Environmentally-induced changes to brain morphology predict cognitive performance

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The relationship between the size and structure of a species' brain and its cognitive capacity has long interested scientists. Generally this work relates interspecific variation in brain anatomy with performance on a variety of cognitive tasks. However, brains are known to show considerable short-term plasticity in response to a range of social, ecological and environmental factors. Despite this, we have a remarkably poor understanding of how this impacts on an animal's cognitive performance. Here, we non-invasively manipulated the relative size of brain regions associated with processing visual and chemical information in fish (the optic tectum and olfactory bulbs, respectively). We then tested performance in a cognitive task in which information from the two sensory modalities was in conflict. Although the fish could effectively utilise both visual and chemical information if presented in isolation, when they received cues from both modalities simultaneously, those with relatively better developed optic tecta showed a greater reliance on visual information, while individuals with relatively better developed olfactory bulbs showed a greater reliance on chemical information. These results suggest that short-term changes in brain structure, possibly resulting from an attempt to minimise the costs of developing unnecessary but energetically expensive brain regions, may have marked effects on cognitive performance.

Keywords: animal cognition, behavioural plasticity, nine-spined stickleback, *Pungitius pungitius*, numerosity

1. Introduction

There has been considerable interest in the relationship between the structure of the brain and a species' cognitive capacity [1], resulting in a substantial body of evidence linking interspecific variation in brain size – or the size of particular functional regions within the brain – to performance in a range of cognitive tasks [2-11]. However, brains are known to show considerable short-term plasticity in response to a range of social, ecological and environmental factors. For example, the structural complexity of the environment has been shown to affect both overall brain size and the development of particular brain regions, while brain morphology is also known to be influenced by social factors such as rearing density, social stimulation and predation risk (reviewed in [12, 13]). However, despite strong evidence that extrinsic factors can impact on brain structure, we have a remarkably poor understanding of how induced variation in brain structure impacts on an animal's cognition, which following [14] we define here broadly as the various ways in which an animal takes in information through the senses, processes, retains and decides to act on it.

Because the brain is the most expensive tissue to develop and maintain [6, 15], energetic constraints may result in brain regions that are important in a given context developing more than those that are of less importance [12]. This is likely to be particularly evident where there is differential availability of information from alternate sensory modalities, which may lead to the relative retardation or enhancement of the specific brain regions responsible for processing this sensory information. Using nine-spined sticklebacks (*Pungitius pungitius*) as a model, we aimed to induce differences in the relative size of brain regions associated with the processing of visual and chemical information (the optic tectum and olfactory bulbs, respectively), by rearing fish in conditions that manipulated the relative efficacy of these different sensory modalities. Fish are ideal for investigating neural plasticity as neurogenesis is extremely pronounced in both juveniles and adults [16-18], potentially affording them considerable scope to differentially develop particular brain regions in response to changing environmental conditions. Following this period of manipulation, we then tested their ability to discriminate between shoals based on the relative number of fish in each [19] – a cognitive task which, under our experimental conditions, required the integration of information from both senses. We predicted that when visual and chemical cues for shoal size were incongruent (i.e. when a shoal appeared large in the visual domain but small in the chemical domain versus a shoal that appeared small in the visual domain but large in the chemical domain), fish reared in conditions designed to promote the relative use of one sensory modality over the other should preferentially utilise this modality to inform their shoal choice, and that this would be consistent with experimentally-induced differences in brain morphology.

2. Methods

Test subjects

Nine-spined sticklebacks were wild-caught using hand nets during October 2013 from a drainage ditch near Lincoln, UK, and juveniles (estimated to be around 3 months old, based on their body size; [20]) selected for use in this study. These fish were randomly divided into two equally-sized treatment groups: (1) visually-unrestricted and (2) visually-restricted, with 3 replicates of each. Each group was housed in an opaque grey 45 L plastic tank filled with dechlorinated tap water. In order to manipulate the transmission of light through the water of fish in the visually-restricted groups, we added 0.16 g L^{-1} black pond dye (Brilliant Black BN; Hydra International Ltd, Milton Keynes, UK), which restricted the visual range to approximately 2 cm. Fish in the visually-unrestricted group were housed in unmanipulated water. Each tank contained an air stone and sponge filter. The temperature was maintained at 12 ± 1°C and the photoperiod was adjusted weekly to match the average natural photoperiod at the source stream. The fish were fed to satiation daily on frozen bloodworm. They were housed under these conditions for approximately 6 months until the start of the experiment.

Experimental design

Experimental and control trials (see below) were run in a rectangular glass tank (65 × 38 × 40 cm) with a water height of 7 cm. Unmanipulated water was used in each case, and water was fully changed between successive trials. Two additional glass tanks (7 × 25 × 45 cm) were placed at either end of the main tank, and housed stimulus shoals of nine-spined sticklebacks. The outward facing walls of the tanks were covered in black plastic to minimise disturbance to the fish. In trials testing the focal fish's ability to utilise visual information (see below), the fish had unrestricted visual access to both stimulus shoals but, because the tanks housing the stimulus fish were physically separated from the main tank, there was no access to other (e.g. chemical or mechanosensory) information. In trials testing their ability to utilise chemical information, visual access to the stimulus shoals was blocked by placing an opaque divider between the main tank and those housing the stimulus shoals. Instead chemical information was provided by dripping stimulus water, containing information consistent with shoals of a particular size, into the experimental tank through burettes located above the centre of tank walls adjacent to the stimulus tanks at a rate of 10 ml min^{-1}. Stimulus water was created by housing 10 fish in 1 L of oxygenated water for 48 h, and then used either undiluted (to simulate 10 fish) or appropriately diluted (to simulate fewer than 10 fish). This ensured that the composition of the chemical stimulus was consistent for each focal fish, but was presented at different concentrations indicative of different shoal sizes. All the fish used in the preparation of the chemical stimulus were unfamiliar to the focal fish and were not involved in these experiments.

