

RESEARCH ARTICLE

Functional diversity of the lateral line system among populations of a native Australian freshwater fish

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ABSTRACT

Fishes use their mechanoreceptive lateral line system to sense nearby objects by detecting slight fluctuations in hydrodynamic motion within their immediate environment. Species of fish from different habitats often display specialisations of the lateral line system, in particular the distribution and abundance of neuromasts, but the lateral line can also exhibit considerable diversity within a species. Here, we provide the first investigation of the lateral line system of the Australian western rainbowfish (*Melanotaenia australis*), a species that occupies a diversity of freshwater habitats across semi-arid northwest Australia. We collected 155 individuals from eight populations and surveyed each habitat for environmental factors that may contribute to lateral line specialisation, including water flow, predation risk, habitat structure and prey availability. Scanning electron microscopy and fluorescent dye labelling were used to describe the lateral line system in *M. australis*, and to examine whether the abundance and arrangement of superficial neuromasts (SNs) varied within and among populations. We found that the SNs of *M. australis* were present in distinct body regions rather than lines. The abundance of SNs within each body region was highly variable, and also differed among populations and individuals. Variation in SN abundance among populations was best explained by habitat structure and the availability of invertebrate prey. Our finding that specific environmental factors explain among-population variation in a key sensory system suggests that the ability to acquire sensory information is specialised for the particular behavioural needs of the animal.

KEY WORDS: Population variation, Altered flow regimes, Sensory evolution, Adaptation

INTRODUCTION

Fishes possess a unique sensory organ, the lateral line system, which allows them to receive both physical and biological information about their environment (reviewed by Mogdans and Bleckmann, 2012). The lateral line system forms the basis of many key survival traits in fishes (Bleckmann and Zelick, 2009; Engelmann et al., 2002) and underlies many behavioural adaptations, including predator avoidance (Montgomery and Macdonald, 1987), social communication (Butler and Maruska, 2016; Partridge and Pitcher, 1980) and orientation to water flow or ‘rheotaxis’ (Baker and

Montgomery, 1999b; Bleckmann and Zelick, 2009; Montgomery et al., 1997). Correspondingly, ecological variables such as predation pressure (McHenry et al., 2009), habitat (Bleckmann et al., 2010; Vanderpham et al., 2013) and water velocity (Wark and Peichel, 2010) may partly explain the diversity in lateral line morphology that is often observed in species occupying different habitats.

The functional link between lateral line morphology, habitat variation and behaviour remains very poorly understood. For example, while it is clear that the lateral line is used by larval zebrafish to respond to suction-feeding predators (McHenry et al., 2009), only one study has shown that exposure to environmental cues, such as predation risk, can affect the development of the lateral line system in fishes (Fischer et al., 2013). Interestingly, it has recently been revealed that variation in an individual’s lateral line morphology can determine the intensity of the rheotactic response (Jiang et al., 2017). Nonetheless, with the exception of the abovementioned studies, we understand surprisingly little about the relationship between lateral line diversity and the ecology and behaviour of fishes.

The lateral line system comprises a series of bundles of hair cells (neuromasts) that extend over the head and the lateral flank of fishes (Carton and Montgomery, 2004; Webb, 1989; Wellenreuther et al., 2010). These neuromasts comprise two distinct types, superficial neuromasts (SNs) and canal neuromasts (CNs), which differ in their performance and function despite similarities in basic structure. SNs are located on the surface of the skin (Carton and Montgomery, 2004) and mostly function to sense the velocity of the surrounding water (Dijkgraaf, 1963). SNs are able to respond to flow that is not orthogonal to their orientation axis, while the response amplitude of the CNs follows a cosine function and is maximised when water flow is in the direction of the canal axis (Janssen, 2004). SNs also facilitate rheotaxis (body orientation into currents), as these cells are constantly stimulated by water flow (Baker and Montgomery, 1999a). The CNs, in contrast, usually occur in a distinct line at the base of a canal running and extending over the head and flank. The CNs have a high threshold sensitivity (i.e. the minimum detectable stimulus) over a broad frequency range (<1 to >100 Hz; van Netten and McHenry, 2013) and are therefore used for both the detection and discrimination of objects, such as predators and prey in the fishes’ local environment (Mogdans and Bleckmann, 2012). Thus, the discrete functional characteristics of these two neuromast types, coupled with their distributions across the body, can provide valuable insights into the sensory requirements of a species occupying a particular habitat.

It has long been recognised that environmental factors such as water flow can result in the evolution of particular functional morphologies of the lateral line system. For example, some studies have found that limnophilic species that live in quieter, slower environments tend to have more SNs than rheophilic species that live in ‘noisier’, fast-paced environments (Bleckmann, 1994;

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Coombs et al., 1988; Dijkgraaf, 1963; Engelmann et al., 2002; Jakubowski, 1967; Janssen, 2004; Teyke, 1990). However, other studies have reported no relationship between SN abundance and water flow in the species' habitat (e.g. Beckmann et al., 2010). It has recently become apparent that the lateral line system can exhibit variation among populations and individuals of a single species. Threespine sticklebacks (*Gasterosteus aculeatus*) inhabiting marine, stream and lake habitats show a similar arrangement of SNs, but fish inhabiting freshwater streams have a higher abundance of SNs than those occupying marine habitats (Wark and Peichel, 2010). Such divergence in neuromast abundance among freshwater (pond) and marine populations has also been reported in ninespine stickleback (*Pungitius pungitius*) (Trokovic et al., 2011). When comparing discrete 'ecotypes' of threespine sticklebacks, fish from limnetic habitats had fewer neuromasts than those from benthic habitats, suggesting that habitat or resource specialisation may drive sensory adaptation (Wark and Peichel, 2010). Variation in predation pressure can also influence lateral line diversity; for example, guppies (*Poecilia reticulata*) inhabiting streams with high risk of predation have a greater abundance of SNs than those occurring in low predation sites (Fischer et al., 2013). Species can also exhibit variation in the canal system of the lateral line; for example, common bully (*Gobiomorphus cotidianus*) from rivers have more canal pores on the head than those collected from lakes (Vanderpham et al., 2013). However, there has been remarkably little assessment of lateral line variation among fish populations, with few studies considering the importance of environmental factors other than water flow speed.

Intraspecific variation in the lateral line system has the potential to facilitate mechanisms of localised sensory adaptation, yet the link between lateral line morphology and a species' ecology and behaviour remains poorly understood. The few studies that have linked lateral line morphology with particular environmental characteristics have used very broad habitat classifications (e.g. marine and freshwater; rheophilic or limnetic; predation risk: high or low) that may have masked the specific environmental factors (and their interactions) that influence neuromast abundance (see Wark and Peichel, 2010). Consequently, a fine-scale approach is required to identify whether multiple environmental factors may promote sensory divergence among populations of the same species. Such an approach is also an essential first step towards understanding how sensory specialisations might optimise key fitness behaviours.

In this study, we use a freshwater fish from northwest Australia, the western rainbowfish [*Melanotaenia australis* (Castelnau 1875)] to investigate whether environmental factors, such as predation risk and flow rate, are associated with within-species variation in the peripheral lateral line system. The western rainbowfish is ubiquitous and occurs in a large variety of freshwater habitats, including springs, ephemeral pools, creeks and lakes (Allen et al., 2002). In the arid Pilbara region, freshwater creeks are highly ephemeral, displaying large variation in water depth and clarity as pools contract during the dry season or over extended drought events (which can last several years) (Siebers et al., 2016). These drought periods are punctuated by infrequent, but sometimes extreme (e.g. owing to cyclonic activity), hydrodynamic events that often cause major flooding. Some pools are maintained through drought periods by continued connection to alluvial groundwater, or are spring-fed, resulting in relatively deep pools (>10 m) that present a temporally stable environment (Skrzypek et al., 2013). Discharge of water associated with mining below the water table has also resulted in permanent surface flows along some previously ephemeral creeks.

The diversity of freshwater habitats present in the Pilbara thus provides a unique opportunity to examine whether fish exhibit specialist adaptations of the lateral line system in response to extreme hydrological variability.

