



## Articles

Visual lateralization is task and age dependent in cuttlefish, *Sepia officinalis*Christelle Jozet-Alves<sup>a,b,\*</sup>, Vincent A. Viblanc<sup>a,b,1</sup>, Sébastien Romagny<sup>a,b</sup>, Matthieu Dacher<sup>a,b,2</sup>, Susan D. Healy<sup>c,d</sup>, Ludovic Dickel<sup>a,b</sup><sup>a</sup> Université de Caen Basse-Normandie, Groupe Mémoire et Plasticité comportementale, Caen, France<sup>b</sup> Université de Caen Basse-Normandie, Centre de Recherches en Environnement Côtier, Luc-sur-Mer, France<sup>c</sup> University of St Andrews, School of Biology, St Andrews, U.K.<sup>d</sup> University of St Andrews, School of Psychology, St Andrews, U.K.

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The behavioural lateralization observed in several invertebrate species appears to parallel the phenomenon seen in vertebrates. However, it is not yet clear whether lateralization in invertebrates varies within an individual across development as it does in vertebrates. We examined when during postembryonic development juvenile cuttlefish begin to show side-turning preferences in a T-shaped apparatus. We also determined whether or not the direction of turning was stimulus dependent by providing, or not providing, shelters in the two choice arms of the apparatus. We found that as the animals aged from 3 to 45 days posthatch they progressively developed a left-turning bias but only when shelters were provided in the apparatus. We suggest that left-turning biases in cuttlefish result from an eye use preference. Emergence of visual lateralization at the time of dispersal may afford juveniles greater behavioural efficiency by enabling them to look out for escape routes while hunting. The left visual field may be associated with rapid responses as in many species of vertebrates. Determining the role of the right visual field of cuttlefish when looking for prey may provide evidence for homology between lateralization in invertebrates and vertebrates.

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Although cerebral lateralization was considered an exclusively human trait for a long time, it has become clear that it is universal and evolutionarily ancient in vertebrates (Vallortigara & Rogers 2005). It is often revealed behaviourally by motor (e.g. handedness) and perceptual (e.g. preferential eye use) asymmetries (e.g. in mammals: Austin & Rogers 2007; in birds: McKenzie et al. 1998; in fishes: Sovrano et al. 1999; in tadpoles: Dadda et al. 2003). The overwhelming evidence of asymmetries in neuroanatomy and neural processing across the animal kingdom has led researchers to consider whether cerebral lateralization has a common ancestry (homology) or whether it is the result of convergent evolution (homoplasy). In domestic chicks, *Gallus gallus domesticus*, the left hemisphere (i.e. right eye) is associated with the response to an identified target such as a prey item (Rogers 1997), while the right hemisphere (i.e. the left eye) is used for controlling rapid responses

(e.g. response to a predator; Rogers et al. 2004). Based on the existence of similar patterns of specialization of the brain hemispheres in several other species (toads: Lippolis et al. 2002; Robins & Rogers 2004; lizards: Bonati et al. 2008, 2010; fishes: Bisazza & de Santi 2003), some authors argue in favour of homology of cerebral lateralization in vertebrates (Vallortigara & Rogers 2005; Rogers 2007; MacNeilage et al. 2009). In contrast, others argue that too few species have been studied for this conclusion to be compelling (e.g. McManus 2005).

In invertebrates, too, cerebral lateralization is probably widespread. The oldest known evidence of behavioural asymmetry dates from the Early Cambrian. Babcock (1993) showed that predation scars are more common on the right than on the left posterior side of the body of fossil trilobites. One interpretation is that these animals were more responsive to predators advancing on the left than on the right, as observed in toads (Lippolis et al. 2002) and dunnarts, *Sminthopsis macroura* (Lippolis et al. 2005). There is also increasing evidence for lateralization in extant invertebrates. For instance, spitting spiders, *Scytodes globula*, use their legs asymmetrically during prey capture (Ades & Ramires 2002) and giant water bugs, *Belostoma flumineum*, make significantly more left turns than right turns in a T-maze (Kight et al. 2008). Additionally, the honeybee, *Apis mellifera*, shows lateralized olfactory

