Evolutionary Ecology Research, 2018, **19**: 487–501

Cerebral lateralization and quantity discrimination abilities in the threespine stickleback (*Gasterosteus aculeatus*)

Marion Mehlis-Rick, Timo Thünken, Markus Krings*,
Simon Käfer[#] and Ingolf P. Rick

Institute for Evolutionary Biology and Ecology, University of Bonn, Bonn, Germany

ABSTRACT

Background: The preferential use of one side of the body during behavioural activities (i.e. behavioural lateralization) is a consequence of functional cerebral asymmetries and can be found throughout the animal kingdom.

Hypothesis: Lateralization provides a selective advantage by conferring greater cognitive abilities on the organism.

Organism: Threespine stickleback (*Gasterosteus aculeatus*).

Methods: We gave individual fish the choice between two shoals differing in group size. We used the following numerical contrasts: 1 vs. 2 (small number/small ratio), 1 vs. 3 (small number/large ratio), 5 vs. 10 (large number/small ratio), and 5 vs. 15 (large number/large ratio). We manipulated eye availability by covering the fish's right eye (left-monocular), covering the fish's left eye (right-monocular), or leaving both eyes uncovered (binocular) during the choice trials. We measured the proportion of time the test fish spent in front of each of the two stimulus shoals.

Results: Sticklebacks generally preferred to shoal with the larger group. This preference was stronger at larger ratios. Quantity discrimination was not significantly restricted by monocular vision. In fact, left-monocular fish even tended to perform better than binocular conspecifics independent of contrast. Furthermore, when we separated the data by contrast, we found a significant effect of eye availability on shoaling preferences for the 5 vs. 10 contrast, i.e. fish with monocular vision performed similarly (right-monocular) or again better (left-monocular) at quantity discrimination compared with individuals that had both eyes uncovered. In comparison, eye occlusion had no significant effect on quantity discrimination in the other three contrasts (1 vs. 2, 1 vs. 3, and 5 vs. 15).

Conclusion: We found that cerebral lateralization is linked to certain numerical skills, which are of adaptive significance in different contexts, including shoaling, foraging, and predator avoidance.

Correspondence: I.P. Rick, Institute for Evolutionary Biology and Ecology, University of Bonn, An der Immenburg 1, 53121 Bonn, Germany. email: irick@evolution.uni-bonn.de

**Present address*: Institute of Biology II, RWTH Aachen, Worringerweg 3, 52074 Aachen, Germany.

[#] *Present address*: Zoological Research Museum Alexander Koenig, Leibniz Institute for Animal Biodiversity, Adenauerallee 160, 53113 Bonn, Germany.

Keywords: cerebral lateralization, cognitive abilities, *Gasterosteus aculeatus*, numerical contrasts, quantity discrimination, shoaling behaviour, threespine stickleback.

INTRODUCTION

Lateralization is an expression of functional cerebral asymmetries and is described as differential processing of information in either hemisphere of the brain (Rogers *et al.*, 2013). Asymmetries of cerebral functions have been documented for a variety of vertebrate (Karenina *et al.*, 2013a; Wiper, 2017) and invertebrate species (Rogers and Andrew, 2002; Rogers and Vallortigara, 2008). In vertebrates, asymmetries in hemispheric functioning are often related to visually guided behaviours, such as asymmetric eye use during social interactions, foraging or predator detection (e.g. Rogers *et al.*, 2004; Bisazza and Dadda, 2005; Dadda and Bisazza, 2006b). This is because in most vertebrates the lateral placement of eyes creates a left and right visual field with visual input being primarily projected to the respective contralateral cerebral hemisphere (Vallortigara and Rogers, 2005).

The strength and direction of lateralization can vary between individuals, populations, and species (e.g. Bisazza *et al.*, 1997; Brown *et al.*, 2007; Brown and Magat, 2011), suggesting that cerebral asymmetries are associated with certain costs, such as processing limitations when inter-hemispheric exchange of information is required (Vallortigara and Rogers, 2005). For example, Dadda *et al.* (2009) presented individuals of the topminnow (*Girardinus falcatus*) with one high-quality shoal composed of a higher number or of larger individuals and one low-quality shoal composed of a lower number or of smaller individuals. Non-lateralized individual fish performed better in discriminating between the two shoals by showing a preference for the high-quality shoal compared with their strongly lateralized conspecifics (Dadda *et al.*, 2009). Furthermore, predictable lateral biases in behaviour at the population level are associated with disadvantages in such contexts as orientation, foraging or predation (e.g. Lippolis *et al.*, 2002; Ghirlanda and Vallortigara, 2004; Brown and Braithwaite, 2005; Ventolini *et al.*, 2005). Consequently, it has been suggested that these disadvantages must be traded off against certain cognitive advantages of behavioural lateralization (Vallortigara and Rogers, 2005), which mainly refer to an enhanced simultaneous processing of different stimulus types in the left and right hemispheres (Rogers and Andrew, 2002; Vallortigara and Rogers, 2005). In fishes, for instance, this has been shown in topminnows, whereby lateralized individuals had better foraging abilities than non-lateralized fish when a predator was present at the same time (Dadda and Bisazza, 2006a). However, depending on the task involved, the benefits of an increased cerebral asymmetry are not always exclusively based on lateralization of information processing. For example, in a further study on topminnows, Sovrano *et al.* (2005) demonstrated that lateralized individuals had improved abilities in a spatial reorientation task compared with non-lateralized fish, which was independent of the direction of lateralization.

A specific form of cognitive abilities is numerical competence – the ability to compare and discriminate between different quantities. Numerical abilities in vertebrates have not only been demonstrated for mammals and birds (e.g. Davis and Bradford, 1986; Roberts and Mitchell, 1994), but also for lower vertebrate groups including amphibians (Uller *et al.*, 2003) and especially fishes (for a review, see Agrillo and Bisazza, 2017). Individuals can benefit from pronounced numerical abilities in different ecological contexts such as foraging (Hauser *et al.*, 2000; Lucon-Xiccato *et al.*, 2015) or when assessing the number of individuals in a group (Hager and Helfman, 1991). The ability to discriminate between the number of conspecifics in social groups is used in agonistic interactions (e.g. McComb *et al.*, 1994) and mating behaviours (Agrillo *et al.*, 2008), but especially in anti-predation contexts (Hager and Helfman, 1991). With regard to the latter, it is beneficial for potential prey individuals to choose a larger group, since these provide enhanced chances to detect predators – the 'many-eyes effect' (see Krause and Ruxton, 2002 and references therein). Furthermore, larger groups make it difficult for a predator to focus on individual prey – the 'confusion effect' (e.g. Landeau and Terborgh, 1986) – and individuals in larger groups benefit from a smaller risk of being caught due to dilution effects (Krause and Ruxton, 2002). Accordingly, numerical abilities in fishes are predominantly studied by focusing on their capability to discriminate between differently sized groups of conspecifics in a shoaling context (e.g. Mehlis *et al.*, 2015; Lucon-Xiccato *et al.*, 2016).

