Environmentally-induced changes to brain morphology predict cognitive performance

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30 The relationship between the size and structure of a species' brain and its cognitive capacity

31 has long interested scientists. Generally this work relates interspecific variation in brain

32 anatomy with performance on a variety of cognitive tasks. However, brains are known to

33 show considerable short-term plasticity in response to a range of social, ecological and

34 environmental factors. Despite this, we have a remarkably poor understanding of how this

35 impacts on an animal's cognitive performance. Here, we non-invasively manipulated the

36 relative size of brain regions associated with processing visual and chemical information in

37 fish (the optic tectum and olfactory bulbs, respectively). We then tested performance in a

38 cognitive task in which information from the two sensory modalities was in conflict.

39 Although the fish could effectively utilise both visual and chemical information if presented

40 in isolation, when they received cues from both modalities simultaneously, those with

41 relatively better developed optic tecta showed a greater reliance on visual information,

42 while individuals with relatively better developed olfactory bulbs showed a greater reliance

43 on chemical information. These results suggest that short-term changes in brain structure,

44 possibly resulting from an attempt to minimise the costs of developing unnecessary but

45 energetically expensive brain regions, may have marked effects on cognitive performance.

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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.

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138 At the start of each trial, the focal fish was placed in a perforated container (5 cm diameter)
139 in the centre of the main tank. Following 1 min of acclimatisation, the container was lifted
140 and the behaviour of the focal fish was monitored using point samples taken every 10 s for 5
141 min. Specifically, we recorded when the fish was in either of two 7.5 cm (i.e. approximately
142 2 body lengths, and so well within the distance that would be considered shoaling; [21])
143 preference zones parallel to the shoal containers at either end of the experimental tank. A
144 fish was considered in the preference area when any part of its body crossed the line.
145 Preference was quantified as the proportion of time spent in the choice zone adjacent to
146 the larger shoal.

Control trials

148 Control trials were conducted in order to determine whether fish from both the visually-
149 unrestricted and visually-restricted groups were able to use chemical and visual information,
150 in isolation, to mediate their shoal choice preferences. We presented randomly selected fish
151 (n = 18 in total, 9 from each treatment group with 3 from each replicate rearing tank) with a
152 series of choices between two stimulus shoals that differed in size. Specifically, each fish
153 received 5 trials in which the size ratio of the two stimulus shoals was either 10:4, 8:4, 7:4,
154 6:4 or 5:4, in each of two conditions: visual information only, or chemical information only
155 (10 trials in total). Based on previous findings from a variety of fish species (e.g. [22-24])
156 these size ratios are assumed to be discriminable under normal circumstances, although
157 with the prediction that discriminatory ability would decrease as the ratio of the number of
158 individuals in each stimulus shoal converges on one (i.e. fish would exhibit a strong
159 preference when shoal sizes were easily distinguishable, but increasingly weaker
160 preferences as shoal sizes became more similar). Trials were presented in a random order,
161 and the side of the tank housing the larger shoal was randomised. There was a 10 min
162 interval between consecutive trials.

Experimental trials

164 The experimental trials aimed to test how fish traded off chemical and visual information
165 when making shoal choice decisions, based on the treatment they came from (visually-
166 restricted or visually-unrestricted). Randomly selected fish (n = 40 in total, 20 from each
167 treatment group with approximately equal numbers from each of the replicate rearing
168 tanks), which had not been used in the control trials, were presented with a series of
169 simultaneous choices between two shoals. These two shoals differed visibly in size, with one
170 always being larger than the other according to the ratios used during the control trials (i.e.
171 10:4, 8:4, 7:4, 6:4 or 5:4). However, in order to test the focal fish's relative utilisation of
172 visual and chemical information in mediating their shoal choice behaviour, chemical
173 information was presented incongruently, such that visual information from one shoal was
174 paired with chemical information indicative of the number of fish present in the other shoal
175 (i.e. focal fish were presented with a shoal that appeared large in the visual domain but
176 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

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

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

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

239 **Ethics**

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

242

243 **3. Results**

244 **Morphometric measurements**

245 There was no significant difference in overall brain volume between fish in the two
246 treatment groups ($F_{1,35} = 0.47$, $p = 0.496$). However, consistent with our predictions, the fish
247 in our experiment differentially developed brain regions involved in processing sensory
248 information: those reared in conditions that increased their relative reliance on chemical
249 information (the visually-restricted group) developed relatively large olfactory bulbs ($F_{1,35} =$
250 36.31, $p < 0.001$), and relatively small optic tecta ($F_{1,35} = 13.61$, $p < 0.001$), compared to fish
251 reared in conditions that allowed them to utilise visual information (the visually-unrestricted

252 group) (Figure 1). No other measured brain regions differed between the groups
253 (telencephalon: $F_{1,35} = 0.07$, $p = 0.792$; dorsal medulla: $F_{1,35} = 0.61$, $p = 0.440$; cerebellum:
254 $F_{1,35} = 0.64$, $p = 0.429$). In line with previous findings in this species, males had significantly
255 larger brains overall than females ($F_{1,35} = 42.33$, $p < 0.001$), as well as significantly larger
256 brain regions (all $p < 0.001$). There were no significant differences between replicate rearing
257 tanks for overall brain volume, or for the volume of any of the measured brain regions (all p
258 > 0.15) except the dorsal medulla ($F_{1,35} = 8.43$, $p = 0.006$), suggesting that each replicate
259 responded to the experimental treatment in a similar way.

