Behavioural lateralisation in a detour test is not repeatable in fishes

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Abstract

Behavioural lateralisation, the asymmetric expression of cognitive functions, is reported to enhance key fitness-relevant traits such as group coordination, multitasking and predator escape. Therefore, studies reporting negative effects on lateralisation in fish due to environmental stressors such as ocean acidification, hypoxia, and pollutants are worrisome. However, such studies tend to use a detour test and focus on population-level measures, without validating whether lateralisation is consistent within individuals across time. We conducted a multi-species, international assessment of the repeatability (R) of lateralisation in four previously studied fish species using a detour test (T-maze), a common method for testing lateralisation. We also re-analysed a published dataset on a fifth species using new statistical methods. We expected the three shoaling species to exhibit greater within-individual consistency in lateralisation than their non-shoaling counterparts given previous reports of stronger lateralisation in group-living fishes. Absolute and relative lateralisation scores were highly non-repeatable in all five species ($0.01<R<0.08$), irrespective of their shoaling status. We carefully reviewed 31 published studies in which the detour test was employed to examine lateralisation in fish and identified statistical issues in all of them. We develop and propose new statistical analyses to test for population- and individual-level lateralisation. The commonly used detour test does not appear to be appropriate for quantifying behavioural lateralisation in fishes, calling into question functional inferences drawn by many published studies, including our own. Potential fitness benefits of lateralisation and anthropogenic effects on lateralisation as a proxy for adaptive brain functioning need to be assessed with alternative paradigms.

Keywords: Behavioural plasticity, laterality, lateralization, repeatability, T-maze

Introduction

Behavioural lateralisation, the asymmetric expression of cognitive functions, has emerged as an important fitness correlate in numerous taxa including invertebrates (e.g. Domenici et al., 2017) and vertebrates (reviewed in Vallortigara & Rogers, 2005). Indeed, cerebral lateralisation is believed to offer advantages in terms of enabling multiple stimuli to be processed simultaneously by different sides of the brain (Vallortigara & Rogers, 2005). Fitness benefits associated with high degrees of lateralisation are thought to include increased cognitive performance (Magat & Brown, 2009; Bibost & Brown, 2014), multitasking (Rogers et al., 2004; Dadda & Bisazza, 2006b), spatial learning (Sovrano et al., 2005), predator recognition learning (Ferrari et al., 2017), schooling performance (Bisazza & Dadda, 2005), coordination of group behaviours (Vallortigara & Rogers, 2005), prey capture success (Kurvers et al., 2017), foraging efficiency (Güntürkün et al., 2000), and escape performance (Dadda et al., 2010b; Lucon-Xiccato et al., 2016). Despite these apparent advantages, most studies report considerable inter- and intra-specific variation in the strength and direction of lateralisation. Several studies also report a high degree of within-individual variation in the strength of lateralisation in individuals measured repeatedly across different contexts, suggesting that this trait may be more labile than previously believed (e.g. Roche et al., 2013; Ferrari et al., 2017). Yet, no studies have systematically measured lateralisation several times per individual, across multiple species, without a change in context, to establish the baseline repeatability of this behaviour (but see (Irving & Brown, 2013) for a single-species study, the data for which are re-analyzed here). Such a validation of the repeatability of lateralisation assessed with a given test is crucial to establish the usefulness of a test for assessing this trait, particularly when differences in individual-level lateralisation strength between ecologically relevant treatments are used to infer effects on fitness.

Behavioural lateralisation in fishes is commonly measured in the laboratory using a detour test (T-maze), wherein the animal moves along a runway until it faces a barrier forcing it to make a choice between turning left or right (Fig. 1). This quick and simple test is now widely used in studies of fish behaviour, evolutionary ecology and ecotoxicology (see Supporting Information Table S1), and is also used to assess lateralisation in various other taxa including birds (Vallortigara et al., 1999), reptiles (Csermely et al., 2010), and molluscs (Domenici et al., 2017). In fishes, lateral bias in a detour test is believed to arise from asymmetries in eye-use, a phenomenon widely documented in animals with laterally-placed eyes and low binocular overlap (Bisazza et al., 1997a). For example, goldbelly topminnows (*Girardinus falcatus*) reported to exhibit a strong side-bias in a detour test also display an eye preference for viewing a neutral *versus* threatening stimulus (Facchin et al., 1999). Similarly, left-right asymmetries in *G. falcatus* assessed in a detour test appear to be consistent with lateral bias measured using other methods (Bisazza et al., 2001). Furthermore, lateralisation score in a detour test is reported to be heritable (Bisazza et al., 2000b; Brown et al., 2007), although lateralisation strength decreases rapidly across generations in artificial selection lines (Bisazza et al., 2007; Facchin et al., 2009). These results have led researchers to assert that the detour test does, indeed, assess inherent asymmetry in an individual’s brain function that influences fitness-relevant behavioural strategies.

Given likely fitness-relevant effects of lateralisation (e.g. Whiteside et al., 2018) and the relative ease of use of the detour test, various studies have evaluated how environmental and anthropogenic stressors such as pollutants and climate change affect lateralisation in fishes, several of which have produced worrying results (Table S1). Most notably, ocean acidification is reported to decrease lateralisation across a range of marine fishes, including tropical (Domenici et al., 2012; Nilsson et al., 2012; Welch et al., 2014) and temperate species

(Jutfelt et al., 2013; Lopes et al., 2016; Maulvault et al., 2018). Such effects are concerning given, for example, the important benefits of lateralisation reported for fishes under high predation risk (e.g. Ferrari et al., 2015; Chivers et al., 2016; Ferrari et al., 2017). Despite this strong interest in behavioural lateralisation and growing use of the detour test in the ecological, cognitive and behavioural sciences, there is a notable absence of studies assessing the repeatability of individual lateralisation scores. Establishing the consistency of lateralisation within individuals is essential for determining the relevance of this trait for evaluating fitness as well as the responses of animals to exogenous stressors.

To evaluate the repeatability of behavioural lateralisation in fishes using a detour test, we studied four species from tropical and temperate environments, including marine and freshwater habitats: two coral reef damselfishes, the ambon damsel (*Pomacentrus amboinensis*) and yellowtail demoiselle (*Neopomacentrus azysron*), the tropical freshwater zebrafish (*Danio rerio*), and the temperate marine goldsinny wrasse (*Ctenolabrus rupestris*) (Fig. 2). All of these species have previously been used in published studies assessing behavioural lateralisation using a detour test (T-maze) similar to the ones used here (Domenici et al., 2012; Nilsson et al., 2012; Sundin & Jutfelt, 2016; Vossen et al., 2016; Ferrari et al., 2017). We also used new (more appropriate) statistical methods to re-analyse a published dataset that included repeated lateralisation measurements for the tropical freshwater guppy (*Poecilia reticulata*) (Irving & Brown, 2013). We predicted a significant repeatability in lateralisation scores comparable in magnitude to that observed for other behavioural traits (Bell et al., 2009). Population-level lateralisation strength was also predicted to be higher in the shoaling species (*N. azysron* and *D. rerio*) compared to territorial species (*P. amboinensis* and *C. rupestris*) as this should help enhance school cohesion (Bisazza et al., 2000a). We had no *a priori* prediction about the direction (left or right) of lateralisation, as among-species differences in response to the same stimulus have been shown previously (Bisazza et al., 2000a). We also examined whether individuals became habituated to the test arena across the four trial series because (1) repeatedly measuring the same individuals in a similar apparatus can decrease measurement error and thus be predicted to increase individual repeatability (Martin & Réale, 2008), and conversely (2) habituation or desensitisation to a stimulus may reduce repeatability by generating different results between trials within an individual (Martin & Réale, 2008). Finally, we carefully reviewed the statistical methods in 31 published studies that examined lateralisation in fishes using the detour test. We develop and propose new statistical approaches to test for population- and individual-level lateralisation.

Materials and methods

Fish collection and husbandry

Sixty *C. rupestris* (1.38-46.52 g) were collected with baited minnow traps from the wharfs at the Sven Lovén Centre for Marine Infrastructure, Kristineberg, Sweden. Experiments on *C. rupestris* were conducted between 22 June and 1 July 2017 (water temperature range in the holding tanks and experimental setup: 14.9-16.1°C). Sixty *N. azysron* (0.74-6.40 g) and 60 *P. amboinensis* (1.0-14.3 g) were collected by SCUBA divers with a barrier net and hand nets on coral reefs nearby the Lizard Island Research Station, Northern Queensland, Australia. Experiments on these two species were conducted between 24 July and 11 August 2017 (water temperature range: 24.3-25.5°C). Sixty *D. rerio* (0.26-1.07 g) were collected in West Bengal, India, in August to September 2016 using hand nets and kept in the Norwegian University of Science and Technology’s (NTNU) aquarium facility, Trondheim, until the experiments were conducted between 10 and 17 October 2017 (water temperature range: 26.9-28.9°C; for details on collection, import and holding conditions, see Morgan *et al.* 2019).

Fish from each species were equally divided into three to four holding tanks (density ~5.5 individuals L^{-1}), provided with artificial plants and/or cut PVC pipes as shelter, and fed *ad libitum* twice daily. *C. rupestris* were fed blue mussels (*Mytilus edulis*) and bloodworms. *N. azysron* and *P. amboinensis* were fed commercial tropical fish flakes. *D. rerio* were fed TetraPro fish flakes. Holding tanks were supplied with flow-through water from the fish's natural habitat for *C. rupestris*, *N. azysron*, and *P. amboinensis*. *D. rerio* were kept in flow-through aquaria at NTNU, with a water exchange rate of ~20% per day, and water quality (conductivity and temperature) monitored daily. Light cycles followed natural conditions at each location. Fish were individually marked using two-colour combinations of Visible Implant Elastomer (Northwest Marine Technology Inc., Shaw Island, WA, USA) implanted postero-dorsally, on both sides of the dorsal fin a minimum of two days prior to the first test.

Lateralisation test

We used a standard detour test to assess behavioural lateralisation (Bisazza et al., 1998a; Dadda et al., 2010b; Jutfelt et al., 2013). Experiments were conducted in the morning between 9:00-12:30 and fish were returned to their respective holding tank between test days. A single fish was introduced into a double-sided, opaque T-maze, consisting of a tank with a runway down the middle and a barrier at both ends (Fig. 1). *C. rupestris* and *D. rerio* were tested in one maze and *N. azysron* and *P. amboinensis* in another (dimensions in Fig. 1). A symmetrical neutral stimulus was affixed to the centre of the barrier to focus the eye-gaze of the fish. This neutral stimulus was changed between each of the four trial series to prevent habituation to the set-up (a cross, two parallel black bars, a cross with a solid circle above it, a cross with a horizontal bar below it). The water height was 10 cm. Experiments were conducted in a closed room and care was taken to ensure that the surrounding environment and lighting were as uniform as possible. We emptied and re-filled the maze with new water between each fish tested to standardise the temperature, maintain normal levels of dissolved oxygen, and to avoid any effect of conspecific stress hormones (e.g. cortisol) on fish behaviour.

To start the experiment, a fish was placed at one end of the runway (maintained in this position by an acrylic divider blocking the entrance to the middle runway) for one minute (Fig. 1). The starting side was determined by flipping a coin and the fish was transferred from its holding tank to the maze in a water-filled container. The divider was then lifted from behind the tank; if the fish did not advance on its own, it was gently pushed with an acrylic paddle to initiate movement down the runway (Jutfelt et al., 2013; Sundin & Jutfelt, 2018). At the end of the runway, the fish faced an opaque barrier forcing it to turn left or right. We ran 10 consecutive trials per fish, recording its turning direction each time. One experimenter conducted any given series of 10 trials, standing behind the fish, in a centred position, and walked from one side of the maze to the other between trials when the fish was behind the barrier (Fig. 1). The side of the maze walked on was random (i.e. haphazardly decided by the experimenter); see the section *Effect of experimenter movement on turning direction in the detour test* in the Supporting Information. Another experimenter recorded the observations on paper except for *D. rerio*, where observations were typed directly into a computer. Experiments were recorded on video with the exception of *C. rupestris*, which was the first species tested and where no video camera was available. Four series of 10 trials were repeated for each individual at intervals of 48 h. Four full series could not be obtained for some individuals (n = 23 of 218), as fish occasionally did not swim down the runway for 10 consecutive runs.

We calculated the relative lateralisation index (L_R) for each individual for each series of 10 trials, where L_R = ((turns to the right − turns to the left) / (turns to the right + turns to the left)) × 100 (Bisazza et al., 1997a). A score of −100 indicates that the fish turned left 10 times out of 10; a score of 100 indicates that the fish turned right 10 times out of 10. L_R can be examined at both the individual and the population (i.e. mean) level, informing whether individuals and/or populations have a side bias, respectively. Since several studies conduct analyses on the absolute lateralisation index (L_A), rather than L_R, we also calculated each individual's L_A for each series, where $L_A = |L_R|$ (Fig. S2). L_A scores of 80 and 100 (i.e. 9 and 10 turns to one direction) are indicative of lateralisation since this number of turns to one side is statistically different from random based on a binomial test.

Re-analysis of published data

To our knowledge, the only published study testing the repeatability of individual lateralisation scores in a detour test was performed on 40 female guppies (*P. reticulata*) (Irving & Brown, 2013). Females were individually marked and tested in a detour test with 24 h or more between trial series. Fish were tested three times in each of two conditions: a barrier presenting a neutral stimulus (an empty aquarium behind a barrier of vertical bars) or a social stimulus (a conspecific in an aquarium behind a barrier of vertical bars) (Fig. 1 in Irving & Brown 2013). For lack of a better statistical approach at the time, the data were analysed using six Spearman rank correlations to assess the repeatability of L_R scores (Irving & Brown, 2013). We re-analysed these data and computed a single intra-class correlation coefficient (*R*) for each of the two test conditions (see *Statistical analysis*).

Statistical analysis

We tested population-level lateralisation with generalised (binomial) linear random-effects models, setting the intercept equal to the grand mean of the sample. Individual-level lateralisation was examined with a chi-square test comparing the observed variance (numerator) to the expected variance (denominator) assuming a normal approximation to the binomial distribution. This is analogous to testing for overdispersion (i.e. are there more observations in the tail ends of the distribution than expected by chance). See the Supporting Information and Table S3 for details and an explanation of issues with tests of lateralisation employed in previous studies.