Studies with human infants suggest that quantity discrimination follows two non-verbal representation systems: the object file system (OFS) and the approximate number system (ANS) (Feigenson and Carey, 2005; Xu *et al.*, 2005). In the OFS, each element is represented as a discrete file in working memory, so that this system should be limited by cognitive capacity (Trick and Pylyshyn, 1994). The so-called set size limit for the OFS is usually around 3–4 (Trick and Pylyshyn, 1994; Ross-Sheehy *et al.*, 2003; Le Corre and Carey, 2007). In comparison, estimation of larger set sizes is done using the ANS. The ANS follows 'Weber's law', whereby the ratio between two quantities is used for discrimination instead of the absolute difference (Gallistel and Gelman, 1992). Some recent findings indicate that these two independently working systems are found in fish as well. For example, guppies (*Poecilia reticulata*) were unable to discriminate between shoal sizes crossing the set size limit (3 vs. 5), whereas they performed well when discriminating between two large set sizes (5 vs. 10) as well as two small set sizes (3 vs. 4) (Piffer *et al.*, 2012). However, in another study, redtail splitfin fish (*Xenotoca eiseni*) also discriminated between quantities crossing the set size limit (Stancher *et al.*, 2013), indicating that the ANS may also be used for smaller set sizes (Cordes *et al.*, 2001) or continuous physical variables (Frommen *et al.*, 2009).

Compared with many other cognitive and behavioural functions that are known to be lateralized (for a review, see Vallortigara and Rogers, 2005), evidence for a lateralization of numerical processing in animals other than humans is limited. Kilian *et al.* (2005) showed that a bottlenose dolphin (*Tursiops truncatus*) discriminated between two stimuli differing in numerosity when only its right eye was available, but not when restricted to its left eye. This suggests the left hemisphere is involved in quantity discrimination. Moreover, when studying individual-level lateralization in guppies, Dadda *et al.* (2015) demonstrated that strongly lateralized individuals have better numerical abilities than non-lateralized fish.

In the present study, we used the threespine stickleback as our model organism, which shows natural shoaling behaviour during the non-reproductive season (fall/winter) (Wootton, 1984). When given the choice, individuals prefer to shoal with the larger of two shoals (see Mehlis *et al.*, 2015 and references therein). To determine whether lateralized processing of perceptual information is associated with numerical abilities, we assessed whether bilateral processing of information is better (or even obligatory) or whether unilateral processing is equally efficient (or even more efficient) for numerical discrimination in a fish. In order to test this, we blocked the sensory input to one hemisphere by the occlusion of the respective contralateral eye. Individual fish that had their right eye covered (left-monocular), their left eye covered (right-monocular), or both eyes uncovered (binocular) were then given the choice between a large and a small shoal. The numerical contrasts, which were successfully discriminated in previous stickleback studies (see also Mehlis *et al.*, 2015 and references therein), were chosen to cover a small number range (1 vs. 2, 1 vs. 3) and a large number range (5 vs. 10, 5 vs. 15), allowing us to test the effects of lateralization across the respective representation systems (OFS, ANS). From a functional perspective, it is advantageous for an individual to prefer the bigger of two small groups yet less important to prefer the bigger of two large groups due to an overall reduced predation risk in the latter scenario (e.g. Roberts, 1996). Consequently, in case lateralized processing of numerical information has some benefits over bilateral processing, one might expect that these benefits are more strongly reflected in enhanced discrimination abilities in the small number range (1 vs. 2 and 1 vs. 3) than in the large number range (5 vs. 10 and 5 vs. 15).

METHODS

Experimental subjects

We collected 400 sub-adult threespine sticklebacks from a pond close to the Institute for Evolutionary Biology and Ecology at the University of Bonn. All fish were caught 3 weeks before the start of the experiments during their non-reproductive season (late winter) using minnow traps and kept in a large outdoor tank (750 litres, flow rate 3 $\text{L} \cdot \text{min}^{-1}$). Shortly before the start of the experiments, we isolated 150 haphazardly collected individuals in six separate holding tanks (50 cm long × 30 cm wide × 30 cm high) and assigned them to be test fish. Each tank accommodated 25 individuals under standardized laboratory conditions (8 hour light/16 hour dark cycle; room temperature 17 ± 1°C). The remaining 250 individuals in the outdoor tank were assigned to be shoal fish. Individuals that showed any signs of beginning breeding coloration were not used as test fish or shoal fish to ensure that reproductive state did not interfere with the social decisions to be made (e.g. Frommen *et al.*, 2012). During the experimental phase, all fish were fed daily in excess early in the morning with defrosted mosquito larvae (*Chironomus* spp.). This was done to avoid confounding effects caused by different hunger levels during the shoal choice experiments (e.g. Frommen *et al.*, 2007).

Experimental set-up

The experimental tank measured 80 cm × 40 cm × 40 cm and was filled to a height of 15 cm with tap water at least a day old. It was divided into three compartments using transparent acrylic glass partitions, i.e. one test fish compartment (48 cm long × 40 cm wide) in the middle and two smaller compartments on each side that contained the stimulus shoals (16 cm × 40 cm each). In front of each stimulus compartment a black line was drawn on the bottom of the tank to indicate preference zones measuring 12 cm × 40 cm each. A fluorescent tube (36 W) was fixed directly above the aquarium to ensure equal illumination. To minimize disturbance from outside, a black curtain was fastened around the whole experimental set-up. Between trials, the water was replaced completely to eliminate olfactory cues from previous trials (e.g. Mehlis *et al.*, 2008).

