Maturation of polarization and luminance contrast sensitivities in cuttlefish (*Sepia officinalis*)

Lelia Cartron¹, Ludovic Dickel¹, Nadav Shashar² and Anne-Sophie Darmaillacq¹,*

¹Université de Caen Basse-Normandie, Groupe Mémoire et Plasticité comportementale (EA 4259), F-14032 Caen, France
²Department of Life Sciences, Eilat Campus, Ben Gurion University of the Negev, P.O. Box 653, Beer-Sheva 84105, Israel

*Author for correspondence (anne-sophie.darmaillacq@unicaen.fr)
SUMMARY

Polarization sensitivity (PS) is a characteristic of the visual system of cephalopods. It has been well documented in adult cuttlefish which use PS in a large range of tasks such as communication, orientation and predation. Since cuttlefish do not benefit from parental care, their visual system (including the ability to detect motion) must be efficient from hatching to enable them to detect prey or predators. We studied the maturation and functionality of polarization sensitivity in newly hatched cuttlefish. In a first experiment, we examined the response of juvenile cuttlefish from hatching to the age of one month, towards a moving, vertically-oriented grating (contrasting and polarized stripes) using an optomotor response apparatus. Cuttlefish showed differences in maturation of polarization vs. luminance contrast motion detection. In a second experiment, we examined the involvement of polarization information in prey preference and detection in cuttlefish of the same age. Cuttlefish preferentially chose not to attack transparent prey whose polarization contrast had been removed with a depolarizing filter. Performances of prey detection based on luminance contrast improved with age. Polarization contrast can help cuttlefish detect transparent prey. Our results suggest that polarization is not a simple modulation of luminance information, but rather that it is processed as a distinct channel of visual information. Both luminance and polarization sensitivity are functional, though not fully matured, in newly hatched cuttlefish and seem to help in prey detection.

Key words: contrast vision, optomotor response, prey detection, predatory behavior, Cephalopods
INTRODUCTION

Visual motion detection plays a crucial role in the early life stages of many organisms, as it is used for predator avoidance (fishes, Fuiman and Magurran, 1994) and prey detection (fish, Luczkovich, 1988; bird, Gall and Fernández-Juricic, 2010). Although motion detection itself is a fundamental trait, occurring with the establishment of visual functions, the acuity of vision and hence the ability to detect small patterns often changes with age and mostly with increase in eye size (Pankhurst et al., 1993; Kiltie, 2000). The optomotor response (OMR) is the unconditioned movement (of the whole body or the head) exhibited by an individual (from insects to humans; McCann and MacGinitie, 1965; Tauber and Koffler, 1966) when it is presented with a large field moving stimulus. Experimentally, this behavior is induced by placing the animal inside a rotating cylinder lined with a pattern made up of contrasting stripes. The OMR has been used to study the ontogeny of visual functions in many animal species (Rahmann et al., 1979; Neave, 1984; Carvalho et al., 2002; Groeger et al., 2005; Rinner et al., 2005) because it allows the testing of a large variety of parameters such as spatial resolution (the capacity to discriminate details) and temporal resolution (the capacity to detect motion). For this purpose, the size of the stripes of the pattern on the rotating cylinder and/or the velocity of rotation can be varied. The OMR allows examination of spectral sensitivity (Cronly-Dillon and Muntz, 1965; Kaiser, 1974; King et al., 1993; Krauss and Neumeyer, 2003) as well as polarization sensitivity (McCann and Arnett, 1972; Wolf et al., 1980; Philipsborn and Labhart, 1990; Dacke et al., 2001; Glantz, 2001).

