RESEARCH ARTICLE

Behavioural responses of threespine stickleback with lateral line asymmetries to experimental mechanosensory stimuli

Nicholas P. Planidin and Thomas E. Reimchen*

ABSTRACT

Behavioural asymmetry, typically referred to as laterality, is widespread among bilaterians and is often associated with asymmetry in brain structure. However, the influence of sensory receptor asymmetry on laterality has undergone limited investigation. Here we used threespine stickleback (*Gasterosteus aculeatus*) to investigate the influence of lateral line asymmetry on laterality during lab simulations of three mechanosensation-dependent behaviours: predator evasion, prey localization and rheotaxis. We recorded the response of stickleback to impacts at the water surface and water flow in photic conditions and low-frequency oscillations in the dark, across four repeat trials. We then compared individuals' laterality with asymmetry in the number of neuromasts on either side of their body. Stickleback hovered with their right side against the arena wall 57% of the time ($P<0.001$) in illuminated surface impact trials and 56% of the time ($P=0.085$) in dark low-frequency stimulation trials. Light regime modulated the effect of neuromast count on laterality, as fish with more neuromasts were more likely to hover with the wall on their right during illumination ($P=0.007$) but were less likely to do so in darkness ($P=0.025$). Population-level laterality diminished in later trials across multiple behaviours and individuals did not show a consistent side bias in any behaviours. Our results demonstrate a complex relationship between sensory structure asymmetry and laterality, suggesting that laterality is modulated by multiple sensory modalities and is temporally dynamic.

KEY WORDS: Laterality, Sensory receptor asymmetry, Side preference, Predator evasion, Rheotaxis, Prey localization

INTRODUCTION

Behavioural laterality, the preferential use of one side of the body for a given task (Rogers et al., 2013), is widespread among vertebrates (Malashichev, 2006; Miletto Petrazzini et al., 2020; Rogers, 2002) and bilateral invertebrates (Frasnelli, 2013; Frasnelli et al., 2012). By specializing in the use of one side of the body, individuals can perform better in tasks such as object discrimination (Güntürkün et al., 2000; Matrai et al., 2019; Mehlis-Rick et al., 2018), predator evasion (Dadda et al., 2010; Heuts, 1999; Lippolis et al., 2002) and object manipulation (Magat and Brown, 2009; McGrew and Marchant, 1999), giving them a fitness advantage (Vallortigara and Rogers, 2020). Additionally, social individuals can influence the side preference of others (Bisazza and Dadda, 2005; Deng and Rogers, 2002; Karenina et al., 2018; Roux et al.,

2016), e.g. mammalian mothers tend to orient with the mother on the left and the infant on the right (Karenina et al., 2017). Whether population laterality – a bias of the majority of individuals in a population to one side – arises through social or non-social mechanisms, it may incur a competitive advantage in interspecific interactions (Frasnelli and Vallortigara, 2018; Ghirlanda and Vallortigara, 2004; Lehman, 1981).

Behavioural laterality can be associated with morphological differences between the two hemispheres of the brain, in which one hemisphere becomes specialized for a given task (Rogers, 2000; Rogers and Andrew, 2002). This morphological asymmetry can improve the efficiency of neural tissue use (Levy, 1977), enable parallel processing (Rogers et al., 2004) and help resolve conflicting sensory information between the two sides (Vallortigara, 2000), all of which may have ramifications for behaviour. There is also a growing body of evidence suggesting that asymmetry in sensory structures influences behavioural laterality (Anfora et al., 2011; Fernandes et al., 2018; Hart et al., 2000; Krings et al., 2019; Lychakov et al., 2006, 2008; Werner and Seifan, 2006) and may play a role in determining which side becomes behaviourally dominant. The ubiquity of subtle morphological asymmetries across taxonomic groups (Clarke et al., 2000; Fey et al., 2020; Hart et al., 2000; Trokovic et al., 2012; Werner and Seifan, 2006) suggests that sensory receptor asymmetry may be a widespread mechanism for the development of behavioural laterality.

This study aimed to determine whether recently documented asymmetry in the lateral line of threespine stickleback (*Gasterosteus aculeatus*) influences behaviour. The lateral line is a mechanosensory organ composed of a series of neuromasts that detect displacement of the surrounding water over the body, either by acceleration-sensitive canal neuromasts or by velocity-sensitive superficial neuromasts (Coombs et al., 2014). Threespine stickleback only have superficial neuromasts (Wark and Peichel, 2010) and therefore are expected to be sensitive to velocity but not acceleration. The lateral line is important for rheotaxis in stickleback (Jiang et al., 2017) and is used by many species of fish during nocturnal foraging (Montgomery and Milton, 1993; Pohlmann et al., 2004; Schwarz et al., 2011); however, investigations of nocturnal foraging in stickleback have predominantly focused on olfaction (Baer et al., 2021; Mussen and Peeke, 2001). Threespine stickleback from ecologically distinct habitats differ in the number of neuromasts on their head and trunk (Ahnelt et al., 2021; Planidin and Reimchen, 2019; Wark and Peichel, 2010) as well as in the bilateral asymmetry of neuromast numbers along their trunk (Planidin and Reimchen, 2021). Stickleback can also exhibit directional asymmetry in bony structures – e.g. lateral plate numbers in a population exposed to asymmetric predator–prey interaction geometry (Bergstrom and Reimchen, 2002, 2003; Reimchen, 1997; Reimchen and Bergstrom, 2009; Reimchen and Nosil, 2001a,b) – and undergo extensive scaring and abrasion throughout their life history due to failed predation events and nest construction in males (Bergstrom and

Department of Biology, PO Box 3020, University of Victoria, Victoria, BC, Canada, V8W 3N5.

*Author for correspondence (reimchen@uvic.ca)

N.P.P., 0000-0003-4182-0841; T.E.R., 0000-0002-5578-8531

Reimchen, 2003; Reimchen, 1988). These aspects of stickleback ecology suggest that interactions between morphological asymmetry and behavioural laterality may have important functional consequences during their life history, regardless of the mechanism that produces asymmetry in the sensory structure.

Population-level laterality differs greatly among behaviours and contexts (Basile et al., 2009; Bisazza et al., 1997, 1998, 1999; Blois-Heulin et al., 2012; De Santi et al., 2001; Domenici et al., 2014; Lai et al., 2015; McLean and Morrell, 2021; Poyser et al., 2006; Yeater et al., 2014); therefore, testing a single population in different contexts will provide a more complete understanding of its laterality and how it relates among behaviours. To sample individual behaviour from a wide degree of contexts, we assessed behavioural laterality in simulations of three different behaviours: predator evasion, rheotaxis and prey localization by mechanosensation. These are known in other species to be associated with lateral line morphology or neuromast count (Yoshizawa et al., 2010; Olszewski et al., 2012; Jiang et al., 2017), making them candidate behaviours for testing the interaction between sensory structure asymmetry and behavioural laterality. Following the testing of each individual four times for each behaviour, we compared lateral line asymmetry with behavioural laterality. By assessing laterality in multiple kinematic metrics during these behaviours, we aimed to understand the degree to which individuals express an overall side preference and the relationship between sensory receptor asymmetry and laterality among behavioural contexts.

MATERIALS AND METHODS
Stickleback collection
We collected 77 adult threespine stickleback (*Gasterosteus aculeatus* L.) from Eagle's Lake, BC, Canada (latitude: 48.5088, longitude: −123.4633) during multiple trips to the field in September of 2021, after the reproductive season for this population. They were captured using minnow traps and transported to the University of Victoria Aquatics Facility in aerated 19 l buckets. All stickleback were housed together in a single 189 l tank at 15°C, with artificial habitat enrichment, on a 12 h:12 h light:dark cycle, and fed on a diet of bloodworms once daily. Fish were haphazardly selected from the housing tank by dip net and transported to the experimental tanks in an aerated 19 l bucket.

Evasion of a simulated predator attack experiment
We tested the evasion of a simulated predator attack, hereafter referred to as predator evasion, by releasing a stream of water adjacent to stickleback in a circular arena (Fig. S1A, Movie 1). Arenas consisted of 19 l buckets with the base cut off, mounted on Plexiglas sheets with silicon. We inserted a mesh 2 cm from the edge of the bucket to allow us to provide stimuli from both sides, even if the stickleback was against the side of the arena (Fig. S1A). We placed a GoPro Hero4 and SJcam4000 below the left and right tanks, respectively (from the observer's perspective), recording 1280×720 footage at 60 frames s^{-1}. We mounted a white sheet above the tanks to provide a consistent background, and after the first four groups, we mounted a mirror at 45 deg above each tank to see the fish without looming over the tank. No behavioural metrics were significantly different between trials conducted with and without the mirror (all $\chi_1^2 \leq 2.43$, $P \geq 0.119$), so their results were pooled for analysis.

We placed fish in the apparatus, and allowed them to acclimate for 5 min. Following acclimation, we dropped 10 ml of water from a stick-mounted turkey baster from a height of 18 cm. We bored a small hole into the baster and attached a rubber hose, so water was consistently released in a continuous stream by removing one's thumb from the top of the hose (Fig. S1A). A 'drop' was repeated every 90 s for 11 drops, with the sixth drop being a control with no water released. We alternated positioning between sides, with the first side counterbalanced within pairs and distance haphazardly determined, usually placed about 2 cm from the fish's midsection. The leftmost tank always received the drop first.

Vibration attraction behaviour experiment
We initially tested 10 stickleback to determine the best frequencies for assessing vibration attraction behaviour (VAB), the tendency for fish to approach an oscillating glass rod in the absence of light, first described by Yoshizawa et al. (2010). The VAB tank consisted of the midsection of a 19 l bucket affixed to a piece of Plexiglas with silicone. The tank was placed on top of cinderblocks, separated by vibration-absorbing foam, illuminated with an ITT IR Illuminator (850 nm) and viewed by an ITT mini Monocular NIGHT-VISION SCOPE mounted onto the lens of a GoPro Hero4, taking a time-lapse video at 0.667 images s^{-1} (Fig. S2A, Movie 2). Oscillations were generated by a Speaker Craft MTR1C, connected via a Yamaha RS-V395 receiver and a Scarlet 2i2 audio interface to a MacBook Pro, generating tones with the oscillator plugin within Ableton Live 9. Sound pressure was converted to mechanical displacement by affixing a glass rod to the speaker diaphragm dust cap, which we centred above the tank. We filled the tank with 6 cm of water and extended the glass rod 1 cm into the water.

We placed a plastic lid on the tank and turned off the room's lights for 5 min prior to testing to allow the stickleback to acclimate to the setup. The infrared light shone through the plastic lid. Each stickleback was exposed to 10 Hz intervals from 20 to 100 Hz for 3 min in random order and 3 min of silence in between stimuli. We selected this frequency range because our speaker was unable to produce oscillations lower than 20 Hz without distortion and as superficial neuromasts are unlikely to be sensitive to stimuli above 100 Hz (Coombs et al., 2014). Following the fifth trial, we refreshed the water, and gave the stickleback another 5 min to re-acclimate.

We calibrated the amplitude and frequency of oscillations with fast Fourier transform analysis (R library *GeneCycle*; https://CRAN.R-project.org/package=GeneCycle) of 240 frames s^{-1} video footage of the glass rod, verifying the rod was oscillating at the same frequency as the speaker and adjusting speaker volume so that displacement was 3 mm for all frequencies: 20 and 60 Hz elicited similar numbers of approaches and more than other frequencies, so they were both used in subsequent testing.

We tested the laterality of VAB using the same setup as for preliminary testing; however, we doubled up the apparatus after testing the first four groups (Fig. S2A). Stickleback were acclimated to the dark for 6 min, with behaviour recorded after the first 3 min as a control, followed by 3 min of exposure to either a 20 or 60 Hz oscillation. We then repeated the same procedure with the other frequency. We initiated video capture at the beginning of acclimation, and the room was kept dark throughout each VAB trial. No behavioural metrics were significantly different after doubling up the testing apparatus (all $\chi_1^2 \leq 3.40$, $P \geq 0.065$), so their results were pooled for analysis.

