

## RESEARCH ARTICLE

# Context-dependent behavioural lateralization in the European pond turtle, *Emys orbicularis* (Testudines, Emydidae)

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## ABSTRACT

Lateralization presents clear advantages in ecological contexts as the dominance of one brain side prevents the simultaneous activation of contrasting responses in organisms with laterally located eyes. This is crucial in selecting a safe refuge during a predatory attack and may strongly affect predator–prey interactions. We explored the possible presence of lateralization in the anti-predatory behaviour of European pond turtles, considering their escape facing a possible predatory attack. Thirty individuals (17 males, 13 females) were exposed to three different environmental situations of gradually increasing predatory threat: escape underwater from an unsafe shelter, diving into the water from a basking site and righting after being overturned. All turtles were tested 20 times for each of the three experiments (60 trials per individual; 1800 overall trials). We recorded multiple behavioural responses in the general context of predation risk. This was done in order to assess both the existence of lateralization and possible correlations among different behaviours as function of lateralization. The number of significant responses to the left side was always prevalent in each of the three simulated anti-predatory situations, suggesting the existence of a lateralized behaviour in this species. At the individual level, the differences we found in the three experiments could be related to different ecological contexts and consequent risk of predation. Our findings, among the few on chelonians, support the possible involvement of right hemisphere activity and, most importantly, reveal how the complexity of a general predatory context can affect the laterality of escape behaviour.

**KEY WORDS:** Lateralization, Predation, Escape behaviour, Righting, Chelonian, Emydidae

## INTRODUCTION

Lateralization seems to be a key property of most vertebrates (Rogers and Andrew, 2002; Rogers and Vallortigara, 2015; Vallortigara and Versace, 2017), with the right and left sides of the brain providing different and integrative functions (Vallortigara et al., 1999a,b). It is considered a plesiomorphic character and it has been argued that the first evolutionary stage of lateralization was related to visual control of predatory behaviour (Andrew, 2002; Rogers et al., 2013). This hypothesis was the basis from which to explore lateralization in natural or semi-natural risky situations, linking this trait to the emergence of adaptive defensive strategies (Vallortigara and Rogers, 2005). At the individual level, a response can be considered lateralized when individuals show a left or right

tendency for a given behaviour, although this difference is not consistent between individuals (Rogers and Andrew, 2002). Lateralization at the population level exists when most or all individuals in a population show the same type of lateralization (e.g. left-biased or right-biased; Versace and Vallortigara, 2015). A peculiar feature of lateralization is that it may occur at different levels of neural organization in opposite directions. For example, in rats it can arise at a cortical or hypothalamic level (Nordeen and Yahr, 1982). This is a possible reason why both individual- and population-level lateralization can be present at the same time. Visual and motor lateralization have been observed in many bird species (Keyes et al., 2000; Weir et al., 2004; Ventolini et al., 2005; Berggren, 2006; Salva et al., 2009) and also in numerous terrestrial and marine mammals (Denenberg et al., 1978; Martin and Niemitz, 2003; Yaman et al., 2003; Böye et al., 2005; Versace et al., 2007; Basile et al., 2009; Robins and Phillips, 2010; Wells and Millsopp, 2009; Karenina et al., 2010; Zucca et al., 2011).

There are many different ecological contexts where lateralization can be observed. The most common are foraging and predatory contexts, where both visual and auditory lateralization have been observed (Cozzutti and Vallortigara, 2001; Palleroni and Hauser, 2003; Templeton and Christensen-Dykema, 2008). It can also be expressed in parent–offspring recognition (Karenina et al., 2010), in courtship behaviour (Ventolini et al., 2005) or in exploratory behaviour and spatial cognition (Vallortigara et al., 1999a, b; Tommasi and Vallortigara, 2004; Vallortigara et al., 2004; Regolin et al., 2005; Reddon and Hurd, 2009; Robins and Phillips, 2010).

Having an asymmetrical brain appears to provide some advantages. For example, lateralized individuals can perform better than non-lateralized ones in many situations (Güntürkün et al., 2000; Rogers et al., 2004; Vallortigara and Rogers, 2005). Another potential advantage offered by a lateralized brain is the avoidance of useless duplication of function in the two hemispheres (Levy, 1977). As reported by Pascual et al. (2004), fruit flies with asymmetrical brains seem to possess a better long-term memory than symmetrical conspecifics. At the individual level, another possible advantage might be multitasking (i.e. the ability to manage more than one activity at the same time), where lateralization allows the two hemispheres to focus on different tasks (Vallortigara, 2006). An interesting case is represented by lateralized chicks, which can perform better than non-lateralized individuals when detecting a predator and distinguishing grains from non-edible pebbles (Rogers et al., 2004). However, lateralized functions might be disadvantageous for survival, as in the case of increased reactivity to predators approaching the individual's left side (Rogers, 2000; Lippolis et al., 2002), which may expose prey to predators on their opposite side. In the context of competition, the agonistic behaviour directed to members of the same group on the animal's left side might also be disadvantageous (Deckel, 1995; Robins et al., 1998; Rogers, 2000; Vallortigara et al., 2001). A similar mechanism may explain the disadvantage for the right side preference when

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responding to prey (Rogers, 2002; Robins and Rogers, 2004; see also Ghirlanda and Vallortigara, 2004; Ghirlanda et al., 2009, for mathematical models of advantages and costs of lateralization).