Cephalopods are colorblind (Brown and Brown, 1958; Hanlon and Messenger, 1996; Marshall and Messenger, 1996; Mäthger et al., 2006). Yet in addition to their contrast sensitivity (grey scale), they are sensitive to the linear polarization of light, a sensitivity (polarization sensitivity; hereafter, “PS”) that arises from the orthogonal distribution of the microvilli of neighboring photoreceptor cells in their retina (reviewed in Mäthger et al., 2009). PS is the capacity to respond to changes in either the $e$-vector orientation (also known as angle of polarization) or the degree of polarization (also referred to as percent polarization or partial polarization) of a light stimulus (Wehner, 2001; Horvath and Varju, 2004; Marshall and Cronin, 2011). Cuttlefish respond to differences as small as 1° (Temple et al., 2012) and thus have highly acute $e$-vector angle discrimination capacities. In addition to its use in target detection by octopus and squid (Moody and Parriss, 1960, 1961; Shashar et al., 1998), PS is used by cuttlefish in a range of tasks such as communication (Shashar et al., 1996; Boal et al., 2004), predation (Shashar et al., 2000) and orientation (Cartron et al., 2012). Cuttlefish are visually driven predators that prey upon various small moving crustaceans (Hanlon and...
Messenger, 1996). Of them, mysid shrimp use transparency for camouflage (Wells, 1962) but their tissues generate localized polarization via scattering, reflection and birefringence, that could allow polarization sensitive predators detect them (Johnsen et al., 2011). Likewise, adult cuttlefish use PS to detect silvery fish that also generate a polarization pattern (Shashar et al., 2000). Other prey, such as crabs, use contrasted pattern of coloration to mimic the background (Todd et al., 2005).

Newly hatched cuttlefish do not benefit from parental care so they need to cope on their own to hunt such silvery and transparent prey (Boletzky et al., 1977). Therefore it is of interest to examine the ontogenetic development of polarization-based visual capacities and to compare them with luminance contrast-based visual capacities. OMR induced in juvenile Sepia officinalis improved in luminance contrast-based visual acuity from 2.5° in minimum separated angle by cuttlefish measuring 1 cm to 0.5° for cuttlefish measuring 8 cm (Groeger et al., 2005). OMR was also used to examine PS in adult cuttlefish of different species, but not yet in hatchlings (Darmaillacq and Shashar, 2008; Talbot and Marshall, 2010a, 2010b). Newly hatched cuttlefish are able to visually discriminate between different crab phenotypes suggesting good detection of prey based on luminance contrast (Guibé et al., 2012).

In the current study, we used both OMR and predatory behavior to examine and to compare the development of polarization and luminance contrast sensitivities in young cuttlefish, from hatching to one month of age. In the first experiment, we observed the OMR to polarization and luminance contrasted patterns in motion. Then, in the second experiment, we examined the importance of polarization information in detecting and attacking prey that use transparency or background matching as camouflage (Stevens and Merilaita, 2009).

**MATERIALS AND METHODS**

**Animals**

Cuttlefish hatched from wild eggs collected in the vicinity of Luc-sur-Mer (France). Eggs, initially laid in clusters, were separated from each other to ensure optimum developmental conditions and were put in shallow tanks at the Centre de Recherches en Environnement Côtière (CREC, Luc-sur-Mer, France). All tanks were supplied with running, oxygenated sea water at 17 ± 1°C. After hatching, the animals were housed in groups and provided with enriched habitats which increases cuttlefish growth rates, has a positive effect on juveniles’ learning abilities (Dickel et al., 2000) and improves the richness of their behavioural repertoire (Poirier et al., 2004, 2005). Animals were fed daily with live shrimp (Crangon crangon) of suitable size. After being tested, cuttlefish younger than one month
were released into their natural environment at low tide in shallow water pools containing potential prey and hiding places (Darmaillacq et al., 2006, 2008). Older animals were kept in the rearing system for further, unrelated, experimentation.

Two experiments were conducted. The first experiment tested an unconditioned response to geometrical moving stimuli (OMR). The second experiment tested predatory behavior in a biologically relevant context.

**Experiment 1: OMR to luminance vs. polarization contrasted patterns**

**Apparatus**

We used an optomotor apparatus consisting of a cylinder (26 cm in diameter, 21 cm high) rotated by a controllable, reversible motor as described in detail in Darmaillacq and Shashar (2008; Fig. 1A). The interior wall of the cylinder was lined with one of the removable striped patterns while the exterior was backed with a light diffuser. A circular glass holding tank (8 cm in diameter, 8.5 cm high; Fig. 1A) was placed on a central stationary platform. A video camera was suspended directly above the glass tank allowing remote viewing and recording of the animal's movements. The apparatus was illuminated with 4 fluorescent lamps outside the cylinder that provided homogeneous lighting of the patterns. The entire apparatus was covered by a black curtain to prevent cuttlefish from using distal cues or being disturbed during the experiments. We tested 2 patterns (Fig. 1B) of alternating stripes 1 cm wide, each covering 6.3 degrees of an arc when viewed from the edge of the holding tank. One pattern consisted of black (0), dark grey (65), white (255), and light grey (190) stripes (BWG pattern; Fig. 1B). The other pattern consisted of polarized stripes with alternating horizontal, 45°, vertical and 135° orientations of linear polarization (Pol pattern; Fig. 1B; for further details, see Darmaillacq and Shashar, 2008). A sheet of white paper was used as a negative control.

