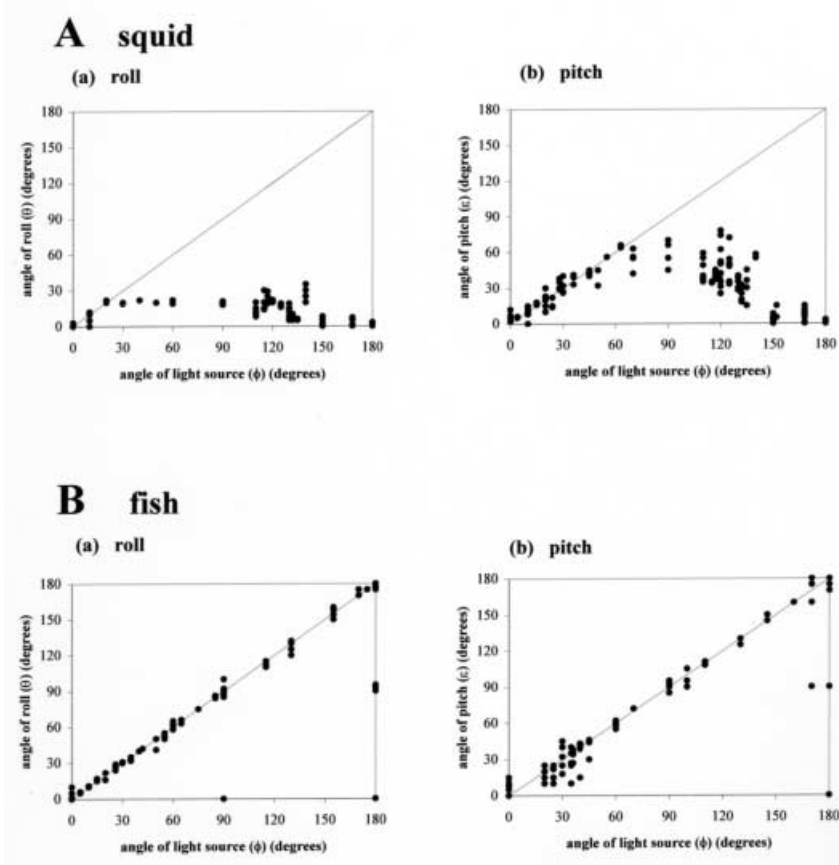


Figure 3. (A) Measurements of the orientation of *Alloteuthis subulata* with light from different directions. (a) Roll plane; (b) pitch plane; (B) measurements of the orientation of *Trachurus trachurus*. (a) Roll plane; (b) pitch plane. ϕ , Angle of the light source; θ , angle of the animal (roll); ϵ , angle of animal (pitch). Each dot represents the orientation of one animal. The line running diagonally represents the angle of roll/pitch expected if the animals were to turn their dorsal sides towards the direction of the brightest light.

When squid were observed from the side, i.e. in the pitch plane, it could be seen that during their continuous forward and backward glides, they underwent some variations in their inclinations with respect to the vertical. Denton (1959) observed that *A. subulata* are not neutrally buoyant, which may explain why these squid constantly move, as they may have to avoid sinking. The swimming movements that *A. subulata* performed when light came from directly above, were essentially as follows. The backward glide was almost always directed in a slightly upward direction (typically between 10 to 40° from the horizontal), whilst the forward glide was nearly horizontal. When the apparatus shown in Figure 1A was rotated so that light was incident from the side, the backward glide of a squid, swimming with its head facing the light source, became steeper, whilst the forward glide was directed downwards. Conversely, if the squid swam with its head facing away from the light source, the backward glide became less steep and the forward glide was directed upwards. The forward glide appeared to be used by the squid to control its orientation, whilst the backward glide appeared to give upward lift. The angles (ϵ) given in Figure 3A (ii) (below) are therefore those measured at the end of the forward glide. Only the orientation of the mantle was measured, as squid have the ability to maintain their heads in a horizontal position during pitch (Preuss & Budelmann, 1995b).

The angles of inclination of squid observed from the side were much more pronounced than the angles in the roll plane (Figure 2C). When the light came from directly above (0°) the squid did not incline their bodies, whilst their orientation approximately matched the angle of the light source for angles up to about 70° (Figure 3Ab). At angles of the light source between 70 to approximately 100° the squid maintained their 70° orientation. At angles greater than 100° the orientation of squid became more and more horizontal and for angles of the light source between 160° and 180° the squid were observed to swim approximately horizontally, with no tilt. The angles of orientation were very close to those expected if the squid were to turn their dorsal sides towards the brightest light for angles of the light source up to approximately 70° ($t=1.45$, $df=12$, $P>0.05$). For angles of the light source greater than 70° the angles of orientation of the squid differed significantly from the expected angles ($t=5.53$, $df=10$, $P<0.001$).

