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Foraging behaviour and brain morphology in recently emerged brook charr, *Salvelinus fontinalis*

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Abstract Recently emerged brook charr (*Salvelinus fontinalis*) foraging in still-water pools along the sides of streams are either active, feeding on insects from the upper portion of the water column away from the stream bank, or sedentary, feeding on crustaceans emerging from the hyporheic zone near the stream bank. We tested whether the frequency of movement displayed by individual brook charr searching for prey in the field was related to the relative volume of the telencephalon, a brain region involved with movement and space use in fishes. Movement of individuals searching for prey was quantified in the field, individuals were captured and volumes of the telencephalon and of the olfactory bulbs, a brain region neighbouring the telencephalon but not implicated in space use, were measured. Individuals with larger telencephalon volumes moved more frequently on average while searching for prey in the field than did individuals with smaller telencephalon volumes. The frequency of movement was unrelated to differences in the volume of the olfactory bulbs, suggesting that the relationship between telencephalon volume and movement was not a consequence of differences in overall brain size. Demonstrating a correlation between foraging behaviour and brain morphology for brook charr exhibiting different foraging tactics suggests

that diversification in brain structure and function could be important aspects of the foraging specialization believed to occur during early stages in the evolution and development of resource polymorphisms.

Keywords Brook trout · Brain volume · Activity · Resource polymorphism · Personality · Telencephalon · Space use

Introduction

Many vertebrate populations consist of subpopulations differing in body size and shape, diet, behaviour and life history traits (resource polymorphism) (Robinson and Wilson 1994; Skúlason and Smith 1995). Resource polymorphisms are well known for freshwater fishes inhabiting high-latitude lakes with well-defined benthic and limnetic prey resources where individuals with deeper bodies feed on insect prey in the littoral zone and individuals with more streamlined bodies feed on zooplankton in the open water (Robinson and Wilson 1994; Skúlason and Smith 1995 and references therein). Examples have been reported for multiple species and populations of sunfishes (Centrarchidae; Robinson and Wilson 1996; Parsons and Robinson 2007), sticklebacks (Gasterosteidae; Schluter and McPhail 1992; Baker et al. 2005) and salmonids (Salmonidae; Malmquist et al. 1992; Bertrand et al. 2008). How phenotypic attributes and environmental features interact to facilitate divergence during the early stages of resource polymorphisms remains poorly understood (Schluter and McPhail 1993; Smith and Skúlason 1996; Shumway 2008), in part because investigations to date have focused on populations where the behavioural and morphological differences between morphs are already well established (e.g. Malmquist et al. 1992; Robinson and Wilson 1996).

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Populations exhibiting behavioural divergence but less morphological divergence are better suited for understanding the initial stages of resource polymorphism (Snorrason and Skúlason 2004; Wilson and McLaughlin 2007).

This study tested whether individual differences in the prey search behaviour of recently emerged brook charr, *Salvelinus fontinalis*, in still-water pools along the sides of streams are linked to differences in the relative volumes of the telencephalon and olfactory bulb regions of an individual's brain. We examined the link between prey search behaviour and brain morphology for two reasons of potential, broad significance. First, divergence in behaviour between morphs is hypothesized to precede divergence in external morphology (Skúlason and Smith 1995; Snorrason and Skúlason 2004; Wilson and McLaughlin 2007), and behavioural differences in foraging and habitat use could reflect corresponding divergence in neural mechanisms that might be revealed by brain morphology. Studies of polymorphic fishes, however, have focused largely on evolved and induced (developed) differences in external morphology, such as jaw, fin and body size and shape (Robinson and Wilson 1994; Skúlason and Smith 1995), and not the structure of internal organs (but see Olsson et al. 2007). In an investigation of how diet affects the behavioural and morphological plasticity of a limnetic morph of threespine stickleback, *Gasterosteus* sp., Day and McPhail (1996) speculated that developmental changes in foraging efficiency might be linked to underlying brain morphology, but the relationships between prey or space use and brain morphology have not been tested in polymorphic fishes and only recently examined in other polymorphic taxa (LaDage et al. 2009).

Second, over the past several decades, numerous studies have reported interspecific and inter-sexual correlations between behaviour and brain morphology, including volumes of specific brain regions (Kotrschal et al. 1998; Healy and Rowe 2007). The relationship between space use and brain morphology has been the topic of much of this research, especially in birds and mammals where spatial abilities have been linked repeatedly to the relative size of the hippocampus (Healy et al. 2005). The interspecific correlations suggest a functional relationship between behaviour and brain morphology such that increased demands on spatial ability lead to the evolution or development of increased sizes of the brain region involved with spatial tasks (LaDage et al. 2009). Examining the relationship at the individual level can usefully complement the broader interspecific patterns because the former are less affected by broad, complex differences in genetic architecture, ecology and evolutionary past.