Rheotaxis experiment
We assessed rheotaxis behaviour in two circular tracks, with water flowing clockwise or counterclockwise (Fig. S3A, Movie 3). We placed two Sicce Mi-Mouse circulation pumps in a reservoir shared by both tanks, feeding into each track by a rubber hose, and we cut an outlet hole out of each track 90 deg upstream of the flow source

and covered it with a fine mesh. We mounted a GoPro Hero3+ above each track and recorded a time-lapse video at 0.667 images s^{-1}. We filled the tracks to 6 cm depth and calibrated flow to average 0.08 m s^{-1} in each track (∼0.2 m s^{-1} at the outer edge and ∼0–0.04 m s^{-1} at the inner edge), similar to the range used by Jiang et al. (2017), which was based on flow rate from a natural stream containing stickleback. We placed stickleback in each track and allowed them to acclimate to still water for 5 min. Then they were acclimated to flow for 5 min, and we then recorded 5 min of behavioural footage. We initiated video capture at the beginning of acclimation, and we started the pumps with a switch hidden from view so as not to disturb the fish's behaviour.

Counter-balance design and repeat testing

We tested 40 fish for each behaviour in four repeat trials in a counter-balanced design (example in Fig. 1; for details see Table S1). We conducted two trials each day for 2 days, with at least a 4 h rest period in between. We tested fish in groups of four (two pairs). We always tested VAB first; if the first pair received 20 Hz then 60 Hz, the other pair would receive 60 Hz then 20 Hz. We reversed the order of stimuli in the subsequent trial, and the day's sequence would be reversed on the second day. We tested one pair for rheotaxis first, with one receiving clockwise flow and the other counterclockwise flow. In the next trial, we reversed the order and tested rheotaxis after predator evasion. The next day we tested flow regimes in the opposite order and the alternative sequence relative to predation evasion testing. We did the same alternation for placement in the two predator evasion arenas and the first side to receive a drop. The sequence of trials was such that each fish completed each behavioural test in each combination of orders. In between experiments, fish were individually housed in 19 l tanks with a plastic aquarium plant and polyvinyl chloride (PVC) pipe shelter, a slow stream of fresh water feeding into the tank, and a plastic cover to reduce stress. We withheld feeding during the 2 day testing period, and the same investigator conducted all behavioural trials to minimize differences among trials.

Microscopy

Within 24 h of behavioural testing, the lateral line of live fish was viewed using fluorescence microscopy. We stained the lateral line using 2-[4-(dimethylamine)styryl]-N-ethylpyridinium iodide (DASPEI) using the procedure from Wark and Peichel (2010). We suspended DASPEI in dH$_2$O to a concentration of 0.038%, then diluted this solution to 0.025% concentration with aquatics facility water. Fish could freely swim in the staining solution for 15 min, then were rinsed with fresh water and anaesthetized in 0.016%

tricaine methylsulfonate (MS-222) solution until motionless and breathing shallowly. Following anaesthesia, fish were placed in a deep Petri dish containing 0.005% MS-222 solution and viewed with an Olympus SZX-ILLD2-100 fluorescence microscope, illuminated by an Olympus U-LH100HGAPO broad-spectrum UV light source and filtered by an Olympus SZX-MGF filter (excitation 460–490 nm; emission 510 nm longpass). All individuals were kept under the microscope for less than 10 min, well within the decay time of DASPEI fluorescence.

Neuromasts were counted first on the left and then on the right side. Counts were divided into continuous rows of neuromasts, hereafter referred to as stitches, as described by Wark and Peichel (2010), but the mandibular (MD) stitch was divided into two sublines (Fig. 2). We scored MD1 on the dentary and MD2 on the preopercular bone separately as they were easily separable, and the MD1 neuromasts were often abraded (Fig. 2). We also extended the main anterior trunk (Ma) stitch to include the eighth lateral plate rather than stopping at the seventh and recorded neuromast counts on the fourth to eighth lateral plates individually. Following viewing, we euthanized the fish with an overdose of 0.025% MS-222, severed their isthmus, and preserved them in 70% ethanol. Following a few days of preservation, we scored fish for lateral plate count, standard length, sex and the presence of parasites. The same investigator conducted all scoring to minimize bias.

Video processing

We converted VAB and rheotaxis images to video using ImageJ macros, cropping out the clips of interest from the full footage and dividing VAB videos into control and stimuli clips. Videos were also cropped to the test tank to minimize future computation. We manually annotated predator evasion videos for the first frame each drop contacted the water surface (impact frame). We then extracted the impact frames and 11 s of footage (1 s before and 10 s after) for each drop using a Python script. We converted impact frames into a video with ImageJ and manually annotated each drop's position using DLTdv8 (Hedrick, 2008).

We annotated the position of the tip of the snout and the midsection between the pectoral fins in all videos using DeepLabCut (Mathis et al., 2018). We manually annotated the initial training frames, selecting up to 20 frames using k-fold selection from 100 predator evasion clips, 50 VAB clips and 10 rheotaxis videos. We iteratively assessed each model's tracking quality and retrained with additional 'outlier' training frames. We corrected errors in the tracking of the final neural networks in DLTdv8.

We corrected for camera distortion and angle by using the edge of each tank as a reference. We annotated 30 points around the
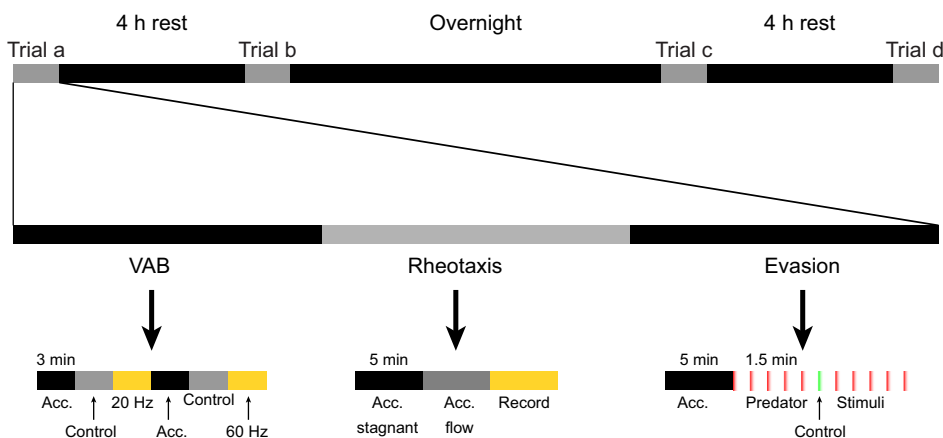
Fig. 1. Example experimental sequence for one group of stickleback being tested for vibration attraction behaviour (VAB), rheotaxis and response to a simulated predatory stimulus (evasion). Acc., acclimation.
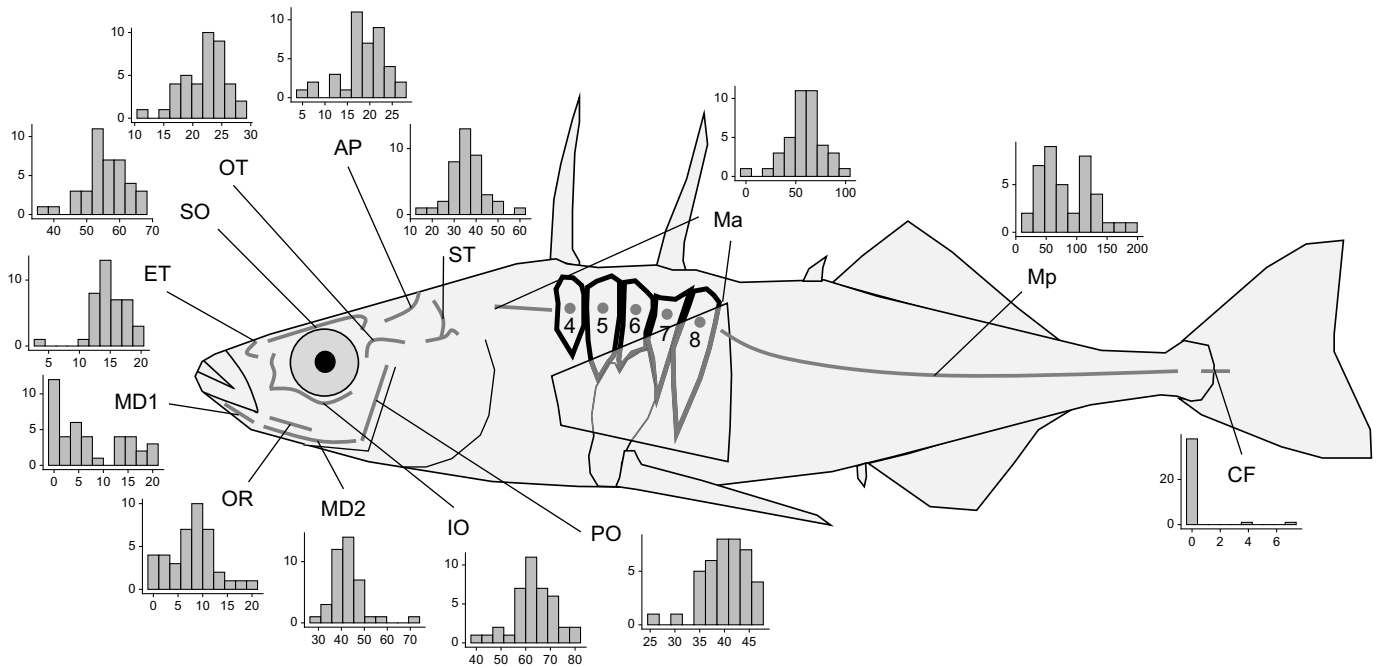
Fig. 2. Diagram of lateral line stitches of threespine stickleback. MD1, mandibular one/dentary; MD2, mandibular two/lower preopercular; OR, oral; IO, infraorbital; PO, preopercular; ET, ethmoid; SO, supraorbital; OT, otic; AP, anterior pit; ST, supratemporal; Ma, main trunk line anterior; Mp, main trunk line posterior; and CF, caudal fin. Numbers (4–8) indicate position of lateral plates. Histograms are the sum of neuromast counts on both sides for a given stitch.

perimeter of each tank for each video and fitted an ellipse to these points; then, we compressed the fish's positions along the major axis of the ellipse to the length of the minor axis (see Fig. S1B).

For predator evasion videos, we divided drops into those on the left and right sides and calculated velocity for each frame (see Fig. S1B for equations). As velocity data are noisy, we used a rolling average with a window size of five frames to calculate maximum velocity. A fish was classified as having initiated an escape response if it reached at least 0.2 m s^{-1}. We chose this threshold as it matched the proportion of C-start escapes observed in previous predator evasion experiments. We also tabulated time to reach 0.2 m s^{-1} (EscTime) using the rolling average velocity.

For VAB videos, we calculated distance travelled as the difference in position of the snout between frames, and angles were calculated relative to the position of the glass rod (see Fig. S2B for equations). An 'approach' occurred when the stickleback got within 5 cm of the glass rod (roughly one body length), with a 3-frame buffer in between approaches to prevent double-counting from noise in position data. Approach side and wall side (the side facing the outer wall of the tank most often) were categorized as left or right, as both were highly bimodal.

As in Jiang et al. (2017), we looked at four metrics of rheotaxis behaviour; net displacement (+upstream, −downstream), cumulative upstream movement, time oriented upstream and flow regime selection (distance from outer edge) (see Fig. S3B for equations). However, we calculated displacement as the change in angle around the track and used distance from the outer edge, rather than calculating the proportion of the fish's movement tangential to the track and partitioning flow regime selection into bins.

Statistical analyses
Predator evasion
We assessed four aspects of predator evasion behaviour for laterality: the side against the outer wall of the arena, whether escape response was initiated, EscTime and maximum velocity

(see Table 1 for data transformations and error structures). We tested the effect of neuromast count $[NC=\Sigma(R_s+L_s)]$ and directional neuromast count asymmetry $[DA=\Sigma(R_s-L_s)]$ on laterality of wall side selection using backwards model selection by Wald's Chi-square test ($\alpha=0.05$) with Eqn 1 and all other behaviours with Eqn 2 (Table 1; Murtaugh, 2009). R_s and L_s are the number of neuromasts on the right and left sides of stitch S, respectively. Previous analyses (Planidin, 2021) found no significant effect of sex, standard length and absolute neuromast count asymmetry $[AA=\Sigma(|R_s-L_s|)]$, lateral plate count and lateral plate asymmetry, so we did not include these traits in the analysis (see Table S2 for a summary of population morphology). Group was not included as a random effect as its variance converged to zero and resulted in a singular fit for most models. We included drop number as a continuous predictor as differences among drop numbers were approximately linear for all behaviours. If NC or DA significantly influenced escape behaviour, we substituted the sum over all stitches with each individual stitch in the reduced model equation, e.g. if there was a significant effect of DA on EscTime, we would model EscTime using R−L neuromast counts for each stitch individually plus any other factors that had a significant effect on EscTime. P-values from individual stitch analysis were Bonferroni corrected ($n=13$) to account for multiple comparisons. Models were fitted with either the *lme4* (Bates et al., 2015) or *glmmTMB* (Brooks et al., 2017) packages within R 3.6.3 (http://www.R-project.org/; see Table 1).