Experimental procedure and data acquisition

Over a period of 10 days, we performed 120 experimental trials using the following four numerical contrasts: 1 vs. 2 ($n = 30$), 1 vs. 3 ($n = 30$), 5 vs. 10 ($n = 30$), and 5 vs. 15 ($n = 30$). Each contrast was tested for each eye condition resulting in the following sample sizes:

both eyes uncovered (BE, $n = 40$), left eye uncovered (LE, $n = 40$) and right eye uncovered (RE, $n = 40$) (for details of the eye-covering procedure, see below). Each combination was replicated ten times in random order to avoid time effects. In addition, we alternated the side at which the large shoal was presented to control for potential side biases.

Immediately before the start of an experimental trial, we haphazardly collected one test fish from one of the six holding tanks and assigned it to one of the three different eye conditions (BE, LE or RE). Test fish were only used once to avoid pseudoreplication. For this purpose, we marked test individuals after the experimental trial by clipping the dorsal spines, which does not lead to any long-lasting impairment (Wedekind and Little, 2004), before returning them to their respective holding tank. Shoal fish originating from the large outdoor tank were returned there in the evening after the day's final trial. They were used more than once during the experimental phase, but never on the same day or in the same combination. Shoals were composed of individuals of similar size. Matching was done by visual inspection. The two shoals were released into the lateral compartments of the experimental tank. Afterwards, we introduced the test fish into a cylinder positioned centrally in the middle of the test fish compartment.

After a period of acclimation of 2 minutes, the cylinder was carefully lifted from behind the curtain using a pulley system. As soon as the test fish crossed the middle line for the first time, it was observed for 10 minutes. Each experimental trial was recorded using a webcam (Creativ WebCam Live!), which was connected to a laptop behind the curtain. We determined the proportion of time the test fish spent in front of each of the two stimulus shoals (preference zone). All videos were viewed blindly with respect to the numerical contrast and the location of the larger shoal (left or right).

Eye covering

The eye covering of test fish was performed quickly (about 30 seconds) out of water using the following steps. First, we carefully dried the area of skin directly surrounding the treated eye with a small piece of paper towelling. Next, we used a small brush to cover the eye and surrounding area with a layer of antiseptic sealing powder (ConvaTec Orahesive Protective Powder), which formed an impermeable barrier and simultaneously bound the next layer. This layer consisted of a mixture of petroleum jelly and magnetite powder (Fe_3O_4), which was topped by a further layer of antiseptic powder for fixation (for details, see Käfer, 2014). The mixture opaquely covered the treated eye for at least 20 minutes in underwater conditions. It could be peeled off in one without leaving a sticky residue on the skin or eye of the treated fish after a maximum of 2 hours. Treated fish did not display any mortality or signs of abnormal behaviour, injury or disease either during or after this treatment. To avoid handling artifacts, both eyes of binocular test fish and the respective uncovered eye of monocular test fish were sham-treated equally as described above but without the application of any substances to their skin and eyes.

Statistical analyses

Statistical analyses were performed using R v.3.0.2 (R Development Core Team, 2013). We excluded four trials [all right eye uncovered (RE): 5 vs. 10 ($n = 2$) and 5 vs. 15 ($n = 2$)] from the final analyses because these test fish visited just one preference zone, resulting in a sample size of $N = 116$.

To determine whether discrimination ability differed from chance (0.5), we used one-sample *t*-tests. In addition, to examine the influence of eye occlusion on quantity discrimination abilities, different linear models (lm) were fitted. In each model, we used the proportion of time the test fish spent in front of the larger shoal relative to that spent in front of both shoals as the dependent variable. In all fitted models (for an overview, see evolutionary-ecology.com/data/3110Appendix.pdf, Table S1), we used either type of vision (binocular or monocular) or eye availability [BE, LE, and RE, followed by *post-hoc* Tukey tests – using the R package lsmeans (see Lenth, 2016)] as the explanatory variable. There was a significant side bias, showing that test fish generally preferred to shoal with individuals located on the left side of the experimental tank ($N = 116$, lm: $\chi^2 = 6.688$, $df = 1$, $P = 0.011$). Although the side at which the larger shoal was presented was alternated between trials, we included the side at which the large shoal was presented as a covariate in all models and never removed it so as to control for the side effect.

Furthermore, we combined our data points both based on ratio (low: 1 vs. 2, 5 vs. 10 and high: 1 vs. 3, 5 vs. 15) and the absolute number of shoaling fish (low: 1 vs. 2, 1 vs. 3 and high: 5 vs. 10, 5 vs. 15) to test the effects of lateralization on quantity discrimination ability across the respective representation systems (OFS, ANS). To elucidate the effect of ratio in relation to type of vision or eye availability on quantity discrimination abilities, we included an interaction term (see models 11 and 12, Table S1). Accordingly, to examine the effect of the absolute number of shoaling fish in relation to type of vision or eye availability on quantity discrimination abilities, we also included an interaction term (see models 18 and 19, Table S1).

In all models, explanatory variables were stepwise-removed in the order of statistical relevance. The residuals of the best explanatory models as well as raw data did not deviate significantly from a normal distribution (according to Shapiro-Wilk tests). All *P*-values were based on two-tailed tests, and the level of significance was set at 0.05.

RESULTS

Effects of eye occlusion on quantity discrimination across and within numerical contrasts

Test fish generally preferred the larger group (one sample *t*-test: $t = 8.038$, $df = 115$, $P < 0.001$, Fig. 1). This was also true for each of the three different eye conditions separately (all $P < 0.001$, Fig. 1). In addition, while there was no significant difference in performance between binocular and monocular fish (lm: $\chi^2 = 1.812$, $df = 1$, $P = 0.181$), there was a trend for eye availability (BE, LE, and RE) to affect quantity discrimination abilities (lm: $\chi^2 = 2.821$, $df = 2$, $P = 0.064$; Fig. 1); and while LE-fish tended to perform better than BE-fish (lm *post-hoc* Tukey: $t = -2.138$, $P = 0.087$; Fig. 1), the other two pair-wise comparisons (BE vs. RE and LE vs. RE) failed to reach statistical significance (both $P > 0.130$; Fig. 1). For detailed test statistics, see Table S1.