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learning (Letzkus et al. 2006), with a better short-term recall of olfactory memory with the right antenna and better long-term recall with the left antenna (Rogers & Vallortigara 2008; Frasnelli et al. 2010). Honeybees also learn to associate a colour stimulus with food better with their right eye than they do with their left eye (Letzkus et al. 2008). These findings parallel the observations in vertebrates where the right visual field often plays a predominant role in foraging and feeding behaviour (e.g. Rogers 1997; Robins & Rogers 2004; Bonati et al. 2008). Similarities in the patterns of cerebral lateralization between vertebrates and invertebrates could merely be a coincidence, or reveal a general pattern of hemispheric specialization conserved throughout evolution, and not only in vertebrates.

The existence of a fundamental template conserved throughout evolution would mean that species possess the same genetic potential for lateralization. However, early experience also plays a role in the development of lateralization. In the domestic chick and pigeon, *Columba livia*, differential visual stimulation between the two eyes prior to hatching generates both lateralization of the visual pathways and an asymmetry in eye use (Rogers & Sink 1988; Skiba et al. 2002). In rodents, both handling between birth and weaning and an enriched environment after weaning induce brain and behavioural asymmetry (Denenberg et al. 1978). Furthermore, exposing rats, *Rattus norvegicus*, to a novel environment daily for several minutes during their first few weeks of life induces a leftward shift in their paw preferences (Tang & Verstynen 2002). In both rodent experiments, the changes observed were stable well into adulthood.

How lateralization develops in invertebrates is still unclear. It is also not clear whether behavioural asymmetries vary among individuals and/or within an individual across development, as occurs in vertebrates. Cuttlefish (a cephalopod mollusc) appear to provide a useful system for examining the ontogenesis of lateralization in invertebrates: the embryos develop entirely within the egg, there is no larval or planktonic stage and newly hatched cuttlefish do not benefit from parental care (Richard 1971). Additionally, incubation and rearing conditions are relatively easy to control in the laboratory. Importantly, adult cuttlefish have an untrained individual-level side-turning preference (preference for turning right or left) in an empty T-maze (Alves et al. 2007). To examine when during ontogeny the lateralization of behaviour seen in adults first occurs, we tested whether juvenile cuttlefish exhibit a right- or a left-turning preference. The deleterious effects of being reared in isolation on neural and behavioural maturation are well known in cuttlefish (Dickel et al. 2006). In our study, cuttlefish were then reared in groups, and as it is impossible to identify or tag juveniles, each individual was released after being tested. Different individuals were tested at 3, 7, 15, 30 and 45 days posthatch in a T-shaped apparatus.

A further aim of the study was to determine whether a side-turning preference in a T-maze was the consequence of an eye use preference in cuttlefish. The adult side-turning preference may be a result of the two eyes sitting laterally on the head (Nixon & Young 2003). When the cuttlefish leave the start box (the stem of the T), they could see the two arms simultaneously on the left and right sides, and the direction of turning to escape could have been made by viewing with either the left or right arm. Asymmetry of eye use seems to be ubiquitous among vertebrates with laterally placed eyes (Vallortigara & Bisazza 2002), and another cephalopod, *Octopus vulgaris*, displays an eye use preference (Byrne et al. 2002, 2004). Demonstrating that visual perception plays a role in determining the direction of turning in cuttlefish would add new comparative data to tackle the question of the existence of a general pattern of hemispheric specialization conserved throughout the animal kingdom. In our study, to determine whether or not the direction of turning was stimulus dependent, cuttlefish were tested

either in an empty apparatus (experiment 1) or with a shelter on each side of the T-maze (experiment 2). We hypothesized that, if side-turning preferences are due to perceptual asymmetry, no lateralization bias would be observed in experiment 1, while in experiment 2, the cuttlefish would preferentially choose the shelter seen with the left eye. This could be consistent with the evidence of a predominant role of the visual field in initiating rapid responses as in vertebrates.