VAB
We tested two aspects of VAB behaviour for the effect of 20 and 60 Hz stimuli: distance travelled and the number of approaches; and two metrics of laterality: wall side and approach side (see Table 1 for data transformations and error structure). We compared non-lateralized and lateralized behaviours with morphological characteristics using Eqns 3 and 4, respectively (Table 1). Analysis of lateralized and non-lateralized behaviours had to be separated for VAB analysis as laterality could not be encoded as an interaction

Table 1. Summary of statistical model structures

Experiment	Equation	Metric	Transform	Residual distribution	Link function
Predation	1	Wall side	None	Binomial	logit
	2	Escape	None	Binomial	logit
	2	Time to escape	Cube root	Gaussian	none
	2	Maximum velocity	ln	Gaussian	none
VAB	3	Distance travelled	Cube root	Gaussian	none
	3	No. of approaches	None	General Poisson	log
	4	Wall side	None	Binomial	logit
	4	Approach side	None	Binomial	logit
Rheotaxis	5	Net displacement	Cube root	Gaussian	none
	5	Upstream movement	ln	Gaussian	none
	5	% Upstream orientation	None	Binomial	logit
	5	Outer edge distance	None	Beta	logit

Model:	
Predation	Eqn 1: Y=NC+DA+drop+*trial*+(1 | *individual ID*)
	Eqn 2: Y=*drop side*×(NC+DA+*wall side*+drop+*trial*)+(1 | *individual ID*)
VAB	Eqn 3: Y=*stimulus*×(NC+*trial*)+(1 | *individual ID*)
	Eqn 4: Y=NC+DA+*trial*+(1 | *individual ID*)
Rheotaxis	Eqn 5: Y=*direction/track*×(NC+DA+trial)+(1 | *individual ID*)

For the models, italicized predictors are categorical whereas non-italicized predictors are continuous. NC, neuromast count; DA, directional asymmetry in neuromast count (R–L).

Transform is the transform applied to the data prior to fitting the model. Gaussian models were fitted with the function *lmer*, binomial models were fitted with *glmer* and general Poisson and beta models were fitted with *glmmTMB*.

effect, e.g. drop side in the predator evasion experiment. We tested individual stitches the same way as with predator evasion.

Rheotaxis
We examined four metrics of rheotaxis behaviour for laterality (see Table 1 for data transformations and error structure). We tested the effect of morphology on lateralized and non-lateralized behaviour using Eqn 7 (Table 1). All models underwent the same backwards model selection procedure, and we tested individual stitches in the same way as predator evasions and VAB trials.

Repeatability of behaviour
We tested the consistency of non-lateralized and lateralized behaviours across the four repeat trials using the *rptR* package (Stoffel et al., 2017). We included trial as a fixed effect and individual ID as the random effect for which repeatability was assessed in all *rptR* models. For the predator evasion experiment, we tested the repeatability of initiating predator evasion, wall side selection and the proportion of escapes initiated in response to drops from the right as binary characteristics. We modelled EscTime and maximum velocity as Gaussian and did not calculate the repeatability of laterality for these behaviours. For the VAB experiment, we tested the repeatability of distance travelled and the number of approaches with Gaussian and Poisson models, respectively. We tested VAB laterality by modelling the number of right-hugging 3 min intervals and right side approaches as proportions. We modelled all rheotaxis behaviours as Gaussian and did not test for repeatability of rheotaxis laterality. We tested correlation in laterality among behaviours exhibiting the greatest laterality with the *psych* package (Revelle, 2020) after summing or averaging the behaviour of each individual.

Ethics statement
This research was undertaken and approved through the University of Victoria Animal Use for Research Protocol 2019-021(1), which follows the guidelines set by the Canadian Council for Animal Care. All individuals were kept in captivity for a maximum of 3 months. They were housed in the same tank and allowed to school in an enriched habitat. All individuals were euthanized as part of experimental procedure.

RESULTS
Statistical output of model selection for all behaviours is presented in Table 2.

Predator evasion
Fish 'hugged' the arena wall with their right side in 56.7% of drops and hugged the left in 43.3% of drops. Fish with fewer neuromasts were more likely to hug the right wall (Fig. 3A), and the preference for hugging the right wall was greatest during trial a (Fig. 3B). Twelve of thirteen stitches negatively correlated with a bias towards right wall hugs, and the main posterior trunk (Mp) stitch had the strongest and only significant correlation (P_{adj}=0.013; β+s.e.=−0.37+0.11; Table S3).

Fish never initiated an escape response during control drops, but 6% of escape responses were initiated before drop impact during drops where water was released. The rate of escape behaviour initiation was highly variable among individuals, ranging from individuals that never initiated an escape response to others which initiated an escape response more than half of the time (Fig. 4A). On average, an escape response was initiated 9.5% [95% confidence interval (CI): 6.6%, 13.3%] of the time. Escape was initiated less frequently in subsequent drops within trials (log-odds+s.e.=−0.25+0.03) and in later trials (Fig. 4C). Fish initiated escape behaviour 10.7% [95% CI: 7.6%, 14.7%] of the time when we placed the drop on the right and 8.2% [95% CI: 5.7%, 11.6%] of the time when the drop was on the left. Fish with more neuromasts initiated an escape response less frequently, with a stronger relationship for drops on the left side (Fig. 4A); however, there was a wide range of response rates for fish with any given neuromast count. Escape responses occurred more frequently with the arena wall on the left and the drop on the right, relative to other wall and drop positions (Fig. 4B). There was a right bias in escape response in all trials except trial d (Fig. 4C). The fish's probability of initiating an escape response did not differ with their DA. Neuromasts counts in 12 of 13 stitches had a negative association with escape

Table 2. Statistical significance of predictors after model selection

	d.f.	Statistical significance (χ^2, P)							
Predation		Wall side		% Escape		EscTime		Max. velocity	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
drop side	1			**4.20**	**0.040**	0.04	0.848	0.65	0.420
NC	1	**7.40**	**0.006**	**7.47**	**0.006**	0.01	0.904	**5.62**	**0.018**
DA	1	0.23	0.633	0.30	0.586	0.00	0.975	**4.30**	**0.038**
wall side	1			0.83	0.364	0.00	0.979	0.56	0.453
drop	1	2.85	0.091	**84.84**	**<0.001**	**4.14**	**0.04**	2.65	0.103
trial	3	**18.10**	**<0.001**	**26.02**	**<0.001**	**58.71**	**<0.001**	**91.96**	**<0.001**
drop side×NC	1			**5.59**	**0.018**	0.12	0.734	0.02	0.887
drop side×DA	1			1.47	0.226	0.00	0.972	0.82	0.366
drop side×wall side	1			**7.29**	**0.007**	0.32	0.573	0.29	0.593
drop side×drop	1			0.14	0.708	0.03	0.869	1.48	0.220
drop side×trial	3			**9.86**	**0.020**	0.26	0.967	1.32	0.724
VAB		Distance travelled		No. of approaches		Wall side		Approach side	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
stimulus	2	0.32	0.854	3.26	0.196				
NC	1	0.03	0.862	0.61	0.433	**4.57**	**0.033**	3.34	0.067
DA	1					0.69	0.406	0.11	0.740
trial	3	**245.59**	**<0.001**	**66.65**	**<0.001**	5.85	0.119	4.58	0.205
stimulus×NC	2	0.44	0.803	1.10	0.578				
stimulus×trial	6	1.34	0.969	0.79	0.990				
Rheotaxis		Net displacement		Edge distance		Upstream movement		% Upstream	
		χ^2	P	χ^2	P	χ^2	P	χ^2	P
direction	1	0.00	1.000	**6.02**	**0.014**	0.65	0.421	2.48	0.115
NC	1	0.10	0.752	1.63	0.201	0.42	0.518	0.92	0.338
DA	1	0.00	0.989	0.24	0.622	0.61	0.436	1.49	0.222
trial	3	**8.73**	**0.033**	6.47	0.091	3.43	0.330	**9.01**	**0.029**
direction×NC	1	0.87	0.352	0.08	0.772	0.04	0.846	0.33	0.568
direction×DA	1	0.27	0.604	0.61	0.436	2.26	0.133	0.09	0.765
direction×trial	3	3.13	0.372	2.66	0.447	1.27	0.736	1.09	0.778

Italicized predictors are categorical whereas non-italicized predictors are continuous. Significant predictors are in bold. Blank cells indicate that a predictor was not included in the model equation for that behaviour (see Table 1). EscTime, escape time.

behaviour, and 11 of 13 had a stronger negative association when drops came from the left side, but there were no significant individual stitches (Table S3).

Average EscTime was 1.2 s [95% CI: 0.98 s, 1.49 s] and ranged from 0.02 to 9.92 s for escape responses initiated after impact. EscTime increased in later trials [log(EscTime)+s.e.: trial a=0.77+0.04; trial b=1.09+0.05; trial c=1.14+0.06; trial d=1.21+0.07; Table 2] and by log(EscTime)+s.e.=0.015+0.07 per drop. EscTime did not differ with wall side, neuromast count or DA, and no predictors had a significant interaction with drop side.

Maximum velocity was 0.39 m s^{-1} [95% CI: 0.34 m s^{-1}, 0.44 m s^{-1}] on average, ranged from 0.2 to 2.2 m s^{-1} and was highly variable both within and among individuals (Fig. 5A). Maximum velocity decreased in later trials (Fig. 5C) but did not change with drop number. Fish with more neuromasts reached a lower maximum velocity on average (Fig. 5A), and fish with more neuromasts on the right reached a higher maximum velocity on average (Fig. 5B), with a relatively small effect size compared with the range of maximum velocities observed. Maximum velocity did not differ with drop side or wall side, and no interactions with drop side were significant. Increased neuromast count in 12 of 13 stitches had a negative association with maximum velocity, with significant associations from the supratemporal (ST) (P_{adj}=0.046; β+s.e.=−0.10+0.04) and Ma (P_{adj}=0.007; β+s.e.=−0.13+0.04) stitches (Table S3). The effect of DA across stitches was inconsistent, with a right bias in eight of 13 lines increasing maximum velocity, including the significantly associated infraorbital (IO) stitch (P_{adj}=0.021; β+s.e.=−0.11+0.04; Table S3).

VAB

In each VAB video, 2.2 approaches [95% CI: 1.9, 2.6] were made on average. There were fewer approaches after trial a [log(approaches)+s.e.: a=1.23+0.10; b=0.63+0.11; c=0.77+0.11; d=0.63+0.11]. Fish travelled 2.24 m [95% CI: 1.77 m, 2.80 m] per 3 min VAB video on average. However, the distance travelled was much higher during trial a relative to other trials [log(distance travelled)+s.e.: a=1.70+0.05; b=1.24+0.05; c=1.20+0.05; d=1.10+0.05]. Neither the 60 Hz stimulus nor the 20 Hz stimulus induced greater travel distance or number of approaches when compared with controls (all $t_{437-622}$≤1.67, P≥0.218). Neuromast count did not affect distance travelled or number of approaches. Oscillatory stimuli did not interact with neuromast count for distance travelled or number of approaches.

Fish tended to orient themselves with the wall on the right more often than the left (proportion wall on right+s.e.=0.56+0.02). Fish with few neuromasts were more likely to orient with the wall on their left, whereas fish with many neuromasts were more likely to orient with the tank wall on their right, with a wide degree of intra-individual variation (Fig. 6). DA did not affect positioning. Nine of the 13 stitches were associated with an increase in the number of right wall hugs, including the significantly associated ethmoid (ET) (P_{adj}=0.029; β+s.e.=0.45+0.15) and Ma (P_{adj}=0.023; β+s.e.=−0.41+0.13) stitches (Table S3).