We computed the repeatability (*R*) in the number of left and right turns (in a series of 10 trials) across trial series for each species using the ‘rpt’ function in the package ‘rptR’ (Stoffel et al., 2017). This analysis on binomial data amounts to testing the repeatability of L_R. We stress that the statistical analyses were conducted on true Bernoulli responses (left or right turn) rather than L_R scores (used in data visualization) because the latter have much less favourable statistical properties. L_R scores are shown in figures for comparison with previous studies. *R* values were computed for primary data collected in this study and for the published data on *P. reticulata* (Irving & Brown, 2013). *R* ranges from 0 (non-repeatable) to 1 (fully repeatable) and provides a standardised measure of the consistency of phenotypes across time or contexts (Nakagawa & Schielzeth, 2010). We specified a binomial error distribution and 1000 bootstrapping and permutation iterations to calculate 95% confidence intervals (CIs). We also computed an ‘adjusted repeatability’ by specifying trial series (1 to 4), fish mass, and start-side of the maze as fixed effects in the models to account for any confounding effects of these variables on *R* (Nakagawa & Schielzeth, 2010). The repeatability of L_A was calculated by specifying a Poisson error distribution. Finally, we tested the effect of experimenter ID and arena start side on L_R using a generalised linear mixed-effects model

(GLMM) in the package 'lme4' (Bates et al., 2014); species, start side, and their interaction were specified as fixed factors in the model while fish ID and experimenter ID were included as random factors. The importance of experimenter ID was tested with a likelihood ratio (LR) test. Models were validated via diagnostic checks with the package 'DHARMa' (Hartig, 2017). Statistical analyses were performed in R3.4.3 (R Core Team, 2017).

Results

Fish exhibited statistically significant individual-level lateralisation in two-thirds of trial series, i.e. 15 of 22 trial series across the four species tested and the species for which data were re-analysed (Table S2 and Fig. S4-S8). Population-level lateralisation was statistically apparent in five of the 22 trial series; two species exhibited a population side bias, but this bias changed with test day for *D. rerio*, and stimulus type for *P. reticulata* (Table S2, Fig. S4-S8).

Importantly, however, L_R was highly variable within individuals and individual identity explained less than 6% of the variance in relative lateralisation across the four species tested, with repeatability estimates ranging from R=0.006 to R=0.028 (Table 1, Fig. 3, Fig. S1). Controlling for the order of trial series, fish mass, and start-side did not change these results (Table 1); fixed factors accounted for less than 4% of the variance in L_R. Similar results were obtained when considering L_A (Fig. S2). The identity of the experimenter (LR test; $\chi^2_{(1)}=0$, $P=0.999$) and the starting side of a series in the experimental arena had no effect on a fish's preference to turn left or right (GLMM; $\chi^2_{(1)}=0.01$, $P=0.917$), irrespective of the species (GLMM; species × start-side interaction; $\chi^2_{(3)}=2.00$, $P=0.575$); see Table 1 and archived analysis script for details.

Similarly to the four species tested here, individual differences among female *P. reticulata* (Irving & Brown, 2013) accounted for very little of the variance in L_R, both when fish were tested using a neutral (R=0.045 [0.015-0.088]) and a social stimulus (R=0.076 [0.028-0.128]) (Fig. S3).

The 95% CIs for the R estimates of L_R in two species (*N. azysron*, *D. rerio*) overlapped zero; in the other three species, 95% CIs were narrow and close to zero, indicating high confidence in very small (albeit non-zero) values of R (Table 1).

Discussion

Numerous studies on fishes and other taxa report that behavioural lateralisation is linked to fitness-enhancing behaviours and can be severely impacted by environmental stressors such as predation, parasitism, pollutants, drugs, and various abiotic parameters, some of which have relied on the detour test methodology (see Table S1 for examples in fishes). We evaluated whether lateralisation assessed using a detour test is consistent in fish species with differing ecologies to establish the baseline repeatability of this behavioural trait and broaden our understanding of its ecological and evolutionary importance. Our validation exercise used robust sample sizes, four species, multiple measurements through time (four repeats at 48 h intervals), and included a re-analysis of data on a fifth species collected seven years ago.

Our results show that behavioural lateralisation as assessed by a detour test is not a repeatable trait in fishes (Fig. 3, Fig. S1-S3). Even though many individuals from all species displayed a strong side-bias (individual-level lateralisation present in 68% of trial series; Table S2, Fig. S4-S8), this turning preference varied markedly across days (Fig. 3, Fig. S1). Of the 69 individuals across all five species displaying an absolute lateralisation (L_A) score of 80 or 100 (nine or 10 turns in either direction) in at least one of the trial series, 52 also had at least one L_A score of 20 (four or six turns in either direction) or zero (five turns each way) when tested again (Fig. S2, S3). Overall, across the five species examined, individual identity explained only a small proportion of the phenotypic variance in lateralisation strength, ranging between 2.8 and 7.6%. These values contrast markedly with the results of several meta-analyses which report much higher repeatability estimates in traits such as behaviour (R = 0.37: Bell, Hankison & Laskowski 2009; R = 0.41: Holtmann, Lagisz & Nakagawa 2017) , cognition (R = 0.15-0.28: Cauchoix *et al.* 2018) , metabolic rates (R = 0.45: Holtmann *et al.* 2017) and hormone levels (R = 0.15: Holtmann *et al*. 2017). A re-analysis of existing data (Fig. S3) supports our findings, and further suggests that individual decisions to turn left or right in the detour test do not provide accurate or precise estimates of eye-preference or cerebral asymmetries in fishes. Our experiments were conducted by two or more researchers at any one time and trials were video recorded except for *C. rupestris*. These recordings, lab notebooks, and the raw data are publicly available (Roche et al., 2019). The fact that multiple labs collaborated to conduct this study across several geographic locations (Australia, Norway, Sweden) further strengthens the robustness of our results (Voelkl et al., 2018).

Only two of the five species exhibited a mean L_R significantly different from zero in at least one trial series, indicative of population-level lateralisation (Table S2, Fig. S4-S8). This population-level side-bias varied between days in the case of *D. rerio,* and stimulus type in the case of *P. reticulata* (see methods in Irving & Brown 2013). We anticipated a greater repeatability of L_R and stronger evidence for a positive or negative mean L_R in the three shoaling species examined (*N. azysron, D. rerio* and *P. reticulata*), as individual- and population-level lateralisation are reported to help social individuals coordinate group behaviours and enhance school cohesion (Bisazza et al., 2000a; Bisazza & Dadda, 2005;

Bibost & Brown, 2013). Bibost & Brown (2013) used a mirror test and found that individual-level lateralisation could influence the geometry of school formation depending on the sex and species of the school. Bisazza & Dadda (2005) used a detour test and reported that lateralised poecilids (*Girardinus falcatus*) exhibit greater school cohesion and coordination than non-lateralised conspecifics; however, schools were composed of only two individuals, which were females from a multi-generation laboratory strain. Bisazza et al. (2000a) also used a detour test and reported significant population-level lateralisation in 10 of the 16 fish species they examined (*n* per species: 7 to 18), yet only six of these were shoaling. Other studies have also reported such population-level side biases in fishes (e.g. Bisazza et al., 1997a; Bisazza et al., 1998a; Facchin et al., 1999; Irving & Brown, 2013; Domenici et al., 2014), yet their association with group living appears ambiguous. For instance, Domenici et al. (2012) and Lopes et al. (2016) failed to observe population-level lateralisation in shoaling *N. azysron* juveniles and *Atherina presbyter* larvae, respectively, despite reporting L_A scores higher than random in their control groups. Similarly, Chivers et al. (Chivers et al., 2016) reported high L_R scores in some shoals of the schooling fusilier *Caesio teres*, but lateralisation strength varied substantially both among and within the four groups tested. Taken together, these results suggest that the detour test is not adequate for assessing lateralisation in fishes and that possible benefits of lateralisation for shoaling species should be investigated using other means.

Implications

Given the large body of literature reporting significant effects of environmental stressors on lateralisation in fishes (Table S1), our results raise several questions that we address below.

Could methodological differences between ours and previous studies explain the lack of repeatability in L_R?

Published studies have used a range of different obstacles to elicit eye-use preference when fish arrive at the end of the runway in a detour test. Some studies use a neutral obstacle, such as an opaque barrier (e.g. Bisazza et al., 2001; Dadda et al., 2010b; Domenici et al., 2012; Roche et al., 2013; Domenici et al., 2014) or a barrier of vertical bars (e.g. Bisazza et al., 1997a; Reddon & Hurd, 2009a; Irving & Brown, 2013; Dadda & Bisazza, 2016). Preferential eye-use is said to occur because fish must explore the unknown space to the side or behind the barrier (Facchin et al., 1999). Other studies have used a non-neutral obstacle with a stimulus, such as a conspecific or an object resembling a predator placed behind a barrier of vertical bars (e.g. Bisazza et al., 1998a; Facchin et al., 1999; Dadda & Bisazza, 2006b; Irving & Brown, 2013). The neutral stimuli (e.g. a cross on an opaque barrier) used in our experiments may not have been valent enough to result in a strong, consistent lateralised response in individuals. Indeed, some research suggests that lateralisation direction and strength is stimulus-dependent (Bisazza et al., 1997a; Sovrano, 2004). It is possible that a predatory stimulus would have increased repeatability of measurements in this test since a consistent behavioural response to a predator may be under stronger directional selection than a neutral stimulus. However, previous studies using different methods for assessing lateralisation in fishes (i.e. mirror tests or circular arena tests) have reported correlations in the strength of individual lateralisation among tests, including between novel/neutral, predator, and social stimuli (Bisazza et al., 2001; Brown et al., 2004). These results suggest that the specific stimulus used to focus gaze could affect the direction of lateralisation (i.e. depending on eye use preference to focus on different stimuli) but should not significantly affect the strength of repeatability in the test. Our results provide evidence for this: we found that L_R was not repeatable across time when experiments were conducted with two different neutral stimuli (this study and re-analysis of Irving & Brown 2013) as well as with a non-neutral stimulus (re-analysis of Irving & Brown 2013). The occurrence of high individual L_R scores in all species in trial series 2, 3, and 4 of the experiment indicates that habituation to the test arena is unlikely (Fig. 3). Importantly, studies have also reported a significant population-level side-bias in species when only an opaque barrier with no stimulus was used (e.g. Bisazza et al., 1998a; Ferrari et al., 2015; Chivers et al., 2016), suggesting that a neutral stimulus should not impede a strongly lateralised response in a detour test. Interestingly, Ferrari et al. (Ferrari et al., 2017) found considerable variability in the lateralisation scores of *P. amboinensis* tested twice on the same day using opaque barriers with no stimuli. These results were interpreted as representing adaptive plasticity in lateralisation strength due to different predation risk scenarios (Ferrari et al., 2017), yet these experiments lacked a control group establishing the consistency of lateralisation strength in untreated individuals. Our results suggest that such variability is likely due to the inherent randomness of turning choice displayed by tested individuals rather than any adaptive behavioural decision. Similarly, a recent reanalysis of a well-cited study on honeybee magnetoreception also revealed random patterns rather than adaptive behaviour as originally suggested (Baltzley & Nabity, 2018).

Numerous other methodological variations exist across published studies that are worth considering in the context of our results. For example, studies differ in their maze design (e.g. single T in Roche *et al.* 2013; Y- *versus* T-entry in Irving & Brown 2013, Jutfelt et al. 2013, and Vila Pouca et al. 2018) and dimensions (Table S1), acclimation time before beginning a series (3 min in Bisazza, Sovrano & Vallortigara 2001, Roche *et al.* 2013; 1 min in Sundin & Jutfelt 2018), wait time between trials within a series (3 min in Roche *et al.* 2013; no wait time in Sundin & Jutfelt 2016 and Sundin & Jutfelt 2018) , and method for encouraging fish to enter runway (e.g. no encouragement in Vossen *et al.* 2016 *versus* encouragement in

Sundin & Jutfelt 2016), to name a few. Although subtle, these differences in protocol or experimental apparatus could considerably influence the results. For instance, Clark et al. (unpublished data) examined lateralisation behaviour in juvenile *Acanthochromis polyacanthus* in a double T-maze and found that a slight asymmetry in the barrier position at one end of their maze induced a strong side-bias in their tested fish. This side-bias was not observed at the other end of the arena where the barrier was centrally placed. Similarly, Sundin et al. (unpublished data) tested wild *P. reticulata* in a T-maze arena, which they later discovered had a small crack in one corner, causing this area of the tank to be slightly darker than the other side. Individuals consistently turned in the direction of this darker area, but no consistent side bias was apparent at the other end of the maze, or in the same individuals when tested in other identically constructed arenas. These observations illustrate the care that must go into the construction of experimental apparatus designed to assess side biases in individuals, as slight construction asymmetry or variations in protocol may dramatically influence the results obtained. Such side biases generated through very slight differences in arena construction or lighting may go overlooked, particularly in analyses of absolute lateralisation.

Could differences in species, sex, and/or life stage influence the repeatability of L_R?

Twenty-seven fish species have so far been tested in 31 published studies using a detour test to either relate lateralisation to fitness-relevant traits or assess the effects of environmental stressors on lateralisation strength (Table S1). Several other fish species also feature in studies simply examining whether individuals or populations are lateralised (e.g. Bisazza et al., 1997b; Bisazza et al., 2000a). The five species examined here (four original and one re-analysed) are phylogenetically diverse, originating from tropical, temperate, marine, and freshwater habitats. Therefore, they constitute a representative sample allowing our results to be generalised with a reasonable degree of confidence.