Control trials

261 There were highly significant differences in preference between shoal size ratios in each of
262 the four control trials (visually-unrestricted, chemical information: $\chi^2(4) = 285.4$, $p < 0.001$;
263 visually-unrestricted, visual information: $\chi^2(4) = 182.2$, $p < 0.001$; visually-restricted,
264 chemical information: $\chi^2(4) = 126.7$, $p < 0.001$; visually-restricted, visual information: $\chi^2(4) =$
265 193.7, $p < 0.001$) (Figure 2). As predicted, fish from both treatments showed significant
266 linear decreases in their preference for the largest shoal as the shoal size ratio became
267 increasingly similar to one, both when only chemical information was available (polynomial
268 contrasts from a GLMM, visually-restricted: quadratic, $z = 2.46$, $p = 0.014$; visually-
269 unrestricted: quadratic, $z = -3.43$, $p < 0.001$; Figure 2a,c) and when only visual information
270 was available (linear contrasts, visually-restricted: linear, $z = -11.68$, $p < 0.001$; visually-
271 unrestricted: quadratic, $z = -4.40$, $p < 0.001$; Figure 2b,d). There was therefore clear
272 evidence that fish from both groups could utilise visual and chemical cues when presented
273 in isolation to make shoal-choice decisions, but that they found this task harder as the
274 shoals became increasingly similar in size.

Experimental trials

276 When visual and chemical information was presented to fish incongruently, there was a
277 significant interaction between treatment and shoal size ratio ($\chi^2(4) = 104.81$, $p < 0.001$),
278 suggesting that different information was salient to the different groups when making
279 decisions (Figure 3). While fish in both the visually-restricted and visually-unrestricted
280 groups showed a non-linear change in their preference over successive shoal size ratios
281 (polynomial contrasts from GLMM, visually-restricted: quadratic, $z = 4.36$, $p < 0.001$;
282 visually-unrestricted: quadratic, $z = -5.39$, $p < 0.001$), these were in opposite directions in
283 each of the two groups: the peak preference for fish in the visually-unrestricted group was
284 for visual information (Figure 3a), while the peak preference for fish in the visually-restricted
285 group was for chemical information (Figure 3b). Specifically, preferences differed
286 significantly between the visually-restricted and visually-unrestricted treatments for the 8:4
287 ($\chi^2(1) = 8.65$, $p = 0.003$) and 7:4 ($\chi^2(1) = 8.30$, $p = 0.004$) shoal size ratios, but not for any of
288 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

327 group level. Only at intermediate shoal size ratios were preferences realised, resulting in the
328 observed non-linear patterns. It is noteworthy that neither the overall brain volume, nor the
329 volume of the other brain regions differed significantly between treatments, although all
330 were larger in males than in females as has been shown previously in this species [27]. The
331 dorsal medulla differed in size between replicates, although the cause of this is not known
332 [34].

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

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

360 In this paper we provide experimental evidence that the size of an individual's brain directly
361 impacts on its performance in a cognitive task [1]. In particular our results emphasise that
362 short-term, environmentally-induced changes in brain structure, possibly resulting from an
363 attempt to minimise the costs of developing unnecessary but energetically expensive brain
364 regions [12, 15], can have a marked impact on an animal's cognitive performance. However,
365 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]).