Trachurus trachurus

Horse mackerel observed in the roll plane (i.e. head-on or tail-on), could be seen to incline their dorsal sides when the tube was rotated. They were very precise at matching the angle of the incident light and could be brought to swim on their sides if the light source was positioned at 90°, as well as to swim upside-down, if the light source was at an angle of 180°. Measurements of angles are

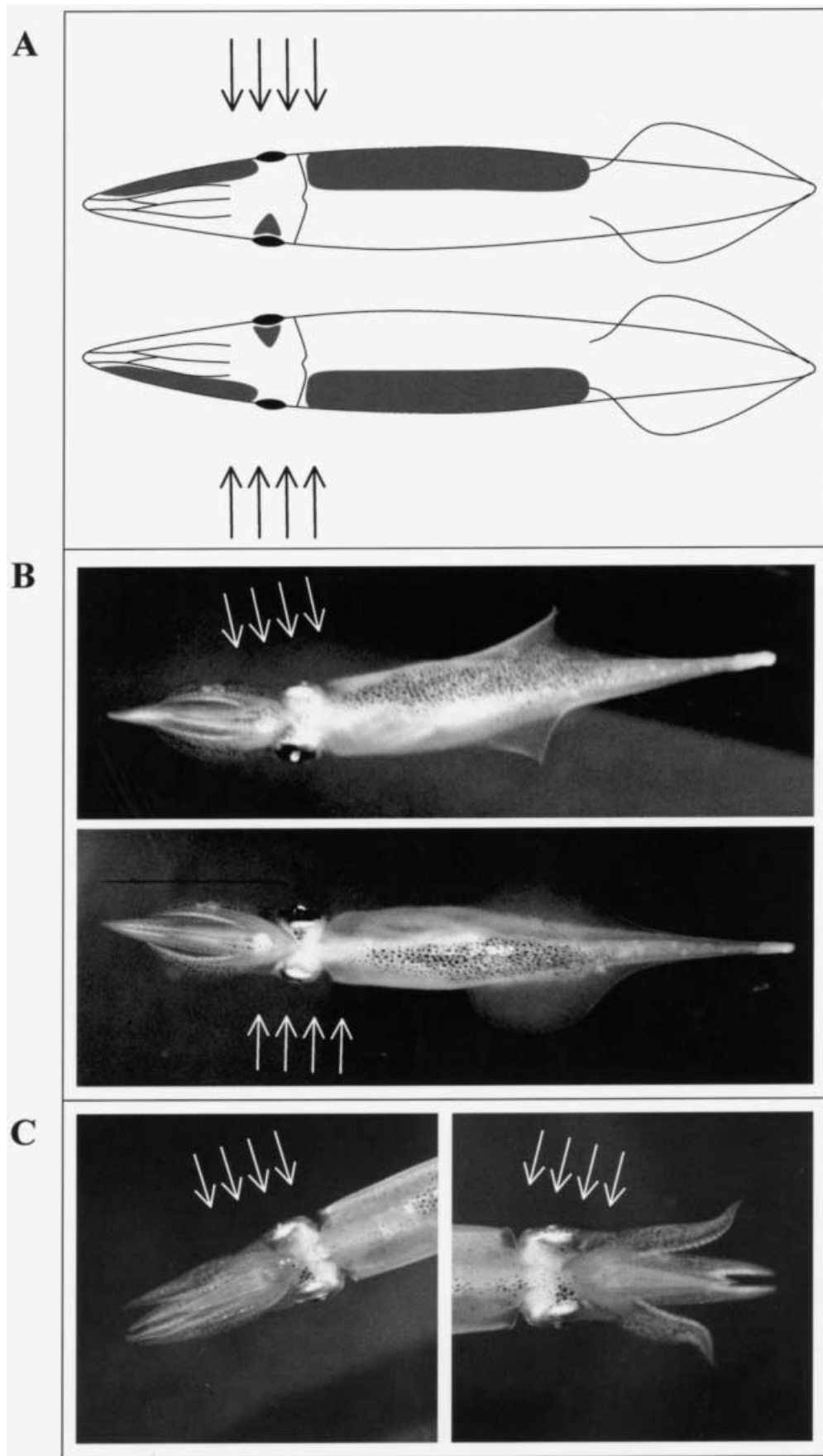


Figure 4. 'Flexible Countershading' pattern in *Alloteuthis subulata*, involving expansion of chromatophores to darken the side of the body facing the brightest light. (A) Diagram showing the pattern in its most commonly observed form; (B) photographs showing the darkening of the side of the mantle facing the light source; (C) photographs of the expanded chromatophores from the area above the fluorescent 'eyespot'. Group of four arrows indicates direction of light.

shown in Figure 3Ba. This shows that the ways in which fish orientate themselves with respect to the angle of the light source are very close to the orientations expected if the fish were to turn their dorsal sides towards the brightest light. There was no significant difference between the expected and the observed angles ($t=0.34$, $df=21$, $P>0.05$).