At the start of each trial, the focal fish was placed in a perforated container (5 cm diameter) in the centre of the main tank. Following 1 min of acclimatisation, the container was lifted and the behaviour of the focal fish was monitored using point samples taken every 10 s for 5 min. Specifically, we recorded when the fish was in either of two 7.5 cm (i.e. approximately 2 body lengths, and so well within the distance that would be considered shoaling; [21]) preference zones parallel to the shoal containers at either end of the experimental tank. A fish was considered in the preference area when any part of its body crossed the line. Preference was quantified as the proportion of time spent in the choice zone adjacent to the larger shoal.

Control trials

Control trials were conducted in order to determine whether fish from both the visually-unrestricted and visually-restricted groups were able to use chemical and visual information, in isolation, to mediate their shoal choice preferences. We presented randomly selected fish (n = 18 in total, 9 from each treatment group with 3 from each replicate rearing tank) with a series of choices between two stimulus shoals that differed in size. Specifically, each fish received 5 trials in which the size ratio of the two stimulus shoals was either 10:4, 8:4, 7:4, 6:4 or 5:4, in each of two conditions: visual information only, or chemical information only (10 trials in total). Based on previous findings from a variety of fish species (e.g. [22-24]) these size ratios are assumed to be discriminable under normal circumstances, although with the prediction that discriminatory ability would decrease as the ratio of the number of individuals in each stimulus shoal converges on one (i.e. fish would exhibit a strong preference when shoal sizes were easily distinguishable, but increasingly weaker preferences as shoal sizes became more similar). Trials were presented in a random order, and the side of the tank housing the larger shoal was randomised. There was a 10 min interval between consecutive trials.

Experimental trials

The experimental trials aimed to test how fish traded off chemical and visual information when making shoal choice decisions, based on the treatment they came from (visually-restricted or visually-unrestricted). Randomly selected fish (n = 40 in total, 20 from each treatment group with approximately equal numbers from each of the replicate rearing tanks), which had not been used in the control trials, were presented with a series of simultaneous choices between two shoals. These two shoals differed visibly in size, with one always being larger than the other according to the ratios used during the control trials (i.e. 10:4, 8:4, 7:4, 6:4 or 5:4). However, in order to test the focal fish's relative utilisation of visual and chemical information in mediating their shoal choice behaviour, chemical information was presented incongruently, such that visual information from one shoal was paired with chemical information indicative of the number of fish present in the other shoal (i.e. focal fish were presented with a shoal that appeared large in the visual domain but small in the chemical domain versus a shoal that appeared small in the visual domain but

large in the chemical domain). Experimental trials were otherwise run following exactly the same protocol as used for the control trials, except that shoaling preference was measured as the proportion of time spent with the visually larger (chemically smaller) shoal.

Morphometric measurements

Immediately following the completion of their experimental trial, fish were euthanized with an overdose of MS222 (tricaine methanesulfonate) and their standard length (from the tip of the mouth to the end of the caudal peduncle) was measured to the nearest 0.01 mm with digital callipers. Brains were then dissected out as described in [25], and fixed in 4% buffered formalin (in 0.1 M phosphate buffered saline) solution for 48 h. After fixation, top- and side-view digital photos were taken, allowing the width, height and length of the whole brain, and five different brain regions (the olfactory bulbs, telencephalon, optic tectum, cerebellum and dorsal medulla), to be measured using ImageJ [25]. We calculated the volume of the different brain regions using an ellipsoid model (e.g. [26]), and estimated total brain volume as the sum of the five constituent regions. Nine-spined sticklebacks are known to have sexually size-dimorphic brains [27], and so sex was determined by visual examination of the gonads.

Statistical analysis

All analyses were conducted in R version 3.3.1 (R Core Development Team). We tested for differences in brain volume, and the volume of individual brain regions, as a function of treatment group (visually-unrestricted or visually-restricted), sex (male or female) and replicate rearing tank (A-F) using a series of general linear models (GLMs) (implemented using the lm function). Models included log10-transformed volume as the dependent variable, and main effects terms of treatment, sex, and rearing tank; three- and two-way interactions between these factors were initially considered, but were all non-significant (all $p > 0.05$) and so dropped from the final models presented here. In the analysis of overall brain size, we included log10-transfomed standard length as a covariate to control for the effects of brain-to-body allometry [28]. In the analyses of individual brain regions, allometry was controlled for by including log10-transformed brain volume (minus the brain region of interest) as the covariate.

In the control trials, preference for the larger shoal was analysed as a function of shoal size ratio using generalised linear mixed models (GLMMs) (using the glmer function in the lme4 package; [29]). Models included a binomial response variable (with a logit link function) of time spent with the larger shoal given the time spent with the smaller shoal; shoal size ratio (10:4, 8:4, 7:4, 6:4 or 5:4) as a fixed factor; and fish identity, sex, and rearing tank as random effects terms. Significance was tested by comparing full models to models that lacked the term of interest, using likelihood ratio tests [30]. Because we predicted a decline in preference as the shoal size ratio approached one (i.e. fish would exhibit a strong preference when shoal sizes were easily distinguishable, but increasingly weaker

preferences as shoal sizes became more similar) we specifically tested for changes in preferences over successive shoal size ratios by fitting polynomial (linear and quadratic) contrasts across levels of the fixed factor [31], rather than focussing on overall preferences. Differences from chance levels of preference for each shoal size ratio were tested using the procedure described by [32], and p-values adjusted using a Bonferroni correction.