We did not include sex in our analyses of repeatability because *C. rupestris* were juveniles, *P. amboinensis* were predominantly female (given the size distribution of tested fish), and sex is difficult to assess non-invasively in the other species. Some studies suggest that sex should always be considered in studies of cerebral lateralisation because male and female brains are organised differently (Bianki & Filippova, 2001). Meta-analyses also suggest that there are important sex-differences in the repeatability of many behavioural traits (Bell et al., 2009), and that including determinants such as participant age and sex increased estimates of temporal repeatability slightly (Cauchoix et al., 2018). Indeed, several studies have reported sex-specific differences in lateralisation strength in fishes (e.g. Bisazza et al., 1998a; Reddon & Hurd, 2008, 2009b; Irving & Brown, 2013; Byrnes et al., 2016), although others have not (e.g. Reddon & Hurd, 2009a; Byrnes et al., 2016; Vossen et al., 2016; Sundin & Jutfelt, 2018). While this is worth exploring in future studies, it is unlikely that sex differences account for the dramatically low repeatability in lateralisation strength measured in all four of our study species (R=0.006 to R=0.028). With the exception of the protogynous *P. amboinensis*, where our sample is likely to be female-biased based on the size distribution of the collected individuals (McCormick, 2016), the remaining species tested have distinct sexes and are not strongly sexually dimorphic. As a result, we assume a roughly even sex-ratio in our samples. If lateralisation in one sex were highly repeatable, we would still expect an R measure considerably higher than observed, with consistency in a turning direction observed in approximately half of the population. This is clearly not observable in our data (Fig. 3, Fig. S1).

The effect of life stage on the strength of lateralisation is another consideration that was not explicitly addressed in our study. Although Bell et al. (2009) found no difference in the repeatability of behaviours between juveniles and adults in general, they note that, among ectotherms, juvenile behaviour is significantly more repeatable. We included a range of sizes in our tested species, which, for *C. rupestris* and *D. rerio,* included juveniles and sub-adults. We also included fish body mass as a fixed factor in our analyses. We found no effect of mass on the strength of lateralisation in any of our tested species. Published studies of detour tests in fishes have tested a range of life stages from pre-settlement larval fish to adults (Table S1). Although there does not seem to be a consistent trend in lateralisation strength owing to life stage, this should be tested more systematically in future studies.

What explains positive results in previous studies?

The high intra-individual variation in lateralisation we observed across test days in all five species examined (Fig. 1, Fig. S3) suggests that individual L_R scores in a detour test are random at any point in time. Therefore, why do numerous studies using detour tests report significant relationships between lateralisation strength and other phenotypic traits or environmental stressors? Unfortunately, many lateralisation studies (including previous publications by authors involved in this study) suffer from low sample sizes (e.g. 20 or fewer individuals per group (Bisazza et al., 2000a; Nilsson et al., 2012; Jutfelt et al., 2013; Roche et al., 2013; Byrnes et al., 2016; Lopes et al., 2016; Ferrari et al., 2017; Sundin & Jutfelt, 2018); Table S1), which considerably increases the likelihood of spurious results (Button et al., 2013; Colquhoun, 2014; Halsey et al., 2015). In addition, all 35 tests (31 studies) identified in Table S3 employed inadequate statistics to test for the presence of lateralised individuals (see *Supporting Information*). Confirmation bias and poor research practices such as p-hacking and selective reporting also contribute to false positives, which are published more readily than negative results (i.e. the publication bias or file-drawer effect) (Nuzzo, 2015; Parker et al., 2016). A recent survey of over 800 researchers revealed that such practices are rife in ecology and evolution, contributing to the ongoing reproducibility crisis (Fraser et al., 2018). Improving our confidence in, and ability to replicate, lateralisation studies requires the implementation of validated methodologies, appropriate statistics, high powered designs (Button et al., 2013), double-blinded protocols (Holman et al., 2015), video recordings (Clark, 2017), open data (Roche et al., 2015), and other transparency measures advocated by the recent Transparency and Openness Promotion (TOP) guidelines (Nosek et al., 2015; Clark et al., 2016).

Conclusion

Behavioural lateralisation is likely to be an ecologically important trait that should continue to be tested and measured. However, the method with which to assess eye-use preference as a proxy of cerebral asymmetry must be thoroughly validated for a species of interest before drawing conclusions about the fitness-consequences of changes in lateralisation direction or strength. Drawing inferences from non-repeatable measurements (or studies) not only obfuscates our understanding of a species' evolutionary ecology but also risks misleading policy and conservation efforts. Our study shows that the detour test as it has traditionally been implemented does not provide accurate, precise, or repeatable estimates of behavioural lateralisation in fishes. However, numerous other methods of assessing eye-use and side preference, including mirror tests, swimming, feeding or attack direction preference, and flume tests, have been applied in a range of species in both field and laboratory settings (see Dadda & Bisazza, 2006a; Dadda et al., 2010a; Takeuchi et al., 2010; Bibost et al., 2013; Bibost & Brown, 2014; Broder & Angeloni, 2014; Brown & Bibost, 2014; Forsatkar et al., 2015; Kurvers et al., 2017). Measurements using these methods and their cross-context repeatability should be validated in accordance with TOP guidelines (Nosek et al., 2015) to establish reproducible protocols that inspire confidence. We also encourage the development of more studies that explicitly examine the link among structural differences between brain hemispheres and individual behaviour in fishes to more concretely validate the link between cerebral and behavioural lateralisation in lower vertebrates (see Bisazza et al., 1998b).

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Animal ethics

Field collections and experiments were approved by the Swedish Board of Agriculture (Dnr 103-2014), Great Barrier Reef Marine Park Authority (G14/36625.1), the James Cook University Animal Ethics Committee in association with AIMS (A2314), and the Norwegian Animal Research Authority (Permit Number: 8578).

Data, code, and materials

The data, script, notebook copies, and videos for this study are archived in the repository figshare following best practices (Roche et al., 2015) and were made available to editors and reviewers upon initial submission: https://doi.org/10.6084/m9.figshare.6881489 (Roche et al., 2019).

Data re-use

Published data (Irving & Brown, 2013) were re-used for this study. The original authors were invited to participate and offered co-authorship.

Competing interests

We have no competing interests.

Author contributions

DGR, MA, JS, TDC, FJ, TN, BSR, and SAB designed the experiments; DGR, MA, RM, JS, AHA, MHF, FJ, MJL, EH, and SAB performed the experiments. JS, FJ and RB contributed vital equipment and funding for the experiments. CB provided the data used in the re-analysis. DGR analysed the data. DGR and SAB wrote the manuscript with input and approval from all authors.

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Table 1. Sample size (*n*), mass range (g), total length range (TL range, cm), and statistics for five species of fish tested to examine the repeatability of behavioural lateralisation. *Poecilia reticulata* were tested with a neutral (1) and a social (2) stimulus (see Irving & Brown, 2013). Estimates are presented for agreement and adjusted repeatability of relative lateralisation (L_R) with 95% CIs in parentheses. Statistics and *P* values are presented for the effect of trial series (1 to 4), body size (total length for *P. reticulata* and mass for all other species), and start-side of the maze on L_R.

	C. rupestris	***N. azysron***	***P. amboinensis***	***D. rerio***	***P. reticulata*[1]**	***P. reticulata* [2]**
n	57	52	60	49	40	40
mass range	1.4-46.5	0.7-6.4	1.0-14.3	0.26-1.07	NA	NA
TL range	4.4-14.9	4.2-8.3	3.8-8.8	2.7-3.6	1.7-3.5	1.7-3.5
R (agreement)	0.028 (0.004-0.052)	0.012 (0.000-0.033)	0.027 (0.008-0.046)	0.006 (0.000-0.023)	0.045 (0.015-0.088)	0.076 (0.028-0.128)
R (adjusted)	0.028 (0.006-0.053)	0.012 (0.000-0.028)	0.022 (0.004-0.038)	0.007 (0.000-0.023)	0.046 (0.011-0.081)	0.077 (0.030-0.126)
trial series	$\chi^2_{(1)} = 0.123$ *P* = 0.725	$\chi^2_{(1)} = 2.379$ *P* = 0.123	$\chi^2_{(1)} = 0.663$ *P* = 0.415	$\chi^2_{(1)} = 3.078$ *P* = 0.080	$\chi^2_{(1)} = 2.757$ *P* = 0.097	$\chi^2_{(1)} = 1.696$ *P* = 0.193
body size	$\chi^2_{(1)} = 0.084$ *P* = 0.772	$\chi^2_{(1)} = 0.890$ *P* = 0.345	$\chi^2_{(1)} = 0.458$ *P* =0.499	$\chi^2_{(1)} = 0.002$ *P* = 0.963	$\chi^2_{(1)} = 3.050$ *P* = 0.081	$\chi^2_{(1)} = 0.942$ *P* = 0.332
start side	$\chi^2_{(1)} = 0.204$ *P* = 0.651	$\chi^2_{(1)} = 0.849$ *P* = 0.357	$\chi^2_{(1)} = 0.354$ *P* = 0.552	$\chi^2_{(1)} = 0.020$ *P* = 0.887	N/A	N/A

Fig. 1 Schematic representation of a double T-maze used to test lateralisation in fishes. A focal fish is acclimated in one side of the arena for one minute behind a barrier (in grey). The barrier is then lifted and the fish is made to swim back and forth in the arena. As it approaches the end of the corridor, the fish faces a barrier and must choose to turn left or right. Decisions to turn left or right are recorded 10 times. Dimensions (mm) of the maze used for *Ctenolabrus rupestris* and *Danio rerio*: 500 (a), 500 (b), 200 (c), 330 (d), 80 (e), 100 (f), and 40 (g); height of walls 150; water level 100. Dimensions (mm) of the maze used for *Neopomacentrus azysron* and *Pomacentrus amboinensis*: 350 (a), 640 (b), 125 (c), 400 (d), 80 (e), 150 (f), and 50 (g); height of walls 370; water level 100.

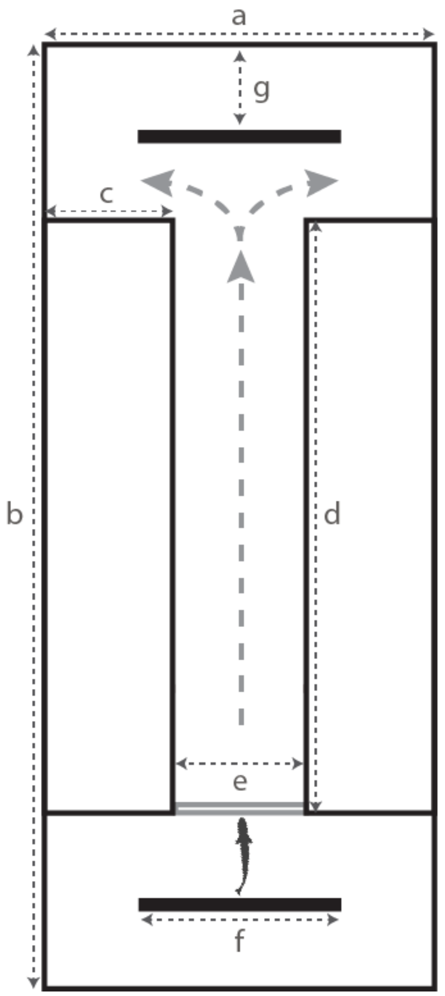


Fig. 2 The four fish species from temperate, tropical, marine, and freshwater habitats tested to determine the repeatability of lateralisation: *Ctenolabrus rupestris* (credit: F. Jutfelt), *Neopomacentrus azysron* (credit: picture.world, https://goo.gl/mTLphF), *Pomacentrus amboinensis* (Gagliano & Depczynski, 2013), and *Danio rerio* (credit: P.H. Olsen, NTNU).