The proportion of left and right approaches to the centre was the same (proportion right approaches+s.e.=0.522+0.19). Fish with more neuromasts tended to approach the centre from the left more frequently (log-odds+s.e.=−0.277+0.151), and DA had no effect.
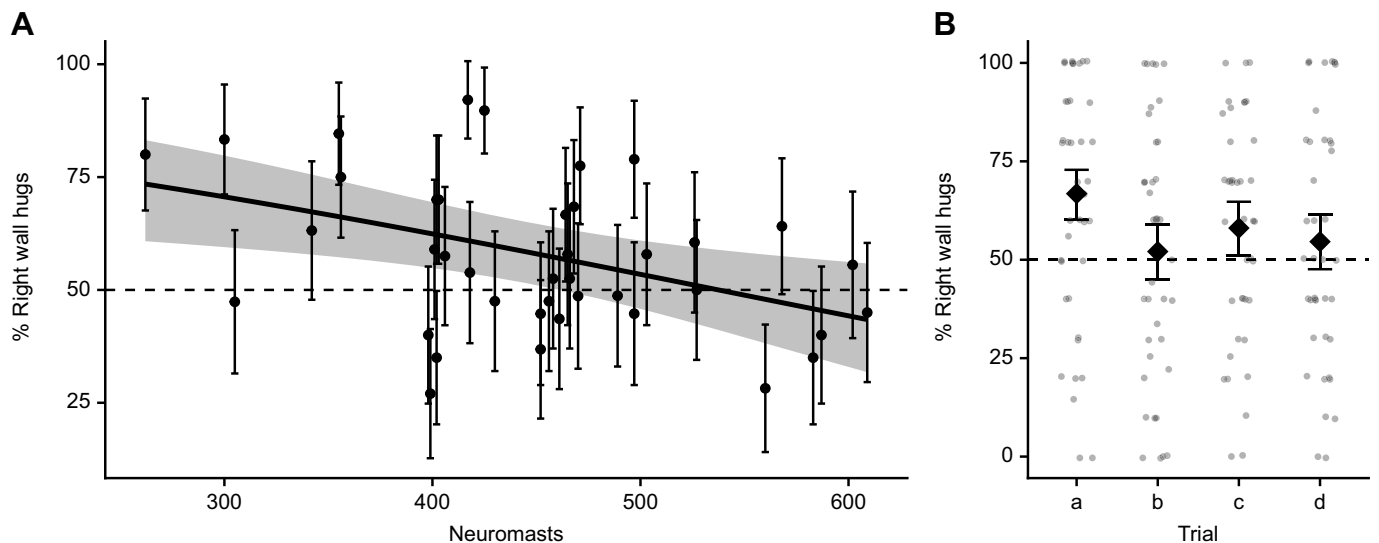
Fig. 3. Change in probability of hugging the right wall with changes in total neuromast count and among trials. (A) Neuromast count: the line is the estimated marginal mean (EMM) with the shaded region indicating the 95% confidence interval (CI), and points are the average response of individuals with 95% CI error bars. (B) Trial: diamonds are estimated marginal means (EMMs) with 95% CI error bars, and circles are the average response of individuals jittered for visibility. The EMM for neuromast count was averaged over all trials. Significance for A: $\chi^2_1=7.40$, $P=0.006$; B: $\chi^2_3=18.1$, $P<0.001$.

Rheotaxis

The position of fish after the rheotaxis trial was on average 0.7 m [95% CI: 0.23 m, 1.65 m] downstream from their initial position. There was less net downstream displacement in trial b compared with all other trials (Fig. 7A). The average distance from the outer edge was 4.70 cm [95% CI: 4.32 cm, 5.08 cm]. Fish tended to position closer to the outer edge in trial a than in other trials (Fig. 7B). Fish faced upstream 78.5% [95% CI: 75.0%, 81.6%] of the time, which did not vary among trials. Cumulative upstream movement was 3.54 m [95% CI: 2.72 m, 4.59 m] on average. Upstream movement was greatest in trial a (Fig. 7C).

Average distance from the outer edge of the tank was greater when water flow was clockwise (clockwise: 5.11 cm [95% CI: 4.73 cm, 5.48 cm]; counterclockwise: 4.48 cm [95% CI: 4.11 cm, 4.86 cm]). Neuromast count and DA did not affect distance from the outer edge or interact with flow direction. Net downstream displacement, cumulative upstream movement and time facing upstream were not influenced by neuromast count or DA, including interactions with flow direction.

Consistency of behaviour

During predator evasion trials, the probability of escape ($R=0.08$ [95% CI: 0.02, 0.11], $P<0.001$) and maximum velocity were repeatable ($R=0.25$ [95% CI: 0.09, 0.45], $P<0.001$) but EscTime was not ($R=0.03$ [95% CI: 0, 0.16], $P=1$). Laterality of escape probability ($R=0.0$ [95% CI: 0, 0], $P=1$) and wall side selection ($R=0.0$ [95% CI: 0, 0] $P=1$) were not repeatable. During VAB trials, distance travelled ($R=0.33$ [95% CI: 0.20, 0.44], $P<0.001$) and number of approaches ($R=0.19$ [95% CI: 0.04, 0.23], $P<0.001$) were repeatable; however, wall side selection (R=0.0 [95% CI: 0, 0.01], $P=0.5$) and approach laterality ($R=0.0$ [95% CI: 0, 0.01], $P=0.5$) was not repeatable. No rheotaxis behaviours were repeatable, i.e. net displacement ($R=0.10$ [95% CI: 0, 0.29], $P=0.34$), cumulative upstream movement ($R=0.09$ [95% CI: 0, 0.29], $P=0.41$), time oriented upstream ($R=0$ [95% CI: 0, 0.17], $P=1$) and distance from the outer edge ($R=0.12$ [95% CI: 0, 0.33], $P=0.26$).

Laterality of individuals during predator avoidance, VAB and rheotaxis trials were not correlated. However, the laterality of wall side selection was correlated with escape laterality, and wall selection during VAB trials was strongly correlated with approach side (Table 3).

DISCUSSION

We found associations between neuromast count and laterality in multiple behaviours. When exposed to an abrupt perturbation of the water simulating a predatory strike, stickleback were more likely to rest with their left side facing the arena wall between drops, and they were more likely to initiate escape behaviour when the stimulus came from the right. Furthermore, stickleback with few neuromasts were more likely to initiate an escape response when the stimuli came from the left side. Stickleback with more neuromasts escaped more slowly, and stickleback with more neuromasts on the right had higher maximum escape velocity. Stickleback did not display VAB towards 20 or 60 Hz stimuli. Despite the lack of VAB, stickleback tended to face the arena's outer wall with their right side, with a stronger right bias in stickleback with more neuromasts. Variance in neuromast count was not associated with rheotaxis behaviour; however, stickleback occupied a slower flow regime when in clockwise flow. No individual stitches stood out as a consistent predictor of predator evasion, VAB or rheotaxis behaviour; rather, most stitches tended to have a similar effect on behaviour. No lateralized behaviours were repeatable, nor was time to reach 0.2 m s^{-1} (EscTime) or any rheotaxis metrics. Many behaviours changed over the course of experimentation, as fish tended to be less active and less lateralized in later trials.

We observed laterality in responses to a simulated predator strike. Stickleback were most likely to respond to drops on the right; however, they were also more likely to place their right side against the arena wall. The preference to expose the left side and respond to stimuli from the right may suggest that the stickleback were more effective at determining that the simulated predatory stimulus was not a true threat when the stimuli came from their left. The notion that initiating escape behaviour was due to a misidentification of the mechanosensory stimuli is further supported by fish with fewer neuromasts responding more often and more vigorously to stimuli. Greater responsiveness to stimulation of neuromasts on the left side
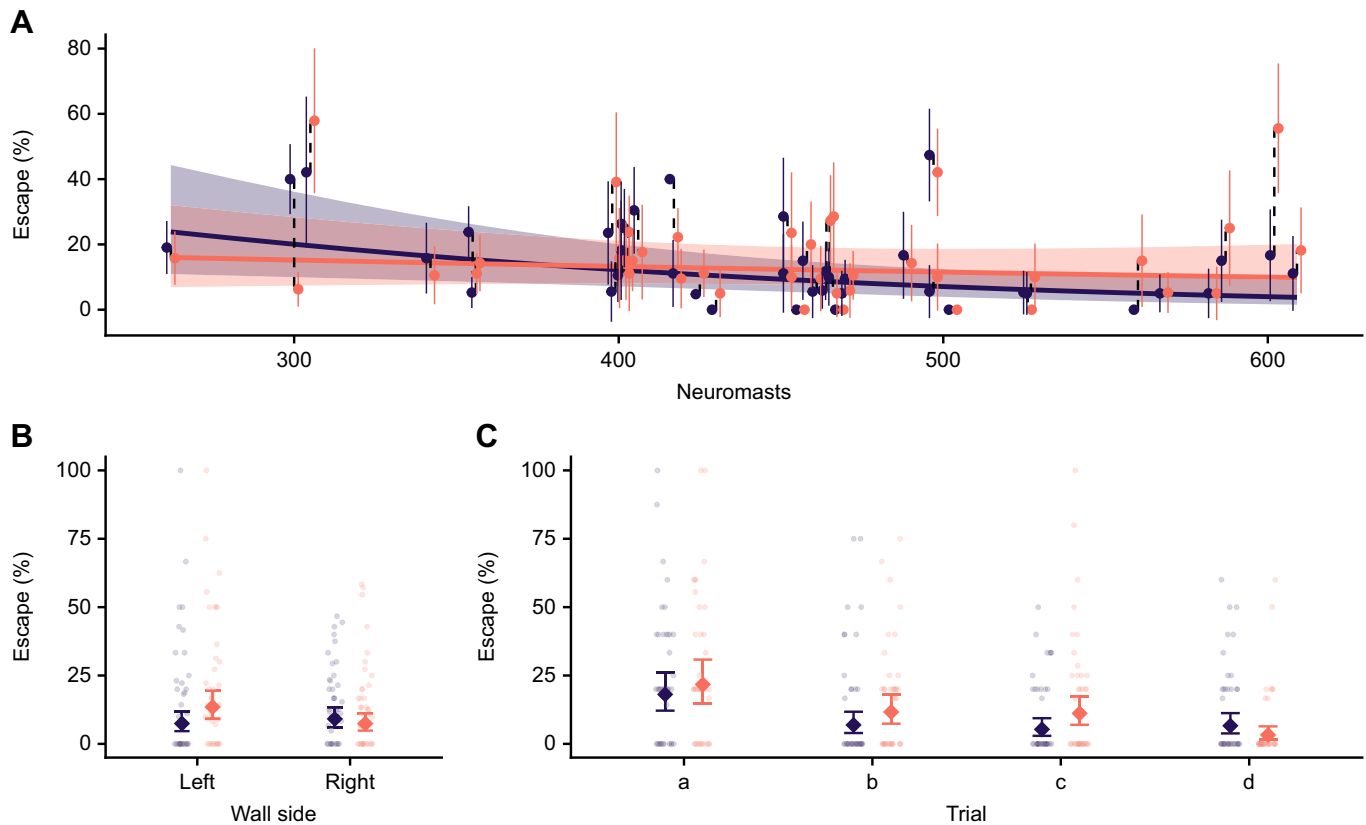
Fig. 4. Change in probability of initiating escape behaviour in response to drops on the right (pink) and left (purple) with changes in total neuromast count, wall side and trial. (A) Neuromast count: lines are EMMs with the shaded region indicating the 95% CI, and points connected by dotted lines are the average response per side for each individual with 95% CI. Individual points have been dodged along the x-axis for easier visualization of differences between the two sides. (B,C) Wall side and trial: diamonds are EMMs with 95% CI error bars, and circles are the average response of individuals jittered for visibility. All EMMs were averaged over all predictors other than the one being visualized. All EMMs were averaged over all (other) categorical predictors, i.e. wall side, trial or both. Significance for A: neuromast χ^2_1=7.47, P=0.006, neuromast×drop side: χ^2_3=5.59, P=0.018; B: drop side χ^2_1=4.20, P=0.040, drop side×wall side χ^2_1=7.29, P=0.007; C: trial χ^2_3=26.02, P<0.001, trial×drop side χ^2_3=9.86, P=0.020.

would explain why neuromasts influenced escape probability more when drops came from the left and the reduced intensity of response when individuals have more neuromasts on the left. Interestingly, a left bias in neuromasts is also associated with feeding behaviour in blind Mexican cavefish (Fernandes et al., 2018). However, the trend we observed differs from the most directly comparable study of predator evasion laterality, in which lungfish were more likely to escape to the left in response to a vertically plunging model predator (Lippolis et al., 2009). The direction and extent of laterality in response to predators is generally variable, differing among species (Heuts, 1999) and life history stages (Cantalupo et al., 1995). There is also disagreement among studies whether lateralized fish initiate escape behaviour faster (Agrillo et al., 2009; Dadda et al., 2010). Overall, there does not seem to be a consistent population-level side bias in escape behaviour among fishes, a trend which occurs in other potentially lateralized behaviours (Bisazza et al., 1997; Bisazza et al., 2000; Sovrano et al., 2001) and across taxa (Jozet-Alves et al., 2012; Lippolis et al., 2002; Lippolis et al., 2005; Romano et al., 2017). It has been suggested that the wide degree of variability in morphological and behavioural laterality among species is adaptive and highly plastic (Hori et al., 2017; Vallortigara et al., 1999), but it is unclear whether this is true of predator evasion laterality. Methodological differences in studies of predator evasion laterality may be inflating observed differences among species and obfuscating true and potentially adaptive differences among species.