Experimental trials were analysed by fitting a GLMM with a binomial response variable (with a logit link function) of time spent with the visually larger (chemically smaller) shoal given the time spent with visually smaller (chemically larger) shoal; shoal size ratio, treatment group, and the interaction between shoal size ratio and treatment as fixed factors; and fish identity, sex and rearing tank as random effects. As for control trials, because we were interested in sequential changes in preference over successive shoal size ratios we fitted polynomial (linear and quadratic) contrasts across the levels of shoal size ratio. Differences from chance levels of preference were tested following [32], and differences between treatments for a given shoal size ratio were tested using GLMMs, with treatment as a single fixed factor but otherwise parameterised as described above. In both cases p-values were adjusted using a Bonferroni correction.

Finally, we considered the performance of individual fish on the experimental task as a function of their olfactory bulb and optic tectum volume. Specifically, we focussed on the two shoal size ratios for which there were the largest overall differences in preference (8:4 and 7:4; see Results) by fitting GLMMs with a binomial response variable (with a logit link function) of time spent with the visually larger (chemically smaller) shoal given the time spent with visually smaller (chemically larger) shoal, and brain region volume as a covariate; treatment, sex and rearing tank were included as random effects. The odds ratio (OR) was used as a measure of effect size [33].

Ethics

The work conducted here strictly complied with the laws of the UK, and the study was approved by the appropriate local ethical review committee at the University of Lincoln.

3. Results

Morphometric measurements

There was no significant difference in overall brain volume between fish in the two treatment groups ($F_{1,35} = 0.47$, $p = 0.496$). However, consistent with our predictions, the fish in our experiment differentially developed brain regions involved in processing sensory information: those reared in conditions that increased their relative reliance on chemical information (the visually-restricted group) developed relatively large olfactory bulbs ($F_{1,35} = 36.31$, $p < 0.001$), and relatively small optic tecta ($F_{1,35} = 13.61$, $p < 0.001$), compared to fish reared in conditions that allowed them to utilise visual information (the visually-unrestricted

group) (Figure 1). No other measured brain regions differed between the groups (telencephalon: $F_{1,35} = 0.07$, $p = 0.792$; dorsal medulla: $F_{1,35} = 0.61$, $p = 0.440$; cerebellum: $F_{1,35} = 0.64$, $p = 0.429$). In line with previous findings in this species, males had significantly larger brains overall than females ($F_{1,35} = 42.33$, $p < 0.001$), as well as significantly larger brain regions (all $p < 0.001$). There were no significant differences between replicate rearing tanks for overall brain volume, or for the volume of any of the measured brain regions (all $p > 0.15$) except the dorsal medulla ($F_{1,35} = 8.43$, $p = 0.006$), suggesting that each replicate responded to the experimental treatment in a similar way.

Control trials

There were highly significant differences in preference between shoal size ratios in each of the four control trials (visually-unrestricted, chemical information: $\chi^2(4) = 285.4$, $p < 0.001$; visually-unrestricted, visual information: $\chi^2(4) = 182.2$, $p < 0.001$; visually-restricted, chemical information: $\chi^2(4) = 126.7$, $p < 0.001$; visually-restricted, visual information: $\chi^2(4) = 193.7$, $p < 0.001$) (Figure 2). As predicted, fish from both treatments showed significant linear decreases in their preference for the largest shoal as the shoal size ratio became increasingly similar to one, both when only chemical information was available (polynomial contrasts from a GLMM, visually-restricted: quadratic, $z = 2.46$, $p = 0.014$; visually-unrestricted: quadratic, $z = -3.43$, $p < 0.001$; Figure 2a,c) and when only visual information was available (linear contrasts, visually-restricted: linear, $z = -11.68$, $p < 0.001$; visually-unrestricted: quadratic, $z = -4.40$, $p < 0.001$; Figure 2b,d). There was therefore clear evidence that fish from both groups could utilise visual and chemical cues when presented in isolation to make shoal-choice decisions, but that they found this task harder as the shoals became increasingly similar in size.

Experimental trials

When visual and chemical information was presented to fish incongruently, there was a significant interaction between treatment and shoal size ratio ($\chi^2(4) = 104.81$, $p < 0.001$), suggesting that different information was salient to the different groups when making decisions (Figure 3). While fish in both the visually-restricted and visually-unrestricted groups showed a non-linear change in their preference over successive shoal size ratios (polynomial contrasts from GLMM, visually-restricted: quadratic, $z = 4.36$, $p < 0.001$; visually-unrestricted: quadratic, $z = -5.39$, $p < 0.001$), these were in opposite directions in each of the two groups: the peak preference for fish in the visually-unrestricted group was for visual information (Figure 3a), while the peak preference for fish in the visually-restricted group was for chemical information (Figure 3b). Specifically, preferences differed significantly between the visually-restricted and visually-unrestricted treatments for the 8:4 ($\chi^2(1) = 8.65$, $p = 0.003$) and 7:4 ($\chi^2(1) = 8.30$, $p = 0.004$) shoal size ratios, but not for any of the other ratios (all $p > 0.05$).