Despite western rainbowfish being common throughout northwest Australia, there have been very few ecological studies of this species, and its lateral line system has never been formally described. In this study, we first describe the morphology of the lateral line system in the western rainbowfish, using a combination of scanning electron microscopy (SEM) and fluorescent labelling (DASPEI) and light microscopy. We captured adult rainbowfish from eight locations across the Pilbara region (subject to available freshwater habitat during the dry season), and quantified the abundance and distribution of neuromasts across the body using fluorescence microscopy. During our field surveys, we evaluated the habitat characteristics of each sample site, including the abundance of surface and benthic invertebrates, water depth, flow rate, turbidity, predation risk and habitat complexity. We then adopted a modelling approach to evaluate the environmental predictors (and their interactions) that best explained the observed population variation in neuromast abundance. Several previous studies have revealed a link between hydrodynamic variability and neuromast abundance (Carton and Montgomery, 2004; Dijkgraaf, 1963; Engelmann et al., 2002, 2003; Schulze, 1870). Thus we expected water velocity to play a primary role in explaining any observed variation in neuromast abundance among populations.

MATERIALS AND METHODS

Study region and model species

The climate of the Pilbara is semi-arid and sub-tropical. Rainfall occurs predominantly in the austral summer, arising from cyclones, monsoonal lows and tropical thunderstorms, which punctuate periods of prolonged drought (Bureau of Meteorology, 2016). The average rainfall for the region is around 350 mm per year but is highly variable both within and among years (Bureau of Meteorology, 2016). Summer temperatures range from 24 to 40°C, and from 11 to 26°C in the winter, such that annual pan evaporation (2500 mm) far exceeds the annual average rainfall (Fellman et al., 2011). During summer periods of heavy rainfall, pools become swollen and can connect and spill out onto the floodplain (Beesley and Prince, 2010). In contrast, during the drier winter months and years with no cyclone activity, waterways can become constricted through evaporation to form a chain of pools along a drainage line (Beesley and Prince, 2010; Fellman et al., 2012; Siebers et al., 2016).

We sampled rainbowfish from two sub-catchments of the Fortescue River ('upper' and 'mid') encompassing sites with a diversity of water flows and habitat complexities. The Fortescue River traverses over 570 km with a catchment area of 480,000 km², with the lower western part of the catchment draining across the plains into the Indian Ocean, while the upper eastern region of the catchment drains from the Hamersley Ranges into the Fortescue Marsh (Barnett and Commander, 1985). The flow regime in the Fortescue River and its tributaries is directly linked to rainfall, with seasonal discharge during the wet summer months of January to March (Rouillard et al., 2015). The area relies on these high rainfall periods to sustain ephemeral pools along the drainage lines, which often reconnect during this time.

Rainbowfishes belong to the family Melanotaenidae, which comprises around 81 species that are endemic to Australia and New Guinea (Unmack et al., 2013). The western rainbowfish is endemic to the Pilbara and Kimberley regions of northwest Australia,

exhibiting considerable variation in body colouration and body shape across its range (Allen et al., 2002; Lostrom et al., 2015; Young et al., 2011). It is presumed that the diet of the western rainbowfish is similar to that of the eastern rainbowfish (*Melanotaenia splendida splendida*), which includes macroalgae, aquatic invertebrates and terrestrial invertebrates (Pusey et al., 2004). Predators of rainbowfish in the Pilbara catchments include low-risk omnivores such as spangled perch (*Leiopotherapon unicolor*) and high-risk predators such as Fortescue grunthers (*Leiopotherapon aheneus*) and barred grunthers (*Amniataba percoides*) (Young et al., 2011).

Sampling rainbowfish populations

Adult rainbowfish were collected from Coondiner Creek and Weeli Wolli Creek (in the upper Fortescue catchment) and from six sites within Millstream-Chichester National Park (in the mid Fortescue catchment) during April–May 2014 (see Table 1 for a summary of site characteristics). Coondiner Creek typically comprises a series of unstable, but hydrologically connected, pools that run along the main gorge line, which are largely reliant on rainfall (Fellman et al., 2011; Siebers et al., 2016). Weeli Wolli Creek encompasses a dense network of tributaries that flow in a northerly direction into the Fortescue Marsh (Dogramaci et al., 2015). The region is subject to significant mining below the water table and around 0.92 Gl of water is pumped annually into Weeli Wolli Creek from the dewatering of adjacent mines, which has significantly changed the flow regime of the creek since discharge began in 2006 (Dogramaci et al., 2015). The discharge creek (Weeli Wolli) runs parallel to the nearby Coondiner Creek, allowing us the opportunity to examine whether hydrological alteration may impact fish lateral line morphology. The freshwater habitats sampled from the mid-Fortescue (in Millstream-Chichester National Park), in contrast, are fed by an underground aquifer that creates a long string of permanent and deep pools (up to 14 m) over a 20 km distance (Skrzypek et al., 2013).

Habitat characterisation

Habitats across all sites were assessed for a range of attributes prior to fish sampling to minimise disturbance. General characteristics of the site, such as the presence or absence of predatory birds (e.g. herons, cormorants), height of recent flood waters (estimated by the height of debris found in nearby trees or along the shore) and the percentage of canopy cover over pools was recorded. We also measured water velocity (m s^{-1}) and turbidity (measured in nephelometric turbidity units, NTU). Benthic habitat type was assessed along transects perpendicular to the bank (or bisecting a pool) in an area where fish

were sighted from the bank. The length of each transect varied, depending on pool width (min. 3 m, max. 8 m). At 0.5 m intervals, a 20 cm quadrant was used to determine the percentage cover of different benthic habitat types, which were categorised according to percentages of coarse (>4 mm) and fine (<4 mm) substrate or gravel, aquatic vegetation and rocks. Photographs of each habitat were taken with an Olympus 1030SW waterproof camera to provide a record of key features of each habitat.

Benthic habitat surveys and site photographs were subsequently used to develop a habitat complexity ranking ranging from 1 to 10. A score of 1 described sites with low diversity in aquatic benthos, little to no aquatic vegetation and largely open water, while a score of 10 was allocated to sites with high habitat diversity, including high cover of aquatic vegetation (such as *Schoenus falucatis*, *Ceratopteris thalictroides*), overhanging vegetation and submerged debris. Sites were evaluated by two independent observers and then a consensus score was given. Following habitat characterisation, a Sontek™ Flow tracker (a handheld acoustic Doppler velocimeter) was used to determine water flow velocity at 0.5 m intervals along each transect and for each of three dimensions (x , y and z). Flow rate was measured (to the nearest 0.0001 m s^{-1}) as a proportion of the total depth from the surface at readings of 0.2 (20%), 0.6 (60%) and 0.8 (80%). These measurements were averaged over the measurement stations (min. 11 stations, max. 16 stations) to give a mean x , y and z velocity, and the level of variation in velocity (the standard deviation of the mean flow measured over a 30 s period) for each site. The flow tracker also recorded the mean temperature at each depth.

The abundance of surface invertebrates present at each site was assessed at the time of fish collection, by sweeping a 250 μm dip net over the surface of the pool in three 10 m sweeps. The net was then emptied into a tray by rinsing with clean creek water, and two observers counted the type and total number of invertebrates collected. The dominant species that were captured included water mites (order Acarina), water striders (order Hemiptera, family Gerridae), mayflies and midge larvae (order Diptera, family Chironomidae). Benthic invertebrates were sampled using a 500 μm D-net and were captured by trampling the substrate within a 1 m^2 area and sweeping the net over the trampled area for 30 s. The contents of the net were then washed through both a 2 mm and a 500 μm steel mesh sieve with clean creek water. Two observers counted the total number of invertebrates collected in the sieves over a 5-min period. Predation pressure was also assessed at the time of fish collection, by an on-site observation of birds that are considered to prey on western rainbowfish (herons and cormorants). In addition, records were made of the abundance of all fish species that were caught or observed at each site

Table 1. Summary of key habitat characteristics for Millstream-Chichester National Park, Coondiner Creek and Weeli Wolli Creek