We then considered the four numerical contrasts separately and found that in the 5 vs. 10 contrast, fish with monocular vision (LE and RE) significantly preferred to join the larger group compared with fish with binocular vision (lm: $\chi^2 = 4.341$, $df = 1$, $P = 0.048$). More specifically, we showed that in this contrast eye availability had a significant effect on quantity discrimination (lm: $\chi^2 = 3.590$, $df = 2$, $P = 0.043$; Fig. 1). LE-fish performed significantly better than fish with binocular vision (BE) (*post-hoc* Tukey: $t = -2.649$,

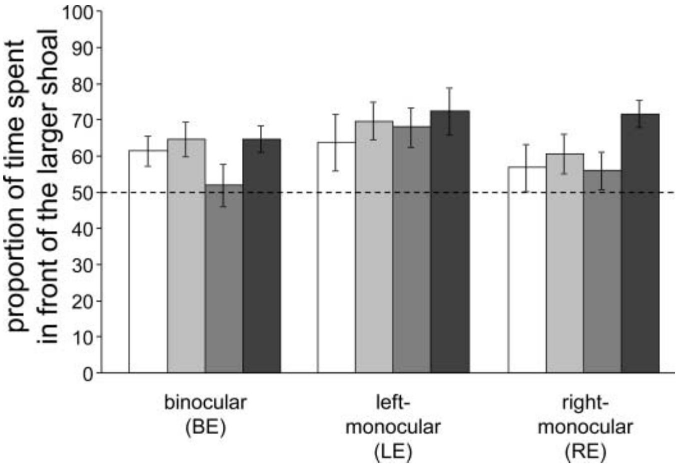

Fig. 1. Proportion of time test fish [left-monocular (LE), right-monocular (RE) or binocular (BE)] spent in front of the larger shoal when presented with the four different numerical contrasts [1 vs. 2 (white), 1 vs. 3 (light grey), 5 vs. 10 (medium grey), 5 vs. 15 (dark grey)]. The horizontal dashed line indicates chance level. Plotted are means and standard errors.

$P = 0.036$; Fig. 1), while the remaining pair-wise comparisons were not significant (both $P > 0.264$; see Fig. 1 and Table S1). We found no significant difference in quantity discrimination ability between binocular and monocular vision when testing 1 vs. 2, 1 vs. 3, and 5 vs. 15 (all $P > 0.237$; see Fig. 1 and Table S1). Furthermore, when looking at the three eye conditions separately, we found no significant influence of eye availability on the ability to discriminate between small and large groups in these numerical contrasts (all $P > 0.424$; see Fig. 1 and Table S1).

Effects of eye occlusion and ratio on quantity discrimination

We found no significant interaction effect between ratio (low: 1 vs. 2, 5 vs. 10 and high: 1 vs. 3, 5 vs. 15) and type of vision (binocular or monocular) on quantity discrimination ability (lm: $\chi^2 = 0.137$, $df = 1$, $P = 0.712$). When we examined the three eye conditions separately, there was also no significant interaction effect between ratio and eye availability (BE-, LE-, and RE-fish) (lm: $\chi^2 = 0.227$, $df = 2$, $P = 0.797$; Fig. 2). However, quantity discrimination was significantly improved when fish were confronted with a high rather than a low ratio (lm: $\chi^2 = 6.156$, $df = 1$, $P = 0.015$; Fig. 2). In more detail, fish with binocular vision performed significantly better when given the choice between shoal combinations of a higher ratio versus a lower ratio (lm: $\chi^2 = 7.979$, $df = 1$, $P = 0.008$; Fig. 2). Among fish with monocular vision, there was a tendency for the ability to discriminate between low and high ratios to be different (lm: $\chi^2 = 2.818$, $df = 1$, $P = 0.097$). In addition, when analysing LE-fish and RE-fish separately, both groups did not differ in their ability to discriminate between low and high ratios (both $P > 0.113$; see Fig. 2 and Table S1).

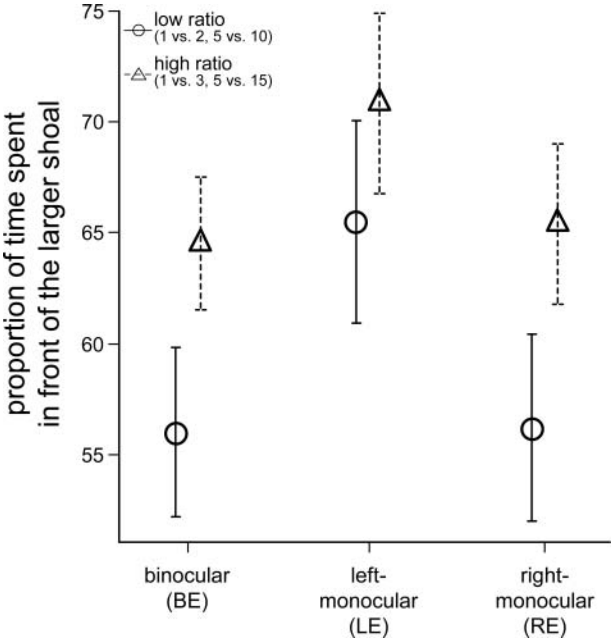

Fig. 2. Proportion of time test fish [left-monocular (LE), right-monocular (RE) or binocular (BE)] spent in front of the larger shoal when presented with a low numerical ratio of 1 : 2 (○: 1 vs. 2, 5 vs. 10) versus a high numerical ratio of 1 : 3 (△: 1 vs. 3, 5 vs. 15). Plotted are means and standard errors.

Effects of eye occlusion and absolute number of shoal members on quantity discrimination

There was no significant interaction effect of the absolute number of shoal members (low: 1 vs. 2, 1 vs. 3 and high: 5 vs. 10, 5 vs. 15) and type of vision (binocular or monocular) on quantity discrimination (lm: $\chi^2 = 2.001$, $df = 1$, $P = 0.160$). When considering the three eye conditions separately, the interaction effects between the absolute number of shoal members and BE-, LE-, and RE-fish were not significant (lm: $\chi^2 = 0.974$, $df = 2$, $P = 0.381$; Fig. 3). Moreover, for both fish with binocular and with monocular vision, there was no significant difference in performance when given the choice between contrasts including a low versus a high absolute number of individuals (all $P > 0.196$; Table S1). Separate analyses for LE-fish and RE-fish revealed that fish from both groups did not display a significant difference in discrimination between quantities regardless of whether the absolute number of individuals was low or high (both $P > 0.387$; see Fig. 3 and Table S1).