Fishes represent excellent candidates for studies examining space use and brain morphology at the individual level. Interspecific comparisons have demonstrated links

between ecology and specific brain regions (e.g. Pollen et al. 2007), resource polymorphism (one form of broad individual variation) occurs relatively frequently in fishes and fish brains continue to develop throughout an individual's life (Kotrschal et al. 1998; Zupanc 2006). Our study focused on the telencephalon because this region of the fish brain, specifically the lateral pallium (the lateral portion of the cerebral hemispheres), has been linked to space use and spatial memory in laboratory experiments with fishes (López et al. 2000; Rodríguez et al. 2002; Broglio et al. 2003). Our study also focused on the olfactory bulb. This neighbouring brain region was used as a control for possible size differences in other regions of the brain, or in overall brain size. We are not aware of any evidence for young salmonid fish that links the olfactory bulbs to small-scale space use or to the use of chemical cues to locate an individual prey item from a background of chemical cues from many prey items, and, in the field, we have not observed recently emerged brook charr displaying the casting and sniffing behaviour commonly associated with olfaction (DeBose and Nevitt 2008).

Our study was conducted on recently emerged brook charr in still-water pools because this study system has been recognized as a model for examining the phenotypic and ecological features facilitating the early stages in resource polymorphisms (McLaughlin et al. 1994; De Kerckhove et al. 2006; Wilson and McLaughlin 2007). These brook charr exhibit individual differences in foraging behaviour that vary in a manner similar to that observed in polymorphic fish populations, yet at this early life stage individuals do not differ consistently in body size and shape (McLaughlin et al. 1994; Wilson and McLaughlin 2007). Some charr are sedentary, feeding on crustacean prey moving out of the hyporheic zone at the edges of pools near the bank (a sit-and-wait tactic). Other charr are active, ranging widely to feeding on insect prey from the upper portion of the water column away from the stream bank (an active search tactic) (Grant and Noakes 1987; McLaughlin et al. 1994). Individuals exhibiting intermediate behaviour are also observed, but less commonly.

Our study tested three predictions. We first tested whether brook charr with larger telencephalon volumes for their body size moved more frequently while searching for prey in the field than brook charr with smaller telencephalon volumes for their body size. This prediction follows from the observations that active brook charr exhibit greater space use while foraging and hence will encounter more physical features and potential landmarks in the shallow pools. It also follows empirical evidence from inter- and intra-specific comparisons of birds and mammals demonstrating that animals experiencing increased demands for spatial memory have a larger

hippocampus (Clayton and Krebs 1994; Lefebvre et al. 1997). The hippocampus of birds and mammals function analogously to the telencephalon of fishes (López et al. 2000; Rodríguez et al. 2002; Broglio et al. 2003). We then tested whether brook charr with larger telencephalon volumes relative to the size of their olfactory bulbs moved more frequently while searching for prey in the field than those with smaller telencephalon volumes relative to the size of their olfactory bulbs. Lastly, we tested the prediction that the volume of the olfactory bulbs for brook charr of a given size was unrelated to movement in the field. Predictions 2 and 3 helped ensure that any relationship between telencephalon volume and movement did not reflect differences in neighbouring brain regions or in overall brain size.

Methods

Field observations and experimental holding conditions

Adult brook charr typically spawn in gravel redds (nests) in cool well-oxygenated streams and lakes from September to late November in southern Ontario. Young-of-the-year (YOY) hatch in mid-winter but remain in the gravel substrate, relying on their yolk sac for nourishment until they emerge at the beginning of April and begin feeding exogenously (see McLaughlin et al. 1992; Scott and Crossman 1998). Between 5 April and 16 May 2004, we observed and captured 89 brook charr (YOY; fork length 2.0–3.0 cm; wet weight 0.050–0.300 g) from the west branch of the Credit River in Erin township, Ontario. Observations and collections were made from seven still-water pools (~1.0–2.0 m² surface area) along a 1-km stretch of river. Observations were made between 9:00 am and 12:00 noon (Eastern Standard Time).