When considering the performance of individual fish in the experimental trials, there were significant positive relationships between optic tectum volume and preference for the visually larger shoal (8:4 shoal size ratio: OR = 1.12, $\chi^2(1) = 3.96$, $p = 0.047$; 7:4 shoal size ratio: OR = 1.21, $\chi^2(1) = 6.99$, $p = 0.008$), suggesting that fish with larger optic tecta were more likely to utilise visual information when making a shoal choice decision (Figure 4a). In contrast, there were negative relationships between olfactory bulb volume and visual preference (8:4 shoal size ratio: OR = 0.75, $\chi^2(1) = 7.06$, $p = 0.008$; 7:4 shoal size ratio: OR = 0.66, $\chi^2(1) = 2.91$, $p = 0.088$), such that fish with larger olfactory bulbs were more likely to use chemical information to inform their shoal choice behaviour (Figure 4b). Finally, if the olfactory bulb/optic tectum ratio is used as the predictor, fish with relatively large olfactory bulbs were more likely to utilise chemical information, while those with relatively small olfactory bulbs were more likely to use visual information (8:4 shoal size ratio: OR = 0.53, $\chi^2(1) = 4.42$, $p = 0.040$; 7:4 shoal size ratio: OR = 0.53, $\chi^2(1) = 19.43$, $p < 0.001$; Figure 4c). These individual-level data are therefore consistent with the patterns observed at the group level, and reveal that fish differentially used information in a manner consistent with the treatment that they been exposed to.

4. Discussion

Our shoal choice experiments provide clear evidence that although fish could use both visual and chemical information in isolation to inform their choice (as indicated by their performance in the control trials), when provided with conflicting information from both sensory modalities simultaneously they exhibited preferences that were entirely consistent with the relative development of the relevant brain regions. Specifically, fish from the visually-restricted condition developed significantly larger olfactory bulbs and significantly smaller optic tecta, and preferentially utilised chemical information over visual information to inform their shoal choice decisions; in contrast, fish from the visually-unrestricted condition developed significantly larger optic tecta and significantly smaller olfactory bulbs, and preferentially utilised visual information. These patterns are also evident when considering the individual-level behavioural data, in which individuals with larger optic tecta preferentially used visual information to inform their shoal choice, while individuals with larger olfactory bulbs tended to use chemical information.

The non-linear preference pattern that was evident across the experimental trials (Figure 3) is likely to be the result of fish finding both the chemical and visual information highly salient when the shoal size differential was largest (i.e. a ratio of 10:4). In contrast, when the shoal size differential was smallest (i.e. as ratios approached 5:4) it is likely that the stimuli were very difficult to discriminate, consistent with the performance of fish in the control trials. In both cases we hypothesise that this resulted in them arbitrarily using one or other of the modalities to inform their shoal choice, resulting in chance levels of preference at the

group level. Only at intermediate shoal size ratios were preferences realised, resulting in the observed non-linear patterns. It is noteworthy that neither the overall brain volume, nor the volume of the other brain regions differed significantly between treatments, although all were larger in males than in females as has been shown previously in this species [27]. The dorsal medulla differed in size between replicates, although the cause of this is not known [34].

While we focussed on two particular sensory modalities in this study, namely the detection of visual and chemical information, sticklebacks are also known to respond to both auditory stimuli [35] and mechanosensory input via their lateral line [36, 37]. While we cannot completely rule out that other senses were impacted by our environmental manipulation (for example, fish reared under the visually-restricted conditions may have made increased use of mechanosensory information for shoaling [37]), the design of the experiment means these are unlikely to have impacted on the general trends were found. It would be informative, though, to consider conditions in which the input to the various different sensory modalities was systematically manipulated, including manipulating the relative availability of chemical and mechanosensory information.

In this study we only considered the overall volume of the various brain regions, and did not investigate whether the variation we observed between treatments was due to increased cell size or increased neuronal density [38], which may be an important distinction in light of work suggesting that cognitive performance depends more on the absolute number of cerebral neurons and their connections [39-41] than the relative size of the brain (or brain region) per se [42]. Understanding the mechanism driving the changes in brain size we observed would also allow us to draw comparisons with similar studies reporting evolutionary (as opposed to plastic) changes to brain architecture in wild stickleback populations [12] which, while superficially similar, may in fact be driven by quite different processes. However, regardless of the underlying mechanism our work provides good experimental support for the assumption that the plastic variation in brain size observed previously in sticklebacks [25, 27, 28, 34, 43] has behavioural relevance. This may be particularly important given that the heritability of relative brain size, and the relative size of the different brain regions, is comparatively low in the closely-related three-spined stickleback (*Gasterosteus aculeatus*) [43], suggesting a large plastic component to brain architecture; plasticity is therefore likely to be an important mechanism by which sticklebacks respond to environmental variation.

In this paper we provide experimental evidence that the size of an individual's brain directly impacts on its performance in a cognitive task [1]. In particular our results emphasise that short-term, environmentally-induced changes in brain structure, possibly resulting from an attempt to minimise the costs of developing unnecessary but energetically expensive brain regions [12, 15], can have a marked impact on an animal's cognitive performance. However, such cerebral plasticity may itself be costly [44], and so the benefits conferred by enhanced

behavioural performance would need to be sufficiently great to overcome them. Although we tested subjects in a group-choice experiment, the ability of animals to differentially utilise sensory information from different modalities (and the constraints placed on this by the relative size of the brain regions responsible for processing this sensory information) is likely to underpin most of its decision-making processes. The implications of this are therefore wide and varied, impacting on behaviours as fundamental and disparate as social interactions, foraging, detecting and evading predators, and locating and selecting mates (reviewed in [45]).

Data accessibility. The data used in the analyses presented here are available in the electronic supplementary material.

Authors' contributions. All authors conceived the idea and designed the experiment. M.R. collected the data, T.P. performed the statistical analyses, and A.W. and T.P. wrote the manuscript with contributions from M.R.

Competing interests. We declare we have no competing interests.

Acknowledgements. We thank D. Simpson for animal husbandry, and W. Hayes and T. Kleinhappel for useful advice and discussions, and two anonymous reviewers for their constructive and helpful comments.