The traditional interpretation of brain lateralization is that it avoids costly duplication of neural circuitry with the same function (Levy, 1977) and decreases the interference between various brain functions (Rogers, 2000). Another hypothesized advantage of lateralization is that dominance of one brain side prevents the simultaneous activation of contrasting responses in organisms with laterally located eyes (Andrew, 1991; Cantalupo et al., 1995; Vallortigara, 2000). This is crucial, for example, in selecting a safe refuge during predator attacks and may strongly affect predator–prey interactions.

Although these aspects have been investigated in other vertebrate groups (Rogers, 2010; Broder and Angeloni, 2014; Ferrari et al., 2015), little is known concerning lateralization in anti-predatory behaviour of reptiles (Bisazza et al., 1998). For example, some species of lizards have been shown to be lateralized with respect to refuge use at both the individual and population level (García-Muñoz et al., 2012, 2013), as well as in escape or anti-predator responses (Bonati et al., 2010; Lustig et al., 2012). Other species of lizards show use of lateralization in the visual field in aggressive interactions (Hews and Worthington, 2001; Hews et al., 2004), predation attempts (Robins et al., 2005; Bonati et al., 2008), predator monitoring (Martín et al., 2010) and maze orientation (Lustig et al., 2013a).

Far less attention has been paid to lateralization in turtles. As the righting response (i.e. returning to an upright position after being flipped onto the back or carapace) is considered a basic behaviour for survival in this group (Ashe, 1970; Domokos and Várkonyi, 2008), it has often been investigated in order to detect possible lateralization. Hermann's tortoises (*Testudo hermanni*) show individual-level bias to turn on the right side when righting (Stancher et al., 2006). Malashichev (2016) found that hatchlings of both green turtles (*Chelonia mydas*) and olive ridley turtles (*Lepidochelys olivacea*) demonstrated individual-level lateralization of the righting response, but only weak population-level lateralization. Although not a righting response, leatherback turtles (*Dermochelys coriacea*) show a population-level right bias in flipper use during nesting (Sieg et al., 2010), suggesting that turtles may express other lateralized behaviours. Concerning freshwater turtles, Smith et al. (2017) found evidence of individual-level laterality in the righting response of the painted turtle (*Chrysemys picta*) but not of the eastern musk turtle (*Sternotherus odoratus*).

In this study, we explored the possible presence of lateralization in escape behaviour of European pond turtles (*Emys orbicularis*), by exposing individuals to different environmental situations: escape underwater from an unsafe shelter, diving into water and righting. We recorded multiple behavioural responses in the general context of predation risk. This was conducted in order to assess both the existence of lateralization in this species in various environmental conditions and the possible correlation among different behaviours as a function of lateralization, i.e. whether lateralization is consistent in these situations. For example, if two behavioural responses reveal a similar left-handed or right-handed lateralization at the individual or population level, it is worth assessing whether they are correlated or not. Connection between different lateralized behaviours in a specific ecological context might provide new insight on the origin and function of lateralization.

## MATERIALS AND METHODS

### Model species and animal collection

We collected adult European pond turtles, *Emys orbicularis* (Linnaeus 1758), in the naturalistic area called Oasi di

Sant'Alessio (circa 20 km south of Milan, Italy), in a typical context of mixed residual damp forest in areas strongly characterized by intensive cultivation. During September 2017, we collected 30 turtles (17 males and 13 females; carapace length  $133.3 \pm 3.2$  and  $154.5 \pm 3.2$  mm, respectively, means  $\pm$  s.e.m.) from an artificial pond characterized by clear water, submerged and riparian aquatic vegetation, and emerging tree trunks that allowed the turtles to bask. The turtles were held in captivity in outdoor plastic aquariums before starting the experiments, and were released to their source pond at the end. The European pond turtles used in this study belong to Oasi di Sant'Alessio, which has permits to hold, breed, capture, manipulate and carry out conservation projects and scientific research on this and many other species found in it.