Prior to an observation period, an observer arrived at a pool and, while sitting at the edge of the stream, watched the fish for 5 to 10 min while allowing them to resume their normal activity. Recently emerged charr at these sites resume activity within 2 min, on average, after the arrival of an observer (Grant and Noakes 1987). A focal individual was then arbitrarily selected for further observation. The behaviour of the focal individual was quantified at 5-s intervals for 10 min using the metronome function on a stopwatch and a countdown timer. The focal individual was considered to be moving during a 5-s interval if it had moved one body length or more by the end of an interval. Intervals not involving an attempt to capture prey, a social interaction or flight to cover were considered to entail search for prey, as was done in earlier studies on this system (McLaughlin et al. 1994, 1999; Wilson and

McLaughlin 2007). This is a common definition for search behaviour (Stephens and Krebs 1987) that is suitable here because all focal fish appeared to be looking for food and were not resting on the stream bottom. Intervals involving search for prey were tallied on a hand counter.

At the end of the observation period, we attempted to capture the focal fish using two aquarium dip nets (18 cm×25 cm). Of 99 focal individuals, 89 were captured. Ten individuals were not captured because they swam outside of our field of vision or passed behind an obstruction (e.g. submerged branch, rock). Immediately after capture, each individual was placed singly into a 1-L glass jar with a mesh top and held in the stream for up to 3 h before being transported to the Hagen Aqualab, University of Guelph. Up to ten fish were collected on any given day.

At Aqualab, fish were held singly in their jars overnight in individual, aerated 38-L rectangular (80×19×25 cm) glass aquaria housed in a walk-in environmental chamber. Room and water temperature were maintained at 12°C and photoperiod at 12:12. Thirty-six individuals were selected at random for this study; the remaining 53 were used in a separate but related component of the study (experiment 4 in Wilson and McLaughlin 2007).

Tissue collection and histology

Fish were anaesthetised with MS-222, sacrificed, measured for fork length to the nearest millimetre and weighed to the nearest microgram. Heads were separated from the body trunk just behind the operculum using a scalpel and placed in individual vials containing Bouin's solution (a fixative) for 24 h. Individual vials were assigned random numbers so that the person (ADMW) measuring the cross-sectional areas of the brain had no knowledge of how each fish behaved in the field. The following day, each head was transferred to separate 100% ethanol baths for two additional 48-h periods and afterward stored in 100% ethanol until tissue sectioning began 2–3 weeks later. Heads were embedded in paraffin prior to sectioning and sagittal sections were cut at 14- μ m intervals. Entire heads were sectioned; removing the brain from the head was impractical due to the small size of the fish (total head length <5 mm). Sections were mounted on glass slides and stained with Harris Hematoxylin and Eosin Y Alcoholic. Hematoxylin stains nuclear material and chromatin (Brown 2002). Eosin stains cytoplasmic material, collagen and connective tissue. Hematoxylin and eosin were selected because these stains are routinely used in gross morphological studies and standardized staining procedures are widely available (Brown 2002).

Determination of telencephalon and olfactory bulb volume

Images of the telencephalon and olfactory bulb (Fig. 1) were digitized and measured for area to the nearest square micrometre using an Olympus microscope (Model BX41TF, Japan) equipped with a CoolSNAP-PRO colour digital camera (Roper Scientific Photometrics, USA) and Image-Pro Plus software (v. 4.5.1.22; Media Cybernetics, Inc. USA). Areas of the telencephalon, left and right cerebral hemispheres and the paired olfactory bulbs (Fig. 1) were quantified for every second stained section (see Perrot-Sinal et al. 1998). Sections were traced twice and the mean area of the tracings was used in further calculations. Volumes (V) of the telencephalon and olfactory bulbs were estimated as:

$$V = I/3(h_1 + \sqrt{h_1} \times \sqrt{h_2} + \sqrt{h_2})$$

where h_1 and h_2 are areas from successive sections and I is the interval between sections (28 μm) (Perrot-Sinal et al. 1998). Volumetric estimates were based on 18 to 35 (mean = 25) sections for each fish, depending on head size. The numbers of sections counted are comparable to those measured in earlier brain analyses (e.g. Jacobs et al. 1990). Quantifying the total volume of the brain was not practical because of the number of sections involved and because the sagittal sections required magnifications below the limits of the microscope imaging system used to make the areal measurements of brain regions.

Statistical analysis

We tested our predictions regarding foraging behaviour and brain structure using multiple regression and partial correlation analyses. Where appropriate to provide context, we used a multiple regression to predict the volume of a brain region expected for individuals of the same fork length, but differing in the number of moves made while searching for prey in the field. We did not categorize fish into groups of sit-and-wait and actively searching individuals for our analyses because of the uncertainty associated with assigning individuals exhibiting intermediate numbers of moves per observation period to a group (Rowland and Qualls 2005) and because the regression approaches applied here have been successful in elucidating the relationships between behaviour, ecology, physiology and morphology in earlier investigations of this study system (McLaughlin and Grant 1994; McLaughlin et al. 1994, 1999).