References

[1] Northcutt, R.G. 2002 Understanding vertebrate brain evolution. *Integr Comp Biol* **42**, 743-756. (doi:10.1093/Icb/42.4.743).
[2] Buechel, S.D., Boussard, A., Kotrschal, A., van der Bijl, W. & Kolm, N. 2018 Brain size affects performance in a reversal-learning test. *Proc R Soc B* **285**. (doi:10.1098/Rspb.2017.2031).
[3] Corral-Lopez, A., Bloch, N.I., Kotrschal, A., van der Bijl, W., Buechel, S.D., Mank, J.E. & Kolm, N. 2017 Female brain size affects the assessment of male attractiveness during mate choice. *Sci Adv* **3**. (doi:10.1126/sciadv.1601990).
[4] Kotrschal, A., Buechel, S.D., Zala, S.M., Corral-Lopez, A., Penn, D.J. & Kolm, N. 2015 Brain size affects female but not male survival under predation threat. *Ecol Lett* **18**, 646-652. (doi:10.1111/ele.12441).
[5] Kotrschal, A., Corral-Lopez, A., Amcoff, M. & Kolm, N. 2015 A larger brain confers a benefit in a spatial mate search learning task in male guppies. *Behav Ecol* **26**, 527-532. (doi:10.1093/beheco/aru227).
[6] Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brannstrom, I., Immler, S., Maklakov, A.A. & Kolm, N. 2013 Artificial Selection on Relative Brain Size in the Guppy

Reveals Costs and Benefits of Evolving a Larger Brain. *Curr Biol* **23**, 168-171. (doi:10.1016/j.cub.2012.11.058).
[7] Lefebvre, L., Nicolakakis, N. & Boire, D. 2002 Tools and brains in birds. *Behaviour* **139**, 939-973. (doi:Doi 10.1163/156853902320387918).
[8] Madden, J. 2001 Sex, bowers and brains. *Proc R Soc B* **268**, 833-838. (doi:DOI 10.1098/rspb.2000.1425).
[9] Reader, S.M. & Laland, K.N. 2002 Social intelligence, innovation, and enhanced brain size in primates. *Proc Natl Acad Sci USA* **99**, 4436-4441. (doi:10.1073/pnas.062041299).
[10] Sol, D. & Lefebvre, L. 2000 Behavioural flexibility predicts invasion success in birds introduced to New Zealand. *Oikos* **90**, 599-605. (doi:DOI 10.1034/j.1600-0706.2000.900317.x).
[11] van der Bijl, W., Thyselius, M., Kotrschal, A. & Kolm, N. 2015 Brain size affects the behavioural response to predators in female guppies (Poecilia reticulata). *Proc R Soc B* **282**, 116-124. (doi:10.1098/Rspb.2015.1132).
[12] Gonda, A., Herczeg, G. & Merila, J. 2013 Evolutionary ecology of intraspecific brain size variation: a review. *Ecol Evol* **3**, 2751-2764. (doi:10.1002/ece3.627).
[13] van Praag, H., Kempermann, G. & Gage, F.H. 2000 Neural consequences of environmental enrichment. *Nat Rev Neurosci* **1**, 191-198. (doi:10.1038/35044558).
[14] Shettleworth, S.J. 2001 Animal cognition and animal behaviour. *Anim Behav* **61**, 277-286. (doi:10.1006/anbe.2000.1606).
[15] Aiello, L.C. & Wheeler, P. 1995 The Expensive-Tissue Hypothesis - the Brain and the Digestive-System in Human and Primate Evolution. *Curr Anthropol* **36**, 199-221. (doi:10.1086/204350).
[16] Zikopoulos, B., Kentouri, M. & Dermon, C.R. 2000 Proliferation zones in the adult brain of a sequential hermaphrodite teleost species (Sparus aurata). *Brain Behav Evol* **56**, 310-322. (doi:Doi 10.1159/000047215).
[17] Ekstrom, P., Johnsson, C.M. & Ohlin, L.M. 2001 Ventricular proliferation zones in the brain of an adult teleost fish and their relation to neuromeres and migration (secondary matrix) zones. *J Comp Neurol* **436**, 92-110. (doi:Doi 10.1002/Cne.1056).
[18] Zupanc, G.K.H., Hinsch, K. & Gage, F.H. 2005 Proliferation, migration, neuronal differentiation, and long-term survival of new cells in the adult zebrafish brain. *J Comp Neurol* **488**, 290-319. (doi:10.1002/cne.20571).
[19] Agrillo, C., Dadda, M. & Bisazza, A. 2007 Quantity discrimination in female mosquitofish. *Anim Cogn* **10**, 63-70. (doi:10.1007/s10071-006-0036-5).
[20] Wootton, R.J. 1984 *A functional biology of sticklebacks*. Beckenham, UK, Croom Helm.
[21] Pitcher, T.J. & Parrish, J.K. 1993 The functions of shoaling behaviour. In *The behaviour of teleost fishes, 2nd ed.* (ed. T.J. Pitcher), pp. 363–439. London, Chapman and Hall.
[22] Buckingham, J.N., Wong, B.B.M. & Rosenthal, G.G. 2007 Shoaling decisions in female swordtails: how do fish gauge group size? *Behaviour* **144**, 1333-1346. (doi:10.1163/156853907782418196).