### Experimental procedures

Before the beginning of trials, we allowed the turtles to bask for at least 1 h, thus attaining optimal body temperature and maximal locomotor performance necessary for rapid escape behaviour. We recorded the escape side chosen by turtles subjected to three alternative simulated predatory attacks: underwater escape from an unsafe shelter, diving escape into the water from a basking site and righting after being overturned. The first situation simulated a possible risk of predation in a submerged environment, where a refuge seemingly becomes not unsafe and the turtle needs to escape by swimming and looking for a safer place. The second reproduced a different threat situation during the turtle's basking activity, inducing it to dive into the water and look for a safe shelter. In the third, we simulated a predatory attack by overturning a turtle onto its carapace and recording the side from which it turned. All turtles were tested 20 times in each of the three experiments, for a total of 60 trials per individual and 1800 overall trials.

We performed all behavioural tests outdoors, on sunny days, in a grass field, with the turtles in opaque plastic containers of the same size (length  $\times$  width  $\times$  height:  $85 \times 60 \times 45$  cm). Each turtle took part in three trials per day. The orientation of the experimental container was randomly changed during the day to eliminate the effect of sunlight, i.e. the formation of shaded and lightened zones, and surrounding environment on behavioural responses. Additionally, the container walls were quite high in order to exclude almost all external landmarks from the turtles' sight. All turtles were carefully inspected prior to the start of the experiments and no detectable physical anomalies or external body asymmetries could be found. In order to avoid confusing effects that could have affected risk perception, all experiments were performed by the same person (D.P.-R.), in a similar way and wearing clothes of the same colour.

### Underwater escape from a shelter

The behavioural response was recorded in opaque plastic containers set to explore an underwater response (Fig. 1). No cover was added, and an opaque PVC cylindrical tube (35 cm length, 15 cm in diameter) was placed underwater in the middle of the proximal short side of the container. This was done in order to induce the turtle into having its body axis aligned with the tube's longitudinal axis when exiting the tube (i.e. at the beginning of the escape position). During the tests, the observer gently put the turtle into the tube and then immediately stimulated it by hitting the external side of the container in order to induce the escape response. Remaining motionless at the back of the apparatus, the observer was constantly aware of his position during the trial, in order to avoid affecting the possible escape direction of the turtle. The longitudinal axis of the container was taken as the reference point of choice. We considered that an individual had opted for one side when the entire body of the