## METHODS

### Animals

The cuttlefish used in these experiments ( $N = 145$ ; mean dorsal mantle length  $\pm$  SEM: 3 days posthatch:  $8.6 \pm 0.4$  mm; 15 days posthatch:  $10.2 \pm 0.5$  mm; 30 days posthatch:  $21.6 \pm 0.7$  mm; 45 days posthatch:  $28.3 \pm 1.0$  mm) all came from eggs laid by several females. Females were originally caught in basket traps in the vicinity of Luc-sur-Mer (France) by technicians of the Centre de Recherches en Environnement Côtier (CREC, Luc-sur-Mer, France). On the boat, females were gently placed in tanks for the journey to the CREC (located just in front of the sea). Females were then kept in large tanks (1500 litres; three or four per tank) with circulating sea water at  $15 \pm 1$  °C, and were fed shrimps, *Crangon crangon*, and crabs, *Carcinus maenas*, ad libitum. We collected all these animals with the authorization of the Commune of Luc-sur-Mer. Females usually die soon after spawning (Mangold 1966).

The eggs (laid by approximately 15 females) were maintained in strainers floating in tanks supplied with running oxygenated sea water maintained at  $18 \pm 1$  °C. As hatching occurs at night (Paulij et al. 1991), cuttlefish were collected at 1000 hours in the morning following hatching (day 0). They were placed together in rectangular PVC tanks ( $50 \times 40$  cm and 7 cm high; 20 cuttlefish per tank) supplied with running oxygenated sea water ( $18 \pm 1$  °C). Rocks, shells and plastic seaweeds were distributed throughout each tank. Cuttlefish were maintained under daylight conditions and they were fed ad libitum with shrimps of suitable size. The number of juveniles tested was approximately the same in each clutch of eggs. After the experiment, juvenile cuttlefish were released at low tide, in pools where prey was abundant. The care, housing and use of the animals in our study were in accordance with the French animal testing laws.

### T-shaped Apparatus

The T-shaped apparatus (Fig. 1) was constructed entirely from white PVC. One arm was used as the start box ( $5 \times 3$  cm and 4 cm high) while the other two arms formed the central alley ( $12 \times 4$  cm and 4 cm high). A Plexiglas sliding door between the start box and

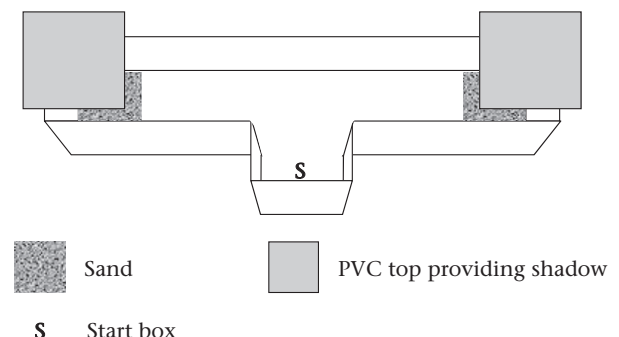


Figure 1. Schematic representation of the test apparatus (not drawn to scale).

the central alley allowed experimenters to keep the cuttlefish in the start box. The apparatus was illuminated by a 100 W halogen lamp positioned 1 m above the centre of the maze and was surrounded by opaque plastic walls to eliminate room cues (30 cm high). We attempted to reduce uncontrolled environmental cues as a source of bias by rotating the apparatus 90° between each day of experimentation in the laboratory room (i.e. four possible orientations of the apparatus, with the same number of cuttlefish tested with each orientation, in each group). Sea water in the apparatus (3 cm deep) was renewed between trials.

Experiment 2 differed from experiment 1 in that we provided a shelter (4 × 3 cm and 4 cm high; Fig. 1) at the end of each choice arm of the T-shaped apparatus. A shelter consisted of sand (1 mm high) and shadow (provided by an opaque sliding PVC top). These shelters are highly attractive for cuttlefish when placed in a T-shaped apparatus (Alves et al. 2007).