Fish left in any position were often observed to swim in the same orientation for approximately 45 min. This dorsal light reflex was relatively rapid for angles of the light source up to about 90°. If the fish were kept within the apparatus in the dark and the light source was subsequently switched on, the fish adjusted their orientation within approximately 5 s. At very steep angles of the light source (e.g. around 180°) the fish took slightly longer to adjust their orientation. Immediately after turning the light source on, the fish were observed to swim with their dorsal sides oriented at 0° and within approximately 10 s they swam upside-down.

Fish that were observed laterally, i.e. in the pitch plane, showed similar responses to squid to changes in the orientation of the light source, but their orientations with respect to the light source and the vertical were much more accurate, i.e. in contrast to squid the fish swam in almost perfectly straight lines. The expected angles of orientation were not significantly different from the observed angles ($t=1.93$, $df=15$, $P>0.05$). As well as adjusting their orientation when viewed head- or tail-on, the fish matched the angle of the light source at all positions when observed from the side (Figure 3Bb).

During experiments there were three fish that remained either at 0° or 90° when light came from 90° or 180° (Figure 3B). Some fish showed abnormal eye developments during the time they were kept in the laboratory and it may be possible that these individuals were partially blind. They have therefore not been included in the statistical test.

'Flexible Countershading' chromatophore pattern

When squid were observed in tanks with light coming from directly above, the animals were most commonly observed to expand the chromatophores on the dorsal side, producing a dark stripe along the dorsal midline (see also Cornwell et al., 1997). This chromatophore pattern changed if light came from the side of the tank. Squid were then observed to expand the chromatophores on the side of the body facing the brightest light. This chromatophore pattern will be termed 'Flexible Countershading'. The pattern was very prominent at angles of the light source between approximately 20 and 110°. There was some variation in the parts of the body that were darkened, but all squid expanded the chromatophores on the side of the mantle facing the light. The following variations in chromatophore expansion were observed: (1) chromatophore expansion covering the area of the mantle containing the internal organs; (2) chromatophore expansion on the entire side of the mantle; (3) chromatophore expansion on arms facing the light; (4) all chromatophores on arms expanded; (5) all chromatophores on arms retracted; (6) chromatophore expansion on dorsal side of head, covering the two 'eyespot's; (7) chromatophore of the dorsal side of head retracted, making the 'eyespot's visible;

and (8) darkening of 'eyespot' opposite to that facing the light. A diagram and photographs of the pattern in its most commonly observed form is shown in Figure 4. If squid were illuminated from below no such pattern was observed, the squid retracted the chromatophores and appeared very transparent.

In order to investigate how quickly this chromatophore pattern could be shown by a squid, the animals were subjected to alternating light sources from each side. It was found that the response time varied between animals, some producing the patterns within as little as 2 s, whilst others responded only after 20 to 30 s.

As some chromatophore patterns appear to be used primarily in camouflage it was decided to investigate whether squid adjusted the counter-shading chromatophore pattern with respect to backgrounds of different intensities. For this experiment the sides of the tank opposite the light source and the bottom of the tank were covered with differently shaded backgrounds: white, grey and black. Whilst being video recorded, each squid was alternately presented with these backgrounds. Most squid ($N=8$) showed a darker chromatophore pattern on a darker background. In others ($N=6$) the expansion of the chromatophores remained roughly the same, irrespective of the background shading.

The intensity of the illumination (39.5 to 738 μWcm^{-2}) did not appear to have any effect: squid maintained the degree of chromatophore expansion, independently of the intensity of the illumination.