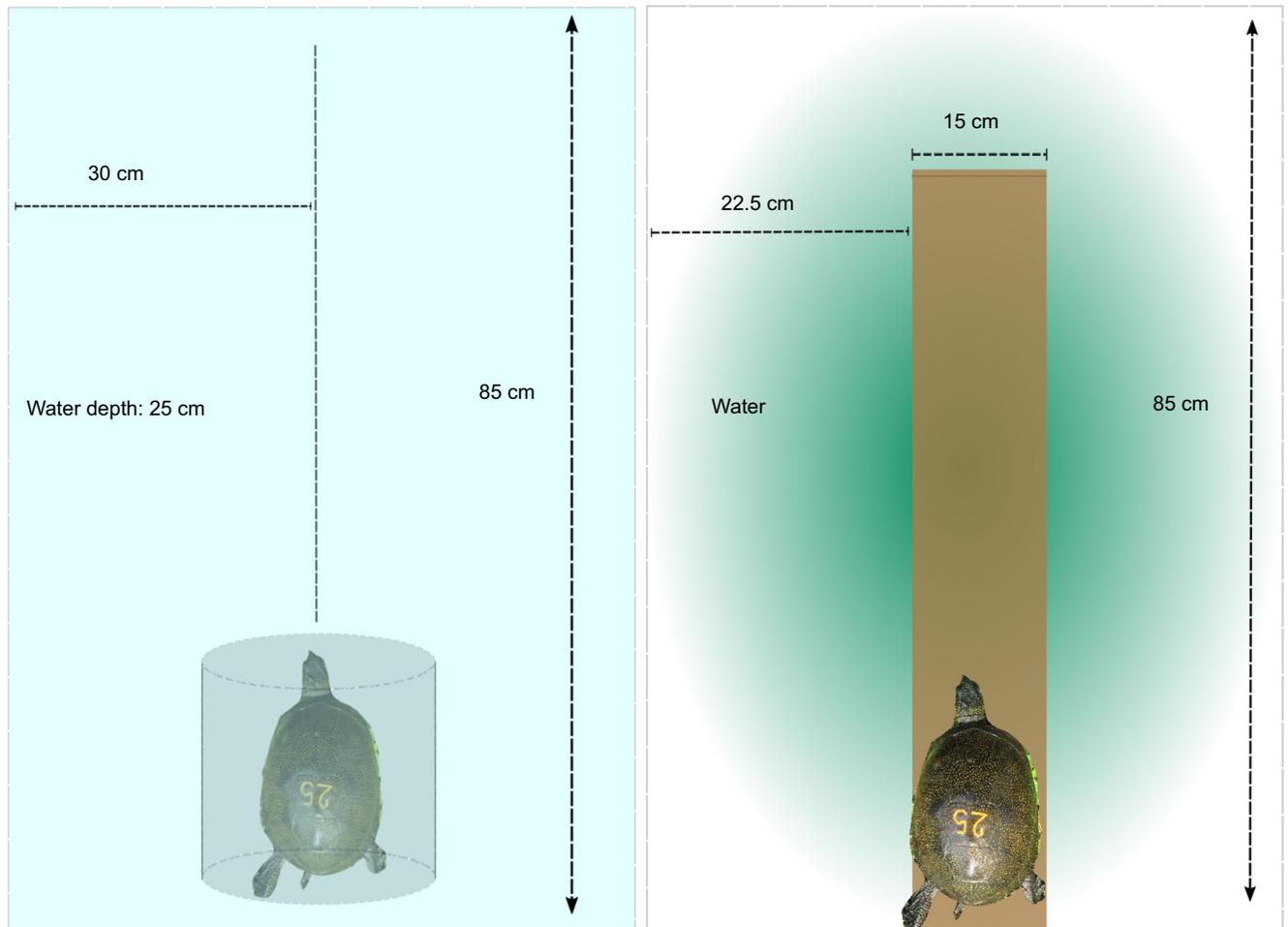


Fig. 1. Experimental set-up for the underwater escape (left) and diving escape (right) response.

turtle crossed the axis. At the end of each trial, we changed the water in order to eliminate the possible effect of chemical signals on the choice of the individuals tested later.

### Diving escape

We simulated predatory attacks toward individual turtles in similar plastic containers to those used for underwater escape responses, filled with 5 cm-deep clean water. In order to simulate a predation threat, we took one turtle from its aquarium and, after handling it gently for 5–10 s, released it in the middle of the experimental container. A flat wooden board (80×20×2 cm) served as a bridge and was positioned along the median longitudinal axis of the container (Fig. 1). During the tests, the observer remained at the back of the container. He gently put the turtle onto the board and produced vibrations, in order to trigger the turtle's escape behaviour, by hitting the centre of the back of the container for a few seconds. Thereafter, and without further handling, the experimenter simulated a persistent waiting predator by remaining motionless in close proximity (<1 m) to the container. The trial ended when the turtle chose a side and dived into the water.

### Righting

Behavioural trials were performed in the same type of plastic container used in the trials described above. However, we prepared it with wire mesh on the base plan, which served as a useful support to facilitate holding by turtles during righting. Each trial consisted of

overturning a turtle (about its sagittal axis) and gently placing it on a circular rubber support (0.5 cm height) in order to obtain a balanced position with respect to the base plan. In order to properly evaluate the righting response, each trial was video-recorded and the direction of righting was scored later. We considered the first leg that turtles attempted to use for righting as the side choice for laterality. After completing each response, the turtle was free to rest and walk around for some minutes before being removed from the container. If the turtle did not move within 2 min, the trial was considered invalid and repeated later.

### Statistical analysis

We used a binomial test to determine individual preference for a specific direction. The level of significance ( $P \leq 0.05$ ) was reached when a turtle chose the same direction 14 times over 20 trials (i.e. an index of 30% for left-prone individuals and 70% for right-prone individuals; see below). To compare the number of left- and right-lateralized individuals, we used Pearson's chi-squared test for count data.

At the population level, lateral asymmetries were analysed by examining an index of laterality (as in Stancher et al., 2006) estimated for each specimen as:  $n_{\text{right}} / (n_{\text{right}} + n_{\text{left}}) \times 100$ , where  $n$  is the number of right/left choices. A value of 50% indicates no preferred direction, values above 50% indicate a preference for the right response and values below 50% show a preference for the left response. Frequency distribution of the laterality index for all

variables is provided in Fig. 2. The difference from the 50% level was assessed using a linear model (*t*-test), with the index of laterality as the response variable, after centring for this value. Normality was controlled with a Shapiro–Wilk test of normality. We used Pearson's product moment correlation coefficient for paired samples to explore possible correlation between behavioural responses. As this method does not allow exclusion of the presence of correlation in null hypothesis significance testing, we computed Bayesian statistics on correlation coefficients, and interpreted the outcome according to Wetzels and Wagenmakers (2012). Statistical analyses were performed using R v.3.5.0 (<https://www.R-project.org/>).