#### Procedure

Each cuttlefish was placed individually in the start box for 30 s before the clear sliding door to the central alley was removed. The cuttlefish was allowed to move freely out of the start box and into either arm of the apparatus. As soon as the cuttlefish reached one of the shelters, it was gently lifted out of the water with a small net and placed back into the start box. If a cuttlefish did not turn into one of the two arms within 1 min, it was removed and placed back into the start box. This procedure was repeated until 10 choices had been made. Each cuttlefish was tested only once (either in experiment 1 or in experiment 2) either at day 3, 7, 15, 30 or 45 posthatch.

#### Data Analysis

##### Laterality index

For each cuttlefish, the arm choice (right versus left) was recorded for each trial (i.e. 10 trials). Lateral asymmetries were analysed by computing for each subject a 'laterality index' (LI; Bisazza & Vallortigara 1997) using the following formula:

$$LI = [(right\ turns - left\ turns)/(right\ turns + left\ turns)] \times 100.$$

Positive values would thus indicate a preference to turn on the right side and negative values a preference to turn on the left side.

Departures from random choices (0%) were estimated by one-sample two-tailed *t* tests performed on the mean values of the LI for each age during each experiment using StatXact (CYTEL Software, Cambridge, MA, U.S.A.).

For the remaining statistical analyses, we used R 2.10 (Free Software Foundation, Vienna, Austria; R Development Core Team 2009). A linear regression of the LI on age was computed to assess the effect of age on the LI. The intercepts of these regressions allowed us to test whether the LI is significantly different from 0 at day 0 (i.e. day of hatching), whereas the slopes allowed us to test whether LI is correlated with age.

##### Individual side-turning preference

Behavioural asymmetries in a population arise either from a systematic bias (i.e. more than 50% of individuals systematically preferring a direction) or from individual differences (Denenberg 1981). Therefore, we also analysed the preference data for each individual.

Side-turning preference was determined using a criterion of nine or more choices of the same arm (significant side-turning preference at 5% level of significance with a binomial test). Then, cuttlefish were categorized into 'left preference', 'right preference' and 'no preference'. Fisher's exact probability tests were used to determine whether or not there was a difference in the number of lateralized cuttlefish during development for each experiment.

## RESULTS

### Experiment 1

#### Laterality index

None of the age groups had a significant group-wide side-turning bias (*t* test: 3 days:  $t_9 = -1.46$ ,  $P = 0.16$ ; 7 days:  $t_9 = -1.37$ ,  $P = 0.19$ ; 15 days:  $t_9 = -1.02$ ,  $P = 0.32$ ; 30 days:  $t_5 = -0.92$ ,  $P = 0.38$ ; 45 days:  $t_9 = 1.66$ ,  $P = 0.12$ ; Fig. 2). However, the intercept of the linear regression of the LI on age was significantly different from 0 and negative (*t* test: intercept:  $t_{45} = -2.12$ ,  $P = 0.04$ ) although the slope was not significant (*t* test: slope:  $t_{45} = 0.38$ ,  $P = 0.71$ ). Thus the cuttlefish displayed a slight left-turning preference at birth and this bias did not change with age.

#### Individual side-turning preference

Whatever their age, few individual juveniles had a significant side-turning preference (Fig. 3). The proportion of juveniles with a side-turning preference did not change through the developmental stages we tested (Fisher's exact test:  $N = 46$ ,  $P = 0.74$ ).