DISCUSSION

Apart from the eyes and the inksac, most body parts of the squid used in this study are highly transparent. Some areas of the body transmit up to 90% of the incident light (Mäthger & Denton, 2001). Horse mackerel, in contrast, are not transparent, and their bodies are densely covered in silvery reflectors (Denton & Nicol, 1966). This represents a problem for these fish if light from the sun falls on them obliquely, because if a fish is oriented vertically with respect to the sea surface the fish would appear very bright on one side and very dark on the other, which would render them obvious to potential predators (Denton & Rowe, 1994). It is therefore essential that a fish inclines its dorsal side to equalize the light falling on its right and left sides, and hence to maximize its chances of being difficult to detect. For squid, this 'dorsal light reflex' does not seem to be as essential to their camouflage as for fish because they are highly transparent and therefore well camouflaged, whatever their orientation. The parts of the squid that are probably most conspicuous are the fluorescent 'eyespot's and the internal organs and it appears likely that using the chromatophores to produce a counter-shading effect is an effective way to conceal these parts of the body. The 'Flexible Countershading' pattern, which is adjusted depending on the direction from which the light comes, may therefore serve similar functions as the 'dorsal light reflex' of fish. The chromatophore pattern may thus be an explanation for why the squid 'dorsal light reflex' is so weak compared to that of fish.

It is also interesting to note that the countershading pattern reported here only occurs on the dorsal side of the mantle, not on the ventral side. This supports the

theory of Lima et al. (2003), who show that the physiological control of chromatophore expansion is more complex on the dorsal than on the ventral side. The chromatophore muscles on the dorsal side have an additional glutamate receptor (NMDA receptor), which enables the chromatophores to be activated for longer. The NMDA receptor is absent in the chromatophore muscles of the ventral side, which only contain the AMPA-like receptors. Lima et al. (2003) show that this physiological difference explains why most of the chromatophore patterns are expressed on the dorsal side.

A very interesting observation was that many squid expanded the chromatophores of the 'eyespot' opposite that facing the brightest light (see Figure 4). This, at first, appears to be the wrong way round. When looking at these squid closely, however, it becomes obvious that the dorsal arches of the eyes are slightly elevated with respect to the rest of the head, so that a predator looking at a squid from an oblique angle would only see the fluorescent layers of the 'eyespot' on the opposite side to that facing the brightest light. This would explain why most squid only expand the chromatophores on that side.

It is not clear why both *Alloteuthis subulata* and *Trachurus trachurus* inclined their dorsal sides towards the light in the pitch plane. The possibility that this may decrease the shadow cast below the body can be eliminated because turning the dorsal side towards the light coming from an oblique angle actually increases the area of the shadow by making it longer. Furthermore, when in a 'head-up' or 'head-down' position at oblique angles of the incident light, fish or squid may not be able to swim at fast speeds for long distances. Mackerel (*Scomber scombrus*) have been known to tilt their bodies with the head facing upwards, whilst swimming at slow speeds of around 0.8 body lengths per second (He & Wardle, 1986). These workers found that head-up swimming provided the negatively buoyant mackerel with lift. However, at normal cruising speeds of approximately 1 body length per second mackerel swam horizontally, with no tilt. The tilting behaviour of both squid and fish in the pitch plane under the influence of light should be investigated further. It would, for example, be interesting to see whether squid and fish in the wild choose to swim with their dorsal sides inclined in the pitch plane if the brightest light comes from behind.

Ferguson & Messenger (1991) reported that lightly anaesthetized *Sepia officinalis*, *Loligo vulgaris* and *Octopus vulgaris* respond to being rotated manually in the roll plane by expanding the chromatophores that face upwards. From their experiments they concluded that what they termed the 'Countershading Reflex' (CSR), was elicited by the gravity receptors in the statocyst. Ferguson et al. (1994) investigated the CSR of *S. officinalis* in more detail and suggested that visual input played a minor role in eliciting the reflex in cuttlefish. Cuttlefish spend the majority of their time on the sea bottom and their camouflage is largely concerned with matching the diverse backgrounds on which they are found. Countershading may therefore not be as important to cuttlefish as to pelagic animals and it is perhaps not surprising that visual input only plays a minor role in the CSR. Squid, in contrast, are pelagic animals for which countershading must play a more important role. Being capable of changing the countershading pattern may be very important,

especially at shallow depths at which the direction of maximum radiance may change frequently, due to clouds obscuring the sun. The experiments described here show clearly that light plays a key role in the production of the 'Flexible Countershading' chromatophore pattern. The difference between the CSR of *S. officinalis* and the 'Flexible Countershading' pattern in *A. subulata* is that the CSR is evoked involuntarily, whilst the 'Flexible Countershading' pattern appears to be entirely under the control of the squid.

I would like to thank the Marine Biological Association of the UK and its Director, Professor S.J. Hawkins, for accommodation, facilities and support. I am grateful to Sir Eric J. Denton for guidance and discussions on this work and Toby F.T. Collins, Pedro A. Lima and John B. Messenger for their comments on this manuscript. This research has been supported by a grant from the Gottlieb Daimler and Karl Benz Foundation, Germany.

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Submitted 24 October 2002. Accepted 2 June 2003.