## RESULTS

A total of 22 turtles showed a form of laterality by choosing an escape side, significantly different from the 50% index value, in at least one of the three simulated anti-predatory situations (Table 1). In particular, 19 individuals chose the left side (nine of these in two different situations and one in all of the three experiments), two chose the right side and only one selected both sides. Eight turtles did not express a significant preference for a side in any of the situations proposed. In the following paragraphs, we discuss the results obtained for each of the experiments performed.

### Underwater escape from a shelter

Among the individuals, 13 out of 30 turtles exhibited a preference to escape underwater in a certain direction. Twelve individuals consistently turned left and one consistently turned right ( $\chi^2=9.31$ , d.f.=1,  $P=0.002$ ). Seventeen turtles did not show a clear preference for any direction (Table 1).

The linear model showed a significant difference from the chance value (50%) of the laterality index (estimate±s.e.  $-10.39\pm 3.10$ , d.f.=29,  $t=-3.34$ ,  $P=0.002$ ) and indicated an overall significant preference for righting to the left (mean±s.e.m.  $39.60\pm 3.10$ ;  $n=30$ ). We did not find any difference between sexes for the index value ( $t=0.68$ , d.f.=28,  $P=0.50$ ).

### Diving escape

Among the individuals, 11 out of 30 turtles exhibited a preference for diving in a certain direction. Nine turtles consistently turned left and two consistently turned right ( $\chi^2=4.45$ , d.f.=1,  $P=0.0348$ ; see below for further evidence of population-level lateralization). Nineteen turtles did not show a clear preference for any direction (Table 1).

The linear model showed a significant difference from the chance value (50%) of the laterality index (estimate±s.e.  $-9.45\pm 3.20$ , d.f.=29,  $t=-2.95$ ,  $P=0.006$ ) and indicated an overall significant preference for righting to the left (mean±s.e.m.  $40.54\pm 3.20$ ;  $n=30$ ). We did not find any difference between sexes for the index value ( $t=0.12$ , d.f.=28,  $P=0.90$ ).

### Righting

Ten turtles consistently showed a preference to overturn (attempt with a leg) by using mainly the left front leg, while we did not observe any preference for the right leg. Twenty individuals did not show a preference for any leg (Table 1).

The linear model showed a significant difference from the chance value (50%) of the laterality index (estimate±s.e.  $-12.0\pm 3.2$ , d.f.=29,  $t=-3.75$ ,  $P=0.0007$ ) and indicated an overall significant preference for righting to the left (mean±s.e.m.  $38.0\pm 3.2$ ;  $n=30$ ). We found males to be more strongly left lateralized than females for the index value (female mean index= $46.53\pm 4.45$ ; male mean index= $31.47\pm 5.91$ ;  $t=10.45$ , d.f.=28,  $P=0.01$ ), and overall 8 males and 2 females showed a clear preference towards the left.

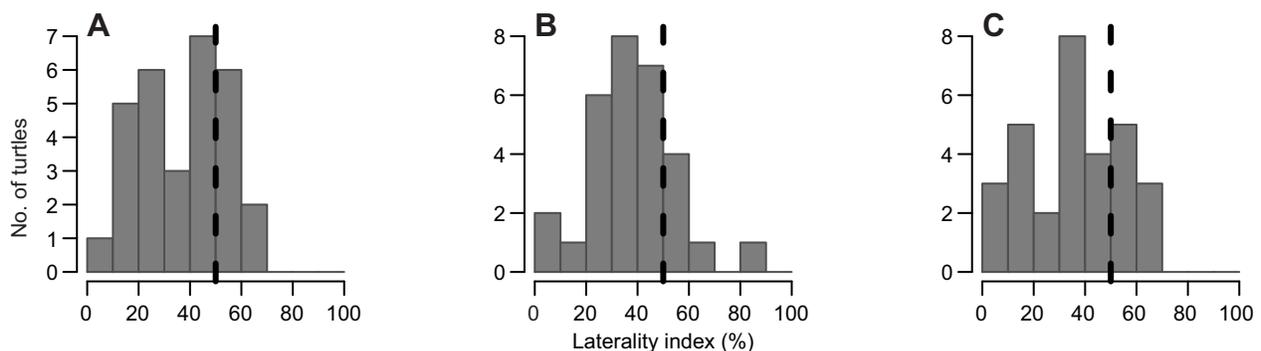
### Correlation between behavioural responses

Considering all individuals, we did not find any correlation between diving and underwater escape ( $r=0.15$ ,  $t=0.84$ , d.f.=28,  $P=0.41$ ,  $n=30$ ; Table 2, Fig. 3).