Region	Site	Habitat complexity	0.2×Flow velocity (m s^{-1})	0.2×s.e.	0.6×Flow velocity (m s^{-1})	0.6×s.e.	Temperature ($^{\circ}\text{C}$)	Benthic invertebrate abundance	Surface invertebrate abundance	Predation risk
Mid-Fortescue	JA	6	0.120	0.001	0.104	0.008	25.4	31	1	Low
	DR	2	0.005	0.001	0.005	0.001	27.2	–	10	High
	OC	9	0.080	0.030	0.057	0.014	25.6	8	5	Low
	PP	6	0.020	0.003	0.08	0.004	23.6	11	9	High
	JL	8	0.305	0.033	0.139	0.018	28.2	20	4	Low
	CP	4	0.004	0.001	0.003	0.001	28.0	4	3	High
	CO	7	–0.002	0.001	0.005	0.001	22.3	12	14	Low
Upper-Fortescue	WW	5	0.177	0.011	0.186	0.013	31.9	–	–	Low

Missing data are where sites were too deep to sample (DR) or where environmental characteristics could not be evaluated due to time constraints (WW). 0.2× and 0.6× refer to mean water velocity at 20% and 60% of the water's depth, measured parallel to direction of flow. The standard error of flow velocity for these depths (0.2×s.e., 0.6×s.e.) is also shown. Population codes: Coondiner Creek (CO), Crossing Pool (CP), Deep Reach (DR), Jayawurrnha (JA), Jirndawurrnha (JL), Out Crossing (OC), Palm Pool (PP) and Weeli Wolli Creek (WW). Habitat complexity score ranges from 0 (homogeneous habitat) to 10 (highly structured).

during the sampling period. Fish were categorised as high- or low-risk predators according to the classification of predation risk to *M. australis* developed by Young et al. (2011).

Fish sampling

At each site, 20–30 adult western rainbowfish of mixed sex [mean \pm s.e.m. standard length (SL)=45.4 \pm 0.60 mm; age unknown] were captured using either a 4 m or 10 m long seine net (both with 6 mm mesh size) depending on the size of the area sampled. Fish were housed for up to 5 days in the field in aerated, 20 l plastic aquaria containing creek water and natural substrate from the collection site. Live fish were then transported to the Biological Sciences Animal Unit at The University of Western Australia by air and placed in aerated aquaria (42 \times 42.5 \times 34 cm) (one population per aquarium) containing gravel, a filter and an artificial plant. The tanks were maintained under fluorescent lighting (12 h:12 h light:dark cycle) and all fish were fed daily on a mixed diet of commercial flake food and *Artemia* nauplii.

Three adults were also collected from each of three sites (Coondiner Creek: pool 7 and pool 1.5; Chichester-Millstream National Park; and Crossing Pool Outflow) and preserved on site for subsequent SEM. These animals were euthanized using an overdose of MS222 (tricaine methanesulfonate; Sigma-Aldrich, St Louis, MO, USA) (200 mg l⁻¹) and then placed in a 50 ml Falcon tube filled with glutaraldehyde fixative (25% glutaraldehyde, 75% distilled water; ProSciotech, QLD, Australia) and were kept cool at approximately 15°C. Bubble wrap was slotted into the Falcon tube to prevent the fish moving around during transport and potentially causing damage to the SNs. Fish that were fixed were used for assessing the number, location and arrangement of neuromasts over the head and body using SEM.

Neuromast characterisation

Live fish were stained with a fluorescent vital dye 2-[4-(dimethylamino)styryl]-N-ethylpyridinium iodide (DASPEI; Life Technologies/Molecular Probes, Eugene, OR, USA) to visualise the neuromasts present on the surface of the body (protocol adapted from Wark and Peichel, 2010). Preliminary trials were conducted at different concentrations of DASPEI for 15 min to determine the optimal concentration for visualisation of the SNs. Each fish was first allowed to swim freely in the aerated DASPEI solution at a concentration of 0.24 g in 1 l water for 15 min. Fish were then anaesthetised in 200 mg l⁻¹ MS222 until light pressure on the caudal fin yielded no response. The fish was then placed right side down in a Petri dish and examined using a fluorescence dissecting microscope (Leica MZ75 fitted with a FITC filter set; Leica Microsystems Inc., Sydney, Australia). Images (8–15 per individual) of the entire body were captured at a magnification of between 0.8 \times and 1.0 \times , using a digital camera (Leica DFC 320). Measurements of the length and sex of each individual were also recorded. Sex was determined based on the following features: males are brighter in colour and have pointed dorsal and anal fins, while females are duller in colour and their dorsal and anal fins are more rounded (Allen, 1995). Following fluorescence photography, fish were revived in fresh, aerated aquarium water and returned to their housing tank. Individual fish from each population underwent the DASPEI staining and photography procedure only once and were subsequently revived and placed in post-experimental stock aquaria. All procedures adhered to the Australian code for the care and use of animals for scientific purposes and were approved by The University of Western Australia Animal Ethics Committee (approval no. RA/3/100/1176).

Once fish from all populations were photographed, the CNs and SNs were classified into distinct regions on the head, trunk and caudal fin, based on the methods of Northcutt (1989) and Webb (1989). Following Northcutt (1989) and Wark and Peichel (2010), the body was divided into 11 distinct regions defined as: rostral, nasal, mandibular, supraorbital, post otic, infraorbital, cheek, operculum, dorsal trunk, ventral trunk and caudal tail (Fig. 1A,B), and we counted the number of SNs in each (hereafter referred to as 'SN abundance'). Any photographs where the number of neuromasts in a particular section was unclear (e.g. owing to suboptimal labelling) were excluded. We did not categorise the SNs into distinct lines because there was large variation in the position, number and distribution of SNs among individuals and populations (Fig. 1B). The abundance of SNs on each body region was quantified for a total of 155 individuals originating from eight populations. The sample size for each population was 20 individuals with the exception of Jayawurrunga (JA), where $n=15$ individuals. This sample size was selected based on previous studies documenting among-individual variation in SN abundance (Wark and Peichel, 2010).

Scanning electron microscopy

Portions of the head, body and tail of each rainbowfish were fixed in Karnovsky's fixative (10 ml of 2.5% glutaraldehyde, 5 ml of 2% paraformaldehyde, 5 ml of 0.13 mol l⁻¹ Sorensen's phosphate buffer, pH 7.2), refrigerated for 3 days and then used for SEM. These samples included both field-collected samples and laboratory fish. The tissue was then washed in a 0.1 mol l⁻¹ phosphate buffer and heated using a microwave oven (250 W for 40 s). Samples were then immersed in increasing concentrations of ethanol (50, 70, 90 and 100%) and heated (as described above) at each concentration. The samples were then placed into a critical point drier for 2.5 h until the tissue was completely dry. Each piece of tissue was then mounted on a stub and sputter-coated with gold palladium. All images were captured with a Zeiss 1555 VP-FESEM (Zeiss Australia, Sydney, Australia) at various magnifications ranging from 78 \times to 16,470 \times .

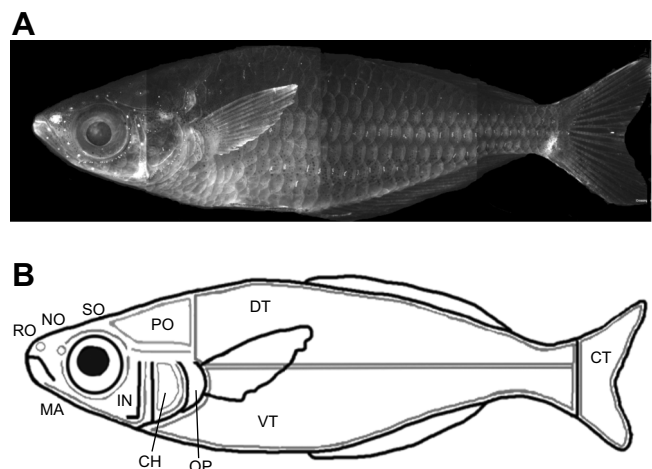


Fig. 1. Arrangements of the superficial neuromast (SN) system over the surface of the body of a western rainbowfish. (A) A representative fish (SL=43 mm) from Crossing Pool with SNs stained with DASPEI dye (photographs superimposed to account for different focal planes). (B) Diagram representing the neuromast groupings into 11 distinct body regions: rostral (RO), nasal (NO), mandibular (MA), supraorbital (SO), post otic (PO), infraorbital (IN), cheek (CH), operculum (OP), dorsal trunk (DT), ventral trunk (VT) and caudal tail (CT).