### Experiment 2

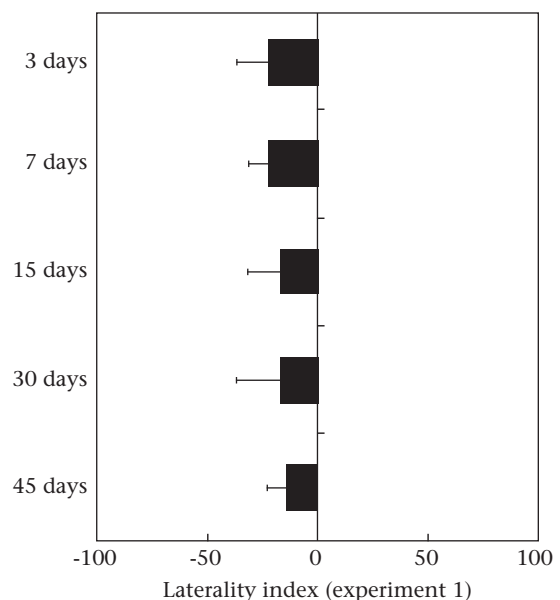
#### Laterality index

There was no significant side-turning bias at 3 or 7 days posthatch (*t* test: 3 days:  $t_{19} = -1.49$ ,  $P = 0.15$ ; 7 days:  $t_{19} = -1.39$ ,  $P = 0.17$ ). However, by 15 days and for both the later ages tested (30 and 45 days posthatch) the animals were significantly side-biased (*t* test: 15 days:  $t_{19} = -2.17$ ,  $P = 0.04$ ; 30 days:  $t_{19} = -2.90$ ,  $P = 0.006$ ; 45 days:  $t_{18} = -2.81$ ,  $P = 0.008$ ; Fig. 4).

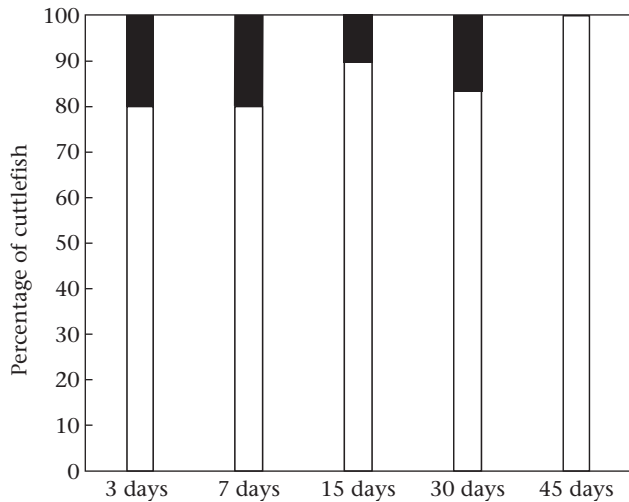
To test whether the bias to turn leftwards progressively increased with age, or appeared at 15 days posthatch, we regressed LI against age. This analysis confirmed that the cuttlefish did not display a left-turning preference at day 0 (*t* test: intercept:  $t_{98} = -1.41$ ,  $P = 0.161$ ). However, the bias to turn leftwards progressively increased as the animals aged (*t* test: slope:  $t_{98} = -2.14$ ,  $P = 0.03$ ).

#### Individual side-turning preference

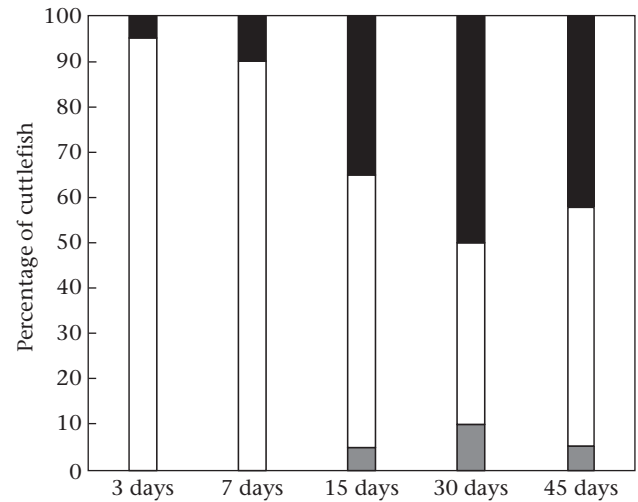
The proportion of juveniles with a side-turning preference changed through development (Fisher's exact test:  $N = 99$ ,



**Figure 2.** Laterality index (calculated over 10 consecutive trials; means  $\pm$  SEM) at different days after hatching during experiment 1 ( $N_{3\text{ days}} = N_{7\text{ days}} = N_{15\text{ days}} = N_{45\text{ days}} = 10$ ;  $N_{30\text{ days}} = 6$ ).