Prior to analysis, fork length and volumes of the telencephalon and olfactory bulbs were \log_{10} -transformed to normalize their distributions (Shapiro–Wilk tests, all $P_s > 0.20$) and to allow regression coefficients to be interpreted as allometric coefficients. The number of moves each individual made per observation period was \log_{10} -transformed after adding 1 to all of the counts to avoid taking the log of 0. Our analyses included fork length rather than body mass as an index of body size. Fork length is a more reliable measurement because it does not include confounding effects associated with

Fig. 1 Mid-sagittal view of the brain of a recently emerged brook charr (*S. fontinalis*) shown as a drawing (a), corresponding enlarged drawing (b) and as an image (c) depicting the olfactory bulb (O) and cerebral hemispheres of the telencephalon (T)

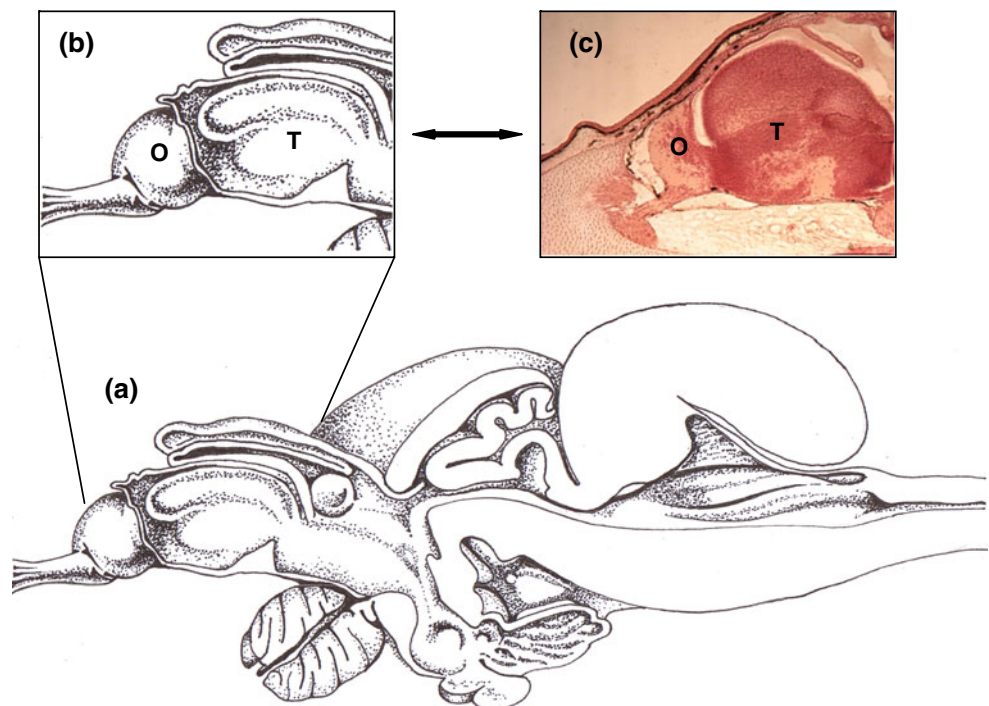


Table 1 Summary statistics for the sub-sample of 36 brook charr used in the analyses of brain histology relative to the 89 brook charr sampled from the field (Wilson and McLaughlin 2007)

Variable	Sub-sample		Field sample	
	Mean	Min–max	Mean	Min–max
Number of moves per 10 min	15.7	0–56	16.0	0–76
Fork length (cm)	2.7	2.0–3.8	2.6	2.0–3.8

condition factor or recent foraging success (McLaughlin et al. 1995). To test our first prediction, we examined the partial regression and correlation between the number of moves made during an observation period and telencephalon volume after adjusting statistically for fork length (Reist 1985). To test our second prediction, we examined the partial regression and correlation between the number of moves made during an observation period and telencephalon volume after adjusting statistically for volume of the olfactory bulbs. To test our last prediction, we examined the partial regression and correlation between the number of moves made during an observation period and volume of the olfactory bulbs after adjusting statistically for fork length. One-tailed tests were used in tests of the first and second predictions, where positive relationships were expected. A two-tailed test was used for the third prediction, where no relationship was expected. A Bonferroni correction was not applied to the probabilities from these tests because our study involved testing a small number of predictions, and not data snooping (Westfall and Young 1993) or dredging (Burnham and Anderson 2002), and because of the mathematical, logical and practical problems associated with the Bonferroni correction (Moran 2003).