[23] Mehlis, M., Thunken, T., Bakker, T. & Frommen, J. 2015 Quantification acuity in spontaneous shoaling decisions of three-spined sticklebacks. *Anim Cogn* **18**, 1125-1131. (doi:10.1007/s10071-015-0884-y).
[24] Agrillo, C., Dadda, M., Serena, G. & Bisazza, A. 2009 Use of Number by Fish. *Plos One* **4**. (doi:10.1371/journal.pone.0004786).
[25] Gonda, A., Herczeg, G. & Merila, J. 2009 Adaptive brain size divergence in nine-spined sticklebacks (Pungitius pungitius)? *J Evol Biol* **22**, 1721-1726. (doi:10.1111/j.1420-9101.2009.01782.x).
[26] Pollen, A.A., Dobberfuhl, A.P., Scace, J., Igulu, M.M., Renn, S.C.P., Shumway, C.A. & Hofmann, H.A. 2007 Environmental complexity and social organization sculpt the brain in Lake Tanganyikan cichlid fish. *Brain Behav Evol* **70**, 21-39. (doi:10.1159/000101067).
[27] Herczeg, G., Valimaki, K., Gonda, A. & Merila, J. 2014 Evidence for sex-specific selection in brain: a case study of the nine-spined stickleback. *J Evol Biol* **27**, 1604-1612. (doi:10.1111/jeb.12409).
[28] Gonda, A., Herczeg, G. & Merila, J. 2011 Population variation in brain size of nine-spined sticklebacks (Pungitius pungitius) - local adaptation or environmentally induced variation? *BMC Evolutionary Biology* **11**. (doi:10.1186/1471-2148-11-75).
[29] Pinheiro, J., Bates, D., DebRoy, S. & Sarkar, D. 2009 *nlme: Linear and Nonlinear Mixed Effects Models*, R package version 3.1–96.
[30] Crawley, M.J. 2007 *The R book*. Chichester, Wiley.
[31] Dean, A.M. & Voss, D. 1999 *Design and Analysis of Experiments*. New York, Springer-Verlag.
[32] Kulkarni, P.M. & Shah, A.K. 1995 Testing the Equality of Several Binomial Proportions to a Prespecified Standard. *Stat Probabil Lett* **25**, 213-219. (doi:Doi 10.1016/0167-7152(94)00224-V).
[33] Bland, J.M. & Altman, D.G. 2000 The odds ratio. *Brit Med J* **320**, 1468-1468. (doi:DOI 10.1136/bmj.320.7247.1468).
[34] Toli, E.A., Noreikiene, K., DeFaveri, J. & Merila, J. 2017 Environmental enrichment, sexual dimorphism, and brain size in sticklebacks. *Ecol Evol* **7**, 1691-1698. (doi:10.1002/ece3.2717).
[35] Purser, J. & Radford, A.N. 2011 Acoustic noise induces attention shifts and reduces foraging performance in three-spined sticklebacks (*Gasterosteus aculeatus*). *Plos One* **6**. (doi:10.1371/journal.pone.0017478).
[36] Wark, A.R. & Peichel, C.L. 2010 Lateral line diversity among ecologically divergent threespine stickleback populations. *J Exp Biol* **213**, 108-117. (doi:10.1242/jeb.031625).
[37] Greenwood, A.K., Wark, A.R., Yoshida, K. & Peichel, C.L. 2013 Genetic and neural modularity underlie the evolution of schooling behavior in threespine sticklebacks. *Curr Biol* **23**, 1884-1888. (doi:10.1016/j.cub.2013.07.058).
[38] Olkowicz, S., Kocourek, M., Lucan, R.K., Portes, M., Fitch, W.T., Herculano-Houzel, S. & Nemec, P. 2016 Birds have primate-like numbers of neurons in the forebrain. *Proc Natl Acad Sci USA* **113**, 7255-7260. (doi:10.1073/pnas.1517131113).

[39] Roth, G. & Dicke, U. 2005 Evolution of the brain and intelligence. *Trends Cogn Sci* **9**, 250-257. (doi:10.1016/j.tics.2005.03.005).
[40] Herculano-Houzel, S. 2011 Brains matter, bodies maybe not: the case for examining neuron numbers irrespective of body size. *Ann Ny Acad Sci* **1225**, 191-199. (doi:10.1111/j.1749-6632.2011.05976.x).
[41] Dicke, U. & Roth, G. 2016 Neuronal factors determining high intelligence. *Philos T R Soc B* **371**. (doi:10.1098/Rstb.2015.0180).
[42] Deaner, R.O., Isler, K., Burkart, J. & van Schaik, C. 2007 Overall brain size, and not encephalization quotient, best predicts cognitive ability across non-human primates. *Brain Behav Evol* **70**, 115-124. (doi:Doi 10.1159/000102973).
[43] Noreikiene, K., Herczeg, G., Gonda, A., Balazs, G., Husby, A. & Merila, J. 2015 Quantitative genetic analysis of brain size variation in sticklebacks: support for the mosaic model of brain evolution. *Proc R Soc B* **282**. (doi:10.1098/Rspb.2015.1008).
[44] Snell-Rood, E.C. 2013 An overview of the evolutionary causes and consequences of behavioural plasticity. *Anim Behav* **85**, 1004-1011. (doi:10.1016/j.anbehav.2012.12.031).
[45] Halfwerk, W. & Slabbekoorn, H. 2015 Pollution going multimodal: the complex impact of the human-altered sensory environment on animal perception and performance. *Biol Lett* **11**. (doi:10.1098/Rsbl.2014.1051).
[46] Pike, T.W. 2017 qboxplot: Quantile-Based Boxplot. R package version 0.2 (https://CRAN.R-project.org/package=qboxplot).

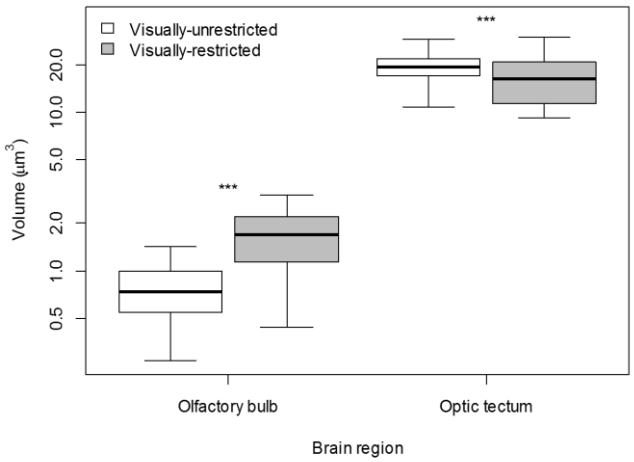


Figure 1. Volumes of two brain regions, the olfactory bulb and the optic tectum, in fish from the visually-unrestricted group (white boxes, *n* = 20) and the visually-restricted group (grey boxes, *n* = 20). In each case, the thick horizontal line represents the median, the boxes the 25th and 75th percentiles and the vertical lines the range of the data. Note the log scale on the vertical axis. Asterisks indicate significant differences between groups for a particular brain region: ***, $p < 0.001$.