**Figure 3.** Percentages of cuttlefish categorized into 'left preference' (black bars) and 'no preference' (white bars) at different days after hatching during experiment 1 (significant side-turning preference at 5% level of significance with a binomial test;  $N_{3 \text{ days}} = N_{7 \text{ days}} = N_{15 \text{ days}} = N_{45 \text{ days}} = 10$ ;  $N_{30 \text{ days}} = 6$ ). No cuttlefish showed 'right preference'.



**Figure 5.** Percentages of cuttlefish categorized into 'left preference' (black bars), 'no preference' (white bars) and 'right preference' (grey bars) at different days after hatching during experiment 2 (significant side-turning preference at 5% level of significance with a binomial test;  $N_{3 \text{ days}} = N_{7 \text{ days}} = N_{15 \text{ days}} = N_{30 \text{ days}} = 20$ ;  $N_{45 \text{ days}} = 19$ ).

$P < 0.001$ ; Fig. 5). The percentage of juveniles with a significant left preference was low until 15 days. The percentage increased from 5–10% at 3–7 days posthatch to 40–50% at 15–30 days posthatch, with little further change after this age.

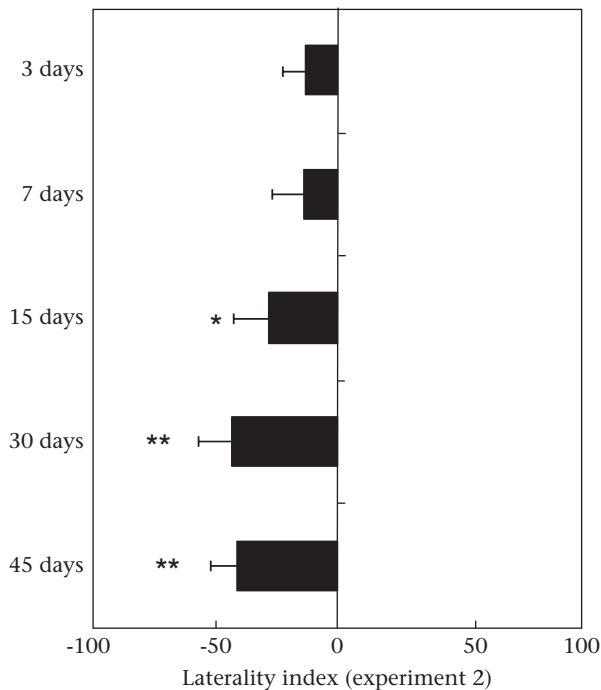
## DISCUSSION

In experiment 1, juvenile cuttlefish showed a slight left-turning preference, which remained unchanged through the developmental stages we tested. In experiment 2, when we provided

shelters in the two choice arms, there was a progressive development of a bias to choose the left arm as the animals aged from 3 to 45 days posthatch. Furthermore, in experiment 2, at 30 and 45 days posthatch, nearly half of the cuttlefish preferred to turn left (most of the other animals had no side preference).

There are two plausible explanations for the left-turning preference observed in experiment 2. First, it may result from a motor lateralization, such as the escape behaviour observed in frogs (Dill 1977) and the C-shape body bending observed in fish (i.e. initial stage of the escape reaction; in roach, *Rutilus rutilus*: Izvekov & Nepomnyashchikh 2010). If this were the case, we would have expected the cuttlefish to turn left preferentially when tested in the T-shaped apparatus in experiment 1. However, there was only a slight left-turning bias in experiment 1, which was not age dependent. Side-turning preferences in cuttlefish may be caused, in part, by a motor bias, but this does not fully explain our results. The second possibility is that the left-turning preference observed in experiment 2 is due to lateralization of the visual system. In fish, there is considerable evidence for motor biases caused by such eye use preference (reviewed in Rogers 2000). In our T-shaped apparatus, when the cuttlefish left the start box, they would have seen the two shelters simultaneously. The visual information received via the left eye would then determine the subsequent motor direction (i.e. turning to the shelter situated on the left) but only when viewing simultaneously two attractive visual stimuli on each side (i.e. the shelters). The existence of an eye use preference in cuttlefish is consistent with the hypothesis that asymmetry of eye use is ubiquitous among species with laterally placed eyes (Vallortigara & Bisazza 2002).