Results

Statistics for the number of moves per 10 min and fork length measured for the 36 fish used in this study were consistent with those of the larger sample of fish observed in the field (Table 1). The frequency distribution of the number of moves per 10 min for the subset of fish used in our brain analysis had a distinct bimodal appearance (Fig. 2) and did not differ from the distribution for the other subset of fish used in the experiments of Wilson and McLaughlin (2007) (Kolmogorov–Smirnov two sample test: $D=0.15$, $P=0.69$). Frequency distributions of fork length, and volumes of the telencephalon and the olfactory bulbs adjusted for fork length, did not have a distinct bimodal appearance (Fig. 3).

Consistent with our predictions, the activity exhibited by brook charr in the field was significantly correlated with the length-adjusted volume of the telencephalon, but not with the length-adjusted volume of the olfactory bulbs. After adjusting statistically for fork length, charr with a larger

telencephalon volume exhibited a greater number of moves per 10 min in the field than did charr with a smaller telencephalon volume (partial $r=0.32$, $df=33$, one-tailed $P=0.03$) (Fig. 4a). Further, a multiple regression relating telencephalon volume to number of moves per 10 min and fork length provided the equation $\log_{10}(\text{telencephalon volume}) = 7.35 + 2.05 \times \log_{10}(\text{fork length}) + 0.05 \times \log_{10}(\text{moves per 10 min} + 1)$ ($R^2=0.87$, $F=110$, $df=2, 33$, $P<0.0001$). From this equation, a brook charr of 2.63-cm fork length (the geometric mean for our sample) exhibiting approximately 20 moves per 10 min, corresponding roughly with the faster mode in Fig. 2, was predicted to have a telencephalon volume 9% larger than that of a charr of the same length exhibiting four moves per 10 min, corresponding roughly with the slower mode in Fig. 2. After adjusting statistically for the volume of the olfactory bulbs, charr with a larger telencephalon volume exhibited a greater number of moves per 10 min in the field than did charr with a smaller telencephalon volume (partial $r=0.41$, $df=33$, one-tailed $P=0.007$) (Fig. 4b). After adjusting statistically for fork length, charr with larger olfactory bulbs were no more active in the field, on average, than those with smaller olfactory bulbs (partial $r=0.10$, $df=33$, one-tailed $P=0.55$) (Fig. 4c).

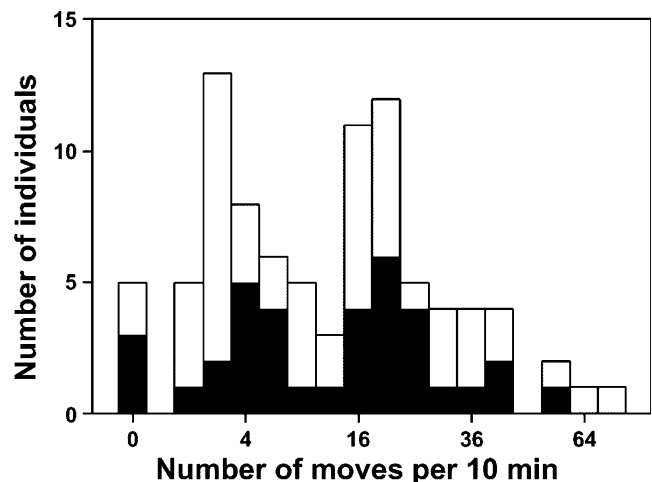


Fig. 2 Frequency distribution summarizing the number of individual, recently emerged brook charr that made a given number of moves while searching for prey in a 10-min field observation. *Shading* depicts the 36 individuals used in the brain analysis. *Shaded and open portions* depict all 89 individuals sampled (Wilson and McLaughlin 2007)