Statistical analyses

Variation in the abundance of SNs was first described by calculating the coefficient of variation (CV) for each population (site) and for each of the 11 distinct body regions. Supraorbital SNs were found on only two individuals, so this body region was excluded from further analyses. We used principle components analysis (PCA; performed on the covariances) to account for correlations among the numbers of SNs in different body regions and to reduce the number of variables in the analyses. This approach (rather than using total neuromast number) allowed us to evaluate whether ecological factors can affect neuromast abundance in specific regions of the body (as revealed by a previous study; Fischer et al., 2013). We accounted for missing data by replacing missing values (i.e. from images with poor labelling) with the population means, which accounted for 1.2% of the total data. Five principal components (PC1–PC5) were extracted with eigenvalues >1, which cumulatively explained 73% of the variance in the data (Table 2).

We used multivariate analysis of covariance (MANCOVA) to examine the effect of population origin (eight sites), sex and body size (SL in mm) on the total number of SNs present in different regions of the body, as described by PC1–PC5. Significant MANCOVA effects were investigated further by conducting subsequent univariate tests for each PC separately. The MANCOVA and subsequent univariate tests were conducted using the statistical software JMP® (version 12.1.0, SAS Institute Inc., Cary, NC, USA). As the MANCOVA revealed a significant variation among populations in the abundance of SNs, it justified further analyses to determine the effect of the environmental variables, and their interactions. We therefore performed a second set of analyses using linear mixed models to specifically evaluate the effect of the environmental predictor variables and their interactions on the abundance of neuromasts in specific body regions (described by the PCs), while controlling for sampling design (population was entered as a random effect). The fixed effects were water velocity (in m s^{-1}), habitat complexity (ordinal factor: ranging from 1 to 10), predation risk (high or low) and the abundance of benthic and surface invertebrates. Unfortunately, turbidity samples were unreliable owing to the presence of a precipitate, and thus excluded from the analyses. In the field, we noted that rainbowfish were most commonly observed in the top 20% of the water column, or approximately 30 cm below the water

surface. Therefore, we based our analyses of water flow rate on the mean measures (for each site transect) taken at 20% of the water depth. We also visualised general patterns of similarity among the sites in terms of their environmental characteristics by plotting the resulting PCs (of the environmental variables) using the software program Primer 6.0 (Primer-E, Ivybridge, UK). Groups closely associated on the PC plots were more similar in environmental conditions.

We used a model selection approach by testing a number of models (considered *a priori*) that could plausibly explain variation in SN abundance over specific body regions. The full list of ‘candidate’ models is listed in Table S1. These models included linear combinations of the fixed effects and the interaction between flow velocity and the abundance of surface invertebrates, because fast flows are typically associated with high availability of drifting invertebrates (Elliott, 1965). Body length and sex were included in the linear mixed models only for body regions that were found to be significant in the preceding (MANCOVA) tests. The linear modelling was performed using the lme4 package (Bates et al., 2012), version 1.1–10, in the software program R (R Foundation for Statistical Computing, Vienna, Austria), version 3.2.2 (‘Fire Safety’). We used Akaike’s information criterion values (corrected for small sample sizes: AICc) to compare the fit of the candidate models. We evaluated all models with a change in AICc relative to the best model (ΔAICc) of <10 and considered models with ΔAICc of <2 to be equally plausible (Symonds and Moussalli, 2011). Model selection was also performed on the basis of the Akaike model weight (w_i), which ranges from 0 to 1 and is a measure of the probability that a given model best describes the data, given the candidate model set (Symonds and Moussalli, 2011). Where there were a number of competing models (ΔAICc of <6), we determined the relative importance of the variables (predictor weight: w_p) by summing the Akaike weights for each predictor in the candidate model set (Symonds and Moussalli, 2011). Model averaging was performed using the ‘AICcmodavg’ package in R (<https://cran.r-project.org/package=AICcmodavg>). Predictor weights vary from 0 to 1, with values approaching 1 indicative that the given parameter is important to the model. We used the R package ‘piecewiseSEM’ to calculate the marginal R^2 for each model, which is the proportion of variance explained by the fixed factors alone (Lefcheck, 2015). The assumptions of the linear models were tested by inspecting the distribution of the residuals.

To understand whether artificially sustained flows due to mining activities at Weeli Wolli Creek may have resulted in changes in the lateral line system of rainbowfish, we used a *t*-test to compare the total number of neuromasts present on the body of fish collected from this site with those collected from nearby Coondiner Creek. Further *t*-tests were conducted to determine whether any divergence in SN abundance between the two sites differed for SNs present on different regions of the body.

RESULTS

The lateral line system of the western rainbowfish

SEM and fluorescence microscopy of DASPEI-labelled SNs revealed that all eight populations of western rainbowfish sampled in this study possessed consistent locations of SNs over 11 distinct body regions (Figs 1 and 2). It was apparent that there was no one baseline for the positions of the neuromasts, i.e. their position was always arranged differently within the designated region. However, the SNs were prolific across the head and body and were found either in small clusters of various shapes or singularly. Clusters of SNs were most often arranged in a crescent shape, although they

Table 2. Loadings matrix for five principal components (PC1–PC5) loaded onto the number of superficial neuromasts (SNs) on different parts of the body

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	2.40	1.48	1.40	1.19	1.05
% variance	21.86	13.42	12.74	10.81	9.54
Rostral	0.15	−0.31	0.58	0.26	−0.29
Nasal	−0.23	−0.19	−0.07	0.81	0.09
Mandibular	0.47	0.22	0.56	−0.24	−0.11
Infraorbital	−0.10	0.16	0.32	0.34	0.70
Supraorbital	−0.24	0.03	−0.60	0.12	−0.30
Operculum	0.57	0.57	−0.04	0.24	−0.10
Cheek	0.43	0.64	0.03	0.15	−0.34
Post otic	0.44	−0.59	0.25	−0.13	−0.18
Dorsal trunk	0.75	−0.25	−0.28	−0.07	0.04
Ventral trunk	0.72	−0.32	−0.35	0.09	0.13
Caudal tail	0.51	0.16	−0.15	−0.41	0.44

The eigenvalue and percentage of variation explained by each PC are also given. PCA was performed on the covariances. Predominant PCs for each body region are shown in bold and indicated by high loadings (>0.5).

also formed patterns such as crosses and abstract groupings (Figs 2 and 3). Canals and canal pores were clearly defined on the head, forming four main lines: the supraorbital, the otic, and the mandibular and infraorbital canals, all with visible clusters of CNs situated around the pore openings (Fig. 1A). The position of these canal lines was highly consistent among individuals and populations. In contrast, no canal pores were clearly visible on the trunk of the body.

A comparison of the level of variation in SNs abundance for the different body regions (Table 3) revealed that the cheek region showed the highest variation in SN abundance ($CV=50\%$; Fig. 3), while the number of SNs in the infraorbital region was highly consistent (i.e. less variable) across samples ($CV=12\%$). Population variation in SN abundance was highest for the Out Crossing site ($CV=26\%$) and lowest for Weeli Wolli Creek ($CV=13\%$; Table 3).

Variation in the complexity of rainbowfish habitats

As expected, environmental characteristics were highly variable among habitats (Fig. S1). For example, the Deep Reach site was a very large, deep body of water (>14 m), in which fish were found swimming freely near the surface and faced few obstacles. In contrast, pools at Coondiner Creek or Jirndawurranha, were quite shallow (< 2 m) and had many obstacles and debris that would

create a complex environment for fish navigation (Table 1; Fig. S1). PCA of the overall habitat characteristics of the sample sites revealed that Deep Reach and Crossing Pool (both mid-Fortescue catchment) were the most similar in habitat structure, complexity, flow rates and depth profiles (Figs S1, 2). The PC plot of the water flow measures recorded at each site (Fig. S2) revealed that Out Crossing had the most variable flow speeds and flow directions, while Crossing Pool and Coondiner Creek had the most stable flow conditions (almost zero flow). Weeli Wolli Creek had particularly high mean flow velocity at 60% water depth, but flow conditions were otherwise comparable to those of unmodified streams (Table 1; Fig. S2).