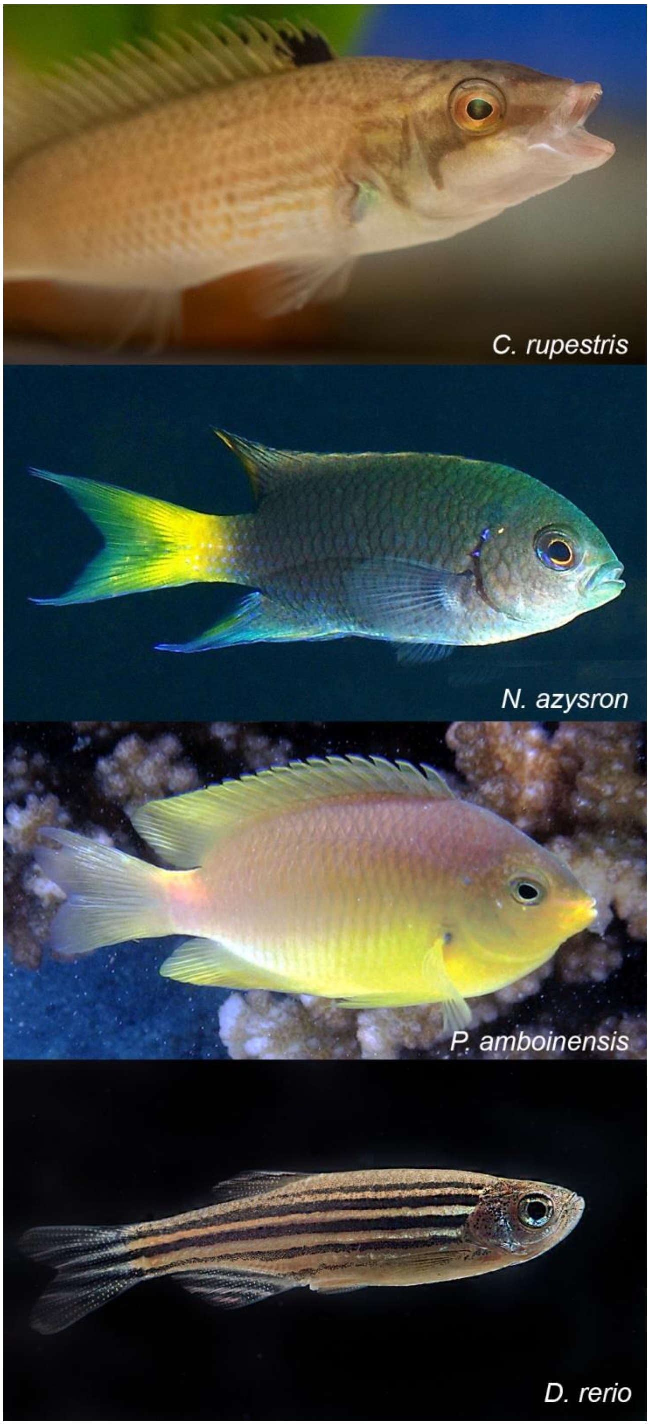
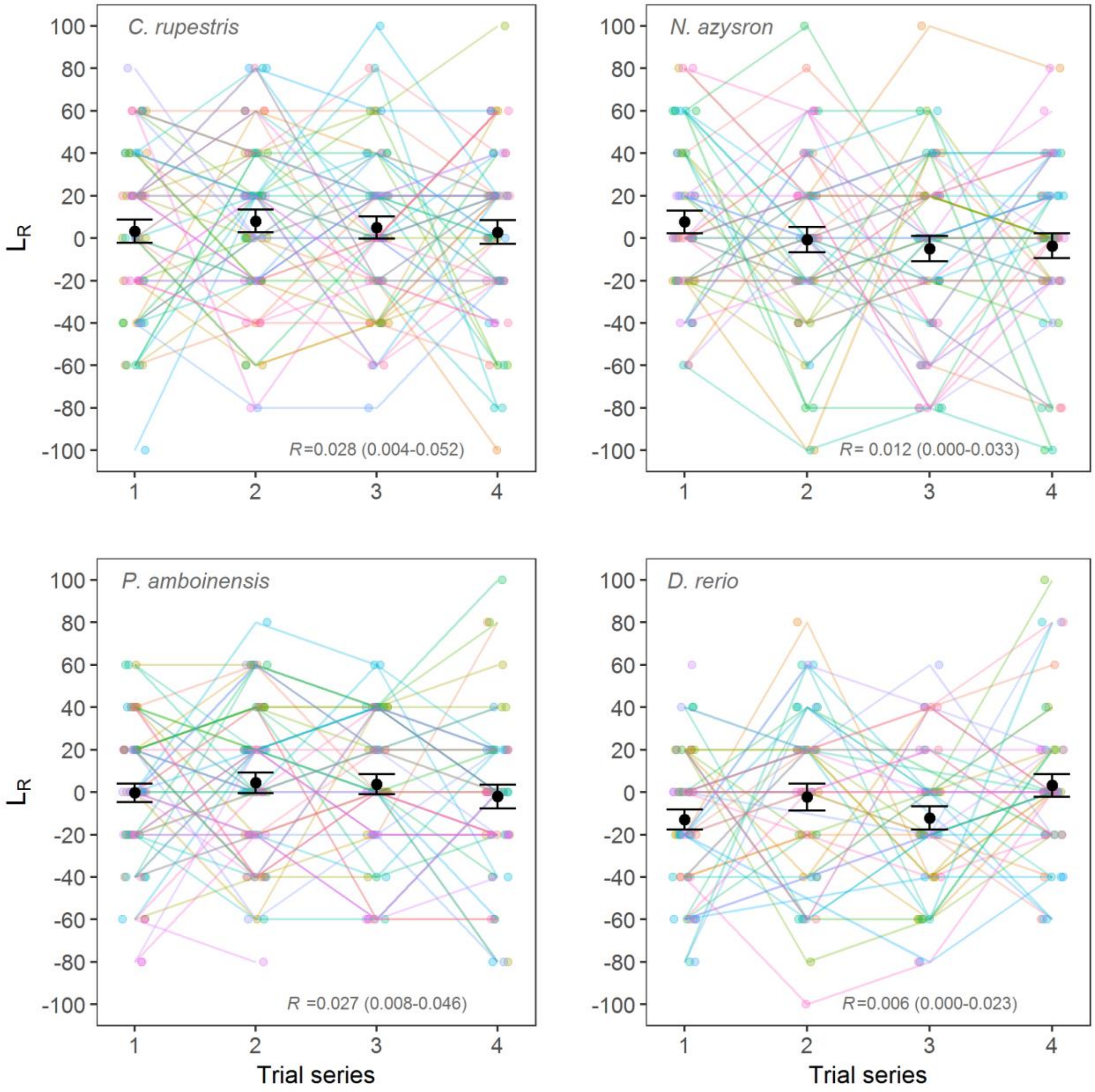


Fig. 3 Relative lateralisation index (L_R) across repeated trials (2 to 4) per individual for four fish species: *Ctenolabrus rupestris* (*n*=57), *Neopomacentrus azysron* (*n*=52), *Pomacentrus amboinensis* (*n*=60), and *Danio rerio* (*n*=49). Individual-level L_R is indicated by the coloured dots connected by lines; the mean (i.e. population-level) L_R and its 95% CI are indicated by black dots and error bars. The repeatability index (*R*) of L_R and its 95% CI are indicated for each species. Four series of 10 trials were conducted. Data points are jittered along the x-axis for presentation purposes.



SUPPORTING INFORMATION

Replication alert: behavioural lateralisation in a detour test is not repeatable in fishes

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Notes on what constitutes a replication

Two main types of replication studies exist: direct replications and conceptual replications, both of which are designed to evaluate the robustness of scientific findings [1-3]. A direct replication is “a study that attempts to recreate the critical elements (e.g., samples, procedures, and measures) of an original study where those elements are understood according to a theoretical commitment based on the current understanding of the phenomenon under study, reflecting current beliefs about what is needed to produce a finding” [3, 4]. As such, a direct replication “does not have to duplicate all aspects of an original study. Rather it must only duplicate those elements that are believed necessary for producing the original effect” [3]. A conceptual replication “tests an extension of the theory to a new context”; for example, “whether an effect extends to a different population given theoretical reasons to assume it will be either significantly weaker or stronger in different groups” [3]. Some researchers believe that conceptual replications are more informative than direct replications because they give better evidence for the generalizability of an effect [see 5].

In the case of our study, we performed both a (near) direct replication (using four species previously tested in a very similar way, from the same location/populations: *N. azysron*, *P. amboinensis, D. rerio, C. rupestris*) and a conceptual replication (using a previously tested species in a different location and with a different variant of the detour test: re-analysis of *P. reticulata*) [see 6, 7-11].

Notes on the use of Visible Implant Elastomer tags

Visible Implant Elastomer (VIE) tagging is a common procedure in fish behaviour/physiology studies, especially when using small individuals (larger fish are usually tagged with T-bar tags or Passive Integrated Transponder [PIT] tags surgically implanted into the abdominal cavity). The tags on our fish were injected using 29G insulin syringes producing a mark that is approximately 0.5 mm in diameter on both sides of the fish’s body. Studies on small fishes (e.g. coho salmon smolts, syngnathids, small European eels, age-0 brown trout) similar in size/mass to the ones we studied indicate that VIE tags had no detectable effect on the behaviour [12-14] and growth/survival [15] of marked versus control fish. We allowed the animals a minimum of 48 h to recover prior to testing, which is ample time according to previous studies [see 16]. Our fish were feeding prior to the first lateralisation test and between subsequent tests, which is a good indication that the tags (and experimental procedures) had a minimal effect on their behaviour.

Effect of experimenter movement on turning direction in the detour test

We reviewed the methods and statistical analyses in the 31 studies listed Table S1 (see stats in Table S3) to see how other authors have addressed this potential issue. We found that none of these studies report on (or account for) the side on which the experimenter walked around the experimental arena. From experience, when experimenters use small arenas, they sometimes remain on one side of the double T-maze and use one arm to guide the fish through the maze rather than walk around the maze. Failure to remove or account for biases in experimenter movements could result in consistent side choices by fishes and thus apparent lateralisation.

Here, the experimenter haphazardly walked on either side of the double T-maze when moving from one end of the maze to the other during a series of ten trials. We did not record the side of the maze on which the experimenter walked during the experiments and this information is difficult to extract from the recorded videos because the experimenter is not consistently in the camera’s field of view.

Note that videos were recorded for all species but C*tenolabrus rupestris*. Since the wall of the arena in which we conducted the trials were opaque and sufficiently high relative to the water depth in the arena (see Fig. 1 caption), we do not believe that the test fish was able to see the experimenter walking around the arena.

To test the effect experimenter movement on turning direction in the detour test, we analyzed data collected in 2015 on burbot (*Lota lota*) where the experimenter (J. Sundin) explicitly recorded the side of the arena she had walked on. These experiments were conducted in June 2015 at the Ar Research Station on Gotland, Sweden. Forty fish were tested (total length 36.2 ± 4.59 mm, mean ± SD) in the same T-maze as was used for *C. rupestris* and *D. rerio* in the present study (see Fig. 1 in the main text for dimensions). Twenty fish were tested in freshwater and 20, in saltwater. The methods were the same as those described in the *Materials and Methods* section of the main text.

We tested the effect of the experimenter's movement on the turning direction of the test fish with a generalised linear random-effects model (glmer function in R) that sets the intercept equal to the grand mean of the data. We specified a binomial distribution of error terms. The model was specified as:

```
g <- glmer(outcome~1+(1|ind), data=dat, family="binomial")
```

where "outcome": 1 = the fish turned towards where the experimenter walked (i.e. RR, LL); 0 = the fish turned away from where the experimenter walked (i.e. RL, LR)

We expect $p = q = 0.5$ (a probability of either outcome being 0.5). If the intercept (β_0) is significantly different from 0, then the side on which the experimenter walked influenced the fish's turning direction. The equation for the logistic regression corresponding to the above model is:

$$\beta_0 = \log(p/(1-p))$$

Based on 340 trials across 40 fish, we found no effect of the experimenter's movement (walking side) on the turning direction of test fish in the detour test (intercept estimate = -0.157 ± 0.110 [SE], $z = -1.43$, $P = 0.152$).

Issues with tests of behavioural lateralisation in the literature

We systematically reviewed the statistical tests employed by studies in Table S1 to determine whether groups of fish exhibit population- and/or individual-level lateralisation. We found that authors often use an arbitrary cut-off or eyeball the distribution of lateralisation scores obtained to determine whether there is evidence of population- or individual-level lateralisation (e.g. see Tables S1 and S3 for a full list of studies). Many studies employ linear models to compare the mean lateralisation scores of control and treatment groups but without testing whether control and treatment groups actually exhibit lateralisation (at the population- or individual-level) (Table S3). This is problematic because populations might have significantly different lateralisation scores without statistically exhibiting lateralisation. Worryingly, the few studies that relied on statistics to explicitly test for lateralisation employed inadequate tests, such as a one-sample t-test or a G-test of goodness-of-fit (this includes previous studies by the authors) [e.g. 6, 8, 17-20] (Table S3). Unfortunately, goodness-of-fit tests such as the G-test result in an inflated type-I error rate when the total sample size is less than 500 and/or an expected number (e.g. the number of right of left turns taken by an individual) is less than five ([21], page 86-89). Given that, in a detour test, each individual is subjected to only 10 decisions whether to turn left or right, the probability of obtaining an expected number of right or left turns smaller than five is 90%. Therefore, using such tests considerably increases the probability of false positives (see [21], page 87).

Testing for lateralisation is not straightforward because it involves multiple binomial experiments with structure. Since a binomial trial (a decision to turn left or right) is repeated 10 times per individual in the detour test, trials conducted on multiple individuals are non-independent. This data structure is different from a simpler situation, such as in a standard binomial experiment (e.g. a coin toss or a trial involving a decision to turn left or right), repeated multiple times by/on a single individual. Here, each trial can be treated as independent.

We have developed and examined the sensitivity of two statistical approaches for testing population- and individual-level lateralisation. A test for detecting lateralisation at the population level requires examining the mean lateralisation score across all individuals in the sample since population-level lateralisation is present when a group of individuals collectively exhibits a side-bias. Specifically, this test involves assessing whether the mean number of turns to the right (or to the left) is significantly different from five, given 10 trials per individual. In contrast, a test for detecting individual-level lateralisation requires examining the sample variance since individual-level lateralisation is present when more individuals exhibit a side-bias than expected by chance (irrespective of whether it is to the left or to the right). For explanations and examples of these two concepts, see Bisazza et al. [22], Domenici et al. [6], and Roche et al. [18].

A robust test to detect population-level lateralisation

Population-level lateralisation can be tested with a generalised linear random-effects model (glmer function in R) that sets the intercept equal to the grand mean of the data. This model can be written as:

```
g <- glmer(X~1+(1|ind), data=dat, family="binomial")
```

where ‘dat’ is a data frame containing three columns: individual ID (‘ind’); trial number (1 to 10); and turning side (column ‘X’) as right (1) or left (0). Specifying the error family as “binomial” sets the probability of success (p) to 0.5. Here, a right turn is considered a success. One *expects* a mean number of 5 turns to the right across all individuals if p = 0.5 (i.e. there is a 50% chance of turning left or right). Testing whether the *observed* mean number of turns to the right differs from the *expected* mean under a binomial error distribution with p = 0.5 simply requires extracting the p-value for the model intercept as such:

```
summary(g)$coefficients[4]
```

If the intercept (β_0) is significantly different from 0 (based on the equation for the logistic regression with p = q = 0.5) [$\beta_0 = \log(p/(1-p))$], there is statistical evidence that the population is lateralised (i.e. has a side-bias).

A robust test to detect individual-level lateralisation

Individual-level lateralisation can be tested with a chi-square test comparing the observed variance (numerator) to the expected variance (denominator) assuming a normal approximation to the binomial distribution. This is analogous to testing for overdispersion (i.e. are there more observations in the tail ends of the distribution than expected by chance), and is achieved as such:

```
chi_sq=((N-1)*var(X1)/(n*.5*.5))
pchisq(chi_sq,df=(N-1),lower.tail=F)
```

where N is the total number of individuals tested; n is the number of trials per individual (i.e. 10); and X1 is a column in a data frame corresponding to the number of right (or left) turns per individual (each row of the data frame represents one individual).

If $P < 0.05$, there is statistical evidence that some individuals are lateralised (i.e. more individuals have an extreme lateralisation score than expected by chance based on a normal approximation to the binomial distribution with p = 0.5).