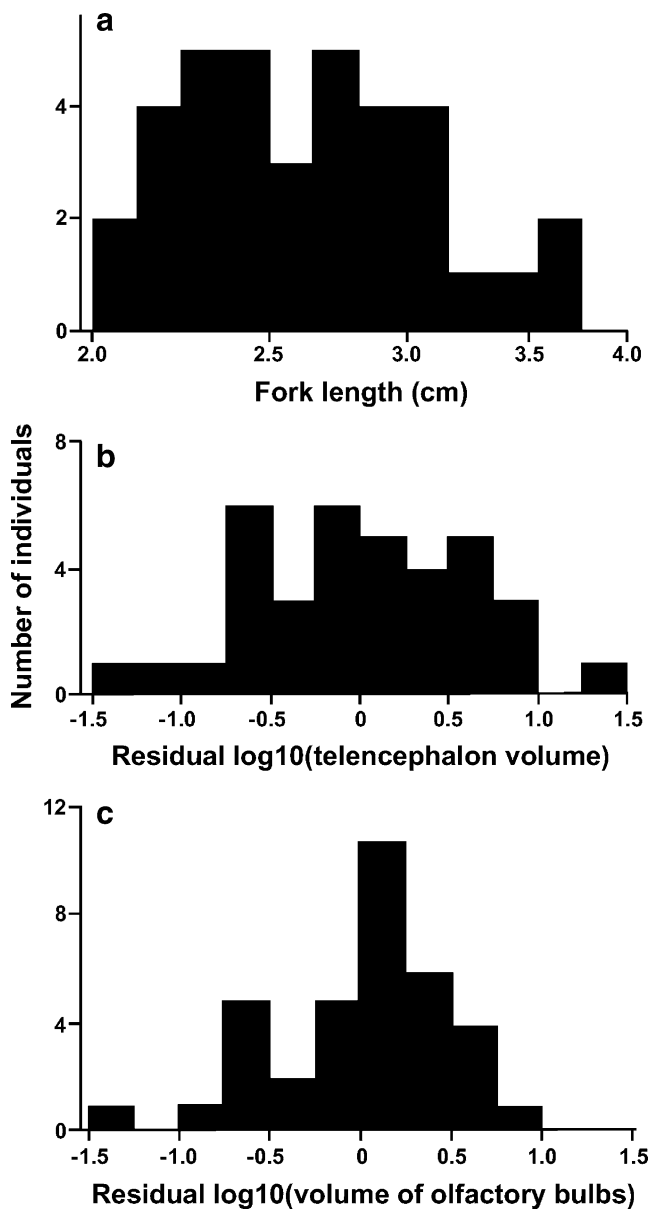


Fig. 3 Frequency distributions summarizing the fork lengths (a) and the residuals of \log_{10} (telencephalon volume) (b) and \log_{10} (volume of the olfactory bulbs) (c) after regressing against \log_{10} (fork length) for the recently emerged brook charr used in this study

Discussion

We provide evidence suggesting a functional link between the frequency of movement exhibited by individual brook charr while searching for prey in the field and the relative size of their telencephalon, a brain region involved with space use and spatial memory in fishes. Individuals possessing larger telencephalons after statistically adjusting for fork length (body size), and for volume of the olfactory bulbs, moved more frequently on average while searching for prey in the field than did individuals with smaller telencephalons. After statistically adjusting for fork length,

the frequency of movement was unrelated to volumetric differences in the olfactory bulbs, suggesting that the relationship between telencephalon volume and movement was not an indirect consequence of differences in overall brain size, although we cannot rule out the possibility of movement while searching for prey being linked to areas of the brain other than the telencephalon and olfactory bulbs.

The magnitude of predicted differences (9%) in mean telencephalon volume for brook charr that made four moves

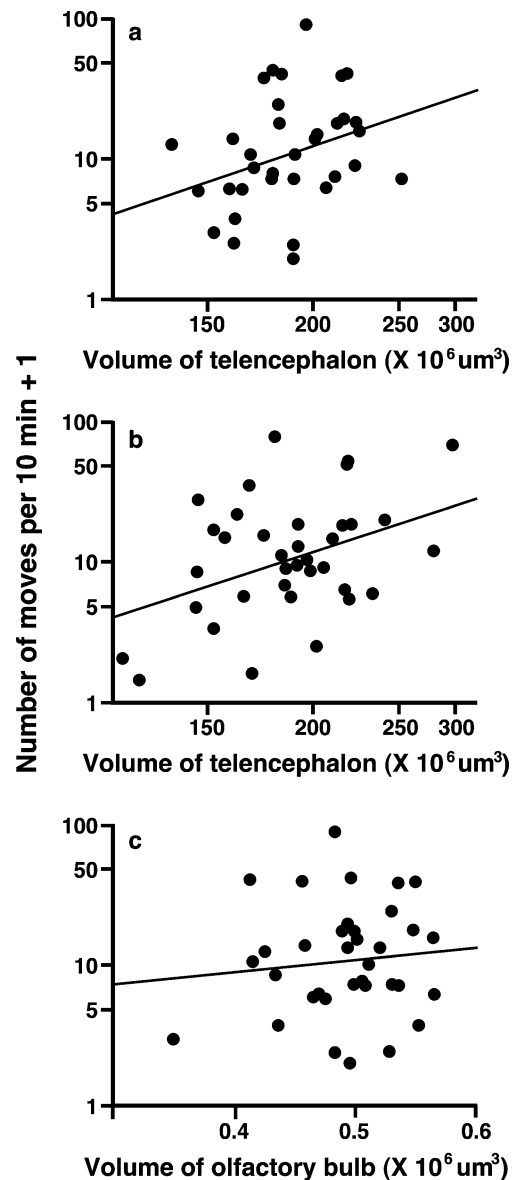


Fig. 4 Leverage plots depicting the relationships between the number of moves made per 10 min by recently emerged brook charr while searching for prey in the field and the volume of their telencephalon after statistically adjusting for fork length (a) and for volume of the olfactory bulbs (b) and depicting the number of moves made per 10 min while searching for prey in the field and volume of the olfactory bulbs after statistically adjusting for fork length (c). *Solid lines* represent partial regression lines