DISCUSSION

In the present study, a general preference for the larger group was observed in binocular as well as monocular fish when pooling the data for the four numerical contrasts. Choosing a larger shoal is a well-known social response of fish because the ecological benefits of group living, such as improved protection against predators (e.g. Magurran, 1990) and enhanced foraging efficiency (Clark and Mangel, 1986), are expected to increase with increasing group size

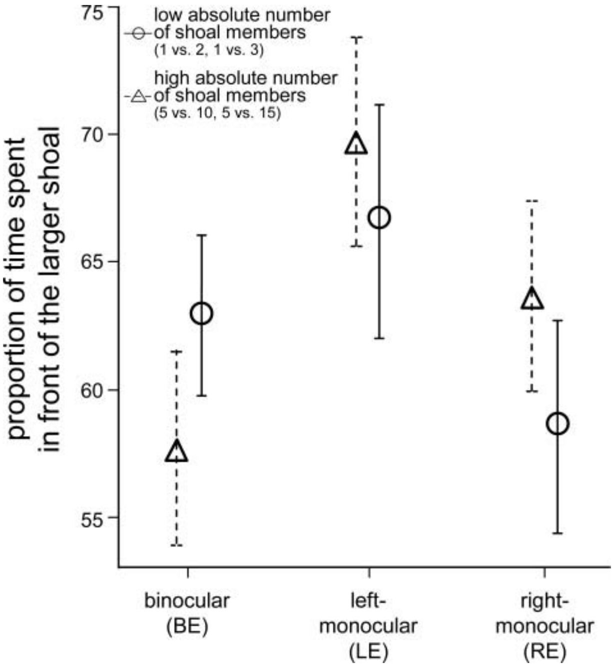

Fig. 3. Proportion of time test fish [left-monocular (LE), right-monocular (RE) or binocular (BE)] spent in front of the larger shoal when presented with a low absolute number of individuals (○: 1 vs. 2, 1 vs. 3) versus a high absolute number of individuals (△: 5 vs. 10, 5 vs. 15). Plotted are means and standard errors.

(Krause and Ruxton, 2002). Thus, in previous studies on numerical abilities in threespine sticklebacks, it has been shown that individuals show a preference for the larger group when presented with two shoals at different numerical contrasts and absolute group sizes, such as 3 vs. 5 (Krause, 1993), 3 vs. 6 (Fischer and Frommen, 2013), 5 vs. 9 and 5 vs. 10 (Krause *et al.*, 1998), 8 vs. 12 (Frommen *et al.*, 2009), or even for larger groups of 15 vs. 60 as well as 20 vs. 60 (Thünken *et al.*, 2014), as well as when only being slightly different [e.g. 6 vs. 7 (Mehlis *et al.*, 2015)].

Furthermore, in accordance with other fish studies (Gómez-Laplaza, 2012) and previous studies of threespine sticklebacks, our findings reveal that quantity discrimination appears to be more difficult when the ratio between shoals is lower. Likewise, fish with binocular vision in the present study performed considerably better at the higher ratio than at the lower ratio. Moreover, performance of fish with monocular vision was not significantly different between the higher and lower ratio. This result appears to be strongly influenced by the improved quantity discrimination of left-monocular fish, especially for the 5 vs. 10 contrast, which we discuss below.

Our results provide experimental evidence that eye availability in sticklebacks is associated with certain cognitive visual processes, i.e. when discriminating between conspecific groups of certain sizes. We found a trend that eye availability influenced test fish performance independent of numerical contrast, implying an overall improved quantity discrimination in left-monocular fish. When considering the specific numerical contrasts, test fish that had one eye covered (monocular) performed similar to (RE-fish) or better than

(LE-fish) binocular fish in the 5 vs. 10 contrast in the quantity discrimination task. Moreover, there were no significant differences in quantity discrimination between monocular and binocular fish in the other three numerical contrasts (1 vs. 2, 1 vs. 3, and 5 vs. 15). However, our findings illustrate that to some extent lateralized processing of visual information might affect quantity discrimination in sticklebacks. This result is in line with the hypothesis that the involvement of binocular vision and the collaboration of both hemispheres might even be disadvantageous in some circumstances. Thus, by avoiding conflicting responses from the two hemispheres, lateralized information processing might be more effective in certain situations, for example, when comparing stimuli differing in quality (Andrew *et al.*, 1982).

In a recent study by Dadda *et al.* (2015), guppies that showed strong lateralization in a mirror test had improved numerical discrimination abilities in a shoal choice and numerical discrimination test compared with non-lateralized individuals. This finding also supports the view that an enhanced cerebral lateralization may be associated with better cognitive efficiency (but see Dadda *et al.*, 2009 for conflicting results). Dadda *et al.* (2015) found that lateralized fish performed better than non-lateralized fish when the set sizes were smaller (e.g. 3 vs. 4) – that is, in the typical range of the object file system (OFS) – whereas larger numbers were not tested in that study. In comparison, the better performance of monocular fish in the 5 vs. 10 contrast in our study demonstrates that cerebral lateralization may also be involved in the processing of larger numbers lying in the range of the approximate number system (ANS), although it should be noted that no significant difference in performance between monocular and binocular fish was observed for the 5 vs. 15 contrast (see below for discussion). Taken together, the results of these studies rather point towards a single ANS system for numerical processing of both small and large numbers. This is in line with our finding that the absolute number of shoal members had no significant influence on quantity discrimination of the test fish, independently of whether type of vision or eye availability was considered or not. Unfortunately, the evidence on the exact mechanism of quantity discrimination in non-human animals remains mixed (e.g. Piffer *et al.*, 2012; Stancher *et al.*, 2013), making it difficult to draw firm conclusions without further testing.