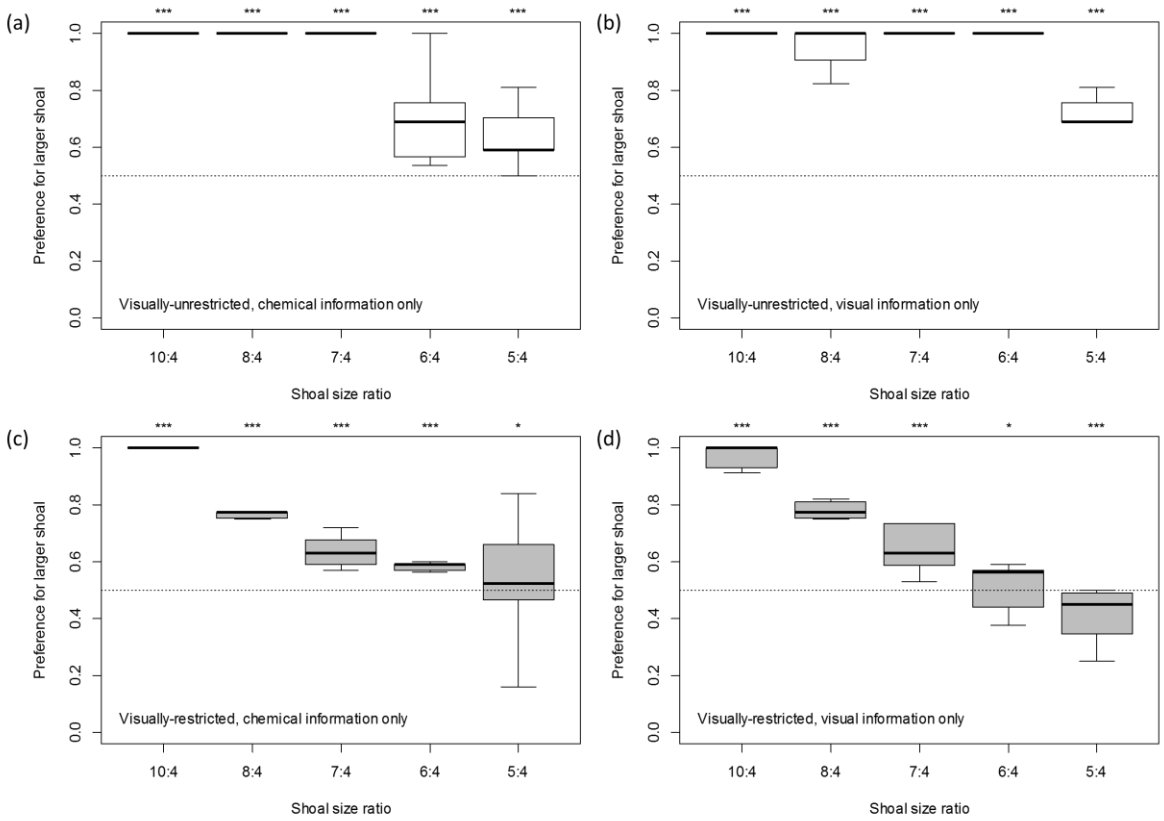
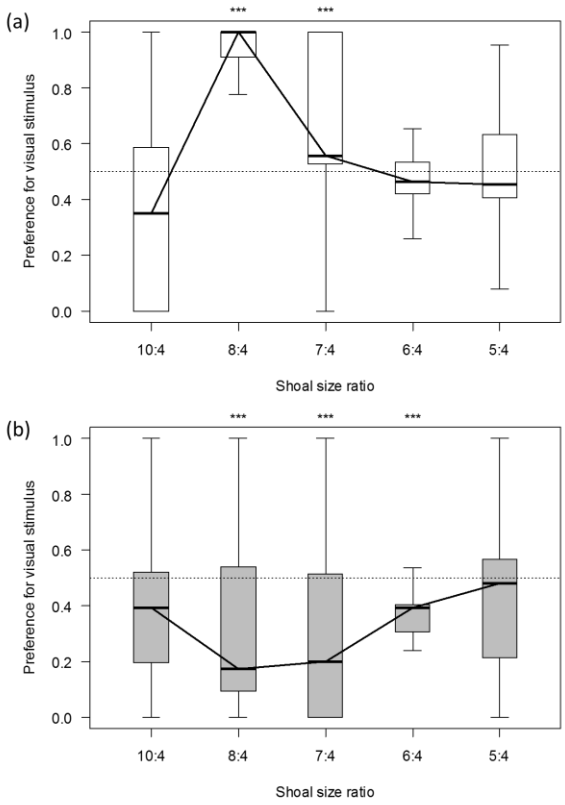


Figure 2. Preferences for the larger of two simultaneously presented shoals (measured as the proportion of time spent with the larger shoal) in fish from the visually-unrestricted group (a, b; white bars, *n* = 9) and the visually-restricted group (c, d; grey bars, *n* = 9), over five different shoal size ratios, when only chemical information was available (a, c) and when only visual information was available (b, d) (see main text for full details). In each case, the thick horizontal line represents the median, the boxes the 40th and 60th percentiles and the vertical lines the range of the data [46]; the dashed horizontal line indicates chance levels of preference. Asterisks above each box denote a significant difference from chance, following Bonferroni correction: ***, $p < 0.001$; *, $p < 0.05$.