per 10 min while searching for prey, roughly corresponding to the sit-and-wait search tactic (Fig. 2), and those that made 20 moves per 10 min, roughly corresponding to the active search tactic (Fig. 2), was large enough to be potentially significant ecologically. By comparison, domesticated mammals typically have brains 8–33% smaller, after statistically adjusting for individual differences in body size, than their wild congeners, and the greatest differences are usually seen in the forebrain (Kruska 1988 and references therein). In rainbow trout, *Oncorhynchus mykiss*, a salmonid fish related to brook charr, hatchery-reared juveniles possessed telencephalon volumes 5–8 % smaller than those of wild-reared juveniles (Marchetti and Nevitt 2003). A 19% reduction in telencephalon volume has been reported for first-generation laboratory-reared Trinidadian guppies, *Poecilia reticulata* (Burns et al. 2009). Lastly, in the lizard *Uta stansburiana*, the relative volume of the dorsal cortical region of the brain in males of the orange colour morph, which defend large territories, is 3% and 14% larger than that of the blue morph, which defends small territories, and the yellow morph, which is non-territorial, respectively (LaDage et al. 2009). Magnitudes of these differences in the size of brain regions are also comparable to the magnitudes of differences observed in fin and body shapes of benthic and limnetic morphs from polymorphic populations of lake fishes (Ehlinger 1990; Robinson and Wilson 1994, 1996) and of individual fish sampled from slow- and fast-running water (Imre et al. 2002 and references therein). In these latter examples involving external morphological traits, differences of this magnitude are often believed to be adaptive (Robinson and Wilson 1994).

Our findings are valuable in three ways having the potential to integrate efforts to understand the early stages of resource polymorphism with emerging findings about brain plasticity and individual differences in behaviour. First, our findings are valuable because a link between foraging behaviour and brain morphology is a key expectation behind functional explanations for the broader correlations observed between spatial behaviour and relative hippocampus size in birds and mammals (Krebs 1990; Healy et al. 1994). To date, examples where differences in the foraging behaviour of individual wild animals have been linked to differences in corresponding brain morphology remain uncommon in general (Krebs et al. 1996; Sherry and Hoshoooley 2009). Yet, relationships between inter-individual differences in brain morphology and in foraging behaviour are expected if brain size and structure are shaped by plastic responses to the environment or by natural selection. Testing whether the differences in telencephalon volume among recently emerged brook charr represent plastic developmental responses or fixed genetic differences remains an important research need.

Second, by linking differences in brain structure to foraging behaviour in the field, our findings for brook charr provide a valuable complement to recent studies of brain plasticity in closely related rainbow trout where volumes of the optic tectum, telencephalon and olfactory bulb are generally smaller for individuals reared in benign (hatchery/laboratory) environments than for individuals reared in the wild (Marchetti and Nevitt 2003; see also Burns et al. 2009). In these studies, initial comparisons of movement behaviour and temperament have been made between treatment groups differing in brain morphology, but how environmental enrichment affects the volume of specific brain regions and, correspondingly, behaviours that might be important ecologically remains unclear. The value of our study is that it draws a link between the size of the telencephalon, which laboratory studies indicate is important in space use and spatial memory (Salas et al. 1996; Ohnishi 1997; Pollen et al. 2007), and a specific aspect of foraging behaviour in brook charr that has been linked to habitat use, diet and biochemical indices of growth and stress in the field (McLaughlin et al. 1994, 1999; Farwell and McLaughlin 2009).

Third, our findings complement recent research elucidating behavioural correlates of domesticated rainbow trout lines selected for low- and high-cortisol responses to confinement stress (Øverli et al. 2005). These responses parallel the distinction between proactive and reactive coping strategies in mammals (Koolhaas et al. 1999) and are often equated with individual differences in temperament or personality (Øverli 2007; Stamps 2007). Behavioural differences in social dominance, foraging and activity have been documented between the lines and are linked to differences in serotonergic activity in several brain regions, including the telencephalon (Øverli et al. 2005; Schjolden et al. 2006). Recently emerged brook charr foraging in still-water pools may provide an ecologically important example where similar patterns of behaviour are observed in wild individuals under natural conditions. The level of activity exhibited by recently emerged brook charr during prey search is correlated with measures of general activity and risk-taking in novel environments (Wilson and McLaughlin 2007). General activity and risk taking are two proposed components of personality (Réale et al. 2007; Wilson and Godin 2009, 2010). Level of activity during prey search is also correlated with baseline titres of cortisol (Farwell and McLaughlin 2009) and brain morphology (this study), suggesting that individuals using different foraging tactics may interpret the environment in the still-water pools differently.