Neuromast abundance in relation to population, sex and body length

The MANCOVA revealed an overall effect of population ($F_{7,145}=6.49$, $P<0.001$) and body length ($F_{1,145}=4.17$, $P=0.001$) on the total number of SNs present on all body regions, but no effect of fish sex ($F_{1,145}=1.69$, $P=0.141$; Table 4A). We reduced the number of variables using PCA. The number of SNs on the operculum, dorsal and ventral trunk, and caudal fin was positively loaded onto PC1 and accounted for 23.7% of the variation in the data. PC2 explained 14.8% of the variation in the data and was

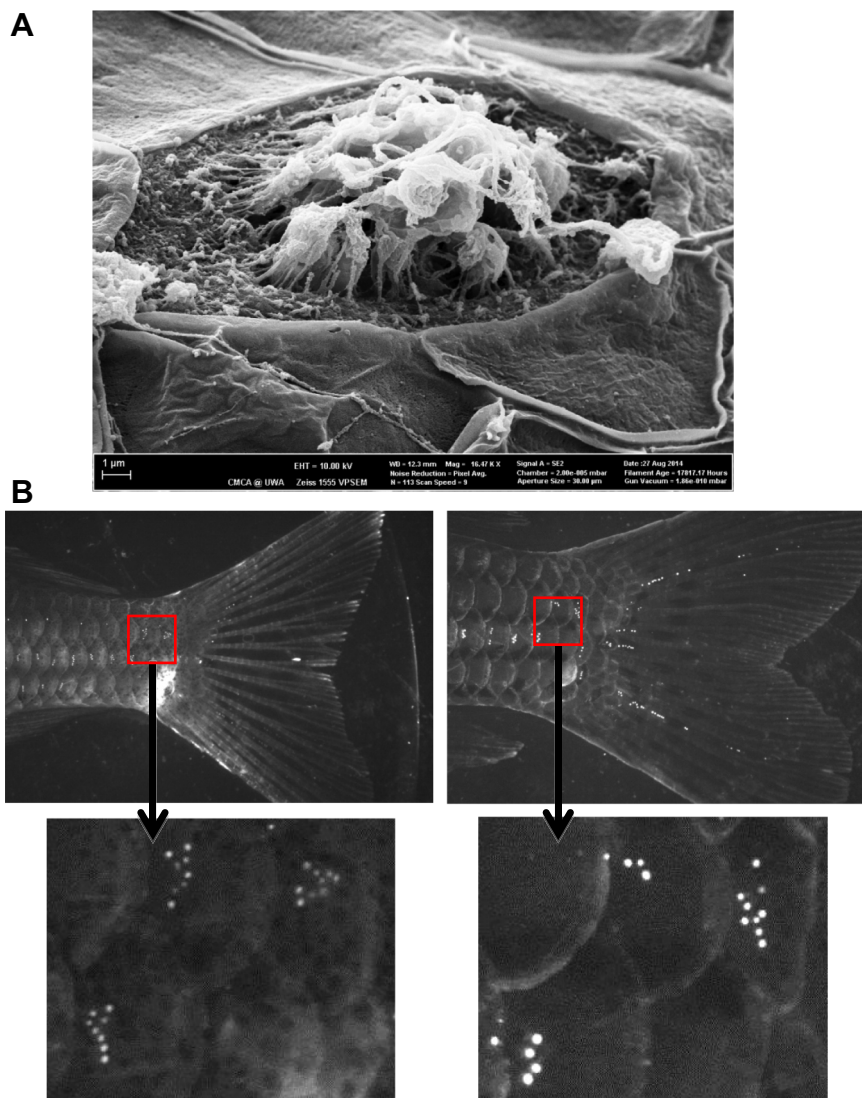


Fig. 2. Morphology and arrangement of SNs in the western rainbowfish. (A) Scanning electron micrograph of an opercular SN showing the aggregation of cilia (magnification=16,470 \times). Note that not all of the cilia are upright/intact owing to low levels of abrasion during transportation from the field. (B) Representative DASPEI images (left: male from Crossing Pool; right: female from Weeli Wolli Creek) showing differences in the arrangement of SNs within the trunk region and the presence of lines of SNs on the caudal fin (top right panel). Bottom images show selected regions of the trunk under increased magnification to reveal the arrangement of SNs in clusters on the scales.

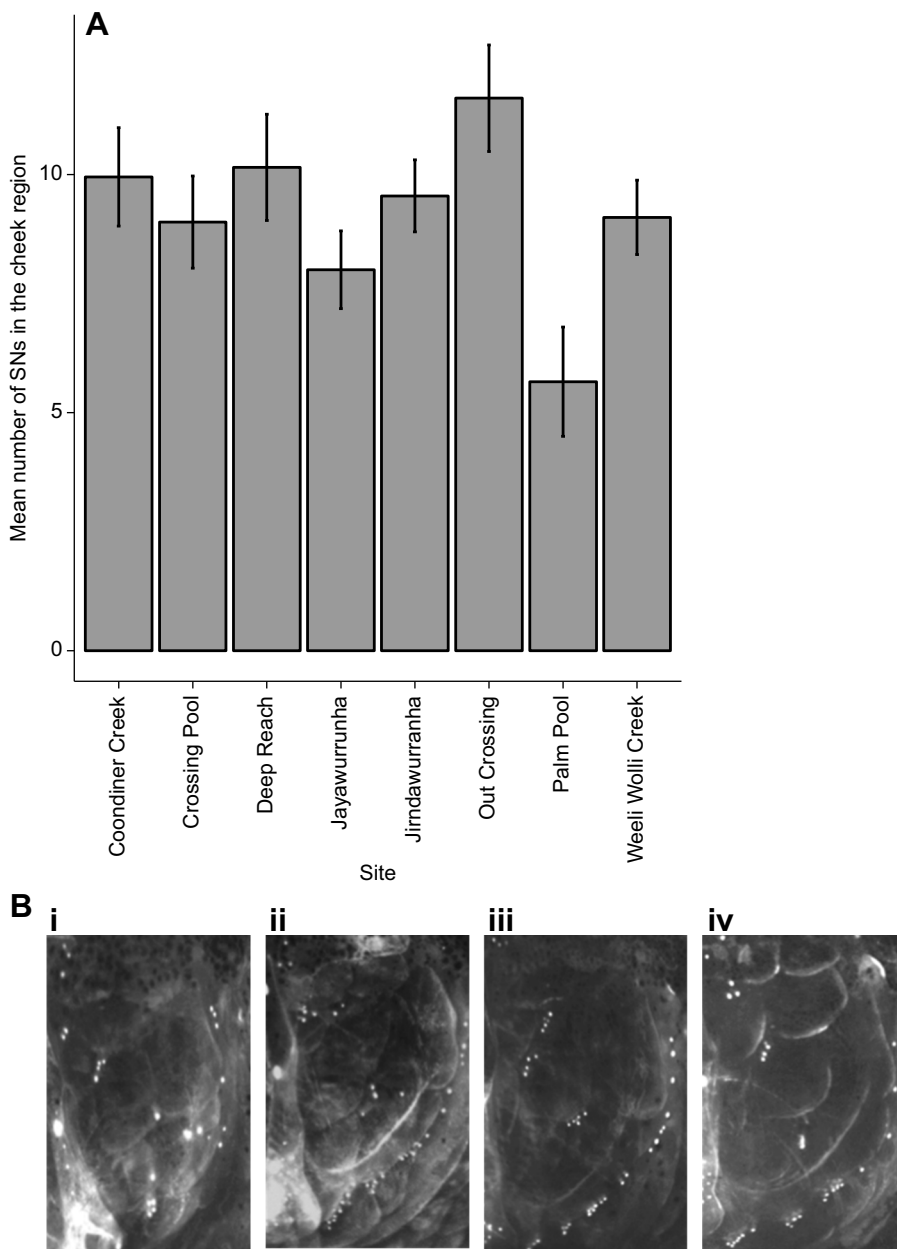


Fig. 3. SN abundance and arrangement varies among rainbowfish collected from different habitats. (A) Population variation in the mean \pm s.e.m. number of SNs present on the cheek (ANOVA: $F_{7,147}=3.16$, $P=0.004$). (B) The DASPEI images show the different arrangements of SNs in the cheek region in fish from (i) Jirndawurrunga, (ii) Crossing Pool, (iii) Deep Reach and (iv) Out Crossing Pool. Note that the line of SNs forms part of the operculum region. Sample size=20 fish per population, with the exception of Jayawurrunga, where $n=15$ fish.

loaded positively by the cheek region and negatively by the post otic region. PC3–PC5 cumulatively explained 34.6% of the variance and loaded positively with the number of SNs in the rostral and mandibular regions (PC3), the nasal region (PC4) and the infraorbital region (PC5). Univariate tests on the PCs revealed that population had a significant effect on SN abundance for all body regions (described by PC1–PC5), while body length had a significant effect for PC1 only (Table 4B). This finding was expected because PC1 encompasses features of body size, particularly the number of SNs on the dorsal and ventral trunk. Body length was therefore included as a covariate in subsequent linear mixed model analyses only for PC1.