In our study, the occurrence of side-turning biases depended on the provision of shelters at the ends of the arms of the T-maze. This suggests that the strength and direction of preferential eye use is not necessarily fixed in an individual and that motivational differences can alter the observed behavioural asymmetries. This observation is consistent with results of studies on fish, in which lateralization is enhanced in females viewing a shoal of males if they have been isolated from them for 2 months (Bisazza et al. 1998). Such alteration of the strength of lateralization was also observed in fish when the motivation to shoal was increased (i.e. shortly after capture in a net; Sovrano et al. 1999). Cuttlefish



**Figure 4.** Laterality index (calculated over 10 consecutive trials; means  $\pm$  SEM) at different days after hatching during experiment 2. Asterisks indicate significant differences from random choices (one-sample two-tailed  $t$  test;  $N_{3 \text{ days}} = N_{7 \text{ days}} = N_{15 \text{ days}} = N_{30 \text{ days}} = 20$ ;  $N_{45 \text{ days}} = 19$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ).



usually avoid open and lit areas when they cannot bury themselves in the substrate (Alves et al. 2007). When placed in our T-maze apparatus, the cuttlefish might have been primarily concerned with seeking shelter. Sand and shadow provided on both sides of the apparatus in experiment 2 could appear to be potential shelters, and increase the motivation to escape from the light. Consequently, shelters are likely to have altered the strength of side-turning preference in experiment 2 compared to experiment 1. As in vertebrates, behavioural asymmetries in cuttlefish seem to be affected by factors independent of lateralized brain mechanisms.

From 30 days posthatch, the cuttlefish that had a side preference (about 50%) moved to the left, which may indicate that this is a population-level phenomenon (Denenberg 1981). This result is unlikely to have been produced by the testing of more juveniles from the same mother, as the number of juveniles tested was approximately the same for each clutch of eggs. It also contrasts with the variation in eye use preferences of *O. vulgaris*, in which the population divides nearly equally into individuals preferring to use their left or their right eye (Byrne et al. 2004). Using mathematical models of the evolution of population-level asymmetries, Ghirlanda & Vallortigara (2004) pointed out that shared directionality in a population might arise as an evolutionary strategy driven by living in a social group, where individuals need to coordinate their asymmetries in behaviour among each other. This might explain why solitary fish species tend to show individual-level preferences in escape behaviour when facing a predator while social species tend to show population-level preferences (Bisazza et al. 2000). This proposal is also supported by the relationship between social behaviour and the evolution of population-level asymmetries in olfactory lateralization in arthropods. In bees, eusocial honeybees and bumblebees, *Bombus terrestris*, show lateralized olfactory learning at the population level, whereas the solitary mason bee *Osmia cornuta* shows lateralized olfactory learning at the individual level (Anfora et al. 2010, 2011). With regard to modern cephalopods, octopuses are the most solitary species, squid the most gregarious, with cuttlefishes in between these two groups (Boal 2006). Our results are somewhat consistent, then, with the notion that sociality is correlated with eye laterality: cuttlefish are moderately social and about half of the animals in our experiments shared side-turning preferences. To our knowledge, no study has yet explored lateralization in squid; however, we could hypothesize that shoaling behaviour in squid must require coordinated responses at the population level to form more cohesive shoals as in fishes (Bisazza & Dadda 2005).