No correlation between righting and either underwater or diving escape was revealed between individuals considering the whole group tested (respectively:  $r=-0.005$ ,  $t=-0.03$ , d.f.=28,  $P=0.97$ ;  $r=0.06$ ,  $t=0.31$ , d.f.=28,  $P=0.75$ ; Table 2, Fig. 3). However, we found a significant correlation for underwater escape and diving considering left-lateralized individuals, i.e. with index value lower than 50% ( $r=-0.68$ ,  $t=-2.26$ , d.f.=8,  $P=0.03$ ,  $n=10$ ; Table 2, Fig. 3). Overall, only two individuals showed a consistent behavioural response in the same direction; only one individual showed a consistent behavioural response for all kind of trials in the same direction (male, index=25 for all responses).

## DISCUSSION

Our results, obtained through a threefold experimental approach, showed that European pond turtles are lateralized in their escape behaviour when facing a possible predatory attack. Even with different individuals, the number of significant responses to the left side was always prevalent in each of the three simulated anti-predatory situations. Moreover, some of the tested individuals showed a consistent response in different experiments, suggesting a



**Fig. 2. Frequency plot showing laterality index (LI) distribution for the three behavioural responses.** (A) Underwater, (B) diving and (C) righting. Vertical axis reports the absolute number of individuals (frequency), showing a 10% range of LI value (i.e. 10 identical intervals). Vertical dashed lines represent LI=50%, i.e. chance level ( $n=30$ ).

**Table 1. Exact binomial test (one sided) for the three escape responses**

Turtle	Sex	Underwater				Diving				Righting			
		Left	Right	Index	<i>P</i>	Left	Right	Index	<i>P</i>	Left	Right	Index	<i>P</i>
1	M	10	10	50	0.59	8	12	60	0.25	7	13	65	0.13
2	F	16	4	20	<b>&lt;0.01</b>	12	8	40	0.25	16	4	20	<b>&lt;0.01</b>
3	F	12	8	40	0.25	15	5	25	<b>0.02</b>	12	8	40	0.25
4	F	6	14	70	<b>0.05</b>	11	9	45	0.41	9	11	55	0.41
5	F	8	12	60	0.25	2	18	90	<b>&lt;0.01</b>	9	11	55	0.41
6	M	11	9	45	0.41	9	11	55	0.41	17	3	15	<b>&lt;0.01</b>
7	M	8	12	60	0.25	15	5	25	<b>0.02</b>	16	4	20	<b>&lt;0.01</b>
8	M	9	11	55	0.41	12	8	40	0.25	17	3	15	<b>&lt;0.01</b>
9	M	15	5	25	<b>0.02</b>	11	9	45	0.41	14	6	30	<b>0.05</b>
10	M	8	12	60	0.25	18	2	10	<b>&lt;0.01</b>	18	2	10	<b>&lt;0.01</b>
11	F	16	4	20	<b>&lt;0.01</b>	8	12	60	0.25	16	4	20	<b>&lt;0.01</b>
12	M	7	13	65	0.13	10	10	50	0.59	13	7	35	0.13
13	F	14	6	30	<b>0.05</b>	19	1	5	<b>&lt;0.01</b>	9	11	55	0.41
14	F	11	9	45	0.41	13	7	35	0.13	7	13	65	0.13
15	F	14	6	30	<b>0.05</b>	13	7	35	0.13	12	8	40	0.25
16	F	10	10	50	0.59	11	9	45	0.41	10	10	50	0.59
17	F	16	4	20	<b>&lt;0.01</b>	17	3	15	<b>&lt;0.01</b>	7	13	65	0.13
18	F	11	9	45	0.41	14	6	30	<b>0.05</b>	10	10	50	0.59
19	F	9	11	55	0.41	9	11	55	0.41	13	7	35	0.13
20	F	20	0	0	<b>&lt;0.01</b>	12	8	40	0.25	9	11	55	0.41
21	M	11	9	45	0.41	15	5	25	<b>0.02</b>	12	8	40	0.25
22	M	11	9	45	0.41	10	10	50	0.59	11	9	45	0.41
23	M	15	5	25	<b>0.02</b>	15	5	25	<b>0.02</b>	15	5	25	<b>0.02</b>
24	M	9	11	55	0.41	10	10	50	0.59	12	8	40	0.25
25	M	17	3	15	<b>&lt;0.01</b>	11	9	45	0.41	18	2	10	<b>&lt;0.01</b>
26	M	14	6	30	<b>0.05</b>	6	14	70	<b>0.05</b>	13	7	35	0.13
27	M	12	8	40	0.25	14	6	30	<b>0.05</b>	18	2	10	<b>&lt;0.01</b>
28	M	12	8	40	0.25	13	7	35	0.13	11	9	45	0.41
29	M	14	6	30	<b>0.05</b>	12	8	40	0.25	13	7	35	0.13
30	M	16	4	20	<b>&lt;0.01</b>	12	8	40	0.25	8	12	60	0.25