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560 Figure 3. Preferences for two simultaneously presented shoals in fish from the visually-
561 unrestricted group (*n* = 20) (a) and the visually-restricted group (*n* = 20) (b). Each fish had a
562 choice between two shoals in which the information regarding shoal size was incongruent: a
563 shoal that appeared large in the visual domain but small in the chemical domain versus a
564 shoal that appeared small in the visual domain but large in the chemical domain, over each
565 of five different shoal size ratios (see text for full details). Preference was measured as the
566 proportion of time spent with the visually larger (chemically smaller) shoal, and so higher
567 values denote a preference for visual information in shoal choice; lower values denote a
568 preference for chemical information. For each box, the thick horizontal lines represent the
569 median, the boxes the 40th and 60th percentiles and the vertical lines the range of the data
570 [46]; medians are joined (thick lines) to illustrate changes in preference over successive
571 shoal size ratios. The thin dashed horizontal line indicates chance levels of preference.
572 Asterisks above a box denote a significant difference from chance, following Bonferroni
573 correction: ***, $p < 0.001$.

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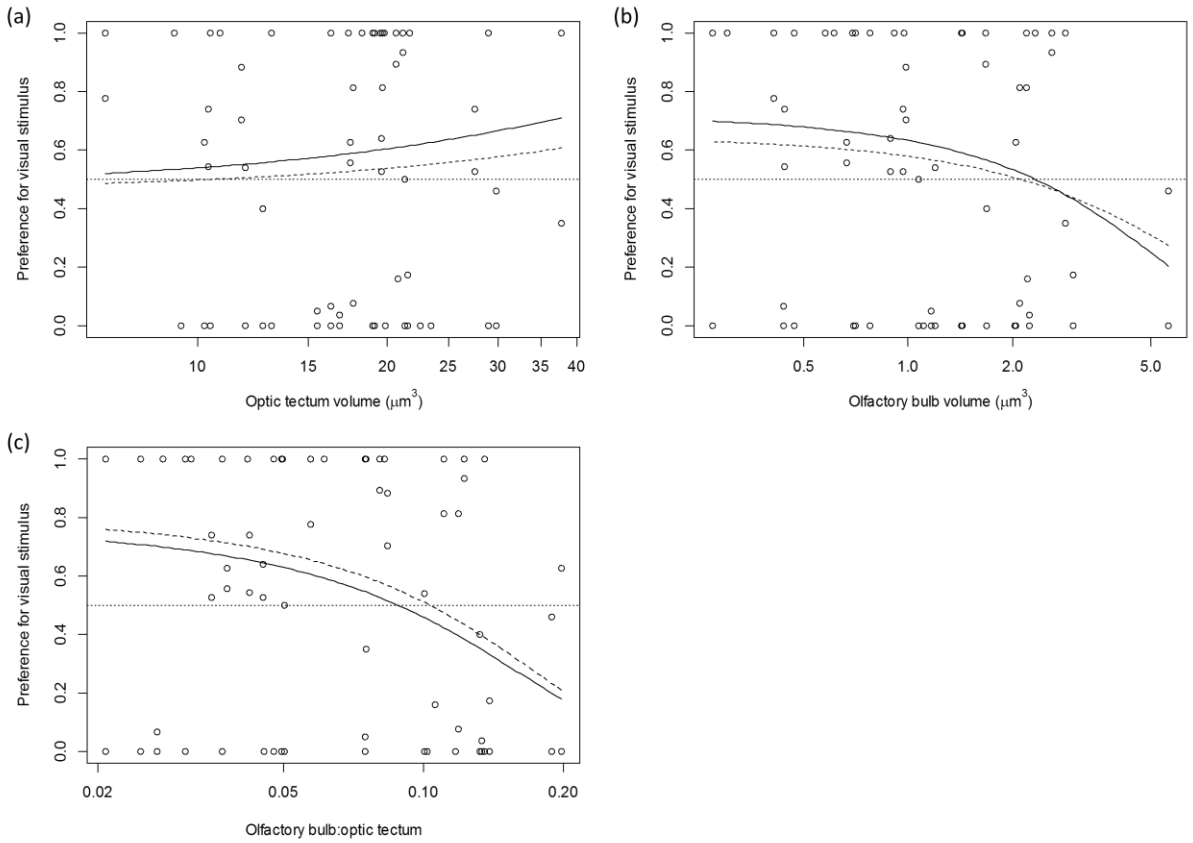

578

579 Figure 4. Individual preferences for two simultaneously presented shoals as a function of (a)
580 optic tectum volume, (b) olfactory bulb volume and (c) olfactory bulb/optic tectum ratio.
581 Each fish had a choice between two shoals in which the information regarding shoal size
582 was incongruent: a shoal that appeared large in the visual domain but small in the chemical
583 domain versus a shoal that appeared small in the visual domain but large in the chemical
584 domain, over each of five different shoal size ratios (see text for full details). Preference was
585 measured as the proportion of time spent with the visually larger (chemically smaller) shoal,
586 and so higher values denote a preference for visual information in shoal choice and lower
587 values denote a preference for chemical information. Data points denote preferences for
588 individual fish, while the curves show the GLMM model fit for shoal size ratios of 8:4
589 (dashed line) and 7:4 (solid line) (n = 20 for each shoal size ratio). The thin dashed horizontal
590 line indicates chance levels of preference. Note the log scale on the horizontal axes.