**Optomotor test**

The experiment started 5 min after the introduction of a cuttlefish into the central holding tank to allow it to calm and settle down. Each pattern was rotated at 4 angular velocities (30, 60, 100 and 130°/s) in both clockwise and counter-clockwise directions. In preliminary experiments using patterns, these speeds ranged from those that elicited a clear positive response (OMR 30-100°/s) to one that no longer elicited any response (130°/s). During a trial, when the cuttlefish followed the direction and speed of the rotating pattern (OMR) with the rotation of their whole body for at least 180° or just with their eyes for at least 4 back and forth motions in the proper direction, the response was considered positive. In this case, the cylinder was stopped to avoid habituation or fatigue. Otherwise the response
was considered negative and the trial ended after 1 min. Each cuttlefish was tested once, in a continuous session consisting of 16 experimental trials (2 patterns x 4 rotation velocities x 2 rotation directions) and 8 control trials with the white sheet (totaling 24 trials). The pattern used for the first 8 trials was chosen randomly. Then, trials were performed with the 4 rotation velocities presented in a random order. For each velocity, two consecutives trials were performed, alternating the directions of rotation. In the next 8 trials, the pattern was switched and tested as described above. Lastly, the white sheet was used to perform negative control trials for each velocity and direction. The inter-trial interval between speeds and directions using the same pattern was 1 min; the inter-trial interval between patterns was 5 min.

Only animals that did not respond to the control presentation and responded to both rotational directions were considered as showing an overall positive response. This requirement may be somewhat harsh as some animals were determined to show a clear handedness preference, responding stronger or exclusively to one handiness of presentation than to another (see also Darmaillacq and Shashar, 2008); as a consequence, the cuttlefish that exhibited handedness were included in the analysis but they were not scored with an overall positive response. None of the cuttlefish responded to the negative control; hence all were included in the analysis.

We used 60 cuttlefish. Ten animals were tested at each age (0, 6, 12, 18, 24 and 30 days). The dorsal mantle length of each cuttlefish was measured after the test.

**Experiment 2: Importance of polarization in prey preference and detection**

*Prey*

In both tests, 2 types of prey were used: mysid shrimp (*Neomysis integer*) which show low luminance contrast and high polarization contrast, and crabs (*Carcinus maenas*) which show high luminance contrast and little or no polarization contrast; as examined with an imaging polarimeter (Shashar, unpublished data). Mysid shrimp were reared at the CREC and were returned there after tests. Crabs were collected at low tide under rocks and were used to feed other cuttlefish after the tests.

*Choice test*

The experimental apparatus was a V-shaped arena made of grey PVC (15 cm long x 10 cm wide x 6 cm deep; Fig. 2). Two glass tubes (4 cm diameter and 6 cm high), each containing five prey of the same species were placed at the opposite of the corner and were illuminated from above by a LED spotlight. One of the tubes was a glass tube transparent to light luminance and polarization information (Pol tube). The other was a glass tube covered
with plastic film transparent to light luminance information but distorted polarization
information (DePol tube), as examined using two Polaroid HN38S linear polarizing filters
(Shashar et al., 2000). The position (left or right) of the tubes was randomly distributed
among cuttlefish. Tubes were occluded by an opaque cover before the beginning of the test.
The cuttlefish was gently transferred from the housing tank to the apparatus. It positioned
itself in the corner of the V, the head facing the tubes. The cuttlefish had 15 min to settle
down and acclimatize to the apparatus. Then, the opaque cover was removed and the
cuttlefish was given 5 min to attack the prey contained in one of the tubes. At the end of the
test, the cuttlefish was put back in an individual rearing tank and fed shrimp, *Crangon*
*crangon*.

Cuttlefish were tested at the age of 7 days (n = 36) and 30 days (n = 36). For each age,
18 cuttlefish were presented with mysid shrimp and the other 18 with crab. Each cuttlefish
was tested only once. The cuttlefish choice was recorded. An attack was recorded when the
cuttlefish shot its tentacles towards a tube containing mysid shrimp or jump on a tube
containing crabs.