Neuromast abundance in relation to environmental variables

The environmental variables explained significant variation in neuromast abundance, as indicated by the finding that the AICc values of the top linear mixed models were all considerably lower than those of the null models (that contained only the random effect)

(Table 5). Specifically, habitat complexity and the abundance of benthic invertebrates appeared in the top model set ($\Delta\text{AICc} < 6$) for all of the PCs and comprised the top model for both PC1 and PC2 (w_i : PC1=0.91; PC2=0.99). For PC3–PC5, other combinations of variables were in the top model set ($\Delta\text{AICc} < 6$), including water flow speed and the abundance of surface invertebrates. Nonetheless, the abundance of benthic invertebrates was included in all of the models considered for PC3 and PC4 and had the highest predictor weight ($w_p=1$) for PC3 and PC4, indicating the importance of this variable.

The effect of the environmental variables was similar for all of the PCs, suggesting that habitat complexity and the abundance of benthic invertebrates affect overall SN abundance, rather than determining SN abundance in specific regions of the body. The same findings were obtained when the analyses were repeated using the summed total of SNs as the dependent variable; the best-fitting model ($w_i=0.98$) out of the candidate model set is one containing the terms habitat complexity and the abundance of benthic

Table 3. Mean values and range for the total number of SNs present in each body region and for each population

		Rostral	Nasal	Mandibular	Infraorbital	Supraorbital	Operculum	Cheek	Post otic	Dorsal	Ventral	Caudal	CV
JA	Mean	8.9	5.9	22.4	15.6	0.2	26.1	8.0	12.6	66.9	185.9	39.2	0.16
	Range	3–15	3–9	7–38	13–19	0–2	19–35	2–13	8–15	8–101	112–270	3–78	
DR	Mean	8.1	4.6	33.4	15.8	0	32.0	10.2	14.2	75.1	185.6	39.3	0.22
	Range	3–15	3–8	20–48	14–19	–	19–42	3–23	9–24	48–107	110–305	15–56	
OC	Mean	8.4	5.7	30.9	16.2	0	31.2	11.6	17.2	89.1	219.1	48.9	0.27
	Range	4–12	2–10	18–52	13–20	–	18–48	5–23	8–29	55–143	148–359	17–78	
PP	Mean	8.4	4.8	28.0	15.6	0	19.4	5.7	19.4	83.3	222.5	50.1	0.22
	Range	4–12	2–10	18–40	13–19	–	4–34	0–15	11–28	44–111	115–327	10–94	
JI	Mean	8.0	3.5	28.0	16.3	0	24.2	9.6	14.9	61.7	135.4	36.5	0.21
	Range	5–12	1–9	11–39	14–18	–	11–45	5–20	9–20	35–79	95–201	13–83	
CP	Mean	8.6	5.2	29.2	16.1	0	28.6	9.0	17.5	74.0	202.9	37.9	0.25
	Range	3–16	3–10	20–37	13–20	–	19–43	2–19	9–27	30–100	118–326	16–84	
CC	Mean	8.2	5.1	27.1	18.0	0	24.1	10.0	13.5	63.1	141.6	43.1	0.16
	Range	0–16	3–7	16–38	13–21	–	9–40	4–23	8–20	37–99	12–203	27–70	
WW	Mean	14.7	4.8	31.5	16.4	0	21.2	9.1	19.4	59.6	163.3	39.2	0.13
	Range	11–27	3–8	6–50	13–21	–	12–35	2–16	13–26	38–79	102–194	19–61	
CV (body)		0.39	0.35	0.27	0.12	9.26	0.31	0.50	0.28	0.29	0.30	0.40	

Population codes: Coondiner Creek (CO), Crossing Pool (CP), Deep Reach (DR), Jayawurrunga (JA), Jirndawurrunga (JI), Out Crossing (OC), Palm Pool (PP) and Weeli Wolli Creek (WW). The coefficient of variation (CV) is also shown for each population. Highlighted in bold are the maximum mean SNs for each body section and highlighted in italics are the minimum and maximum SN counts for each body section.

invertebrates. The overall relationship between these variables and total SN abundance was negative (Fig. 4A,B), suggesting that homogeneous habitats with relatively low food availability were associated with an increased abundance of SNs.

SN abundance in a creek with modified water flows

A comparison of the total number of SNs in fish collected from a creek subjected to continuous and high flows (Weeli Wolli Creek) with fish from a nearby unaffected creek (Coondiner Creek) revealed that fish exposed to the high water flows had more SNs over the surface of the body than those captured from the natural creek, where water flows were close to zero at the time of collection ($t_{38}=-2.23$, $P=0.032$; mean±s.e.m. total SN abundance: Weeli Wolli: 398.3±9.7; Coondiner: 367.8±9.7). Subsequent t -tests to

compare SN abundance in specific regions of the body revealed that fish from the modified flow site had a greater abundance of SNs in the rostral ($t_{38}=-5.54$, $P<0.001$), post otic ($t_{38}=-5.50$, $P<0.001$) and ventral trunk regions ($t_{38}=-2.06$, $P=0.047$), and fewer SNs in the infraorbital region ($t_{38}=2.37$, $P=0.023$), than fish from an adjacent creek with natural water flows.

DISCUSSION

Our investigation of the peripheral lateral line system of a freshwater fish occupying habitats with diverse and dynamic environmental characteristics has revealed that the abundance of SNs varied over specific regions of the body, and also varied substantially among individuals and populations. Our fine-scale modelling approach revealed that population differences in overall neuromast abundance were best explained by variation in habitat complexity and the abundance of benthic invertebrate prey. We did not find that SN abundance in specific regions of the body was correlated with any of the environmental variables measured, suggesting that habitat characteristics may promote changes in overall sensory sensitivity rather than localised specialisation. We speculate that the observed variation in lateral line morphology may facilitate particular behaviours in a given habitat, such as navigation and the detection of water movements from invertebrate prey. Nonetheless, our best-fitting model explained less than 50% of the variation in neuromast number and arrangement, suggesting that other factors or constraints may explain lateral line system diversity.

The lateral line system of the western rainbowfish

This investigation into the canal structure of the western rainbowfish revealed four canals over the head; the mandibular, the otic, the supraorbital and the infraorbital canals. Although we were unable to find any evidence of a (pored) canal system along the trunk of *M. australis*, the absence of trunk canal neuromasts appears to be characteristic of benthic, planktivorous or schooling fishes (Webb, 1989). As the western rainbowfish is a shoaling species, one possible explanation for the proposed absence of the canal trunk system is that it is an adaptation for shoaling.

This study found that SNs abundance was highly variable within the 11 distinct body regions, and also varied within and among fish from different populations. The areas on the body that were most

Table 4. Results of (A) MANCOVA testing for an effect of population, body length (SL) and sex on principal components 1–5 (PC1–PC5), which describe the total number of SNs found on the body of wild-caught rainbowfish, and (B) univariate ANCOVAs showing the effects of population and body length on the PCs (the non-significant effect of sex was removed)

A				
Effect		d.f.	<i>F</i>	<i>P</i>
Population		7, 145	6.49	<0.001
SL		1, 145	4.17	0.001
Sex		1, 145	1.69	0.141
B				
PC	Effect	d.f.	<i>F</i>	<i>P</i>
1	Population	7, 146	8.51	<0.001
	SL	1, 146	16.77	<0.001
2	Population	7, 146	9.41	<0.001
	SL	1, 146	0.23	0.63
3	Population	7, 146	8.98	<0.001
	SL	1, 146	0.05	0.83
4	Population	7, 146	5.45	<0.001
	SL	1, 146	3.62	0.06
5	Population	7, 146	2.40	0.024
	SL	1, 146	0.24	0.63

Significant effects are shown in bold.