Sensitivity analysis

We ran simulations to examine the tests' sensitivity and probability of type I and type II errors. Both tests were robust to a changes in N, n, and p across a wide range of values. The R code to carry out the tests and run the sensitivity analysis are publicly available on figshare: https://doi.org/10.6084/m9.figshare.6881489

Alternatives

Note that: 1) individual-level lateralisation can also be assessed with a GLMM rather than a chi-square test but the GLMM approach is less powerful; and 2) population-level lateralisation can also be assessed with a Z-test rather than a GLMM – both have similar power but the Z-test does not account for structure in the data (i.e. it ignores individual ID). We provide these alternatives but recommend the two tests detailed in the paragraphs above.

Table S1. Fish studies documenting links between phenotypic traits or environmental stressors and lateralisation assessed using a detour test. Studies that used a detour test only to assess whether individuals or populations are lateralised are not included. Similarly, studies that used methods other than a detour test to assess lateralisation are excluded. Information is provided on: the type of environmental stressor or phenotypic trait examined (stressor / trait) for each species; life stage; body size (T = treatment group, C = control group); sample size (*n*); environment (env.; FW = fresh water, SW = salt water); whether or not individuals are shoaling/schooling (shoal / school; yes or no); habitat (temperate or tropical); sex (♀ = females, ♂ = males, ♀♂ = both sexes); whether population-level lateralisation was assessed (pop. lat.; R = right biased, L = left biased); the effect of the environmental stressor on the strength of lateralisation or the relationship between lateralisation and a given trait (effect; ‘+’ = increase or positive, ‘−’ = decrease or negative); whether relative (L_R) and/or absolute (L_A) lateralisation were examined (L_R / L_A); the dimensions of the T-maze in cm (dimensions; L = length of the runway, W = width of the runway, B = length of the barrier); reference (ref.).

stressor / trait	**species**	**life stage**	**body size**	***n***	**env.**	**shoal / school**	**habitat**	**sex**	**pop. lat.**	**effect**	**L_R / L_A**	**dimensions**	**ref.**
aggression	*Archocentrus nigrofasciatus*	adult	NA	C=26 T=14	FW	no	tropical	♀♂	no	♀ + right ♂ + left	L_R + L_A	L=75, W=10, B=15	[23]
aquatic acidification	*Acanthochromis polyacanthus*	juvenile	NA	C=105 T=250	SW	yes	tropical	NA	yes (L)	−	L_R + L_A	L=25, W=3, B=12	[24]
	Argyrosomus regius	juvenile	6.8±0.6 cm 2.6±0.8 g	10	SW		temperate	NA	yes (L)	−	L_R + L_A	NA	[25]
	Atherina presbyter	larval	20.48±0.30 mm	C=46 T=60	SW	yes	temperate	NA	yes (L)	−	L_R + L_A	L=50, W=3, B=NA	[26]
	Ctenolabrus rupestris	adult	C: 94.2±8.46 93.7±8.25 mm	C&T= 17-24	SW	no	temperate	NA	no	none	L_R + L_A	L=33, W=9, B=NA	[11]
	Danio rerio	adult	NA	C&T= 7-11	FW	yes	tropical	♀♂	no	+	L_R + L_A	L=33, W=9, B=NA	[7]
	Gadus morhua	juvenile	5.8±0.3 to 13.0±2.4 g	C=21 T=17	SW	yes	temperate	NA	yes (L)	none	L_R + L_A	L=33, W=9, B=NA	[27]
	Gasterosteus aculeatus	adult	~1.6 - 1.9 g	C&T= 20-25	SW	yes	temperate	♀	no	−	L_A	L=33, W=9, B=NA	[28]

	Gasterosteus aculeatus	adult	46.5±2.2 mm 1.3±0.2 g	C=11-12 T=12-13	SW	yes	temperate	NA	no	–	L_A	L=33, W=9, B=NA	[29]
	Gasterosteus aculeatus	adult	C: 46.5±8.9 T: 46.2±6.8 mm	C=14 T=13	SW	yes	temperate	♀♂	yes (R)	–	L_R + L_A	NA	[30]
	Gobiusculus flavescens	adult	0.73-0.86 g	C=11-30 T=19-25	SW	yes	temperate	♀♂	yes (R)	none	L_R + L_A	L=33, W=9, B=NA	[31]
	Neopomacentrus azysron	larval	~12 mm	C=15-30 T=15-30	SW	yes	tropical	NA	NA	–	L_A	L=25, W=3, B=NA	[8]
	Neopomacentrus azysron	larval	12.1±0.0 mm	C=70 T=68	SW	yes	tropical	♀♂	no	–	L_R + L_A	L=25, W=3, B=12	[6]
	Pomacentrus wardi	juvenile	NA	24-58	SW	yes	tropical	♀♂	yes (R)	reversed	L_R + L_A	L=25, W=3, B=12	[17]
boldness	*Archocentrus nigrofasciatus*	adult	NA	100	FW	no	tropical	♀♂	no	+	L_R + L_A	L=75, W=10, B=15	[20]
	Heterodontus portusjacksoni	juvenile	~30 cm	17	SW	no	tropical	NA	no	none	L_R + L_A	NA	[19]
	Poecilia reticulata	adult	♀: 28.4±0.4 ♂: 19.2±0.2 mm	80	FW	yes	tropical	♀♂	yes (R)	none	L_R + L_A	L=25, W=10, B=7.5	[32]
cognition	*Cyprinus carpio*	adult	4.6-6.9 g 7.0-7.8 cm	C=8 T=8	FW	no	temperate	NA	no	none	L_R	L=30, W=12, B=30	[33]
	Girardinus falcatus	adult	NA	12-16	FW	yes	tropical	♀	no	+	L_R	NA	[34]
	Girardinus falcatus	adult	NA	6-9	FW	yes	tropical	♀	no	+	L_R	NA	[35]
	Gambusia holbrooki	adult	NA	4-7	FW	yes	subtropical	♀	no	+	L_R	L=40, W=7, B=NA	[36]

cortisol	*Pomacentrus amboinensis*	juvenile	NA	C=25 T=25	SW	no	tropical	NA	no	−	L_R	L=25, W=3, B=12	[9]
growth rate	*Geophagus brasiliensis*	adult	7.0-14.4 cm	26	FW	no	tropical	♀♂	no	+	$L_R + L_A$	L=75, W=10, B=NA	[37]
hypoxia	*Leptocottus armatus*	adult	12.4±0.3 cm (mean±SE)	C=42 T=42	SW	no	temperate	NA	yes (R)	−	$L_R + L_A$	L=75, W=10, B=20	[38]
parasitism	*Scolopsis bilineatus*	adult	T: 11.8±2.5 C: 14.8±1.5 cm (mean±SD)	C=25 T=16	SW	no	tropical	NA	no	+	$L_R + L_A$	L=70, W=15, B=25	[18]
pesticide exposure	*Acanthurus triostegus*	larval	NA[1]	C=10 T=10	SW	yes	tropical	NA	no	−	L_R	NA	[39]
Pharma-ceuticals	*Argyrosomus regius*	juvenile	C: 5.4-6.8 T: 5.4-6.6 cm	C=10 T=10	SW		temperate	NA	yes (L)	−	$L_R + L_A$	NA	[25]
predation	*Brachyrhaphis episcopi*	adult	~2.5 cm	T_1=32 T_2=27	FW	no	tropical	♀♂	no	+	$L_R + L_A$	L=47, W=10, B=NA	[40]
	Poecilia reticulata	adult	NA	C=65 T=68	FW	yes	tropical	♂	no	+	$L_R + L_A$	L=NA, W=8, B=15	[10]
	Caesio teres	juvenile	3.59±0.03 cm (mean±SE)	6-35	SW	yes	tropical	NA	yes (R)	+	$L_R + L_A$	L=25, W=3, B=12	[41]
	Pomacentrus amboinensis	juvenile	NA	T_1=10 T_2=15	SW	no	tropical	NA	no	+	$L_R + L_A$	L=25, W=3, B=12	[9]
	Pomacentrus chrysurus	juvenile	NA[2]	C=30 T=30	SW	no	tropical	NA	NA	+	L_A	L=25, W=3, B=12	[42]
schooling	*Girardinus falcatus*	adult	NA	17-37	FW	yes	tropical	NA	no	+	L_R	NA	[43]
temperature	*Pomacentrus wardi*	juvenile	NA	C=20 T=20	SW	yes	tropical	♀♂	yes (R)	−	$L_R + L_A$	L=25, W=3, B=12	[17]

	Argyrosomus regius	juvenile	C: 5.4-6.8 T: 7.0-8.6 cm	C=10 T=10	SW	temperate	NA	yes (L)	−	$L_R + L_A$	NA	[25]

[1] 0.21-0.25 cm at capture; [2] settlement stage

Table S2. Statistics for tests of individual- and population-level lateralisation for five species of fish: *Ctenolabrus rupestris* (*n*=57), *Neopomacentrus azysron* (*n*=52), *Pomacentrus amboinensis* (*n*=60), *Danio rerio* (*n*=49), and *Poecilia reticulata* (*n*=40). Individuals were tested on four occasions (trials 1-4), except for *P. reticulata*, which were tested three times using both a neutral and a social stimulus (see [32]). A chi-square statistic (ind. χ^2) and *P*-value (ind. *P*) are presented for tests of individual-level lateralisation; *P*<0.05 indicates lateralisation. A z-value (pop. z) and *P*-value (pop. *P*) are presented for tests of population-level lateralisation; *P*<0.05 indicates lateralisation. Statistical significance is indicated in bold (α=0.05).

Species	**trial**	**ind. χ^2**	**ind. *P***	**$\bar{X}$**	**pop. z**	**pop. *P***
C. rupestris	1	**89.42**	**0.002**	5.16	-0.60	0.546
	2	**91.88**	**0.002**	5.40	-1.53	0.126
	3	**81.80**	**0.011**	5.25	-0.99	0.322
	4	**93.14**	**0.001**	5.14	-0.52	0.603
N. azysron	1	**70.31**	**0.025**	4.98	-1.45	0.149
	2	**92.70**	**0.000**	5.22	0.15	0.885
	3	**93.50**	**0.000**	5.19	0.87	0.386
	4	**84.55**	**0.001**	4.90	0.64	0.521
P. amboinensis	1	68.39	0.189	5.38	0.08	0.939
	2	**80.05**	**0.029**	4.96	-0.93	0.354
	3	60.05	0.207	4.75	-0.82	0.412
	4	**73.80**	**0.010**	4.82	0.37	0.712
D. rerio	1	51.59	0.299	**4.35**	**2.71**	**0.007**
	2	**70.57**	**0.004**	4.88	0.39	0.696
	3	49.50	0.144	**4.39**	**2.24**	**0.025**
	4	58.36	0.072	5.16	-0.59	0.557
P. reticulata (neutral stimulus)	1	**78.04**	**0.000**	**3.65**	**-3.72**	**0.000**
	2	49.56	0.120	**4.05**	**-3.35**	**0.001**
	3	38.79	0.479	**4.23**	**-3.09**	**0.002**
P. reticulata (social stimulus)	1	**58.84**	**0.022**	4.65	-1.16	0.245
	2	**74.04**	**0.001**	5.15	0.47	0.642
	3	**78.55**	**0.000**	5.12	0.37	0.709

Table S3. Statistical analyses used by studies listed in Table S1.

[23] Reddon & Hurd 2008 – aggression Tested the effect of sex and aggressor status on relative and absolute lateralisation (stated as laterality index). Statistical methods are not described, but **R^2 and F-statistic are given**.
[24] Welch et al. 2014 – aquatic acidification Tested juveniles across nine treatment groups. Differences between treatment groups (parents and offspring exposed to control, intermediate and high CO2 in a full factorial design) in absolute lateralisation, was tested using a general **linear mixed-effects model** (**LMM**; LA ~ Parental treatment * Offspring treatment + various random effects). Auhors state that relative lateralisation was used to compare turning bias among the nine treatments using **Pearson's Chi-square test** but no test statistics are presented for L_R in the main text or the supplementary material.
[25] Maulvault et al. 2018 – aquatic acidification, warming, and pharmaceuticals The effects of venlafaxine (in water and in feed), high temperature, and CO2 in various combinations on absolute and relative lateralisation were tested using **generalised linear mixed-effects models (GLMMs)** with a Gaussian error distribution. Tank was specified as random factor (not specified given whether fish from 2 or 3 tanks were used in the test) and the authors employed a model selection approach using AIC.
[26] Lopes et al. 2016 – aquatic acidification Tested the effect of high CO2 exposure for 7 and 21 days on relative and absolute lateralisation with **GLMMs**. Treatment and time were entered as fixed effects and tank as a random effect. Model selection was performed using AIC.
[11] Sundin & Jutfelt 2016 – aquatic acidification Tested control and fish exposed to high CO2, at exposure day 9, 19, and 21 (all fish tested in control water for the last run). Used **generalised linear models (GLMs)** with a binomial error structure and relative and absolute lateralisation as response variables (number of turns to the left over total number of left and right turns (corresponding to relative lateralisation), and maximum number of turns to the preferred side over total number of left and right turns (corresponding to absolute lateralisation), and treatment, test run (for run 1 and 2, run 3 was analysed separately), and the interaction between them as fixed effects.
[7] Vossen et al. 2016 – aquatic acidification Tested eight treatment groups (control CO_2, no gabazine females; control CO_2, no gabazine males; high CO_2, no gabazine females; high CO_2, no gabazine males; control CO_2, gabazine females; control CO_2, gabazine males; high CO_2, gabazine females; and high CO_2, gabazine males). Relative lateralisation (proportion of right turns) analysed using a **GLMM with binomial errors** with CO_2 exposure level, gabazine treatment, and sex as fixed effects, and exposure tank and number of days exposed as random effects.
[27] Jutfelt & Hedgärde 2015 – aquatic acidification Tested juveniles after 29-30 days exposure to control or high CO_2. Used a **nested ANOVA** (tank nested under treatment) on relative and absolute lateralisation to test for differences among treatment groups.
[28] Jutfelt et al. 2013 – aquatic acidification