The association between escape behaviour and neuromast count that we observed may be influenced by visual sensory information. Despite using water to minimize predator-like visual stimuli during testing, visual and mechanosensory information were present during escape response trials, and at least 6% of escape responses were entirely visually mediated, i.e. initiated prior to impact at the water's surface. Irregularities in the posture of the observer and reflections from the bottom of the tank or water surface also may have introduced variability into the perceived position of the predatory stimuli, reducing the power of our experimental observations. During preliminary testing, we were unable to elicit escape responses from stickleback in the dark. Similarly, goldfish rarely initiate escape responses to only auditory stimuli; however, the integration of auditory and visual stimuli increases their escape response beyond what would be expected if the two sensory modalities were operating independently (McIntyre and Preuss, 2019). The integration of both mechanosensory and visual information in the initiation of an escape response affects our interpretation of side dominance. Innervation of Mauthner cells by mechanosensory afferents is predominantly ipsilateral (Mirjany and Faber, 2011), whereas innervation of the optic tectum (Schwassmann and Kruger, 1965) and nucleus isthmi (Northmore, 1991) by the optic nerve is predominantly contralateral. These opposing innervation patterns mean that an individual with a dominant right brain hemisphere would have a dominant left eye and or a dominant right side of the lateral line, putting laterality in
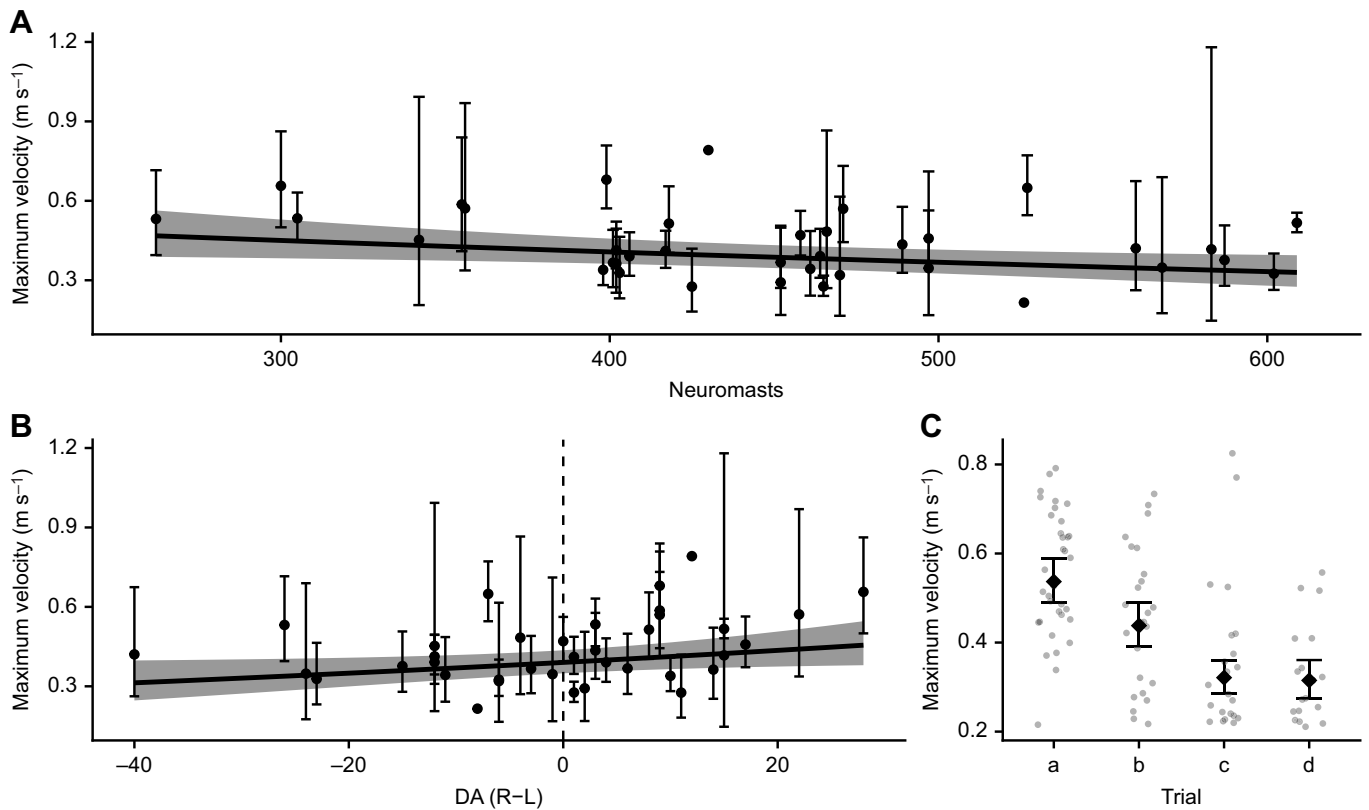
Fig. 5. Change in maximum velocity with changes in neuromast count and directional asymmetry (DA), and among trials. (A,B) Neuromast count and DA: lines are EMMs and shaded regions are 95% CIs, averaged over all predictors other than the one being visualized. Points are the average response of individuals with 95% CI. DA was calculated as the sum of right−left (R−L) neuromast counts for each neuromast stitch. (C) Trial: diamonds are EMMs with 95% CI error bars, and points are the average maximum velocity of each individual per trial, jittered for visibility. EMMs for neuromast count and directional asymmetry were averaged over all trials. Significance for A: χ^2_1=5.62, P=0.018; B: χ^2_1=4.30, P=0.038; C: χ^2_3=91.96, P=0.001.

the two sensory modalities at odds. Furthermore, both modalities show evidence of integration of both ipsilateral and contralateral stimuli (Gebhardt et al., 2019; Mirjany and Faber, 2011), suggesting that laterality in these sensory modalities cannot be explained by simple dominance of function in one side of the brain. Laterality in different sensory modalities can be independent (McGreevy and Rogers, 2005; Oltedal and Hugdahl, 2017) or related (Harshaw et al., 2021). To understand the role that sensory structures play in the development of a lateralized escape response, we require a detailed understanding of how sensory information is integrated between the two hemispheres of the brain because of the multi-sensory nature of this behaviour.

Lateralized behaviour may directly produce asymmetry in neuromast counts. During fluorescence microscopy, it was clear that the MD1 and Mp stitches often had damaged or missing neuromasts, suggesting that fish that tended to escape towards
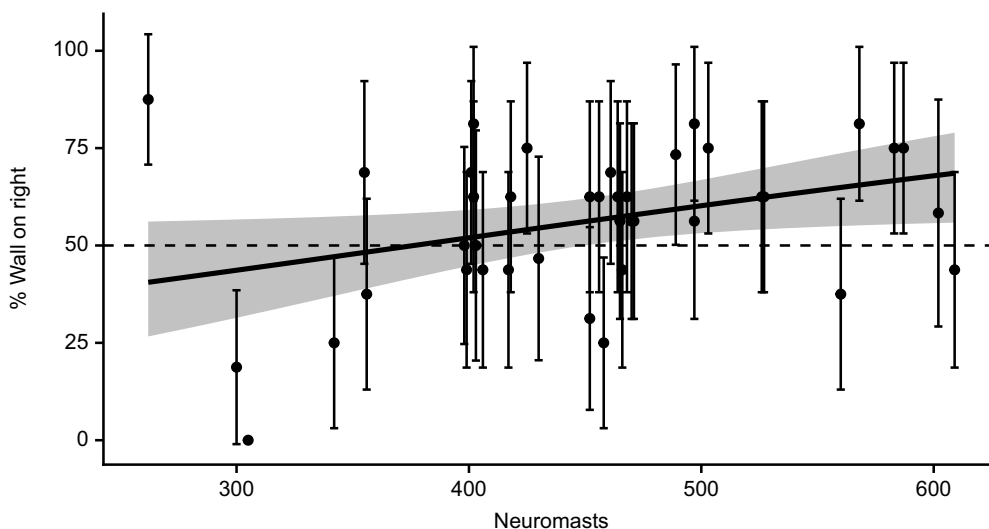
Fig. 6. Changes in the proportion of time with the outer wall on the right with changes in neuromast count. The line is the EMM with the shaded region indicating the 95% CI, and points are the average response of an individual with 95% CI. The EMM was averaged over all trials. Significance: χ^2_1=4.57, P=0.033.
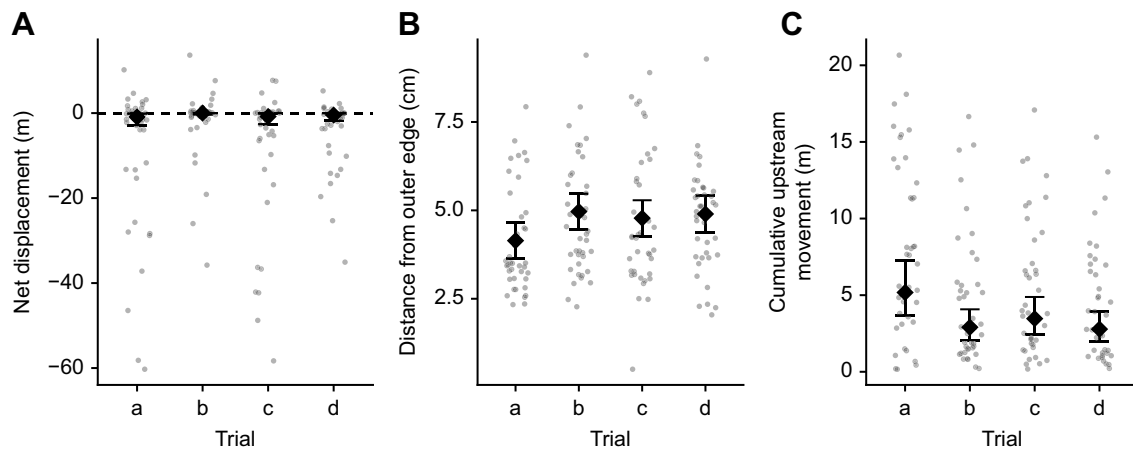
Fig. 7. Changes in fish position among rheotaxis trials. (A) Net displacement, (B) distance from the outer edge and (C) upstream movement. Diamonds are EMMs with 95% CI error bars, and circles are the average response of individuals jittered for visibility. Significance for A: $\chi^2_3=8.73$, $P=0.033$; B: $\chi^2_3=6.47$, $P=0.091$; C: $\chi^2_3=9.01$, $P=0.029$.

the arena's outer wall would have lost neuromasts on that side. If abrasion of the MD1 and Mp lines drove all associations between laterality and neuromast count, we would expect them to be outliers in the individual stitch analyses; however, as the MD1 and Mp stitches did not consistently drive associations between neuromast count and behaviour, abrasion is not likely to be the sole cause of the observed correlation between morphology and behaviour. Behaviourally induced receptor asymmetry may further bias the behaviour of that individual to escape with the same trajectory – positive feedback – or bias the individual to respond in the opposite direction – negative feedback – in the future. These sensory–behaviour feedback loops may be particularly important for long-lived populations of stickleback, which often incur scars and abrasions during interaction with lateralized predators (Bergstrom and Reimchen, 2003; Reimchen and Bergstrom, 2009). Asymmetry in other morphological characteristics, such as trunk muscle volume (Heuts, 1999), may also influence asymmetry in neuromasts, whether by behavioural or developmental interactions. By incorporating a feedback mechanism between sensory structures and behavioural laterality, we may better understand how lateralized behaviour changes over the life history of individuals.