Fish with binocular vision in the present study performed poorly in the 5 vs. 10 contrast, although some studies on sticklebacks and other fish species have revealed that individuals are able to distinguish between groups of 5 vs. 10 individuals in a shoaling context (e.g. Krause *et al.*, 1998). Differences in experimental design between studies may have led to these contradictory results. For example, the experimental set-up and procedure as well as the measure of group choice used in the study of Krause *et al.* (1998) differed markedly from the approach used in the present study. Furthermore, Gómez-Laplaza and Gerlai (2012) demonstrated that juvenile angelfish (*Pterophyllum scalare*) could not discriminate between 5 vs. 10 individuals when controlling for differences in stimulus fish activity between groups, indicating that the exact mechanisms underlying quantity discrimination in shoal choice assays are often not clear. Consequently, it cannot completely be ruled out that, especially for the 5 vs. 10 contrast in the present study, discrimination between groups was, to some extent, influenced by other variables, such as activity or density (see Frommen *et al.*, 2009; Gómez-Laplaza and Gerlai, 2013). Not controlling for potentially confounding continuous variables might have led to limited discrimination abilities in binocular fish (e.g. due to inter-hemispheric conflict), while lateralized processing in monocular individuals (in LE-fish at least) might have responded less to such non-numerical features leading to improved quantity discrimination. In addition, although we anticipated that all four numerical contrasts could be successfully discriminated (see Mehlis *et al.*, 2015 and references therein), lateralization effects were only found for the 5 vs. 10 contrast, which might be considered the cognitively most difficult discrimination task in our study. Nevertheless, to gain better insight into the association between lateralized processing of information and quantity discrimination abilities, future research should incorporate other numerical contrasts, for example ones that are closer to the acuity threshold in sticklebacks (see also Mehlis *et al.*, 2015).

Although the experiments performed by Dadda *et al.* (2015) addressed within-population variation in lateralization and not lateralization at the population level as in our study on monocular viewing conditions, there are similarities with regard to the direction of lateralization. For instance, when we further subdivided the data by eye condition (left-monocular, right-monocular, binocular), we found no significant difference in quantity discrimination between left-monocular and right-monocular fish for the 5 vs. 10 contrast or any of the other contrasts. Furthermore, in the study of Dadda *et al.* (2015), left- and right-lateralized fish did not differ significantly in their numerical performance, so that both studies suggest that the strength rather than the direction of lateralization is associated with numerical abilities.

Our results, however, show that left-monocular fish performed significantly better at quantity discrimination than fish with both eyes uncovered, whereas right-monocular fish did not. This finding could point to a specific role of the left hemi-visual field and associated right hemisphere in the visual processing of quantities. There is some evidence that the left eye–right brain system is used predominantly for social recognition in gregarious vertebrate species (e.g. Bisazza *et al.*, 2002; Sovrano and Andrew, 2006; but see Sovrano *et al.*, 1999, 2001; Bisazza and Brown, 2011). For example, in a study on the Amur sleeper (*Perccottus glenii*), individual fish showed a population-level preference to use their left eye when inspecting social stimuli such as their own mirror image, a group of conspecifics or a conspecific model (Karenina *et al.*, 2013b). Evidence for a left eye–right hemisphere preference in the processing of numerical information has been reported in humans where non-verbal numerical skills are based on a right-hemispheric dominance (e.g. Boles, 1986; Pasini and Tessari, 2001). Moreover, lesion and behavioural studies on domestic chicks point to increased activation of the right hemisphere at least if visual stimuli include coherent spatial and numerical information (for a review, see Rugani *et al.*, 2015; see also Vallortigara *et al.*, 2010). In contrast, monocular testing of a binocularly trained bottlenose dolphin to discriminate between two quantities yielded the opposite result – better performance of the right eye–left hemisphere system (Kilian *et al.*, 2005). Taken together, although the improved numerical performance in left-monocular sticklebacks versus binocular fish in the present study indicates right hemispheric dominance, the result is weakened by the absence of a significant difference between left-monocular and right-monocular fish.

It is worth noting that, in general, the effects observed in the present study were relatively moderate, potentially because our analysis was based on a small sample size (ten individuals per numerical contrast and eye condition), and maybe more importantly, due to the fact that we had no knowledge of the inter-individual variation in lateralization of our test fish. Thus, additional studies are needed that, for example, incorporate a larger sample size together with detailed knowledge of the individual and population-level strength and direction of lateralization.

In conclusion, we found evidence linking quantity discrimination to lateralization in stickleback fishes, i.e. experimentally manipulated monocular individuals showed improved abilities compared with binocular individuals when discriminating between two differently sized groups of conspecifics. Although lateralization effects on quantity discrimination were limited to one of the four numerical contrasts tested and additional studies are needed for more detailed investigation, our results suggest that unilateral processing may be more efficient than bilateral processing at least in specific numerical tasks, possibly by preventing a conflict between the left and right hemispheres, thereby increasing cognitive efficiency (Andrew *et al.*, 1982). We also provide some evidence for a left eye–right hemisphere advantage in quantity discrimination, with the right hemisphere dominant in tasks that require numerical processing (see Rugani *et al.*, 2015). Our findings show that lateralization can be linked to certain cognitive functions such as enhanced numerical abilities, which are of adaptive significance in ecologically relevant contexts like shoaling behaviour, foraging, and predator avoidance.

ACKNOWLEDGEMENTS

This article is dedicated to Theo C.M. Bakker on the occasion of his retirement. Marion Mehlis-Rick, Timo Thünken, and Ingolf P. Rick would like to express their gratitude for his long-term support. In addition, we thank the members of the former research group of Professor Bakker for discussion and advice, especially Meike Hiermes, Joachim G. Frommen, Ricarda Modarressie, and Sebastian A. Baldauf. Finally, we would like to thank Stéfanie Goth and Jan Vieth for their help in performing the experiments. Markus Krings was supported by a Cusanus fellowship. Timo Thünken was supported by a DFG grant (TH 1615/3-1).

REFERENCES

Agrillo, C. and Bisazza, A. 2017. The contribution of fish studies to the ‘number sense’ debate. *Behav. Brain Sci.*, **40**: 16–17.

Agrillo, C., Dadda, M. and Serena, G. 2008. Choice of female groups by male mosquitofish (*Gambusia holbrooki*). *Ethology*, **114**: 479–488.

Andrew, R.J., Mench, J. and Rainey, C. 1982. Right–left asymmetry of response to visual stimuli in the domestic chick. In *Analysis of Visual Behaviour* (D.J. Ingle, M.A. Goodale and R.J.W. Mansfield, eds.), pp. 197–209. Cambridge, MA: MIT Press.

Bisazza, A. and Brown, C. 2011. Lateralization of cognitive functions in fish. In *Fish Cognition and Behaviour* (C. Brown, K. Laland and J. Krause, eds.), pp. 298–324. Chichester: Wiley-Blackwell.

Bisazza, A. and Dadda, M. 2005. Enhanced schooling performance in lateralized fishes. *Proc. R. Soc. Lond. B: Biol. Sci.*, **272**: 1677–1681.

Bisazza, A., Pignatti, R. and Vallortigara, G. 1997. Laterality in detour behaviour: interspecific variation in poeciliid fish. *Anim. Behav.*, **54**: 1273–1281.