Tested control and high CO_2 treatments at 20 and 40 days of exposure. Among-treatment effects on relative and absolute lateralisation were tested at two time points using **independent samples t-test with unequal variances**.
[29] Lai et al. 2015 – aquatic acidification Tested control and high CO_2 treatments at 40 and 50 days of exposure (following 30 minutes of gabazine treatment). Absolute lateralisation between control and high-CO_2 fish on day 40 was analysed using a **two-tailed t-test**. A **two-way ANOVA** was used to examine the effects of high CO_2 and gabazine on day 50 followed by Sidak's multiple comparison test as the post-hoc test.
[30] Näslund et al. 2015 – aquatic acidification Tested control and high CO_2 at 20 days of exposure. Absolute lateralisation between the two groups was analysed using a **permutation test using Boss Permutations Tester**.
[31] Sundin & Jutfelt 2018 – aquatic acidification Tested the effect of CO_2 exposure on relative lateralisation across two years. Individuals were tested at 12-19 days of exposure. The effect of CO_2 on relative lateralisation was tested using a **GLMM with binomial errors**. **Wilcoxon-signed rank tests** were used to test against an expected value of 0 to investigate whether the fish were significantly left- or right-biased.
[8] Nilsson et al. 2012 – aquatic acidification Tested the effect of CO_2 and gabazine exposure in a full factorial design. It appears that separate within-treatment tests of absolute lateralisation were performed using **G-tests** (i.e. separate tests were performed for the group treated with control or CO_2 water). Differences before *versus* after gabazine treatment were tested using **t-tests** (also separately for each control/CO_2 treatment).
[6] Domenici et al. 2012 – aquatic acidification Tested the effect of four days of CO_2 exposure, using relative and absolute lateralisation. "In addition, a random simulation (RS) was generated based on 10 random binary choices (i.e. left or right) per individual ($n = 70$). This simulation was generated in order to test if any of the samples yielded left–right proportions that were not different from that expected by random choice." Within-group preference for left *versus* right turns was tested using **one sample t-tests**, among-group relative and absolute lateralisation was tested using **Kruskal–Wallis tests** (testing all three groups: control, CO_2, and hypothetical random choice group) followed by **Dunn's multiple comparison tests**. The distributions of the control and the elevated-CO_2 individuals were compared with a theoretical binomial distribution using a **G-test**.
[17] Domenici et al. 2014 – aquatic acidification Tested effect of seven days of CO_2 exposure and elevated temperature on fish from four treatments (control CO_2/control temperature, control CO_2/high temperature, high CO_2/control temperature, high CO_2/high temperature), using both relative and absolute lateralisation. Within-group preference for left *versus* right turn (relative lateralisation) was tested using **one sample t-tests**, among-group relative and absolute lateralisation was tested using **two-way ANOVAs** followed by **Tukey HSD tests**.
[20] Reddon & Hurd 2009 – boldness Tested for laterality index (LI) and absolute LI (corresponding to relative and absolute lateralisation). Both were analysed using **one-sample t-tests**.

[19] Byrnes et al. 2016 – boldness Tested for correlation between laterality index and laterality strength (corresponding to relative and absolute lateralisation) and personality. Population-level departures from random choice in laterality direction and overall non-lateralisation were examined using **two-tailed and one-tailed Wilcoxon signed rank tests**, respectively. General **linear models (LMs)** were used to examine the influence of sex, population, time in captivity, total length, boldness, and stress reactivity on lateralisation direction and strength.
[32] Irving & Brown 2013 – boldness Used **repeated measures ANOVA** to examine the effect of stimulus (neutral or control; repeated measure) and sex on relative lateralisation. **Regression** was used to examine relationships between relative (and absolute) lateralisation and boldness, activity, and sociability.
[33] Garina et al. 2016 – cognition Tested fish divided among three experimental groups (serotonin-modulating anticonsolidation protein and two controls). Fish were tested on four different days, five times per day. The median number of right turns out of all five turns was used to assess lateralisation. Changes in turning direction and latency were analysed using **Friedman's test and coefficient of concordance**. The median number of right turns was compared pair-wise between days within each experimental group, as well as on the same day between different experimental groups using **Mann–Whitney U tests**. The median values for each experimental group were calculated across days and compared pair-wise using the same test.
[34] Dadda & Bisazza 2006 – cognition Tested fish from three lines that had been selected for lateralisation (left detour fish, right detour fish, and non-lateralised fish). Data were analysed using **two-way ANOVAs** where laterality type and presence/absence of the male were included as the independent factors, and average time to retrieve food was included as the dependant variable. Lateralisation was not tested directly in the paper; therefore, no statistics on absolute or relative laterality are presented.
[35] Sovrano et al 2005 – cognition Tested fish from lines that had been selected for lateralisation: right detour (RD) fish, left detour (LD) fish, and non-lateralised (NL) fish. Data were analysed using **ANOVAs** firstly to see if laterality affected the proportion of correct choices and, secondly, to see if laterality affected the frequency of choice corner. Laterality type (RD, LD, and NL) was first included as a between-subjects factor, and trial as within-subjects factor. The RD and LD were then grouped into lateralised fish (L) and reanalysed using an **ANOVA.** Lateralisation was not tested directly in the paper; therefore, no statistics on absolute or relative laterality are presented.
[36] Dadda & Bisazza 2006 – cognition Tested fish from lines that had been selected for lateralisation (right detour fish, left detour fish, and non-lateralised fish). Data were analysed using **ANOVAs** (e.g. to test if eye-preference differed between lateralisation groups) and **t-tests** (e.g. to test eye-preference within each lateralisation group). Lateralisation was not tested directly in the paper; therefore, no statistics on absolute or relative laterality are presented.
[9] Ferrari et al. 2017 – cortisol

Tested absolute lateralisation using a **two-way blocked ANOVA**, testing the effect of cortisol (sham *versus* cortisol), and blocking for testing day (random factor).
[37] Reddon et al. 2009 – growth rate Tested fish for absolute and relative lateralisation. Population- and individual-level lateralisation were tested using **one sample t-tests**.
[38] Lucon-Xiccato et al. 2014 – hypoxia Tested relative and absolute lateralisation. Both measurements were analysed after an arcsine transformation. Relative lateralisation was tested within each group using **one sample t-tests**. **Independent sample t-tests** were used to compare mean relative and absolute lateralisation indices between treatment groups.
[18] Roche et al. 2013 – parasitism Tested two groups (unparasitised and parasitised) for relative and absolute lateralisation. Used **goodness-of-fit G-tests** to test within-group relative lateralisation and a **GLM** to test for differences between groups. Between-group absolute lateralisation was tested using a **Mann–Whitney U test** and between parasitised and parasite-removed fish, using a **Wilcoxon paired-sample test**. A **GLM** was used to test turning direction with respect to the side the parasite was attached on parasitised and parasite-removed fish.
[39] Besson et al. 2017 – pesticide exposure Tested eye-use in fish exposed to one of four stimuli, for relative lateralisation. Within-stimulus relative lateralisation was tested using two-tailed **Mann-Whitney U tests**, differences between stimuli were tested using non-parametric tests (**Wilcoxon rank sum test and Kruskall-Wallis test**). Additionally, fish were tested for the effect of lateralisation on predator avoidance, using **Kruskal-Wallis test** (lateralisation was tested and fish were divided into three groups: left tending, right tending, and unbiased – it is not specified which criterion was used). Finally, relative lateralisation was tested for fish divided among two treatments (chlorpyrifos and control) and four different stimuli using **Mann-Whitney U test** as above.
[25] Malvault et al. 2018 – pharmaceuticals See above.
[40] Brown et al. 2007 – predation Tested first generation descendants, from either a high-predation population, or a low-predation population, as well as wild-caught adult females from high-predation or low-predation population, as representatives of the respective wild populations. Fish were tested for relative and absolute lateralisation when sequentially presented with three different stimuli: control, novel object, and unfamiliar same-sex conspecific. **Repeated measures ANOVA** was used to test the response of wild-caught female fish to the three treatments, the difference between the wild-caught females and the female laboratory-reared offspring, and the differences between males and females in the laboratory-reared fish only.
[10] Broder & Angeloni 2014 – predation Tested males from four different populations (high predation populations and low predation populations) exposed to one of two treatments (predator or no predator exposure). Relative and absolute lateralisation were tested using '**mixed-effects**' **ANOVAs**.
[41] Chivers et al. 2016 – predation Tested fish from two treatments (low-risk and high-risk). Relative and absolute lateralisation were tested using a **two-way nested ANOVA** where fish were nested within

tanks, and tanks within risk group. Lateralisation scores of the low- and high-risk fish were also compared to pre-treatment scores using a **one-way ANOVA** followed by post hoc **Tukey tests**.
[9] Ferrari et al. 2017 – predation Tested fish from two treatment groups differing in perceived predation risk pattern for relative and absolute lateralisation using a **two-way repeated measures ANOVA**, investigating the effect of risk peak time (noon *versus* evening) on the absolute lateralisation of fish tested both at noon and in the evening.
[42] Ferrari et al. 2015 – predation Tested fish divided among three treatment groups (no treatment [‘before’ treatment], high-risk environment, and low-risk environment) for absolute lateralisation using a **one-way ANOVA**.
[43] Bisazza & Dadda 2005 – schooling Tested fish originating from lines selected for lateralisation: left detour fish, right detour fish, a mixture of right and left detour fish, and non-lateralised fish. No additional tests on laterality are included in the paper; therefore, no statistics on absolute or relative laterality are presented.
[17] Domenici et al. 2014 – temperature See above.
[25] Malvault et al. 2018 – temperature See above.

Fig S1. Relative lateralisation index (L_R) across repeated trials per individual for four fish species: *Ctenolabrus rupestris* (*n*=57), *Neopomacentrus azysron* (*n*=52), *Pomacentrus amboinensis* (*n*=60), and *Danio rerio* (*n*=49). Individuals are in ascending order of their mean L_R along the x-axis. The repeatability index (*R*) of L_R and its 95% CI are indicated for each species.

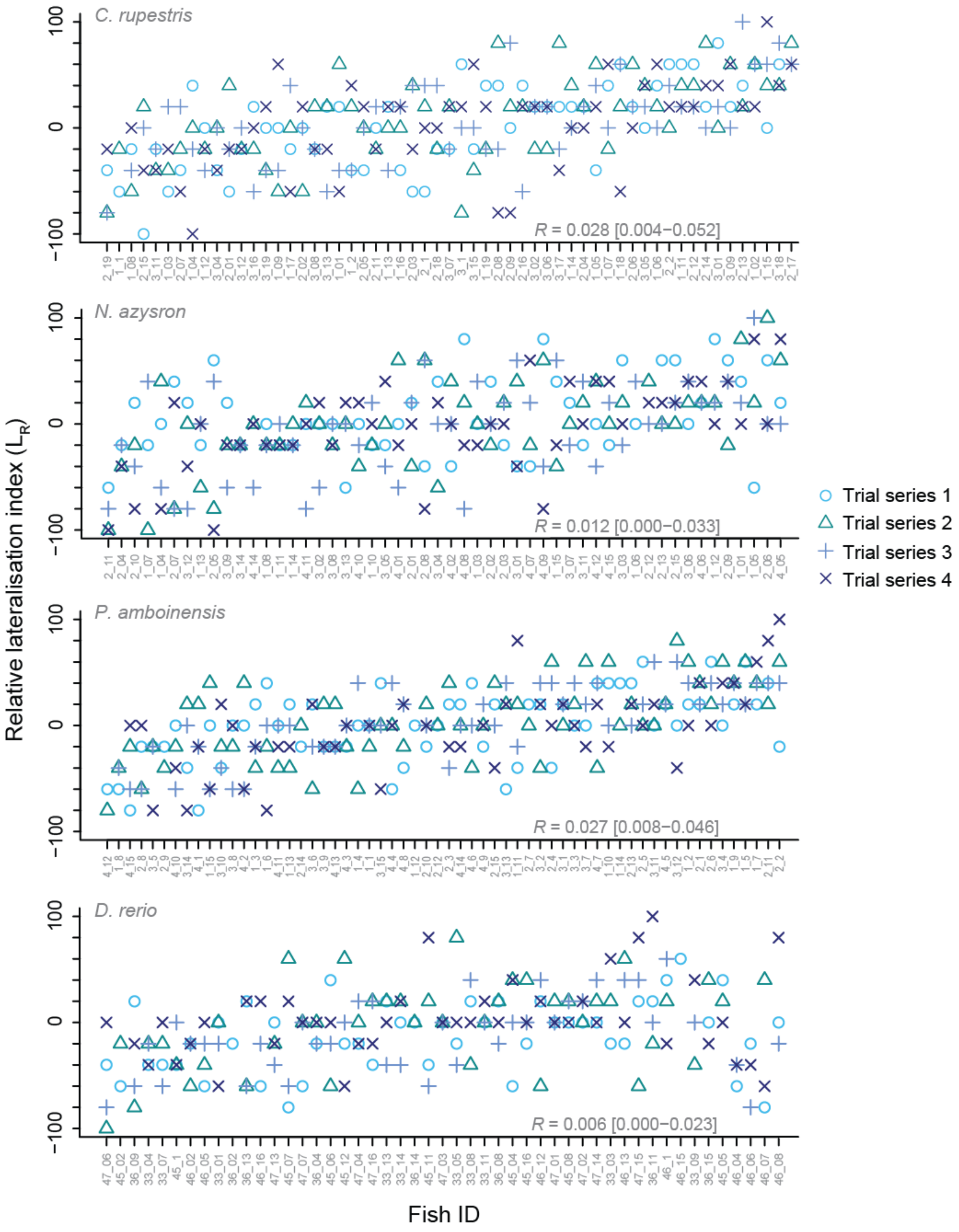


Fig. S2 Absolute lateralisation index (L_A) across repeated trials for four fish species: *Ctenolabrus rupestris* (*n*=57), *Neopomacentrus azysron* (*n*=52), *Pomacentrus amboinensis* (*n*=60), and *Danio rerio* (*n*=49). Individual-level L_A is indicated by the coloured dots connected by lines; the mean (i.e. population-level) L_A and its 95% CI are indicated by black dots and error bars. The repeatability index (*R*) of L_A and its 95% CI are indicated for each species. *R* was computed using the 'rpt' function in the package 'rptR' [44], with a Poisson error distribution. Four series of 10 trials were conducted. Data points are jittered along the x-axis for presentation purposes.