Significant preferences for one direction are indicated in bold.

possible spontaneous tendency to not escape randomly from a predator, but to select a preferential side for escape, as found in other taxa (Bonati et al., 2010; García-Muñoz et al., 2012; Lustig et al., 2013b). Many studies have shown evidence of a left-lateralized hemisphere in contexts requiring focused attention for controlling feeding cues and searching for food resources, by using the right eye (Rogers, 1997; Robins, 2006; Bonati et al., 2008; Lippolis et al., 2009). In contrast, threatening situations due to possible predation, aggressive cues or environment exploration are usually associated with the right hemisphere, with a consequent specialization of the left eye input, as shown in toads, fishes and birds (Vallortigara, 2000; Robins, 2006; MacNeilage et al., 2009). In reptiles, a left lateralization was observed in common wall lizards (*Podarcis muralis*), which are able to process the predatory input perception by

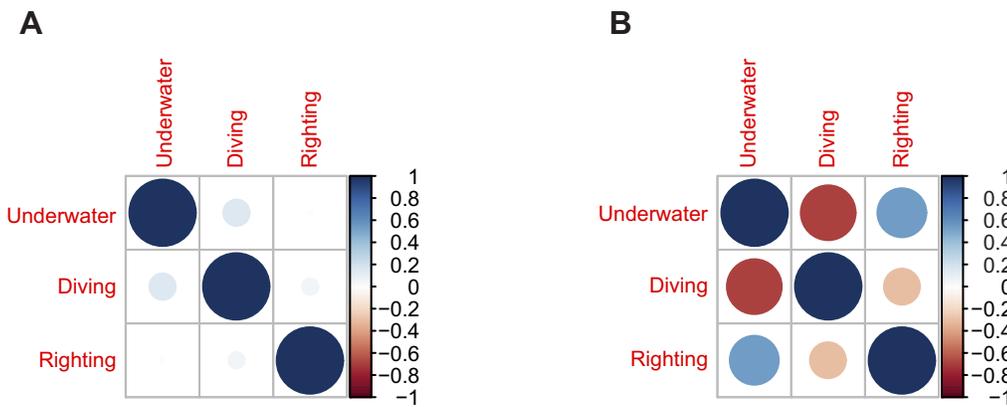
using the left eye, with a consequent involvement of the right brain hemisphere in detecting anti-predatory cues (Martín et al., 2010). A similar pattern was present in aggressive rejections displayed by females of the striped plateau lizard (*Sceloporus virgatus*), with a left-eye bias for conspecific aggression (Hews et al., 2004).

Among reptiles, chelonians have been investigated only marginally for laterality: sea turtles (*C. mydas* and *L. olivacea*: Malashichev, 2016), terrestrial tortoises (*T. hermanni*: Stancher et al., 2006) and recently in freshwater turtles (Smith et al., 2017). In this group, the righting response has been shown to vary among individuals in terms of the latency to start the action and the time required to overturn, with some evidence for heritable variation in these traits (e.g. Steyermark and Spotila, 2001; Delmas et al., 2007). It also appears that the ability to quickly right is tied to survival and growth rate, at least in *Trachemys scripta elegans* (Delmas et al., 2007), with some turtles demonstrating laterality in the righting response. However, conflicting results have emerged from these studies, with terrestrial Hermann's tortoises preferentially turning to the right side (*T. hermanni*: Stancher et al., 2006) and sea turtles showing a not well defined pattern of lateralization, with the number preferring the left side not differing significantly from the number choosing the right (Malashichev, 2016). Concerning freshwater turtles, Smith et al. (2017) detected laterality exclusively at the individual level in the righting response of *C. picta*, but not at the population level, and not at all in *S. odoratus*. Painted turtles tended to turn more to the right side than the left, but only when combining all individuals in three categories and with a small number of replicates (Smith et al., 2017). In our study, European pond turtles seemed to be quite consistent in their preferred escape side, with

**Table 2. Coefficient of correlation (*r*) between behavioural responses and *P*-value from Pearson product moment correlation for paired samples**

Behavioural variables	Full sample ( <i>n</i> =30)			LI<50% ( <i>n</i> =10)		
	<i>r</i>	<i>P</i>	Bf	<i>r</i>	<i>P</i>	Bf
Underwater–diving	0.15	0.41	0.20	−0.68	<b>0.03</b>	2.46
Underwater–righting	−0.005	0.97	0.14	0.54	0.10	0.87
Diving–righting	0.06	0.75	0.15	−0.29	0.41	0.32

Two analyses were performed on laterality index values (LI): one for the full sample (*n*=30) and the other for a restricted group with LI<50% (*n*=10). Bf represents Bayes factor and shows substantial evidence for the null hypothesis if inside the interval [0.1–0.33] or anecdotal evidence for an alternative hypothesis if inside the interval [1–3].