Despite marine stickleback nocturnally foraging on brine shrimp (Mussen and Peeke, 2001), which produce regular oscillations by beating their legs (Lent, 1971), we found that stickleback do not exhibit VAB behaviour at the frequencies we tested.

However, the primary frequency of oscillations produced by prey may be outside of the range we examined during preliminary trials (20–100 Hz), as is the case with brine shrimp, which produce oscillations between 5 and 10 Hz (Lent, 1971). There is also the possibility that reflections off the outer wall of the tank obfuscated the signal being produced by the centre rod; however, Yoshizawa et al. (2010) utilized a similar assay to demonstrate VAB in a species with a greater proliferation of neuromasts, which is likely to be more sensitive to these weaker reflections. The lack of attraction to vibrations by stickleback is not wholly unexpected, given that only cave varieties of *Astyanax mexicanus* exhibit this behaviour (Yoshizawa et al., 2010). While we might expect that stickleback in low light environments, e.g. stained lakes, would have a similar adaptation for finding prey in the absence of visual information, these populations typically have undergone a reduction in the number of neuromasts (Planidin and Reimchen, 2019) rather than the proliferation seen in cavefish and deep sea species (Marranzino and Webb, 2018; Marshall, 1996; Yoshizawa et al., 2010). These same low-light populations of stickleback also exhibit increased levels of asymmetry in the lateral line (Planidin and Reimchen, 2021), suggesting that morphological asymmetry in sensory structures may exert a greater influence on the laterality of these fish.

When navigating in the dark, stickleback tended to hug the wall with their right sides. This side bias is the same as in the predator avoidance test and matches the laterality of cavefish exploring novel

Table 3. Correlation in laterality of behaviours

	(Rheo.) edge dist.	(Pred.) wall side	(Pred.) esc. side	(VAB) wall side	(VAB) appr. side
Correlation					
(Rheo.) edge dist.	–				
(Pred.) wall side	−0.17 [−0.46,0.16]	–			
(Pred.) esc. side	0.05 [−0.29,0.38]	**0.36 [0.03,0.62]**	–		
(VAB) wall side	−0.2 [−0.49,0.12]	0.15 [−0.,18,0.45]	0.03 [−0.31,0.36]	–	
(VAB) appr. side	−0.14 [−0.44,0.18]	−0.11 [−0.42,0.21]	0.03 [−0.3,0.36]	**−0.47 [−0.68,−0.17]**	–
P-value					
(Rheo.) edge dist.	–				
(Pred.) wall side	0.31	–			
(Pred.) esc. side	0.77	**0.03**	–		
(VAB) wall side	0.22	0.37	0.87	–	
(VAB) appr. side	0.39	0.5	0.85	**<0.001**	–

Correlation coefficients are shown with 95% confidence intervals in brackets. Significantly correlated behaviours are in bold.

objects in the dark (Burt de Perera and Braithwaite, 2005). The relationship between neuromast count and right-biased 'wall hugs' is opposite in predator evasion and VAB trials, in which the environment differed in light regime and the presence of a threat. As early light exposure (Dadda and Bisazza, 2012; George et al., 2021; Rogers and Krebs, 1996; Sovrano et al., 2016) and the presence of predatory stimuli (De Santi et al., 2000; Jozet-Alves and Hebert, 2013) can influence laterality, differences in both of these environmental factors are plausible causes for changes in the relationship between neuromast count and laterality between predator evasion and VAB experiments. Cavefish utilize mechanosensation for navigation in the dark (Holzman et al., 2014) and may have a greater potential to develop lateralized mechanosensory navigation behaviour than stickleback, as a result of having a higher density of facial neuromasts. Eagle's Lake stickleback, a population with a relatively high number of neuromasts, do not exhibit specialized mechanosensory-mediated foraging, suggesting that stickleback lack this behaviour.

Perhaps our most surprising finding is the lack of association between neuromast count and rheotaxis behaviour. Jiang et al. (2017) demonstrated that ablation of the entire lateral line in threespine stickleback affects rheotaxis behaviour and that natural variation in neuromast count within populations also correlates with rheotaxis behaviour. In contrast, we found that natural variation in neuromast count does not correlate with rheotaxis behaviour under similar flow regimes to those used by Jiang et al. (2017). There are several mechanisms through which these differences may have arisen. Firstly, habitat differences – e.g. flow regime of inlet streams – in the populations assessed may have led to divergent results. However, the range of natural variation in neuromast counts examined is comparable between studies (Jiang et al., 2017; Fig. 2). Secondly, experimental protocol may have influenced the results; for example, if there were major differences in the turbulence of the flow regime, which may influence the ability of superficial neuromasts to detect abiotic flows (Coombs et al., 2014). Jiang et al. (2017) also tested for a different kind of association, using canonical correlation analysis (CCA) to examine the multivariate interaction between natural variation in neuromast count and rheotaxis behaviour. In contrast, we looked for a linear relationship with total neuromast count. While the lateral line is important for rheotaxis in stickleback, natural variation in neuromast count within populations likely does not have a consistent linear relationship with rheotaxis.

We found that population-level laterality changed in repeat tests at different rates among behaviours. Wall hug laterality was lost after the first predator evasion trial (illuminated) but remained consistent during VAB trials (dark), and laterality in escape response initiation diminished slowly over time. Laterality differs in response to novel versus familiar stimuli across taxonomic groups (Burt de Perera and Braithwaite, 2005; Fourie et al., 2021; Hausberger et al., 2021; Regolin and Vallortigara, 1996; Robins and Rogers, 2006; Wilson and McLaughlin, 2007). Therefore, habituation to a stimulus consistently alters the laterality of related behaviours. As laterality is more prevalent during first exposures, laterality may be the default state of behaviour, and symmetry may be learned over time. The exception to this would be if there is a positive feedback mechanism supporting further lateralization. While the changes in laterality we observed cannot be viewed independently from the overall reduction in activity level in subsequent trials, our findings suggest that the valence of stimuli modulates the strength of this learning response, e.g. laterality was reduced over time

when exposed to predatory stimuli but not when in the dark. Furthermore, changes in laterality between repeat tests of the same individual, whether within the same day or over the ontogeny of an individual (Bisazza et al., 1997, 1998; Burt de Perera and Braithwaite, 2005; Cantalupo et al., 1995; Sovrano et al., 2001), may emerge from habituation rather than developmental effects or genetic selection.

Our findings indicate that stickleback do not exhibit repeatable individual-level laterality across multiple behaviours. Further, individuals did not display consistent behavioural phenotypes for non-lateralized behaviours, i.e. time to initiate escape response and all of the rheotaxis assays. Furthermore, the repeatability metric we and others (Roche et al., 2020) used does not consider learning. A population that is 100% left-lateralized in the first of four trials, then exhibits randomized laterality in the following three trials generates zero repeatability. Roche et al. (2020) call into question the validity of the T-maze assay for lateralized behaviour, but the T-maze assay has typically been used as an assessment of an individual's innate laterality. Even with a highly novel stimulus, laterality may arise from learning from similar events in an individual's life history and not necessarily be truly innate. Our findings suggest that innate or previously learned laterality can only be observed in the first few encounters with a stimulus before habituation, with differences among stimuli of variable salience or novelty. This rapid habituation then raises the question of whether laterality prior to or after a novel encounter is more critical to the survival of an individual, to which the answer seems largely dependent on the specific behavioural context. In the evasion of a predator, success in the first encounter is pivotal, whereas foraging may be less important to succeed in the first attempt.

Laterality of individual sticklebacks did not correlate among any of the behavioural assays, but bias in how individuals oriented themselves during trials influenced the laterality of their subsequent behaviour. The effect of orientation on behavioural laterality suggests that environmental geometry that may bias individuals towards a certain orientation can largely determine the laterality of behaviours in the wild. This also means that temporal autocorrelation may be an important factor when determining the absolute degree of laterality. If individuals are slow moving and change their orientation infrequently, they will be more likely to exhibit consistently lateralized behaviours during testing. If individuals are fast moving and change their orientation frequently, they will be more likely to exhibit inconsistent laterality. Given that our fish's activity levels were reduced after the first trial in many of the behaviours we observed, changes in laterality in subsequent trials may be due to this mechanism.

We found evidence of laterality in the positioning of stickleback within a circular arena but no association between asymmetry in the lateral line and laterality in mechanosensory-related behaviours. Stickleback preferred to hover with the wall on their right, and neuromast count and light regime modulated this tendency. Thus, sensory structure morphology plays a role in the development of lateralized behaviour, potentially including interactions across multiple sensory modalities. We observed rapid habituation and subsequent loss of laterality across multiple behaviours, suggesting that laterality, while deeply rooted in evolutionary history (Wiper, 2017), can exhibit plasticity with respect to directionality. The structure of the lateral line in stickleback appears to have a nuanced association with their behavioural laterality, a trend which could possibly occur across sensory modalities and taxonomic groups.

References

Agrillo, C., Dadda, M. and Bisazza, A. (2009). Escape behaviour elicited by a visual stimulus. A comparison between lateralised and non-lateralised female topminnows. *Laterality* 14, 300-314. doi:10.1080/13576500802396693

Ahnelt, H., Ramler, D., Madsen, M. Ø., Jensen, L. F. and Windhager, S. (2021). Diversity and sexual dimorphism in the head lateral line system in North Sea populations of threespine sticklebacks, *Gasterosteus aculeatus* (Teleostei: Gasterosteidae). *Zoomorphology* 140, 103-117. doi:10.1007/s00435-020-00513-1

Anfora, G., Rigosi, E., Frasnelli, E., Ruga, V., Trona, F. and Vallortigara, G. (2011). Lateralization in the invertebrate brain: left-right asymmetry of olfaction in bumble bee, *Bombus terrestris*. *PLoS ONE* 6, e18903. doi:10.1371/journal.pone.0018903

Baer, J., Gugele, S. M., Bretzel, J., DeWeber, J. T. and Brinker, A. (2021). All day-long: sticklebacks effectively forage on whitefish eggs during all light conditions. *PLoS ONE* 16, e0255497. doi:10.1371/journal.pone.0255497

Basile, M., Boivin, S., Boutin, A., Blois-Heulin, C., Hausberger, M. and Lemasson, A. (2009). Socially dependent auditory laterality in domestic horses (*Equus caballus*). *Anim. Cogn.* 12, 611-619. doi:10.1007/s10071-009-0220-5

Bates, D., Mächler, M., Bolker, B. and Walker, S. (2015). Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67, 1-48. doi:10.18637/jss.v067.i01

Bergstrom, C. A. and Reimchen, T. E. (2002). Geographical variation in asymmetry in *Gasterosteus aculeatus*. *Biol. J. Linn. Soc.* 77, 9-22. doi:10.1046/j.1095-8312.2002.00078.x

Bergstrom, C. A. and Reimchen, T. E. (2003). Asymmetry in structural defenses: insights into selective predation in the wild. *Evolution* 57, 2128-2138. doi:10.1111/j.0014-3820.2003.tb00390.x

Bisazza, A. and Dadda, M. (2005). Enhanced schooling performance in lateralized fishes. *Proc. R. Soc. B Biol. Sci.* 272, 1677-1681. doi:10.1098/rspb.2005.3145

Bisazza, A., Pignatti, R. and Vallortigara, G. (1997). Laterality in detour behaviour: interspecific variation in poeciliid. *Anim. Behav.* 54, 1273-1281. doi:10.1006/anbe.1997.0522

Bisazza, A., Facchin, L., Pignatti, R. and Vallortigara, G. (1998). Lateralization of detour behaviour in poeciliid fish: the effect of species, gender and sexual motivation. *Behav. Brain Res.* 91, 157-164. doi:10.1016/S0166-4328(97)00114-9