**Latency test**

This test was designed to check that cuttlefish were able to detect prey in a DePol tube
and to compare the detection latencies in both conditions (Pol vs. DePol).

The experimental apparatus was V-shaped arena made of grey PVC (15 cm long x 6
cm wide x 6 cm deep). One tube (Pol or DePol) containing 5 prey items was placed at the
opposite of the corner. Each cuttlefish was tested successively with the Pol tube and the
DePol tube presented in a random order with 5h between presentations, during which time
cuttlefish were returned to their home tank. The acclimation procedure was the same as the
one described above. The latency of prey detection was recorded. The detection was assessed
by the binocular convergence of the cuttlefish eyes and the positioning of the arms pointed
towards the prey (Messenger, 1973). Cuttlefish were tested at the age of 7 days (n = 36) and
30 days (n = 36). For each age, half of the cuttlefish were presented with mysid shrimp and
half with crab.

**Statistical analysis**

Data were analyzed using StatXact 7 (Cytel Studio software). All analyses used a
significance threshold of $\alpha = 0.05$. Pearson’s chi square tests were used to compare the
percentage of cuttlefish showing an OMR with the same pattern between the different ages. If
the null hypothesis was rejected, Fisher’s exact tests were used for post-hoc pairwise
comparisons. Mc Nemar tests were used to compare the percentage of cuttlefish showing an OMR at the same speed and age between the two different patterns (Siegel and Castellan, 1988). Permutation tests were used to compare the detection latencies. The preference for attacking prey in the polarization or luminance contrast condition was analysed with chi-square exact tests. Fisher’s exacts test was used to examine whether preference differed between prey groups and between age groups.

RESULTS

Experiment 1: OMR to luminance vs. polarization contrasted patterns

When presented with an intensity pattern (BWG), the percentage of cuttlefish showing an OMR at a rotating velocity of 30°/s and 60°/s did not significantly vary with age (Fig 3; 30°/s, $\chi^2_{(5\ ddl)} = 10.34, p = 0.2$; 60°/s, $\chi^2_{(5\ ddl)} = 7.451, p = 0.3$). At 30°/s, this percentage was already very high at hatching (80%) and then reached 100% at 6 days. At 60°/s, 60% of the cuttlefish showed an OMR at hatching and 6 days of age and 100% was observed from 24 days. At the velocity of 100°/s, the percentage of cuttlefish showing an OMR significantly increased from 10% at hatching to 90% at 30 days ($\chi^2 = 12.8, p = 0.001$). At the control velocity of 130°/s, none of the cuttlefish showed an OMR, regardless of age.

With the Pol pattern, the percentage of cuttlefish showing an OMR increased at each velocity with age (30°/S, $\chi^2_{5} = 21.67, p = 0.0003$; 60°/s, $\chi^2_{5} = 11.67, p = 0.04$; 100°/s, $\chi^2_{5} = 17.92, p = 0.003$). At 30°/s, 20% of the cuttlefish responded at hatching and the percentage increased to reach 100% at 30 days ($\chi^2 = 13.33, p < 0.001$). At 60°/s, the percentage increased from 10% of the cuttlefish showing an OMR at hatching to 80% at 30 days ($\chi^2 = 9.899, p = 0.006$). At 100°/s, none of the cuttlefish showed an OMR at hatching and 6 days but the percentage of cuttlefish responding reached 60% at 30 days ($\chi^2 = 8.571, p = 0.01$).

The two patterns did not elicit the same percentage of cuttlefish showing an OMR. Indeed, at the rotating velocity of 30°/s, fewer cuttlefish responded to the Pol pattern than to the BWG pattern at hatching, 6 and 12 days (hatching, $p = 0.004$; 6 days, $p = 0.007$; 12 days, $p = 0.026$). From 18 to 30 days, these percentages were no longer significantly different (18 days, $p = 0.1$; 24 days, $p = 0.5$). At the velocity of 60°/s, fewer cuttlefish showed an OMR to the Pol pattern than to the BWG one from hatching to 24 days (hatching, $p = 0.007$; 6 days, $p = 0.05$; 12, 18 and 24 days, $p = 0.026$). At 100°/s, the percentage of cuttlefish showing an OMR with the Pol and BWG pattern was both low and not different from hatching to 12 days (hatching, $p = 0.5$; 6 and 12 days, $p = 0.2$). More cuttlefish responded to the BWG than to the Pol pattern at 18 and 24 days of age ($p = 0.026$ and $p = 0.05$, respectively).
Experiment 2: Importance of polarization in prey preference and detection