Table 5. Linear mixed models giving estimates of the fixed effects (±s.e.) of the environmental predictors on PC1–PC5

Variable	Site variation (%)	Effect	k	AICc	$\Delta AICc$	w_i	Estimate±s.e.	Marginal R^2
PC1	0	Habitat complexity+Benthic inverts+SL	9	397.8	0.00	0.91	Habitat complexity: 1.16±0.29 (L); 1.47±0.36 (Q); 0.93±0.26 (C); 0.17±0.35 (4)	0.42
							Benthic inverts: −0.02±0.02 SL: 0.09±0.02	
PC2	10.1	Surface inverts+Benthic inverts+SL	6	403.9	6.09	0.04	Surface inverts: −0.12±0.06 Benthic inverts: −0.07±0.03 SL: 0.09±0.02	0.31
	17.4	Benthic inverts+SL	5	404.5	6.67	0.03	Benthic inverts: −0.05±0.03 SL: 0.09±0.02	0.23
	16.0	Flow+Benthic inverts	6	406.2	8.41	0.01	Flow: −1.96±2.76 Benthic inverts: −0.03±0.04 SL: 0.09±0.02	0.24
	23.0	Null model	3	553.1	155.3	0	–	–
PC3	0	Habitat complexity+Benthic inverts	8	344.6	0	0.99	Habitat complexity: 0.47±0.22 (L); 0.41±0.29 (Q); −0.84±0.21 (C); 1.22±0.26 (4)	0.33
							Benthic inverts: −0.07±0.02 0.01±0.03	0.01
	27.7	Benthic inverts	4	354.4	9.83	0.01	Habitat complexity: 0.06±0.19 (L); 0.04±0.24 (Q); −0.46±0.17 (C); 0.43±0.22 (4)	–
	0	Null model	3	465.3	119.7	0	–	–
PC4	0	Habitat complexity+Benthic inverts	8	303.6	0	0.50	Benthic inverts: −0.01±0.01 Flow: 1.44±1.10	0.11
							Benthic inverts: −0.02±0.01 Surface inverts: 0.02±0.03 Benthic inverts: −0.01±0.02	0.01
	5.4	Benthic inverts	4	305.1	1.51	0.24	Habitat complexity: −0.30±0.23 (L); 0.96±0.30 (Q); 0.41±0.21 (C); 1.20±0.27 (4)	–
	3.3	Flow+Benthic inverts	5	305.7	2.18	0.17	Benthic inverts: 0.07±0.02 Flow: −2.82±1.84	0.19
PC5	0	Surface inverts+Benthic inverts	5	307.0	3.40	0.09	Benthic inverts: 0.04±0.02 Surface inverts: −0.01±0.06 Benthic inverts: 0.01±0.03	0.01
	30.2	Null model	3	443.1	139.6	0	–	–
	0	Habitat complexity+Benthic inverts	8	353.8	0	0.88	Habitat complexity: 0.09±0.03 Benthic inverts: 0.01±0.01	0.10
	13.4	Benthic inverts	4	359.5	5.61	0.05	Habitat complexity: 0.15±0.22 (L); −0.78±0.28 (Q); 0.22±0.20 (C); 0.22±0.25 (4)	0.11
PC5	9.3	Flow+Benthic inverts	5	359.6	5.79	0.05	Benthic inverts: −0.03±0.02 Flow: −1.38±1.28	0.01
	13.9	Surface inverts+Benthic inverts	5	361.6	7.79	0.02	–	–
	15.4	Null model	3	457.9	123.5	0	–	–
	0	Surface inverts+Benthic inverts	5	330.5	0	0.91	Surface inverts: 0.09±0.03 Benthic inverts: 0.01±0.01	0.10
PC5	0	Habitat complexity+Benthic inverts	8	336.4	5.96	0.05	Habitat complexity: 0.15±0.22 (L); −0.78±0.28 (Q); 0.22±0.20 (C); 0.22±0.25 (4)	0.11
							Benthic inverts: −0.03±0.02 Flow: −1.38±1.28	0.01
	5.0	Benthic inverts	4	337.7	7.22	0.02	–	–
	3.4	Flow+Benthic inverts	5	338.8	8.37	0.01	–	–
PC5	5.4	Null model	3	444.4	113.9	0	–	–

Site was entered as a random effect in all models and standard length (SL) was only included as a covariate for PC1. The variance that is explained by site is given (as a proportion of the total variance), k is the number of parameters in the model and AICc and $\Delta AICc$ compare the fit of the models for each PC. Only models with $\Delta AICc < 10$ are shown. The null model (random effect only) is included in each case for comparison. The marginal R^2 is the proportion of variance explained by the fixed factors alone.

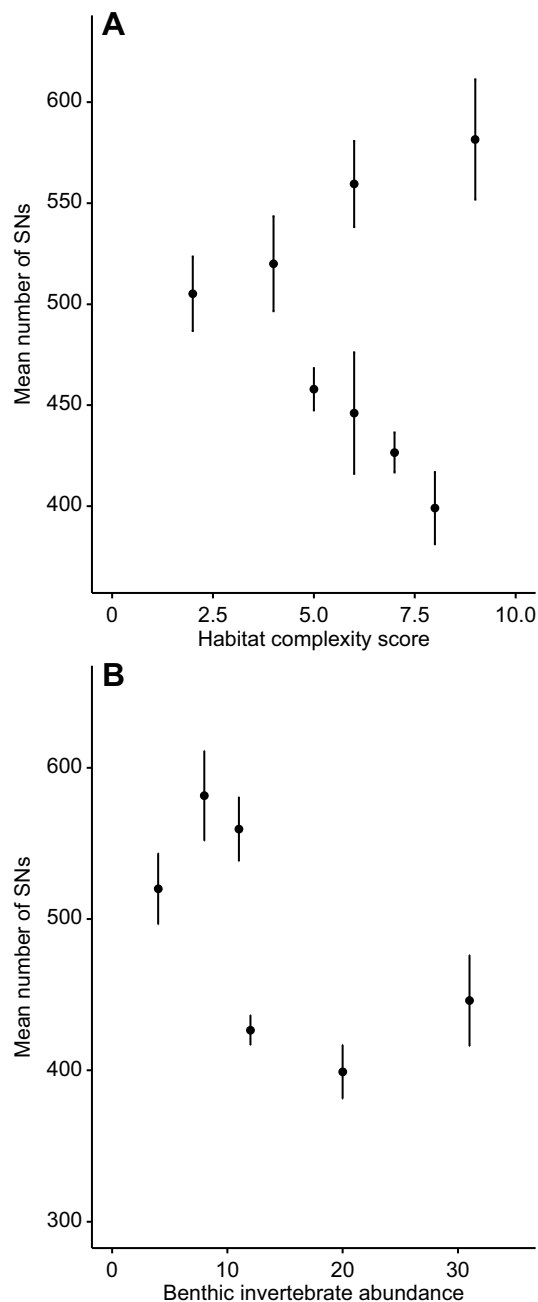


Fig. 4. Relationship between mean number of SNs present on all regions of the body and habitat complexity and benthic invertebrate abundance. (A) Habitat complexity score ranges from 0 (homogeneous habitat) to 10 (highly structured). (B) The abundance of benthic invertebrate prey present. Error bars indicate ± 1 s.e. Sample size=20 fish per population, with the exception of site JA (Jayawurrunga), where $n=15$ fish.

densely packed with SNs were the nasal and rostral regions. As these are the areas that make first contact with oncoming flow, it is likely that this is an adaptation for the early assessment of hydrodynamic flow. Interestingly, we found that variation in SN abundance over specific regions of the body was not tied to any of the environmental variables measured, suggesting that localised variation in mechanosensitivity is likely explained by other factors. Only recently have researchers begun to link the distribution of neuromasts over the body with a particular behavioural function. For example, Yoshizawa et al. (2010) used targeted SN ablation to

reveal that SNs in the face region (specifically the supraorbital area) of Pachón cavefish (*Astyanax mexicanus*) are responsible for detecting water vibrations, which helps them capture prey in the dark. Another study with *A. mexicanus* revealed that ablation of SNs in the cranial and trunk regions affects sleep patterns in Pachón cavefish but not other populations of cavefish, or surface fish (Jaggard et al., 2017). These findings not only reveal a functional relationship between sleep and sensory processing by the lateral line, but also point to independent evolutionary origins of the underlying neural mechanisms (Jaggard et al., 2017). Experiments such as these, which combine the use of targeted lateral line ablation with specific behavioural responses, are an essential and powerful tool for unravelling neuromast function.