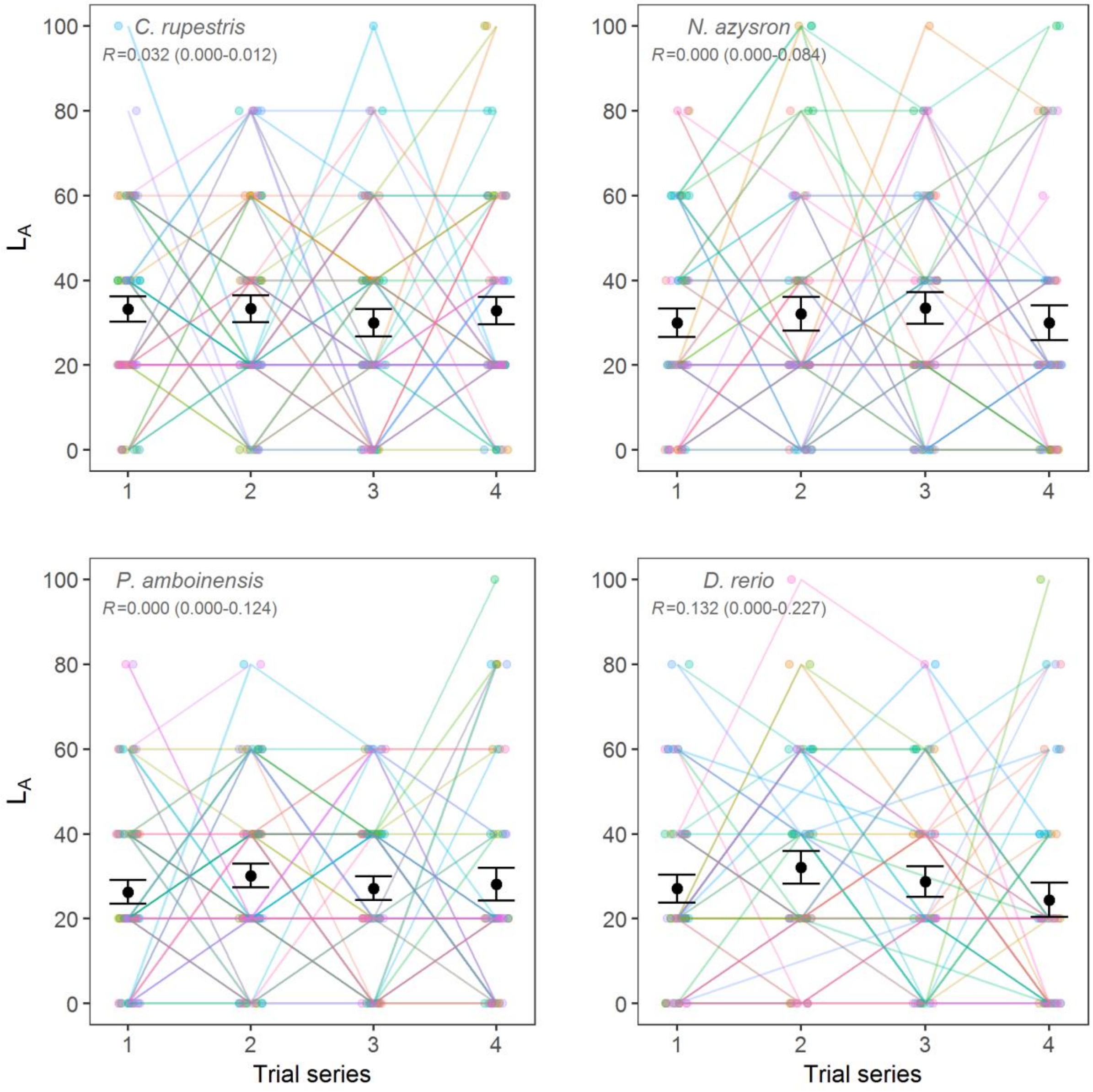


Fig. S3 Relative lateralisation index (L_R) of female guppies (*Poecilia reticulata*) (*n*=40) across three repeated trial series per individual in two different contexts: (**A**) the barrier of the T-maze consists of a neutral stimulus (an empty aquarium behind barrier of vertical bars); (**B**) the barrier of the T-maze consists of a social stimulus (an aquarium containing a conspecific behind a barrier of vertical bars); see Fig. 1 in [17]. Data are from Irving & Brown [32]. Individual-level L_R is indicated by the coloured dots connected by lines; the mean (i.e. population-level) L_R and its 95% CI are indicated by black dots and error bars. The repeatability index (*R*) of L_R and its 95% CI are indicated at the bottom of each panel. Three series of 10 trials were conducted on each individual. Data points are jittered along the x-axis for presentation purposes.

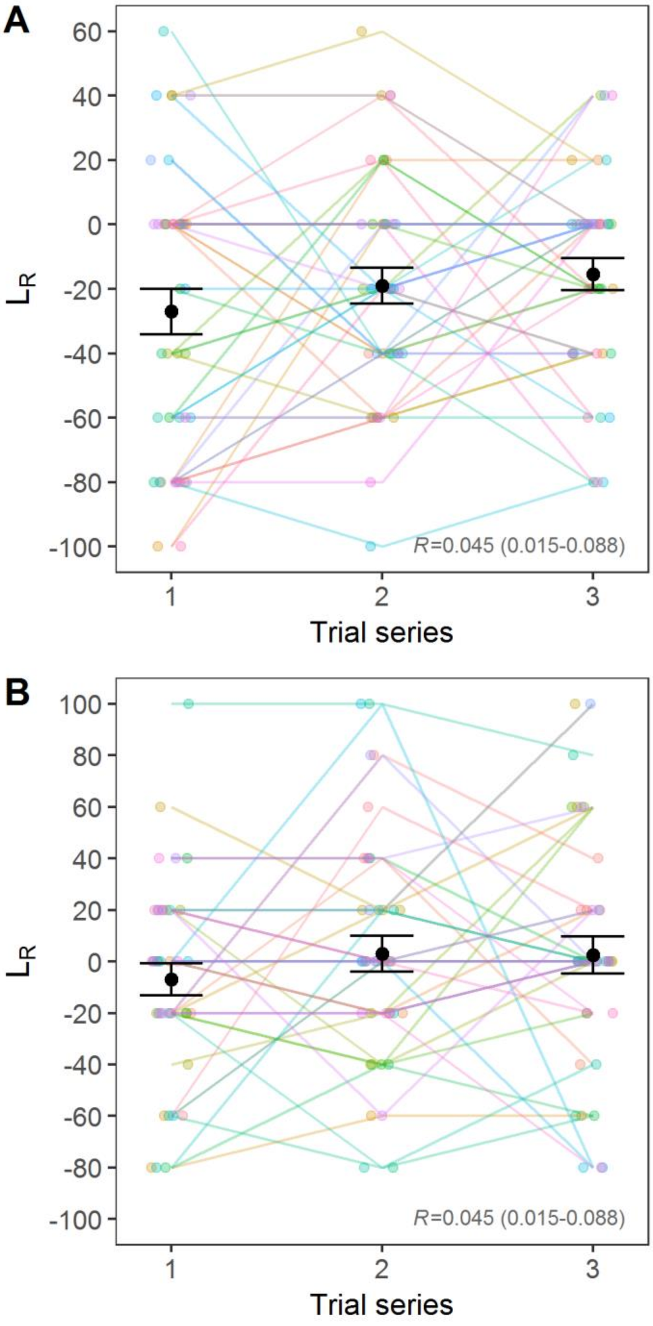


Fig. S4 Frequency distribution of the number of right turns taken by *Ctenolabrus rupestris* (*n*=57) in a detour test (total of 10 turns per individual). The same individuals were tested on four occasions (A, B, C, D) at 48 h intervals. The dashed line represents the mean number of right turns. See Table S2 for statistics.

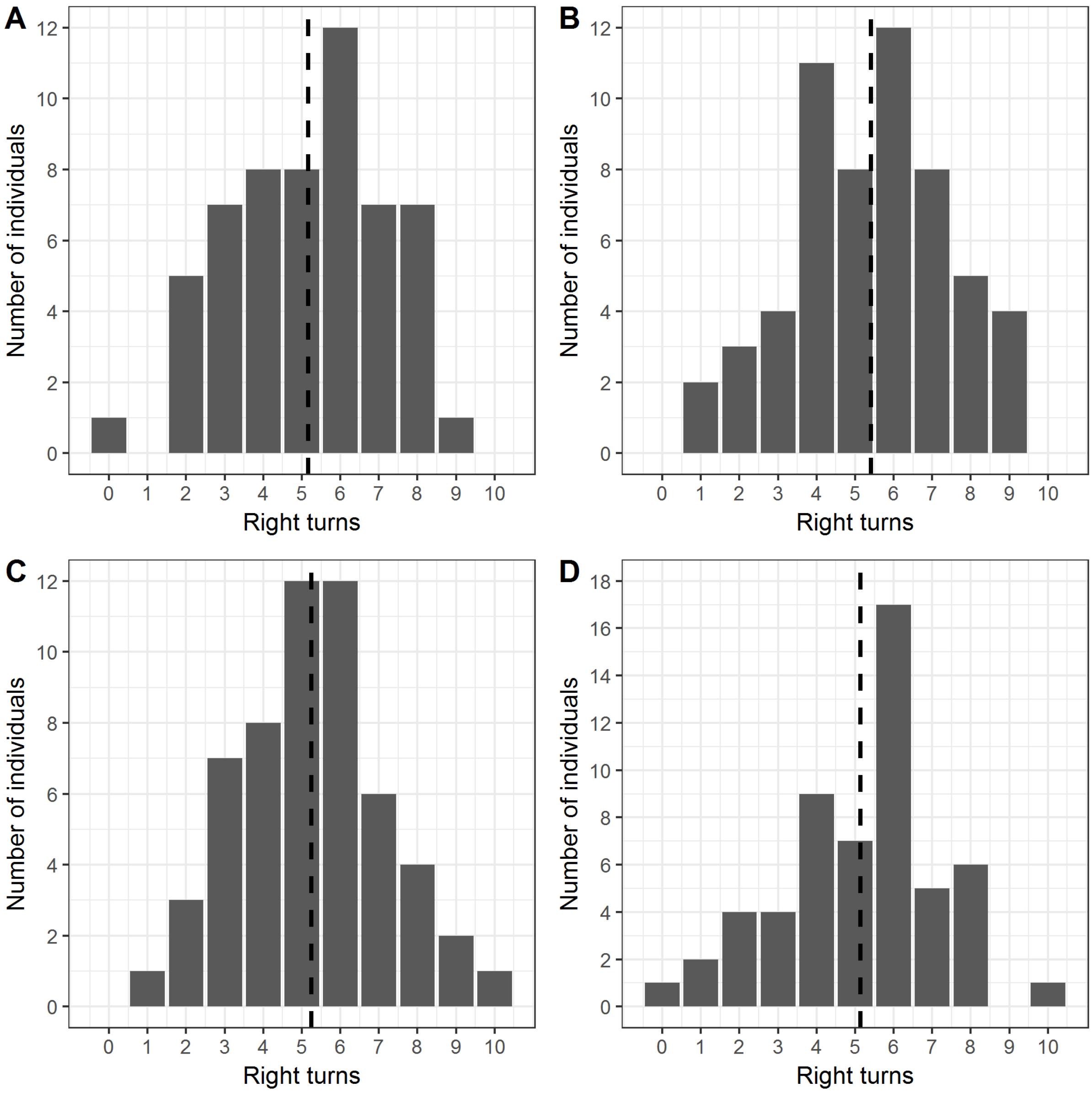


Fig. S5 Frequency distribution of the number of right turns taken by *Neopomacetrus azysron* (*n*=52) in a detour test (total of 10 turns per individual). The same individuals were tested on four occasions (A, B, C, D) at 48 h intervals. The dashed line represents the mean number of right turns. See Table S2 for statistics.