Choice test

At 7 and 30 days after hatching, cuttlefish more frequently attacked mysid shrimp in the Pol tube over the DePol tube (7 days, $\chi^2 = 5.6, p = 0.03$; 30 days, $\chi^2 = 8, p = 0.008$; Fig 4), but did not attack crabs in either tube significantly more often (7 days, $\chi^2 = 0.2, p = 0.8$; 30 days, $\chi^2 = 0.8, p = 0.5$). At 30 days, cuttlefish significantly preferred mysid shrimp the Pol but made no difference between crabs in the Pol and DePol tubes ($\chi^2 = 7.5, p = 0.015$).

Latency test

At 7 days of age, cuttlefish detected mysid shrimp faster when placed in the Pol tube than in the DePol tube ($p = 0.0006$; Fig 5). At 30 days, the latency of detection did not significantly differ between conditions ($p = 0.59$). In both conditions, cuttlefish detected mysid shrimp faster at 30 days than at 7 days (shrimp in a Pol tube, $p = 0.04$; in a DePol tube, $p < 0.001$; Fig 5). At both 7 and 30 days of age, there was no difference in the latency of detection for crabs placed in the Pol tube or in the DePol tube (7 days, $p = 0.95$; 30 days, $p = 0.72$). However, cuttlefish detected the crabs faster at 30 days than at 7 days in both conditions (crabs in a DePol tube, $p = 0.012$; in a Pol tube, $p = 0.006$; Fig 5).

DISCUSSION

In the first experiment, most of the cuttlefish showed an OMR to the luminance (BWG) pattern rotating from hatching at the lowest velocity (30°/s) where each stripe covered 6.3° of an arc. This result is in accordance with the findings of Groeger et al. (2005): under well illuminated conditions, newly hatched cuttlefish detected stripes covering at least 2.5° of an arc. At higher velocities, nearly all animals detected and followed the pattern at 30 days of age (Fig. 3). These results suggest that the temporal resolution of juvenile contrast sensitivity increases with age. The polarization sensitivity develops more slowly. Indeed, only few newly hatched cuttlefish showed an OMR to the Pol pattern at the low velocities of 30 and 60°/s. The number of responding cuttlefish slowly increased with age. Further, the responses at young ages were stronger at the low rotation speeds (30°/s and 60°/s) than the faster one (100°/s), but this was not the case with the older cuttlefish (but for the 130°/s rotation speed). These results suggest a maturation of the temporal resolution for polarization input as well. At 30 days, at least half of the cuttlefish responded to the polarization pattern at all velocities, but for the highest rotation rate of 130°/s (Fig 3). Previous studies demonstrated an OMR to
polarized stripes in other mature cuttlefish species, using a velocity of 12°/s and with 2.5 cm
stripes width (Talbot and Marshall, 2010a, 2010b), which raises the possibility that our
cuttlefish would have responded to slower rotating patterns as well. However, Darmillaq
and Shashar (2008), did not succeed in eliciting an OMR to a polarized pattern in adult Sepia
elongata, using velocities ranging from 34 to 178°/s, although S. elongata possess orthogonal
photoreceptors in their retina suggesting the ability for polarization detection. In animals that
are known to see colors, patterns composed of two colored stripes of equal luminance do not
necessarily elicit OMR (Schaerer and Neumeyer, 1996; Anstis et al., 1998; Krauss and
Neumeyer, 2003). Therefore, other techniques of investigations were needed to determine if
the ability for PS does exist in newly hatched cuttlefish. For this purpose, we used a
biologically relevant context: prey detection and attack.