Bisazza, A., De santi, A. and Vallortigara, G. (1999). Laterality and cooperation: mosquitofish move closer to a predator when the companion is on their left side. *Anim. Behav.* 57, 1145-1149. doi:10.1006/anbe.1998.1075

Bisazza, A., Cantalupo, C., Capocchiano, M. and Vallortigara, G. (2000). Population lateralisation and social behaviour: a study with 16 species of fish. *Laterality* 5, 269-284. doi:10.1080/713754381

Blois-Heulin, C., Crével, M., Böye, M. and Lemasson, A. (2012). Visual laterality in dolphins: importance of the familiarity of stimuli. *BMC Neurosci.* 13, 9. doi:10.1186/1471-2202-13-9

Brooks, M. E., Kristensen, K., Benthem, K. J., van, Magnusson, A., Berg, C. W., Nielsen, A., Skaug, H. J., Maechler, M. and Bolker, B. M. (2017). glmmTMB balances speed and flexibility among packages for zero-inflated generalized linear mixed modeling. *R J.* 9, 378-400. doi:10.32614/RJ-2017-066

Burt de Perera, T. and Braithwaite, V. A. (2005). Laterality in a non-visual sensory modality — the lateral line of fish. *Curr. Biol.* 15, R241-R242. doi:10.1016/j.cub.2005.03.035

Cantalupo, C., Bisazza, A. and Vallortigara, G. (1995). Lateralization of predator-evasion response in a teleost fish (*Girardinus falcatus*). *Neuropsychologia* 33, 1637-1646. doi:10.1016/0028-3932(95)00043-7

Clarke, G. M., Yen, J. L. and McKenzie, J. A. (2000). Wings and bristles: character specificity of the asymmetry phenotype in insecticide-resistant strains of *Lucilia cuprina*. *P. Roy. Soc. Lond. B Bio.* 267, 1815-1818. doi:10.1098/rspb.2000.1215

Coombs, S., Bleckmann, H., Fay, R. R. and Popper, A. N. (eds) (2014). *The Lateral Line System*, pp. 22-168. New York: Springer-Verlag.

Dadda, M. and Bisazza, A. (2012). Prenatal light exposure affects development of behavioural lateralization in a livebearing fish. *Behav. Process.* 91, 115-118. doi:10.1016/j.beproc.2012.06.008

Dadda, M., Koolhaas, W. H. and Domenici, P. (2010). Behavioural asymmetry affects escape performance in a teleost fish. *Biol. Lett.* 6, 414-417. doi:10.1098/rsbl.2009.0904

De Santi, A., Bisazza, A., Cappelletti, M. and Vallortigara, G. (2000). Prior exposure to a predator influences lateralization of cooperative predator inspection in the guppy, *Poecilia reticulata*. *Ital. J. Zoolog.* 67, 175-178. doi:10.1080/11250000009356312

De Santi, A., Sovrano, V. A., Bisazza, A. and Vallortigara, G. (2001). Mosquitofish display differential left- and right-eye use during mirror image scrutiny and predator inspection responses. *Anim. Behav.* 61, 305-310. doi:10.1006/anbe.2000.1566

Deng, C. and Rogers, L. J. (2002). Social recognition and approach in the chick: lateralization and effect of visual experience. *Anim. Behav.* 63, 697-706. doi:10.1006/anbe.2001.1942

Domenici, P., Allan, B. J. M., Watson, S.-A., McCormick, M. I. and Munday, P. L. (2014). Shifting from right to left: the combined effect of elevated CO2 and temperature on behavioural lateralization in a coral reef fish. *PLoS ONE* 9, e87969. doi:10.1371/journal.pone.0087969

Fernandes, V. F. L., Macaspac, C., Lu, L. and Yoshizawa, M. (2018). Evolution of the developmental plasticity and a coupling between left mechanosensory neuromasts and an adaptive foraging behavior. *Dev. Biol.* 441, 262-271. doi:10.1016/j.ydbio.2018.05.012

Fey, D. P., Greszkiewicz, M., Jakubowska, M., Lejk, A. M., Otremba, Z., Andrulewicz, E. and Urban-Malinga, B. (2020). Otolith fluctuating asymmetry in larval trout, *Oncorhynchus mykiss* Walbaum, as an indication of organism bilateral instability affected by static and alternating magnetic fields. *Sci. Total Environ.* 707, 135489. doi:10.1016/j.scitotenv.2019.135489

Fourie, B., Berezina, E., Giljov, A. and Karenina, K. (2021). Visual lateralization in artiodactyls: a brief summary of research and new evidence on saiga antelope. *Laterality* 26, 106-129. doi:10.1080/1357650X.2020.1852245

Frasnelli, E. (2013). Brain and behavioral lateralization in invertebrates. *Front. Psychol.* 4, 939. doi:10.3389/fpsyg.2013.00939

Frasnelli, E. and Vallortigara, G. (2018). Individual-level and population-level lateralization: two sides of the same coin. *Symmetry* 10, 739. doi:10.3390/sym10120739

Frasnelli, E., Vallortigara, G. and Rogers, L. J. (2012). Left–right asymmetries of behaviour and nervous system in invertebrates. *Neurosci. Biobehav. R.* 36, 1273-1291. doi:10.1016/j.neubiorev.2012.02.006

Gebhardt, C., Auer, T. O., Henriques, P. M., Rajan, G., Duroure, K., Bianco, I. H. and Del Bene, F. (2019). An interhemispheric neural circuit allowing binocular integration in the optic tectum. *Nat. Commun.* 10, 5471. doi:10.1038/s41467-019-13484-9

George, I., Lerch, N., Jozet-Alves, C. and Lumineau, S. (2021). Effect of embryonic light exposure on laterality and sociality in quail chicks (*Coturnix coturnix japonica*). *Appl. Anim. Behav. Sci.* 236, 105270. doi:10.1016/j.applanim.2021.105270

Ghirlanda, S. and Vallortigara, G. (2004). The evolution of brain lateralization: a game-theoretical analysis of population structure. *Proc. R. Soc. B-Biol. Sci.* 271, 853-857. doi:10.1098/rspb.2003.2669

Güntürkün, O., Diekamp, B., Manns, M., Nottelmann, F., Prior, H., Schwarz, A. and Skiba, M. (2000). Asymmetry pays: visual lateralization improves discrimination success in pigeons. *Curr. Biol.* 10, 1079-1081. doi:10.1016/S0960-9822(00)00671-0

Harshaw, C., Barasch Ford, C. and Lickliter, R. (2021). Hearing better with the right eye? The lateralization of multisensory processing affects auditory learning in Northern bobwhite quail (*Colinus Virginianus*) chicks. *Appl. Anim. Behav. Sci.* 236, 105274. doi:10.1016/j.applanim.2021.105274

Hart, N. S., Partridge, J. C. and Cuthill, I. C. (2000). Retinal asymmetry in birds. *Curr. Biol.* 10, 115-117. doi:10.1016/S0960-9822(00)00297-9

Hausberger, M., Henry, L., Rethoré, B., Pougnault, L., Kremers, D., Rössler, C., Aubry, J., Cousillas, H., Boye, M. and Lemasson, A. (2021). When perceptual laterality vanishes with curiosity: a study in dolphins and starlings. *Laterality* 26, 238-259. doi:10.1080/1357650X.2021.1890758

Hedrick, T. L. (2008). Software techniques for two- and three-dimensional kinematic measurements of biological and biomimetic systems. *Bioinspir. Biomim.* 3, 034001. doi:10.1088/1748-3182/3/3/034001

Heuts, B. A. (1999). Lateralization of trunk muscle volume, and lateralization of swimming turns of fish responding to external stimuli. *Behav. Process.* 47, 113-124. doi:10.1016/S0376-6357(99)00056-X

Holzman, R., Perkol-Finkel, S. and Zilman, G. (2014). Mexican blind cavefish use mouth suction to detect obstacles. *J. Exp. Biol.* 217, 1955-1962. doi:10.1242/jeb.098384

Hori, M., Nakajima, M., Hata, H., Yasugi, M., Takahashi, S., Nakae, M., Yamaoka, K., Kohda, M., Kitamura, J., Maehata, M. et al. (2017). Laterality is universal

among fishes but increasingly cryptic among derived groups. *Zool. Sci.* **34**, 267-274. doi:10.2108/zs160196

Jiang, Y., Peichel, C. L., Torrance, L., Rizvi, Z., Thompson, S., Palivela, V. V., Pham, H., Ling, F., Bolnick, D. I. (2017). Sensory trait variation contributes to biased dispersal of threespine stickleback in flowing water. *J. Evol. Biol.* **30**, 681-695. doi:10.1111/jeb.13035

Jozet-Alves, C. and Hebert, M. (2013). Embryonic exposure to predator odour modulates visual lateralization in cuttlefish. *Proc. R. Soc. B-Biol. Sci.* **280**, 20122575. doi:10.1098/rspb.2012.2575

Jozet-Alves, C., Viblanc, V. A., Romagny, S., Dacher, M., Healy, S. D. and Dickel, L. (2012). Visual lateralization is task and age dependent in cuttlefish, *Sepia officinalis*. *Anim. Behav.* **83**, 1313-1318. doi:10.1016/j.anbehav.2012.02.023

Karenina, K., Giljov, A., Ingram, J., Rowntree, V. J. and Malashichev, Y. (2017). Lateralization of mother–infant interactions in a diverse range of mammal species. *Nat. Ecol. Evol.* **1**, 1-4. doi:10.1038/s41559-016-0030

Karenina, K., Giljov, A., de Silva, S. and Malashichev, Y. (2018). Social lateralization in wild Asian elephants: visual preferences of mothers and offspring. *Behav. Ecol. Sociobiol.* **72**, UNSP 21. doi:10.1007/s00265-018-2440-7

Krings, M., Müller-Limberger, E. and Wagner, H. (2019). EvoDevo in owl ear asymmetry—The little owl (*Athene noctua*). *Zoology* **132**, 1-5. doi:10.1016/j.zool.2018.10.002

Lai, F., Jutfelt, F. and Nilsson, G. E. (2015). Altered neurotransmitter function in CO2-exposed stickleback (*Gasterosteus aculeatus*): a temperate model species for ocean acidification research. *Conserv. Physiol.* **3**, cov018. doi:10.1093/conphys/cov018

Lehman, R. A. W. (1981). Lateralized asymmetry of behavior in animals at the population and individual level. *Behav. Brain Sci.* **4**, 28-28. doi:10.1017/S0140525X00007421

Lent, C. M. (1971). Metachronal limb movements by *Artemia salina*: synchrony of male and female during coupling. *Science* **173**, 1247-1248. doi:10.1126/science.173.4003.1247

Levy, J. (1977). The mammalian brain and the adaptive advantage of cerebral asymmetry. *Ann. N.Y. Acad. Sci.* **299**, 264-272. doi:10.1111/j.1749-6632.1977.tb41913.x

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. doi:10.1080/13576500143000221

Lippolis, G., Westman, W., McAllan, B. and Rogers, L. (2005). Lateralisation of escape responses in the stripe-faced dunnart, *Sminthopsis macroura* (Dasyuridae: Marsupialia). *Laterality* **10**, 457-470. doi:10.1080/13576500442000210

Lippolis, G., Joss, J. M. P. and Rogers, L. J. (2009). Australian lungfish (*Neoceratodus forsteri*): a missing link in the evolution of complementary side biases for predator avoidance and prey capture. *Brain. Behav. Evolut.* **73**, 295-303. doi:10.1159/000230674

Lychakov, D. V., Rebane, Y. T., Lombarte, A., Fuiman, L. A. and Takabayashi, A. (2006). Fish otolith asymmetry: morphometry and modeling. *Hearing Res.* **219**, 1-11. doi:10.1016/j.heares.2006.03.019

Lychakov, D. V., Rebane, Y. T., Lombarte, A., Demestre, M. and Fuiman, L. A. (2008). Saccular otolith mass asymmetry in adult flatfishes. *J. Fish Biol.* **72**, 2579-2594. doi:10.1111/j.1095-8649.2008.01869.x

Magat, M. and Brown, C. (2009). Laterality enhances cognition in Australian parrots. *Proc. Biol. Sci.* **276**, 4155-4162. doi:10.1098/rspb.2009.1397

Malashichev, Y. B. (2006). *Behavioural and Morphological Asymmetries in Vertebrates*. CRC Press.