These links reinforce earlier speculation that individual differences in perceptual ability could facilitate the behavioural divergence believed to occur during the early stages of resource polymorphism (Wilson and McLaughlin 2007).

Differences in general activity and risk-taking early in life, as observed in young brook charr, can influence the kinds of habitats and prey that individuals encounter. In addition, authors of a recent review summarized a wide variety of physiological and internal morphological processes that can reinforce early individual differences in behaviour (Stamps 2007; Biro and Stamps 2008). These processes remain largely unexplored within the context of resource polymorphisms (Skúlason and Smith 1995; Snorrason and Skúlason 2004), although Olsson et al. (2007) recently demonstrated that littoral zone morphs of Eurasian perch (*Perca fluviatilis* L.) possess longer relative gut lengths than pelagic morphs. These processes also remain underexamined within the context of mechanisms generating the diversifying selection that would favour alternative behavioural types (De Kerckhove et al. 2006). Much of the emphasis on mechanisms for resource polymorphism has focused on competition among individuals for spatially separated resources. A more comprehensive examination of mechanisms involved during the early stages of resource polymorphism is needed because behaviour can facilitate or resist, and precede or follow, a morphological change (Huey et al. 2003), empirical tests of the diversifying role of competition are rare and infer behaviour from diet (Svanback and Bolnick 2007) and, in theory, physiological and morphological processes that resist behavioural change could increase the strength of disruptive selection for resource polymorphisms (Egas et al. 2004).

There are limitations of our study, of course, and recognizing these are important for ensuring that our findings are interpreted cautiously and for identifying avenues for future research. One limitation is that our analyses have been correlational in nature. As such, we cannot determine whether the differences in telencephalon volume represent plastic responses to differences in foraging behaviour or corresponding environmental conditions in the field or to fixed genetic or developmental differences in telencephalon volume that predispose individuals to adopt foraging tactics differing in the level of activity during prey search. Also, without further experimentation, we cannot eliminate the possibility that the correlation between telencephalon volume and foraging behaviour is an indirect consequence of correlated but unmeasured behaviours (e.g. diet choice) or brain regions (Healy and Rowe 2007). A second limitation is that our measure of brain morphology (volume) is crude, and corroborative analyses involving more refined measurements such as counts of cell types or neural connections (Coggeshall 1992) would be beneficial in understanding the mechanistic bases for the differences observed in telencephalon volume. On one hand, empirical comparisons like ours have been valuable for linking the ecologies of species, and individuals within species, to brain structure and function (Krebs et al. 1989; Jacobs et al. 1990; Kotrschal et al. 1998). On the other hand, there is a recognized need for experiments investi-

gating the behavioural and neural mechanisms responsible for the structural differences observed (Healy et al. 2005; Burns et al. 2009). A third limitation is that our sample of individuals was modest in size. As such, statistical power is a concern for our test of the relationship between field activity and volume of the olfactory bulbs (prediction 3). However, we detected a correlation between activity in the field and telencephalon volume (predictions 1 and 2) that was robust across tests adjusting for measures of body and brain size. A final limitation is that the magnitude of the correlation between activity in the field and telencephalon volume was moderate. In the early stages of resource polymorphism, relationships between behaviour, ecology and morphology are expected to be subtle (De Kerckhove et al. 2006; Wilson and McLaughlin 2007), and hence correlations are smaller and more challenging to detect than in comparisons of distinct morphs or across species because the range of phenotypic variation is smaller (Smith 1980).

Despite these limitations, our study provides a novel and valuable example where the movement behaviour of individual animals foraging in the wild is linked to corresponding differences in brain structure, in a manner expected based on form and function. This example suggests that taxa exhibiting resource polymorphisms could provide rich opportunities for understanding the neural mechanisms behind differences in foraging behaviour observed for natural populations. This has certainly been true for systems exhibiting reproductive polymorphisms (e.g. Bass 1996; Rowland and Emlen 2009). Conversely, our example also suggests that studies of brain structure and function in polymorphic taxa could provide rich opportunities for understanding the behavioural specialization believed to occur during the early stages in the evolution and development of resource polymorphism (Snorrason and Skúlason 2004).

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