Bisazza, A., De Santi, A., Bonso, S. and Sovrano, V.A. 2002. Frogs and toads in front of a mirror: lateralisation of response to social stimuli in tadpoles of five anuran species. *Behav. Brain Res.*, **134**: 417–424.

Boles, D.B. 1986. Hemispheric differences in the judgment of number. *Neuropsychologia*, **24**: 511–519.

Brown, C. and Braithwaite, V.A. 2005. Effects of predation pressure on the cognitive ability of the poeciliid *Brachyraphis episcopi*. *Behav. Ecol.*, **16**: 482–487.

Brown, C. and Magat, M. 2011. The evolution of lateralized foot use in parrots: a phylogenetic approach. *Behav. Ecol.*, **22**: 1201–1208.

Brown, C., Western, J. and Braithwaite, V.A. 2007. The influence of early experience on, and inheritance of, cerebral lateralization. *Anim. Behav.*, **74**: 231–238.

Clark, C.W. and Mangel, M. 1986. The evolutionary advantages of group foraging. *Theor. Popul. Biol.*, **30**: 45–75.

Cordes, S., Gelman, R., Gallistel, C.R. and Whalen, J. 2001. Variability signatures distinguish verbal from nonverbal counting for both large and small numbers. *Psychon. Bull. Rev.*, **8**: 698–707.

Dadda, M. and Bisazza, A. 2006a. Does brain asymmetry allow efficient performance of simultaneous tasks? *Anim. Behav.*, **72**: 523–529.

Dadda, M. and Bisazza, A. 2006b. Lateralized female topminnows can forage and attend to a harassing male simultaneously. *Behav. Ecol.*, **17**: 358–363.

Dadda, M., Zandona, E., Agrillo, C. and Bisazza, A. 2009. The costs of hemispheric specialization in a fish. *Proc. R. Soc. Lond. B: Biol. Sci.*, **276**: 4399–4407.

Dadda, M., Agrillo, C., Bisazza, A. and Brown, C. 2015. Laterality enhances numerical skills in the guppy, *Poecilia reticulata*. *Front. Behav. Neurosci.*, **9**: 285.

Davis, H. and Bradford, S.A. 1986. Counting behavior by rats in a simulated natural environment. *Ethology*, **73**: 265–280.

Feigenson, L. and Carey, S. 2005. On the limits of infants' quantification of small object arrays. *Cognition*, **97**: 295–313.

Fischer, S. and Frommen, J.G. 2013. Eutrophication alters social preferences in three-spined sticklebacks (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.*, **67**: 293–299.

Frommen, J.G., Luz, C. and Bakker, T.C.M. 2007. Nutritional state influences shoaling preference for familiars. *Zoology*, **110**: 369–376.

Frommen, J.G., Hiermes, M. and Bakker, T.C.M. 2009. Disentangling the effects of group size and density on shoaling decisions of three-spined sticklebacks (*Gasterosteus aculeatus*). *Behav. Ecol. Sociobiol.*, **63**: 1141–1148.

Frommen, J.G., Bakker, T.C.M., Proscurcin, L.C. and Mehlis, M. 2012. Gravidity-associated shoaling decisions in three-spined sticklebacks (*Gasterosteus aculeatus*). *Ethology*, **118**: 1149–1156.

Gallistel, C.R. and Gelman, R. 1992. Preverbal and verbal counting and computation. *Cognition*, **44**: 43–74.

Ghirlanda, S. and Vallortigara, G. 2004. The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proc. R. Soc. Lond. B: Biol. Sci.*, **271**: 853–857.

Gómez-Laplaza, L.M. 2012. Shoal size and shoaling in fish. *Ind. J. Soc. Nat. Sci.*, **1**: 7–18.

Gómez-Laplaza, L.M. and Gerlai, R. 2012. Activity counts: the effect of swimming activity on quantity discrimination in fish. *Front. Psychol.*, **3**: 484.

Gómez-Laplaza, L.M. and Gerlai, R. 2013. Quantification abilities in angelfish (*Pterophyllum scalare*): the influence of continuous variables. *Anim. Cogn.*, **16**: 373–383.

Hager, M.C. and Helfman, G.S. 1991. Safety in numbers – shoal size choice by minnows under predatory threat. *Behav. Ecol. Sociobiol.*, **29**: 271–276.

Hauser, M.D., Carey, S. and Hauser, L.B. 2000. Spontaneous number representation in semi-free-ranging rhesus monkeys. *Proc. R. Soc. Lond. B: Biol. Sci.*, **267**: 829–833.

Käfer, S. 2014. Influence of brain lateralization on shoaling behavior, female mate choice and aggression in the threespine stickleback (*Gasterosteus aculeatus*). Master's thesis, University of Bonn.

Karenina, K., Giljov, A., Ivkovich, T., Burdin, A. and Malashichev, Y. 2013a. Lateralization of spatial relationships between wild mother and infant orcas, *Orcinus orca*. *Anim. Behav.*, **86**: 1225–1231.

Karenina, K.A., Giljov, A.N. and Malashichev, Y.B. 2013b. Eye as a key element of conspecific image eliciting lateralized response in fish. *Anim. Cogn.*, **16**: 287–300.

Kilian, A., von Fersen, L. and Gunturkun, O. 2005. Left hemispheric advantage for numerical abilities in the bottlenose dolphin. *Behav. Process.*, **68**: 179–184.

Krause, J. 1993. The influence of hunger on shoal size choice by three-spined sticklebacks, *Gasterosteus aculeatus*. *J. Fish Biol.*, **43**: 775–780.

Krause, J. and Ruxton, G.D. 2002. *Living in Groups*. Oxford: Oxford University Press.

Krause, J., Godin, J.G.J. and Rubenstein, D. 1998. Group choice as a function of group size differences and assessment time in fish: the influence of species vulnerability to predation. *Ethology*, **104**: 68–74.

Landeau, L. and Terborgh, J. 1986. Oddity and the 'confusion effect' in predation. *Anim. Behav.*, **34**: 1372–1380.

Le Corre, M. and Carey, S. 2007. One, two, three, four, nothing more: an investigation of the conceptual sources of the verbal counting principles. *Cognition*, **105**: 395–438.

Lenth, R.V. 2016. Least-squares means: the R package lsmeans. *J. Stat. Softw.*, **69**: 1–33.