**Fig. 3. Correlation plot for the three behavioural variables recorded.**

(A) All individuals ( $n=30$ ). (B) Individuals with  $LI < 50\%$  ( $n=10$ ).

lateralized individuals choosing the left side more often. This could be due to many variables such as carapace shape or different environments and ecological pressures that characterize different groups. The shape of the shell is crucial in determining the strategy of self-righting as flatter turtles use their neck more than their feet in turning their body, in contrast to more rounded ones (Domokos and Várkonyi, 2008; Smith et al., 2017). In our population, all individuals mainly used their feet and nails as a hook on the ground to right themselves, while the neck served as a support to provide more pressure to turn. Therefore, the difference we found with respect to previous studies of freshwater turtles could be influenced by different processes involved in righting mechanisms.

Our experimental approach aimed to simulate three different threatening situations with a risk gradient based on the ecological needs of a semi-aquatic species such as *E. orbicularis*. This species shows a clear activity in terrestrial environments and usually basks on rocks and along shorelines. Typical predators include wild boars, mustelids, rodents and birds, especially for newborns and young turtles (Zuffi et al., 2011). In the underwater experiment, turtles were in a lower risk context, placed in their most congenial environment and subject to a slight predation threat. In the second experimental set (diving), the turtles were placed out of the water, reproducing a classic basking situation with a heightened risk of predation and the chance of a quick escape into the water. Finally, the righting test represented the situation of greatest threat, with animals overturned and placed on the ground, thus simulating an upcoming predation. At the individual level, the differences we found in the three experiments could be related to different contexts and consequent risk of predation. Only one turtle showed a consistent response in all situations, although usually animals significantly selecting an escape side were not the same in different situations. Interestingly, we detected a significant negative correlation between underwater and diving responses from a subgroup of individuals with index values lower than 50% (i.e. left-lateralized individuals). This finding, despite being based on weak evidence (see Table 2), suggests some flexibility in the anti-predatory behaviour of these freshwater turtles, allowing them to diversify the escape response in different contexts. Such a plastic response might avoid providing landmarks in favour of predator learning ability and increase the chances of escape in risky conditions, as shown in many reptiles (Cooper and Blumstein, 2015).

Another factor possibly affecting anti-predatory responses is related to sex. In the righting experiment, we found a greater left-lateralization in males than in females, suggesting that in a

higher risk context males are more consistent in their escape direction choice. However, no difference involving sex was found in the two low-risk situations, as reported in the escape behaviour of common wall lizards (Bonati et al., 2010). We can thus hypothesize that females facing a situation of high risk prefer to carefully evaluate which side to turn when fleeing. Other studies showed differences in the escape behaviour between sexes in reptiles (Ibáñez et al., 2014; Jacobson et al., 2016; Samia et al., 2016), linking them to sex-specific environmental constraints affecting the reproductive effort of females more than males.

In the last few years, studies on laterality in reptiles, and in particular in turtles, have been increasing, but remain scarce. These data do not allow a full understanding of the mechanisms involved in the expression and pattern of lateralization. Nevertheless, we have now begun to grasp the relevance of some related factors, such as species, sex, body shape and environmental context. Our results show a general preference of turtles for the left side when subjected to a potential predatory threat. However further experiments are required in order to discover which information is used by them to escape towards the left side. According to the data collected on *P. muralis* (Bonati et al., 2010), we might conclude that the turtle's choice is based on the right eye assessment. Conversely there may be different explanations for the leftward bias escape, for example: (1) the left eye is prompt in detecting a safer escape direction and (2) right limbs are more efficient for locomotion or more sensitive to ground vibrations. The preferences we observed for the left limb when turning around lead us to propose a general bias for the use of the left limb. Nonetheless, this hypothesis needs to be supported by suitable experiments. As a fitness-related trait, laterality for escape behaviour is modulated by the general environmental context and can be expressed with different intensity according to the specific situation. This suggests the existence of a trade-off preventing the emergence of a population-level lateral fixed response in different situations. However, further studies should follow an integrated approach, including multiple behavioural responses in the same environmental context, in order to clarify which mechanisms are involved in the evolution of behavioural lateralized traits and the relationship among them.

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#### Competing interests

The authors declare no competing or financial interests.

## Author contributions

Conceptualization: D.P.-R., A.G.; Methodology: D.P.-R., A.G.; Software: A.G.; Validation: D.P.-R.; Investigation: D.P.-R., A.G.; Data curation: A.G.; Writing - original draft: A.G.; Writing - review & editing: D.P.-R., A.G.; Supervision: D.P.-R.

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