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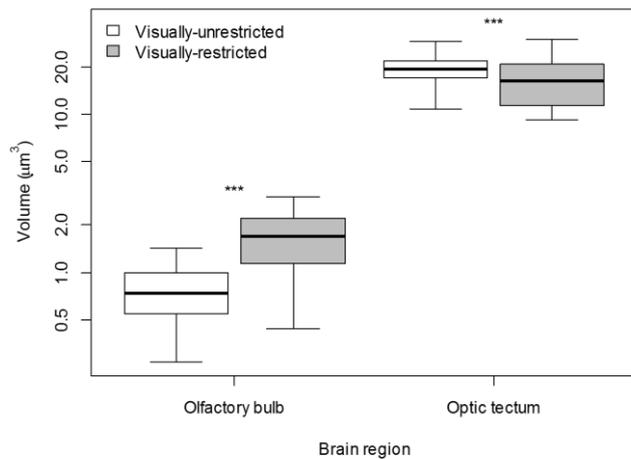
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Figure 1. Volumes of two brain regions, the olfactory bulb and the optic tectum, in fish from the visually-unrestricted group (white boxes, $n = 20$) and the visually-restricted group (grey boxes, $n = 20$). In each case, the thick horizontal line 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: ***, $p < 0.001$.
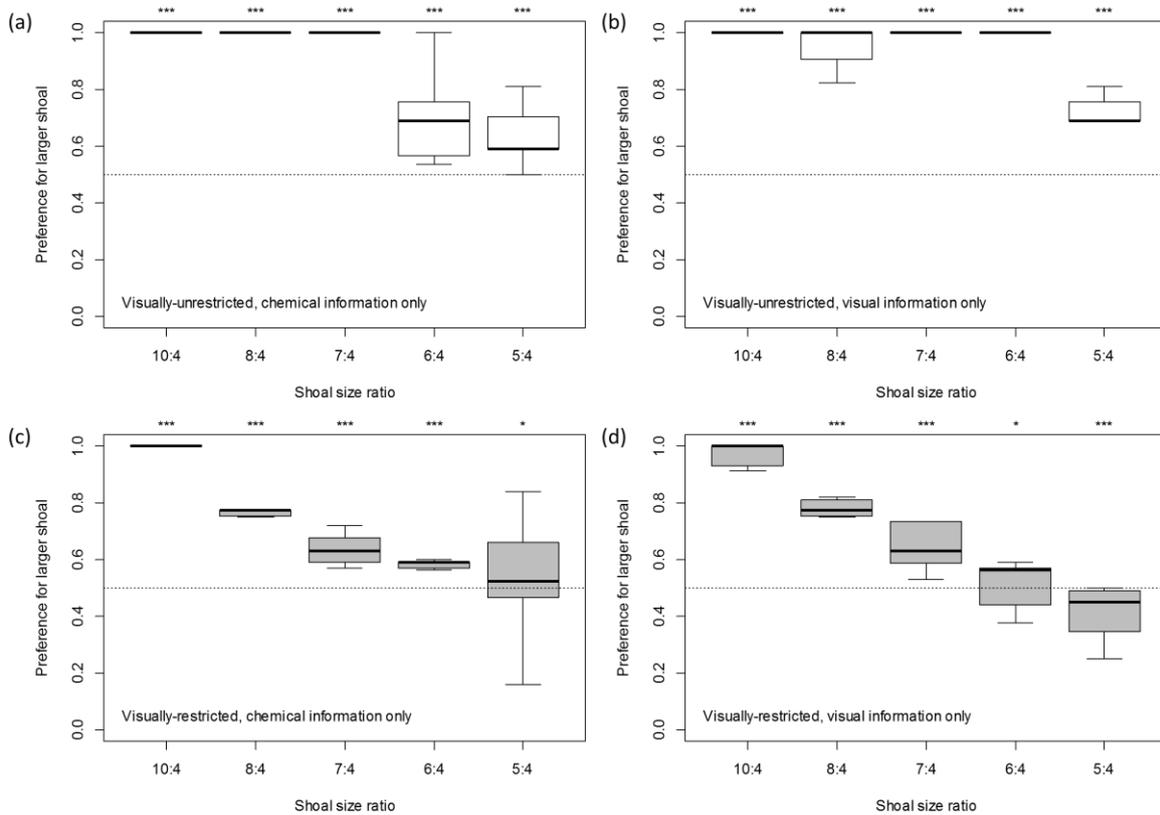
Figure 2. Preferences for the larger of two simultaneously presented shoals (measured as the proportion of time spent with the larger shoal) in fish from the visually-unrestricted group (a, b; white bars, n = 9) and the visually-restricted group (c, d; grey bars, n = 9), over five different shoal size 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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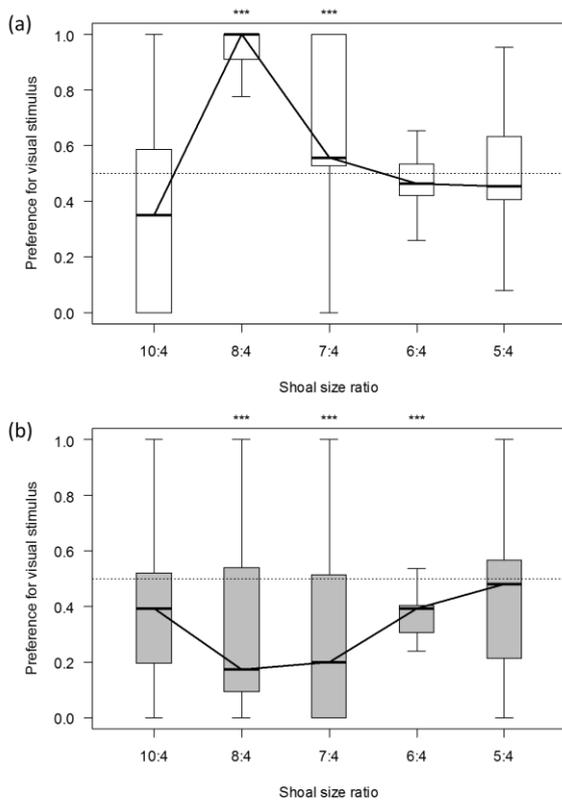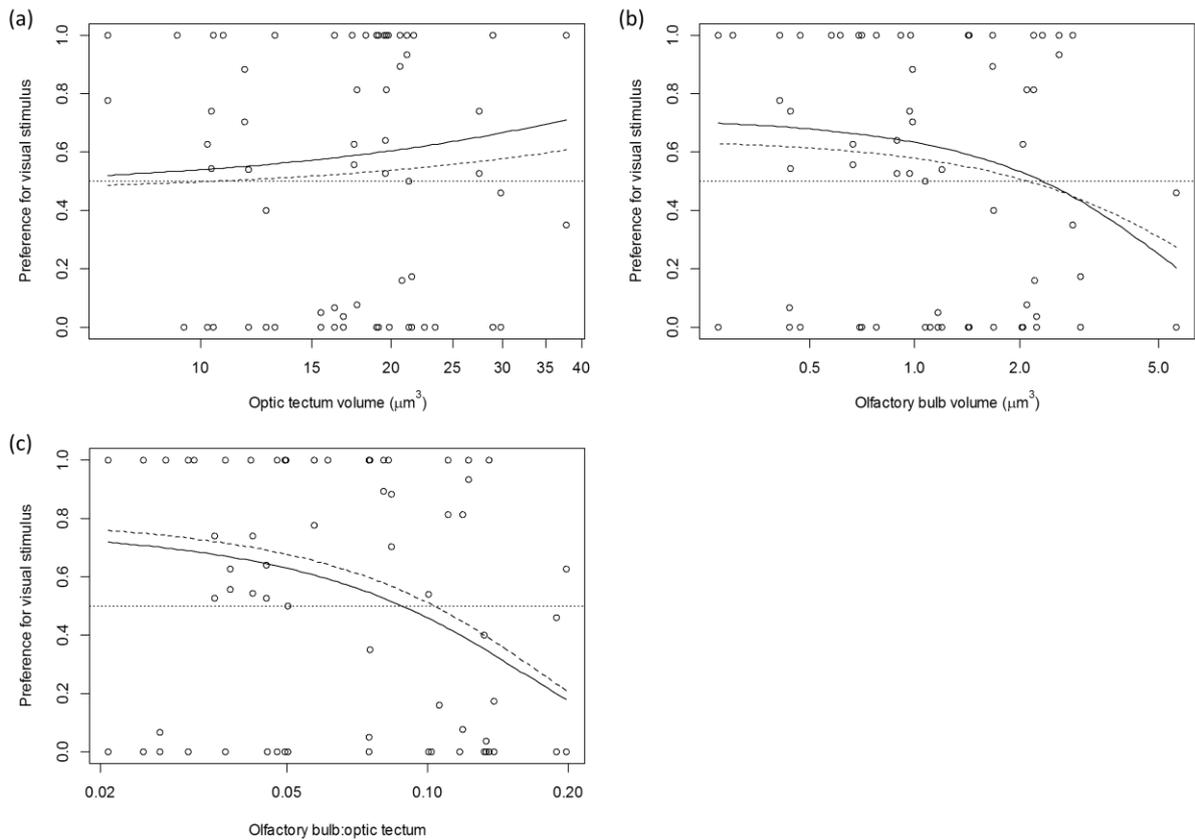560 Figure 3. Preferences for two simultaneously presented shoals in fish from the visually-
561 unrestricted group (*n* = 20) (a) and the visually-restricted group (*n* = 20) (b). Each fish had a
562 choice between two shoals in which the information regarding shoal size was incongruent: a
563 shoal 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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Figure 4. Individual preferences for two simultaneously presented shoals as a function of (a) optic tectum volume, (b) olfactory bulb volume and (c) olfactory bulb/optic tectum ratio. Each fish had a choice between two shoals in which the information regarding shoal size was incongruent: 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, over each of five different shoal size ratios (see text for full details). Preference was measured as the proportion of time spent with the visually larger (chemically smaller) shoal, and so higher values denote a preference for visual information in shoal choice and lower values denote a preference for chemical information. Data points denote preferences for individual fish, while the curves show the GLMM model fit for shoal size ratios of 8:4 (dashed line) and 7:4 (solid line) (n = 20 for each shoal size ratio). The thin dashed horizontal line indicates chance levels of preference. Note the log scale on the horizontal axes.