In vertebrates, the ontogenesis of lateralization is markedly influenced by early experience. In birds, differential visual stimulation between the two eyes during embryonic development is necessary to generate visual lateralization (reviewed in Rogers 2007). In cuttlefish, an embryo can perceive visual stimulation coming from their surrounding environment through the capsule of its egg (Darmaillacq et al. 2008). However, as embryos move freely inside the egg, the visual system is not asymmetrically stimulated. As visual lateralization appears progressively during the first few weeks after hatching in cuttlefish, it is possibly influenced by early experience during postembryonic development. In fish, lateralized behaviour patterns fluctuate between populations of the same species exposed to differing levels of predation pressure (Brown et al. 2004). In cuttlefish, social interactions probably occur during the first few weeks of life, when juveniles live around the egg mass (Dickel et al. 2006). This could induce a directional organization in the two sides of the brain. The side-turning preference observed in juveniles when they look for shelters may be a consequence of the brain partitioning induced by social life. This is a possibility that requires testing (e.g. rearing with a low or large number of congeners).

In our study, eye use preference appeared progressively through postembryonic development. Visual and motor biases seem to have their own developmental courses in cuttlefish. Indeed, juveniles showed a slight left-turning bias until 45 days posthatch in experiment 1, and adults have individual-level side-turning preference in an empty T-shaped apparatus (Alves et al. 2007). Motor lateralization seems to develop later during the life of cuttlefish compared to visual lateralization. The emergence of visual lateralization in juvenile cuttlefish coincides with other known ontogenetic events such as the progressive diversification of their diet (Darmaillacq et al. 2004), increasing efficiency in matching the background (Hanlon & Messenger 1988) and dispersal (Dickel et al. 2006), although it is not clear what role, if any, increasing lateralization plays in these behaviours. One potential advantage of visual lateralization might be the ability to process two types of information simultaneously. For example, in topminnows, *Girardinus falcatus*, lateralized females can forage for food and attend to harassing males simultaneously (Dadda & Bisazza 2006), while in chicks, lateralized individuals are better at carrying out more than one task simultaneously than are nonlateralized individuals (e.g. hunting and looking for predators; Rogers et al. 2004; Rosa Salva et al. 2007). Between 30 and 60 days after hatching, the young cuttlefish leave their shelter to diversify their diet (Dickel et al. 2006) and become exposed to high predation pressure. The emergence of visual lateralization coincides with their dispersal, when juveniles start to need rapid processing of visual information from the environment to avoid predators and look for escape routes while hunting. It is possible that the lateralization enables the young cuttlefish to deal with these competing pressures more effectively.

Consistent with the interpretation that the left visual field is generally associated with rapid responses to threat or detection of escape routes in vertebrates, the cuttlefish preferred to hide in the shelter seen with the left eye (Rogers 2007). In zebrafish, *Danio rerio*, fry, Andrew et al. (2009) showed that there is an asymmetry of response according to which eye sees alternating stripes, a pattern suggesting a potential refuge. Fish showed a close approach to the pattern when seen with the left eye. Our findings raise an intriguing question: is the right visual field of cuttlefish associated with the response to an identified target (such as a prey item) as in vertebrates (Vallortigara & Rogers 2005) and honeybees (Letzkus et al. 2008)? Similarities between vertebrates and invertebrates may reveal a general pattern of hemibrain specialization conserved throughout evolution. Studying the implication of the right visual field of cuttlefish while hunting could provide important insights into whether there is a possible homology between lateralization in invertebrates and vertebrates. Either way, our results are consistent with that hypothesis.

In conclusion, our results add to increasing evidence that visual lateralization occurs and is common in invertebrate species. Furthermore, the cuttlefish's visual system parallels several observations in vertebrates in which the left visual field plays a predominant role in defensive behaviour. The present study is also, to our knowledge, the first study on the ontogenesis of lateralization in an invertebrate. Studying lateralization in cuttlefish will open up new possibilities for conducting controlled experiments on the influence of early experience on lateralization (absence of parental care) and its neural correlates. Finally, our study points out the importance of taking into account side-biased behaviours when designing behavioural experiments using cephalopods.

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