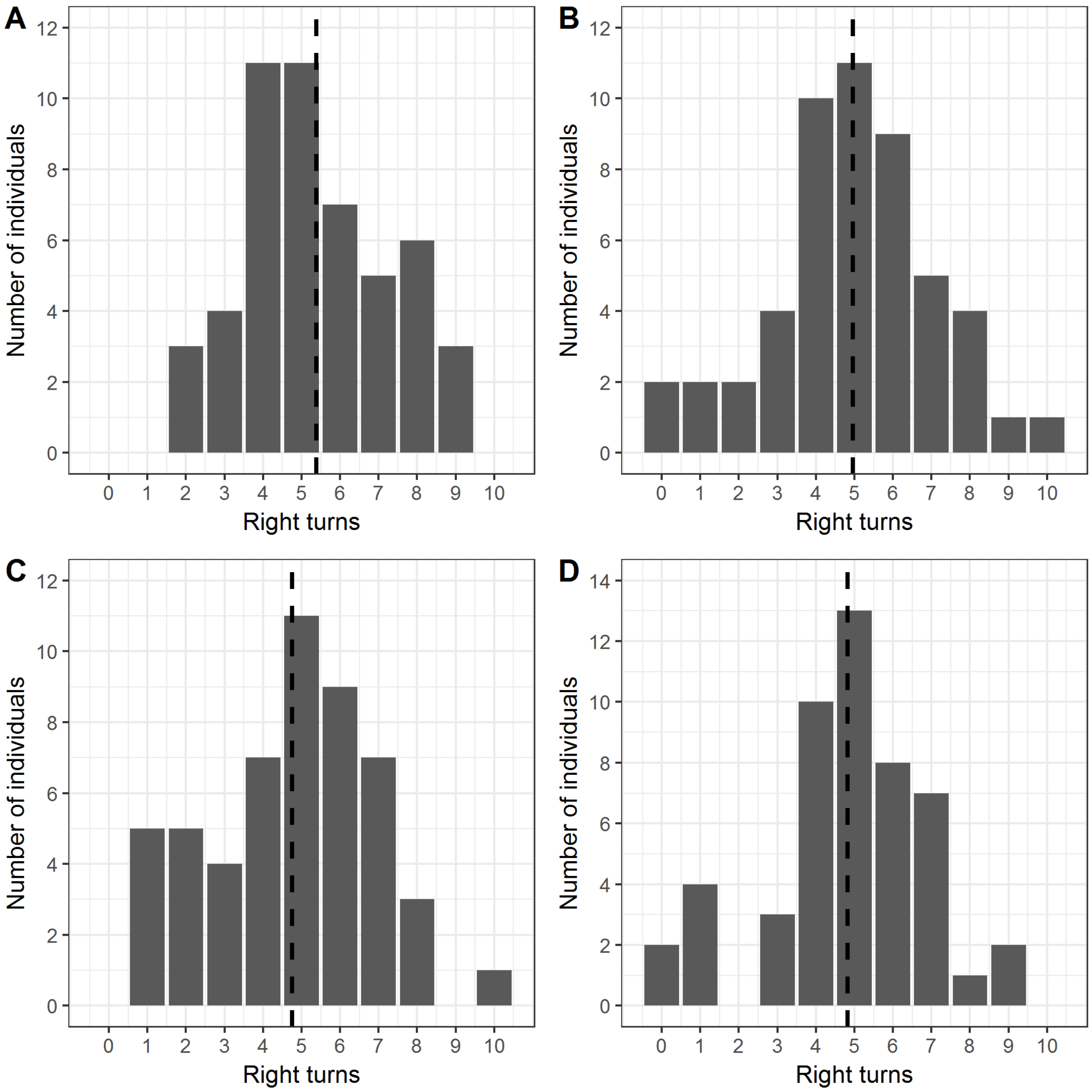


Fig. S6 Frequency distribution of the number of right turns taken by *Pomacentrus amboinensis* (*n*=60) in a detour test (total of 10 turns per individual). The same individuals were tested on four occasions (A, B, C, D) at 48 h intervals. The dashed line represents the mean number of right turns. See Table S2 for statistics.

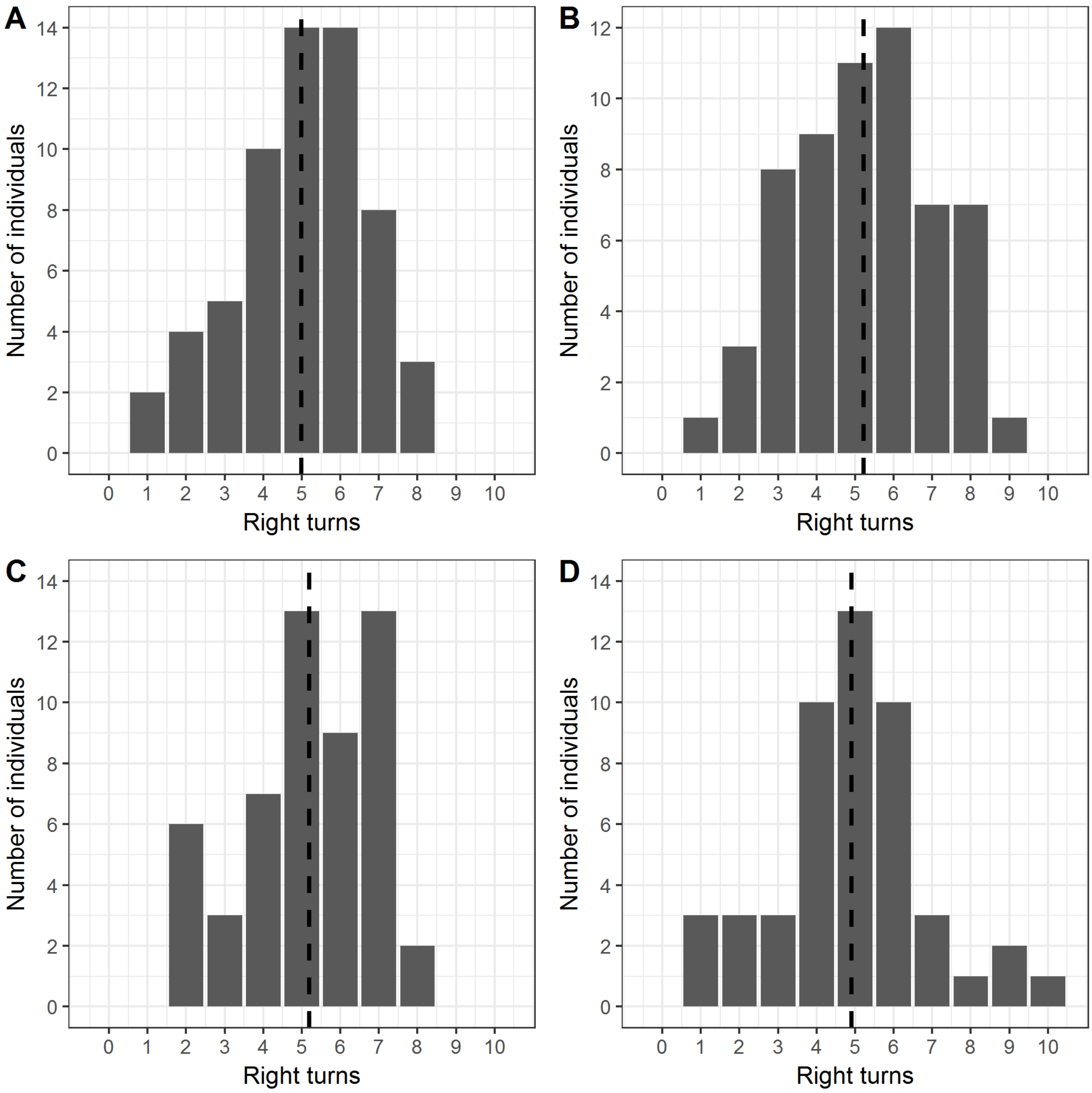


Fig. S7 Frequency distribution of the number of right turns taken by *Danio rerio* (*n*=49) in a detour test (total of 10 turns per individual). The same individuals were tested on four occasions (A, B, C, D) at 48 h intervals. The dashed line represents the mean number of right turns. See Table S2 for statistics.

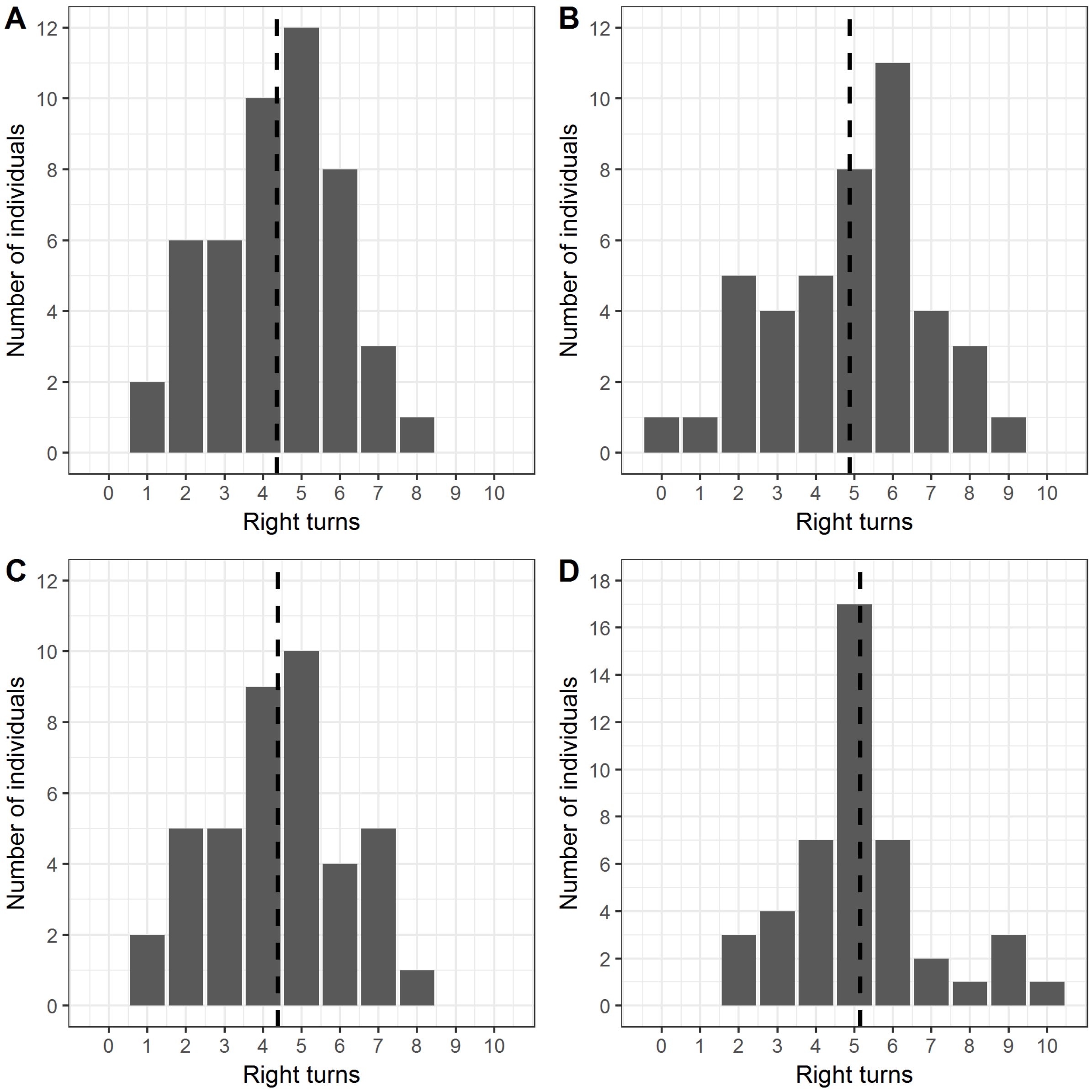
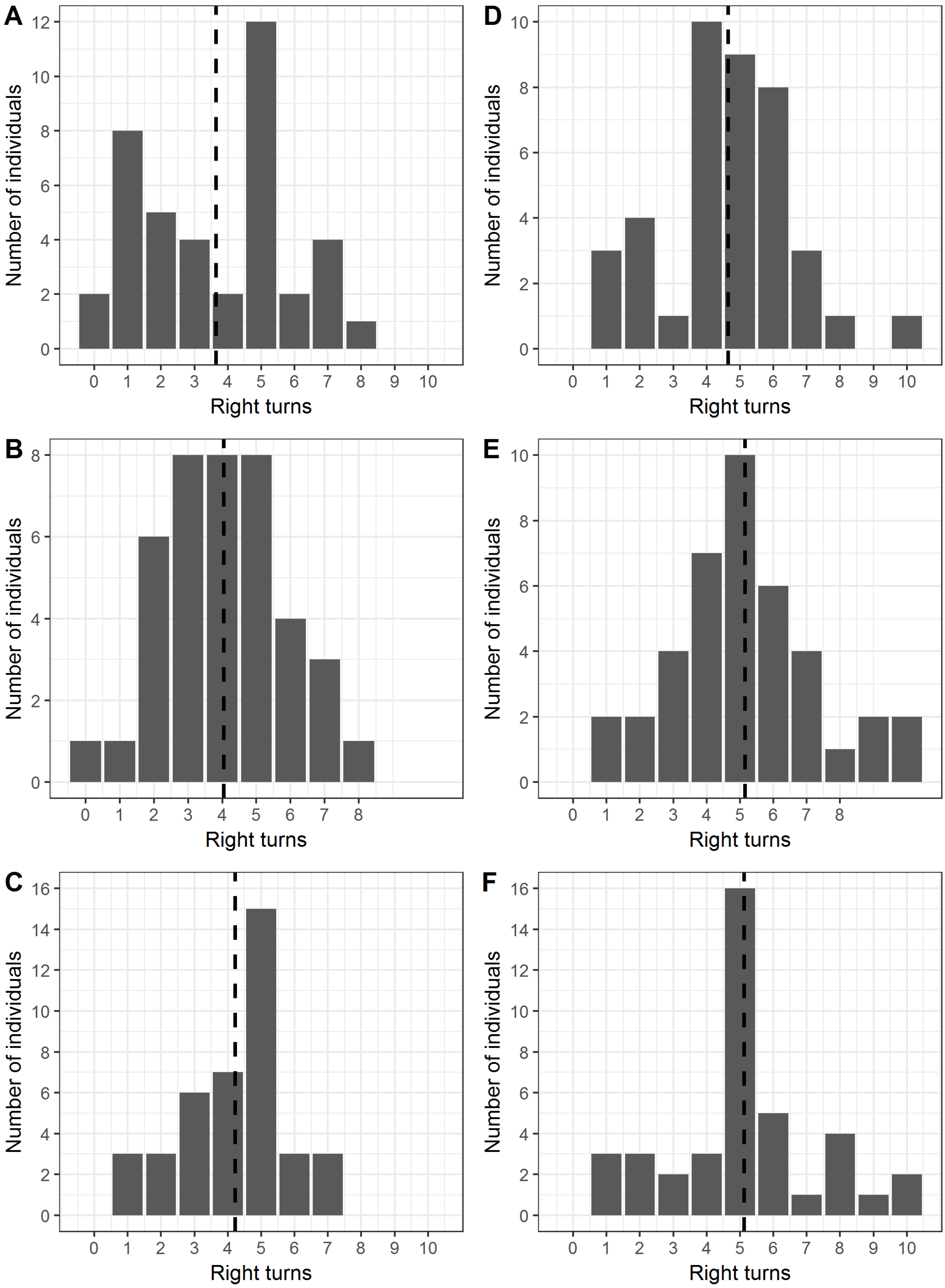


Fig. S8 Frequency distribution of the number of right turns taken by *Poecilia reticulata* (*n*=40) in a detour test (total of 10 turns per individual). The same individuals were tested on three occasions using either a neutral (A, B, C) or a social (D, E, F) stimulus. The dashed line represents the mean number of right turns. See Table S2 for statistics.



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