In the second experiment, 7-day-old cuttlefish preferred to attack mysid shrimp when
polarization information was available (Pol tube; Fig. 4). Detection of mysid shrimp was
faster when contained in the Pol tube rather than in the DePol tube (Fig. 5). In 30-day-old
cuttlefish, we still observed this preference but cuttlefish did not detect faster mysid shrimp in
the Pol tube. Indeed, detection latency of mysid shrimp when placed in the DePol tube
improved. On the contrary, 7 and 30-day-old cuttlefish did not show a preference for crab in
Pol and DePol tube, and the latency of detection was the same in both conditions. The latency
of detection decreased with age. Those results suggest a hierarchical order of image
processing. When the prey is highly luminance contrasted, such as the crab, PS is not
involved in its detection or recognition. However, when luminance contrast is low (such as
with the mysid shrimp), PS kicks in. PS has been shown to enhance the detection of
transparent prey in squid paralarvae (Shashar et al., 1998). Dickel et al. (1997) showed a rapid
increase in triggering of predation on mysid shrimp during the first week of life in cuttlefish.
This might suggest a post-embryonic development of PS allowing increase in prey detection
and prey-catching success. Our results confirm that PS helps newly hatched cuttlefish detect
transparent prey. Moreover, they show that luminance contrast sensitivity improves during
their first month of life. PS overcomes this poor sensitivity in helping cuttlefish be more
efficient to detect prey that generate a polarization contrast from their background, such as
shrimp.

The second experiment shows that polarization was perceived by cuttlefish as young
as 7 days of age and that prey detection based on luminance contrast improves with age. Yet,
the percentage of cuttlefish showing an OMR to the polarization pattern was lower than for
the luminance pattern at all ages and velocities of rotation. These apparently puzzling results
could be explained by the higher speed of motion for the rotating pattern compared to the nearly stationary prey. In such a case, using a slowly moving pattern (Talbot and Marshall, 2010a, 2010b) might elicit stronger responses even in very young animals. Alternatively these apparently contradicting results can be due to differences in the size of the receptive fields of the retina needed to detect each type of signal. At least two photoreceptors are needed to detect motion based on luminance differences alone. However, for simple polarization detection (without motion), at least two photoreceptors with their microvilli oriented orthogonally to each other are needed (Wehner, 2001; Horvath and Varju, 2004; Marshall and Cronin, 2011). Therefore, it is likely that more photoreceptors should be necessary for polarization-based motion detection than for luminance-based motion detection. Another hypothesis is that polarization and luminance signals are processed separately and may have different roles in vision. Polarization contrast could be more useful to detect form as prey than motion, although it could have a function in both as well as in other tasks. In insects, or at least in locust, polarized and unpolarized information are coded differently and are processed by different type of neurons in the optic lobe (Labhart and Meyer, 2002; Pfeiffer et al., 2005). In cephalopods, little is known about the coding of luminance and polarization information in the optic lobe. However, the current and previous behavioural observations and previous ones (Grable et al., 2002) suggest that polarization is not a simple modulation of luminance information, but rather that it is processed as a distinct channel of visual information. Further studies using electrophysiological and immunochemistry procedures are needed to determine the neural pathways for polarization and luminance information processing.

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REFERENCES


Figures captions

Figure 1: A) Optomotor apparatus. B) Picture taken from above: 1) the BWG pattern; 2a) the Pol pattern; 2b) the Pol pattern seen through a polarizer.

Figure 2: Apparatus for choice test. Prey items were mysid shrimp or crabs placed in a transparent tube that let pass through only light luminance (DePol tube) or light luminance and contrast (Pol tube).

Figure 3: Percent of the cuttlefish (n=10 per group) that showed an optomotor response (OMR) to BWG (luminance only; in black) or Pol (polarization; in grey) patterns rotating at velocities of A) 30°/s, B) 60°/s and C) 100°/s. Asterisk indicates a significant difference using a Mac Nemar test between the percentage of cuttlefish showing an OMR with the BWG and the Pol patterns (*p < 0.05).

Figure 4: Preference between prey by 7 and 30 days old cuttlefish. Prey items were either mysid shrimp or crabs presented either in the DePol tube (in black) or the Pol tube (in grey).

Figure 5: Mean latencies in seconds (± SEM) of prey detection by 7 and 30 days old cuttlefish. Prey items were mysid shrimp or crabs presented in the DePol tube (in black) or the Pol tube (in grey). *p < 0.05, **p < 0.01, ***p < 0.001 permutation test.
Mean latencies of detection (s) ± SEM

- **Mysid**
  - 7 days: Black bar
  - 30 days: Grey bar

- **Crab**
  - 7 days: Black bar
  - 30 days: Grey bar

Significance levels:
- *p < 0.05
- **p < 0.01
- ***p < 0.001