Abundance of SNs of western rainbowfish in relation to environmental variables

Our finding that habitat complexity and the availability of benthic prey explain variation in lateral line diversity is consistent with the notion that differences in resource utilisation can result in distinct functional morphologies in fishes that relate to traits such as body size, shape and colour, the structure of the gills, teeth and jaws, and the number and arrangement of lateral plates (reviewed by Robinson and Wilson, 1994; Schluter, 1996; Skúlason and Smith, 1995). Although few studies have linked variation in neuromast abundance with behaviour, a recent study by Jiang et al. (2017) has revealed that neuromast number is correlated with the strength of the rheotactic response in sticklebacks. Specifically, sticklebacks with more neuromasts displayed a stronger negative rheotactic response (they were displaced downstream, spent more time swimming upstream and downstream, and spent less time orientated upstream) than those with fewer neuromasts (Jiang et al., 2017). While it is known that SNs play a role in numerous other behaviours including prey capture (Carton and Montgomery, 2004; Montgomery et al., 2002), predator avoidance (Montgomery et al., 2002) and social communication (Butler and Maruska, 2016; Partridge and Pitcher, 1980), we are aware of no other studies (besides Jiang et al., 2017) that have correlated neuromast abundance with the expression of a particular behavioural trait.

The general lack of studies linking variation in lateral line morphology with behaviour makes it difficult to predict the functional outcome of our findings. Nonetheless, if the behaviour of individuals with ablated neuromasts can be used as a proxy for the behaviour of individuals with low neuromast abundance (Jiang et al., 2017), then we can predict, from previous studies, that low numbers of neuromasts are associated with reduced navigational ability (Flammang and Lauder, 2013) and a reduction in the response to water disturbances (Yoshizawa et al., 2010) relative to individuals with high numbers of neuromasts. We speculate here that the increased number of SNs in rainbowfish populations from open-water habitats thus facilitates navigation in an environment in which sensory information from nearby obstacles is limited. A large number of neuromasts may also aid prey detection in environments in which food is relatively scarce. However, it is important to note that lateral line function varies among species, even for key behaviours such as rheotaxis (e.g. Van Trump and McHenry, 2013); further studies are thus required to test whether variation in neuromast abundance affects navigational performance and prey detection in rainbowfish (and other species). It is interesting to note that all studies to date have focused on neuromast abundance, while ignoring other aspects of morphology (e.g. neuromast arrangement and orientation) that may play a part (Jiang et al., 2017).

It is important to recognise that it is unlikely that lateral line morphology is influenced by a single selective agent and it is more probable that combinations of environmental variables (including those that we did not evaluate) act on multiple traits. We note that our study was based on a small sample size (eight populations), which reflects the limited availability of freshwater habitat across the arid study region at the time of sampling. Given the extreme temporal variation in conditions (e.g. turbidity, pool depth, water velocity) that is typical of much of northern and inland Australia, both within and among years, the habitats characterised are unlikely to represent the full range of environmental conditions to which fish are exposed.

In summary, this investigation has revealed that multiple environmental factors can explain some of the observed lateral line system diversity within a single species. Thus habitat disturbances, for example, owing to altered water flows, have the potential to alter sensory morphology. There is growing recognition that human-induced environmental change can cause disruption to senses such as olfaction (Munday et al., 2009) and vision (Chung et al., 2014). A future challenge is to determine whether the lateral line system exhibits some level of resilience to habitat disturbance (e.g. through developmental plasticity), or whether human impacts are likely to disrupt this sensory system and cause cascading effects for fitness-related behaviours.

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Competing interests

The authors declare no competing or financial interests.

Author contributions

Conceptualization: P.F.G., S.P.C., J.L.K.; Methodology: S.P.C., J.L.K.; Formal analysis: L.S., J.L.K.; Investigation: L.S., J.L.K.; Writing - original draft: L.S.; Writing - review & editing: P.F.G., P.M.D., J.H., S.P.C., J.L.K.; Supervision: J.H., S.P.C., J.L.K.; Project administration: P.F.G., P.M.D., S.P.C., J.L.K.; Funding acquisition: P.F.G., P.M.D., S.P.C., J.L.K.

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Data availability

Data are publicly available at Datacite: doi:10.4225/23/58c7a336d7f18

Supplementary information

Supplementary information available online at <http://jeb.biologists.org/lookup/doi/10.1242/jeb.151530.supplemental>

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Supplementary Material: Spiller et al.

Table S1. Full model set used to test for an effect of the environmental predictors (entered as fixed effects) on the PCs, which explain variation in the number of superficial neuromasts over specific regions of the body. Body length (SL) was included as a covariate in all models for PC1.

Model 1 = Predation risk

Model 2 = Habitat complexity

Model 3 = Flow velocity

Model 4 = Turbidity

Model 5 = Benthic invertebrates

Model 6 = Surface invertebrates

Model 7 = Habitat complexity + Flow velocity

Model 8 = Flow velocity * Surface invertebrates

Model 9 = Flow velocity + Predation risk

Model 10 = Flow velocity + Turbidity

Model 11 = Surface invertebrates + Benthic invertebrates

Model 12 = Flow velocity * Benthic invertebrates

Model 13 = Turbidity + Predation risk

Model 14 = random effect (site) only

Fig. S1. A plot of the principle components showing variation in the combined habitat characteristics among the sample sites. Data from one population (Weeli Wolli Creek) were excluded from the analysis owing to an incomplete dataset.

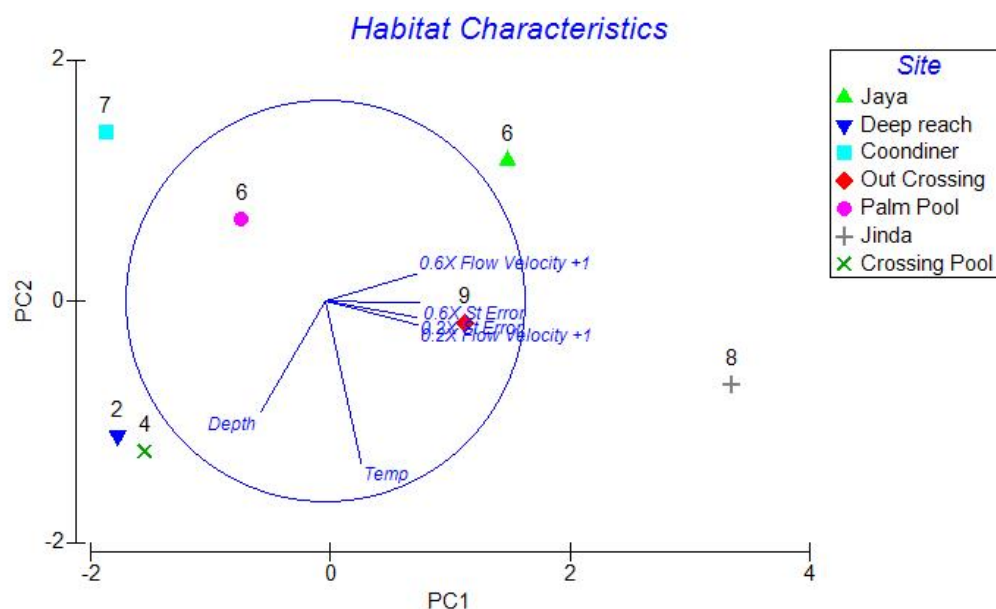


Fig. S2. A plot of the principle components for the flow measurements at the three different depths (0.2, 0.6, 0.8) for all three-flow directions (X, Y and Z) for Millstream National Park, Coondiner Creek and Weeli Wolli Creek.