Lippolis, G., Bisazza, A., Rogers, L.J. and Vallortigara, G. 2002. Lateralisation of predator avoidance responses in three species of toads. *Laterality*, **7**: 163–183.

Lucon-Xiccato, T., Miletto Petrazzini, M.E., Agrillo, C. and Bisazza, A. 2015. Guppies discriminate between two quantities of food items but prioritize item size over total amount. *Anim. Behav.*, **107**: 183–191.

Lucon-Xiccato, T., Dadda, M. and Bisazza, A. 2016. Sex differences in discrimination of shoal size in the guppy (*Poecilia reticulata*). *Ethology*, **122**: 481–491.

Magurran, A.E. 1990. The adaptive significance of schooling as an antipredator defense in fish. *Ann. Zool. Fenn.*, **27**: 51–66.

McComb, K., Packer, C. and Pusey, A. 1994. Roaring and numerical assessment in contests between groups of female lions, *Panthera leo*. *Anim. Behav.*, **47**: 379–387.

Mehlis, M., Bakker, T.C.M. and Frommen, J.G. 2008. Smells like sib spirit: kin recognition in three-spined sticklebacks (*Gasterosteus aculeatus*) is mediated by olfactory cues. *Anim. Cogn.*, **11**: 643–650.

Mehlis, M., Thünken, T., Bakker, T.C.M. and Frommen, J.G. 2015. Quantification acuity in spontaneous shoaling decisions of three-spined sticklebacks. *Anim. Cogn.*, **18**: 1125–1131.

Pasini, M. and Tessari, A. 2001. Hemispheric specialization in quantification processes. *Psychol. Res.*, **65**: 57–63.

Piffer, L., Agrillo, C. and Hyde, D.C. 2012. Small and large number discrimination in guppies. *Anim. Cogn.*, **15**: 215–221.

R Development Core Team. 2013. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing [http://www.R-project.org/].

Roberts, G. 1996. Why individual vigilance declines as group size increases. *Anim. Behav.*, **51**: 1077–1086.

Roberts, W.A. and Mitchell, S. 1994. Can a pigeon simultaneously process temporal and numerical information. *J. Exp. Psychol. Anim. Behav. Process.*, **20**: 66–78.

Rogers, L.J. and Andrew, R.J. 2002. *Comparative Vertebrate Lateralization*. Cambridge: Cambridge University Press.

Rogers, L.J. and Vallortigara, G. 2008. From antenna to antenna: lateral shift of olfactory memory recall by honeybees. *PLoS One*, **3**: e2340.

Rogers, L.J., Zucca, P. and Vallortigara, G. 2004. Advantages of having a lateralized brain. *Proc. R. Soc. Lond. B: Biol. Sci.*, **271**: S420–S422.

Rogers, L.J., Vallortigara, G. and Andrew, R.J. 2013. *Divided Brains: The Biology and Behaviour of Brain Asymmetries*. Cambridge: Cambridge University Press.

Ross-Sheehy, S., Oakes, L.M. and Luck, S.J. 2003. The development of visual short-term memory capacity in infants. *Child Dev.*, **74**: 1807–1822.

Rugani, R., Vallortigara, G. and Regolin, L. 2015. At the root of the left–right asymmetries in spatial-numerical processing: from domestic chicks to human subjects. *J. Cogn. Psychol.*, **27**: 388–399.

Sovrano, V.A. and Andrew, R.J. 2006. Eye use during viewing a reflection: behavioural lateralisation in zebrafish larvae. *Behav. Brain Res.*, **167**: 226–231.

Sovrano, V.A., Rainoldi, C., Bisazza, A. and Vallortigara, G. 1999. Roots of brain specializations: preferential left-eye use during mirror-image inspection in six species of teleost fish. *Behav. Brain Res.*, **106**: 175–180.

Sovrano, V.A., Bisazza, A. and Vallortigara, G. 2001. Lateralization of response to social stimuli in fishes: a comparison between different methods and species. *Physiol. Behav.*, **74**: 237–244.

Sovrano, V.A., Dadda, M. and Bisazza, A. 2005. Lateralized fish perform better than nonlateralized fish in spatial reorientation tasks. *Behav. Brain Res.*, **163**: 122–127.

Stancher, G., Sovrano, V.A., Potrich, D. and Vallortigara, G. 2013. Discrimination of small quantities by fish (redtail splitfin, *Xenotoca eiseni*). *Anim. Cogn.*, **16**: 307–312.

Thünken, T., Eigster, M. and Frommen, J.G. 2014. Context-dependent group size preferences in large shoals of three-spined sticklebacks. *Anim. Behav.*, **90**: 205–210.

Trick, L.M. and Pylyshyn, Z.W. 1994. Why are small and large numbers enumerated differently? A limited-capacity preattentive stage in vision. *Psychol. Rev.*, **101**: 80–102.

Uller, C., Jaeger, R., Guidry, G. and Martin, C. 2003. Salamanders (*Plethodon cinereus*) go for more: rudiments of number in an amphibian. *Anim. Cogn.*, **6**: 105–112.

Vallortigara, G. and Rogers, L.J. 2005. Survival with an asymmetrical brain: advantages and disadvantages of cerebral lateralization. *Behav. Brain Sci.*, **28**: 575–633.

Vallortigara, G., Regolin, L., Chiandetti, C. and Rugani, R. 2010. Rudiments of mind: insights through the chick model on number and space cognition in animals. *Comp. Cogn. Behav. Rev.*, **5**: 78–99.

Ventolini, N., Ferrero, E.A., Sponza, S., Chiesa, A.D., Zucca, P. and Vallortigara, G. 2005. Laterality in the wild: preferential hemifield use during predatory and sexual behaviour in the black-winged stilt. *Anim. Behav.*, **69**: 1077–1084.

Wedekind, C. and Little, T.J. 2004. The clearance of hidden cestode infection triggered by an independent activation of host defense in a teleost fish. *J. Parasitol.*, **90**: 1329–1331.

Wiper, M.L. 2017. Evolutionary and mechanistic drivers of laterality: a review and new synthesis. *Laterality*, **22**: 740–770.

Wootton, R.J. 1984. *A Functional Biology of Sticklebacks*. London: Croom Helm.

Xu, F., Spelke, E.S. and Goddard, S. 2005. Number sense in human infants. *Dev. Sci.*, **8**: 88–101.