Marranzino, A. N. and Webb, J. F. (2018). Flow sensing in the deep sea: the lateral line system of stomiiform fishes. *Zool. J. Linnean. Soc.* **183**, 945-965. doi:10.1093/zoolinnean/zlx090

Marshall, N. J. (1996). Vision and sensory physiology. The lateral line systems of three deep-sea fish. *J. Fish Biol.* **49**, 239-258. doi:10.1111/j.1095-8649.1996.tb06079.x

Mathis, A., Mamidanna, P., Cury, K. M., Abe, T., Murthy, V. N., Mathis, M. W. and Bethge, M. (2018). DeepLabCut: markerless pose estimation of user-defined body parts with deep learning. *Nat. Neurosci.* **21**, 1281-1289. doi:10.1038/s41593-018-0209-y

Matrai, E., Hoffmann-Kuhnt, M. and Kwok, S. T. (2019). Lateralization in accuracy, reaction time and behavioral processes in a visual discrimination task in an Indo-Pacific bottlenose dolphin (*Tursiops aduncus*). *Behav. Processes* **162**, 112-118. doi:10.1016/j.beproc.2019.02.003

McGreevy, P. D. and Rogers, L. J. (2005). Motor and sensory laterality in thoroughbred horses. *Appl. Anim. Behav. Sci.* **92**, 337-352. doi:10.1016/j.applanim.2004.11.012

McGrew, W. and Marchant, L. (1999). Laterality of hand use pays off in foraging success for wild chimpanzees. *Primates* **40**, 509-513. doi:10.1007/BF02557586

McIntyre, C. and Preuss, T. (2019). Influence of stimulus intensity on multimodal integration in the startle escape system of goldfish. *Front. Neural Circuits* **13**, 7. doi:10.3389/fncir.2019.00007

McLean, S. and Morrell, L. J. (2021). Sex differences in laterality are associated with reproduction in three-spine stickleback. *Am. Nat.* **197**, 708-718. doi:10.1086/714138

Mehlis-Rick, M., Thuenken, T., Krings, M., Kaefer, S. and Rick, I. P. (2018). Cerebral lateralization and quantity discrimination abilities in the threespine stickleback (*Gasterosteus aculeatus*). *Evol. Ecol. Res.* **19**, 487-501.

Miletto Petrazzini, M. E., Sovrano, V. A., Vallortigara, G. and Messina, A. (2020). Brain and behavioral asymmetry: a lesson from fish. *Front. Neuroanat.* **14**, 11. doi:10.3389/fnana.2020.00011

Mirjany, M. and Faber, D. S. (2011). Characteristics of the anterior lateral line nerve input to the Mauthner cell. *J. Exp. Biol.* **214**, 3368-3377. doi:10.1242/jeb.056226

Montgomery, J. C. and Milton, R. C. (1993). Use of the lateral line for feeding in the torrentfish (*Cheimarrichthys fosteri*). *N. Z. J. Zool.* **20**, 121-125. doi:10.1080/03014223.1993.10422868

Murtaugh, P. A. (2009). Performance of several variable-selection methods applied to real ecological data. *Ecol. Lett.* **12**, 1061-1068.

Mussen, T. D. and Peeke, H. V. S. (2001). Nocturnal feeding in the marine threespine stickleback (*Gasterosteus aculeatus* L.): modulation by chemical stimulation. *Behaviour* **138**, 857-871. doi:10.1163/156853901753172683

Northmore, D. P. M. (1991). Visual responses of nucleus isthmi in a teleost fish (*Lepomis macrochirus*). *Vis. Res.* **31**, 525-535. doi:10.1016/0042-6989(91)90103-C

Oltedal, L. and Hugdahl, K. (2017). Opposite brain laterality in analogous auditory and visual tests. *Laterality* **22**, 690-702. doi:10.1080/1357650X.2016.1269335

Olszewski, J., Haehnel, M., Taguchi, M. and Liao, J. C. (2012). Zebrafish larvae exhibit rheotaxis and can escape a continuous suction source using their lateral line. *PLoS ONE* **7**, e36661.

Planidin, N. P. (2021). Asymmetry in the lateral line of threespine stickleback, Gasterosteus aculeatus: ecology, evolution and behaviour.

Planidin, N. P. and Reimchen, T. E. (2019). Spatial, sexual, and rapid temporal differentiation in neuromast expression on lateral plates of Haida Gwaii threespine stickleback (*Gasterosteus aculeatus*). *Can. J. Zool.* **97**, 988-996. doi:10.1139/cjz-2019-0005

Planidin, N. P. and Reimchen, T. E. (2021). Ecological predictors of lateral line asymmetry in stickleback (*Gasterosteus aculeatus*). *Evol. Ecol.* **35**, 609-629. doi:10.1007/s10682-021-10117-w

Pohlmann, K., Atema, J. and Breithaupt, T. (2004). The importance of the lateral line in nocturnal predation of piscivorous catfish. *J. Exp. Biol.* **207**, 2971-2978. doi:10.1242/jeb.01129

Poyser, F., Caldwell, C. and Cobb, M. (2006). Dog paw preference shows lability and sex differences. *Behav. Process.* **73**, 216-221. doi:10.1016/j.beproc.2006.05.011

Regolin, L. and Vallortigara, G. (1996). Lateral asymmetries during responses to novel-coloured objects in the domestic chick: a developmental study. *Behav. Process.* **37**, 67-74. doi:10.1016/0376-6357(95)00076-3

Reimchen, T. E. (1988). Inefficient predators and prey injuries in a population of giant stickleback. *Can. J. Zool.* **66**, 2036-2044. doi:10.1139/z88-299

Reimchen, T. E. (1997). Parasitism of asymmetrical pelvic phenotypes in stickleback. *Can. J. Zool.* **75**, 2084-2094. doi:10.1139/z97-843

Reimchen, T. E. and Bergstrom, C. A. (2009). The ecology of asymmetry in stickleback defense structures. *Evolution* **63**, 115-126. doi:10.1111/j.1558-5646.2008.00520.x

Reimchen, T. E. and Nosil, P. (2001a). Lateral plate asymmetry, diet and parasitism in threespine stickleback. *J. Evol. Biol.* **14**, 632-645. doi:10.1046/j.1420-9101.2001.00305.x

Reimchen, T. E. and Nosil, P. (2001b). Dietary differences between phenotypes with symmetrical and asymmetrical pelvis in the stickleback *Gasterosteus aculeatus*. *Can. J. Zool.* **79**, 533-539. doi:10.1139/z01-006

Revelle, W. (2020). *Psych: Procedures for Psychological, Psychometric, and Personality Research*. Evanston, Illinois: Northwestern University.

Robins, A. and Rogers, L. J. (2006). Complementary and lateralized forms of processing in *Bufo marinus* for novel and familiar prey. *Neurobiol. Learn. Mem.* **86**, 214-227. doi:10.1016/j.nlm.2006.03.002

Roche, D. G., Amcoff, M., Morgan, R., Sundin, J., Andreassen, A. H., Finnøen, M. H., Lawrence, M. J., Henderson, E., Norin, T., Speers-Roesch, B. et al. (2020). Behavioural lateralization in a detour test is not repeatable in fishes. *Anim. Behav.* **167**, 55-64. doi:10.1016/j.anbehav.2020.06.025

Rogers, L. J. (2000). Evolution of hemispheric specialization: advantages and disadvantages. *Brain Lang.* **73**, 236-253. doi:10.1006/brln.2000.2305

Rogers, L. J. (2002). Lateralization in vertebrates: its early evolution, general pattern, and development. *Adv. Study Behav.* **31**, 107-161. doi:10.1016/S0065-3454(02)80007-9

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

Rogers, L. J. and Krebs, G. A. (1996). Exposure to different wavelengths of light and the development of structural and functional asymmetries in the chicken. *Behav. Brain Res.* **80**, 65-73. doi:10.1016/0166-4328(96)00021-6

Rogers, L. J., Zucca, P. and Vallortigara, G. (2004). Advantages of having a lateralized brain. *Proc. R. Soc. B-Biol. Sci.* **271**, S420-S422. doi:10.1098/rsbl.2004.0200

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

Romano, D., Benelli, G. and Stefanini, C. (2017). Escape and surveillance asymmetries in locusts exposed to a Guinea fowl-mimicking robot predator. *Sci. Rep.* **7**, 12825. doi:10.1038/s41598-017-12941-z

Roux, N., Duran, E., Lanyon, R. G., Frédérich, B., Berthe, C., Besson, M., Dixson, D. L. and Lecchini, D. (2016). Brain lateralization involved in visual recognition of conspecifics in coral reef fish at recruitment. *Anim. Behav.* **117**, 3-8. doi:10.1016/j.anbehav.2016.04.011

Schwarz, J. S., Reichenbach, T. and Hudspeth, A. J. (2011). A hydrodynamic sensory antenna used by killifish for nocturnal hunting. *J. Exp. Biol.* **214**, 1857-1866. doi:10.1242/jeb.051714

Schwassmann, H. O. and Kruger, L. (1965). Organization of the visual projection upon the optic tectum of some freshwater fish. *J. Comp. Neurol.* **124**, 113-126. doi:10.1002/cne.901240109

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. doi:10.1016/S0031-9384(01)00552-2

Sovrano, V. A., Bertolucci, C., Frigato, E., Foà, A. and Rogers, L. J. (2016). Influence of exposure in ovo to different light wavelengths on the lateralization of social response in zebrafish larvae. *Physiol. Behav.* **157**, 258-264. doi:10.1016/j.physbeh.2016.02.016

Stoffel, M. A., Nakagawa, S. and Schielzeth, H. (2017). rptR: repeatability estimation and variance decomposition by generalized linear mixed-effects models. *Methods Ecol. Evol.* **8**, 1639-1644. doi:10.1111/2041-210X.12797

Trokovic, N., Herczeg, G., Ab Ghani, N. I., Shikano, T. and Merila, J. (2012). High levels of fluctuating asymmetry in isolated stickleback populations. *BMC Evol. Biol.* **12**, 115. doi:10.1186/1471-2148-12-115

Vallortigara, G. (2000). Comparative neuropsychology of the dual brain: a stroll through animals' left and right perceptual worlds. *Brain Lang.* **73**, 189-219. doi:10.1006/brln.2000.2303

Vallortigara, G. and Rogers, L. J. (2020). A function for the bicameral mind. *Cortex* **124**, 274-285. doi:10.1016/j.cortex.2019.11.018

Vallortigara, G., Rogers, L. J. and Bisazza, A. (1999). Possible evolutionary origins of cognitive brain lateralization. *Brain Res. Rev.* **30**, 164-175. doi:10.1016/S0165-0173(99)00012-0

Wark, A. R. and Peichel, C. L. (2010). Lateral line diversity among ecologically divergent threespine stickleback populations. *J. Exp. Biol.* **213**, 108-117. doi:10.1242/jeb.031625

Werner, Y. L. and Seifan, T. (2006). Eye size in geckos: asymmetry, allometry, sexual dimorphism, and behavioral correlates. *J. Morphol.* **267**, 1486-1500. doi:10.1002/jmor.10499

Wilson, A. D. M. and McLaughlin, R. L. (2007). Behavioural syndromes in brook charr, *Salvelinus fontinalis*: prey-search in the field corresponds with space use in novel laboratory situations. *Anim. Behav.* **74**, 689-698. doi:10.1016/j.anbehav.2007.01.009

Wiper, M. L. (2017). Evolutionary and mechanistic drivers of laterality: a review and new synthesis. *Laterality* **22**, 740-770. doi:10.1080/1357650X.2017.1291658

Yeater, D. B., Hill, H. M., Baus, N., Farnell, H. and Kuczaj, S. A. (2014). Visual laterality in belugas (*Delphinapterus leucas*) and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*) when viewing familiar and unfamiliar humans. *Anim. Cogn.* **17**, 1245-1259. doi:10.1007/s10071-014-0756-x

Yoshizawa, M., Gorički, Š., Soares, D. and Jeffery, W. R. (2010). Evolution of a behavioral shift mediated by superficial neuromasts helps cavefish find food in darkness. *Curr. Biol.* **20**, 1631-1636. doi:10.1016/j.cub.2010.07.017