Rearing Environment Affects the Brain Size of Guppies: Lab-Reared Guppies have Smaller Brains than Wild-Caught Guppies

James G. Burns, Arthy Saravanan & F. Helen Rodd

Department of Ecology and Evolutionary Biology, University of Toronto, Toronto, Canada

Correspondence
James G. Burns, Laboratoire Evolution, Génomes et Spéciation, CNRS - Bat 13, Avenue de la Terrasse, F-91190 Gif sur Yvette, France. E-mail: james.burns@legs.cnrs-gif.fr

Received: July 23, 2008
Initial acceptance: September 11, 2008
Final acceptance: September 11, 2008
(G. Beckers)

doi: 10.1111/j.1439-0310.2008.01585.x

Abstract

Animals bred for captivity often have smaller brains and behave differently than their wild counterparts. These differences in brain size have been attributed to genetic changes resulting from, for example, inbreeding depression and pleiotropic effects of artificial selection for traits such as docility. A critical question, though, is whether these differences in brain size are due to plastic responses to the environment, not just genetic changes. We observed a large reduction in brain size in first generation, lab-reared female guppies compared with wild-caught ones (19% smaller telencephalon, 17% smaller optic tectum). We then reared first-generation, lab-born guppies in environments varying in spatial complexity and size in an attempt to isolate factors that might increase brain size and change temperament, but no significant differences in phenotype were observed. The results of these experiments show that, although the environmental factors responsible for the effect have not been found, even first generation lab-reared individuals can have smaller brains than wild individuals.

Introduction

Animals bred in captivity, be it for the laboratory, the farm or human companionship, often have smaller brains and behave differently than their wild counterparts. Domesticated mammals, such as Norwegian rats (*Rattus norvegicus*), pigs (*Sus scrofa* f. dom.), sheep (*Ovis aries*), and cats (*Felis catus*) typically have brains 8–33% smaller than their wild congeners (when corrected for body size), with the greatest reduction usually seen in the forebrain (Kruska 1988). Differences in brain size have been attributed to genetic changes resulting from selection over multiple generations in the captive setting, inbreeding depression, pleiotropic effects of artificial selection for traits such as docility and increased rates of reproduction, and relaxed selection pressure (Price 1999). A critical question, though, is whether differences in brain size between wild and captive animals are, at least in part, due to plasticity, not just genetic changes. For instance, an effect of plasticity is indicated by Kihslinger et al. (2006) who found that, in salmon (*Oncorhynchus tshawytscha*) from the same genetic stock and generation, hatchery-reared individuals had smaller brains than wild-caught individuals. This contrasts with data from feral animals; these domesticated strains of animals that have re-entered the wild tend to retain the small brains of their domesticated predecessors (Kruska 2005; O'Regan & Kitchener 2005). Thus, while there is clear evidence for a genetic basis to domestication, what remains unknown is how quickly phenotypes can change when animals are brought into captivity. Here, we address this issue by studying whether brain size responds plastically to rearing environment by comparing wild-caught Trinidadian guppies (*Poecilia reticulata*) to first-generation guppies reared in laboratory environments varying in size and spatial complexity.

Ethology **115** (2009) 122–133

Brain morphology is plastic, as evidenced by studies on animals raised in the laboratory in environments varying in complexity. In general, animals that developed in enriched environments tend to have larger brain structures, greater rates of neurogenesis and higher learning ability than those that developed in un-enriched environments (reviewed in Rosenzweig & Bennett 1996; van Praag et al. 2000). What makes an environment 'enriched' may be species-specific, but can entail greater structural complexity, more foraging opportunities, greater spatial area, or more social interactions (Newberry 1995).

Only a few studies, though, have examined differences in brain size and behavior between wild animals and captive-bred animals from enriched and un-enriched environments. The results vary. Kihslinger et al. (2006) compared brain sizes of wild chinook salmon to individuals reared in conventional (i.e. un-enriched) or enriched hatcheries and found larger telencephalic lobes and olfactory bulbs in wild fish, but no significant differences between the hatchery treatments. Similarly, Kihslinger & Nevitt (2006) compared brain sizes of wild, juvenile steelhead salmon (*Oncorhynchus mykiss*) to fish from two hatchery treatments, one with small stones on the bottom and one without; wild fish had larger total brain volumes than fish from both hatchery treatments, but the cerebella was larger in the wild and hatchery fish reared with small stones as substrate when compared with fish raised without stones. On the other hand, Stuermer & Wetzel (2006) found that domesticated Mongolian gerbils (*Meriones unguiculatus*) had lower brain weights than wild gerbils, but the first generation of wild gerbils bred in the lab did not have lower brain weights than their wild-caught kin. We cannot presently say whether these differences between studies are due to, for example, taxa-specific effects or differences in the complexity and stimulation provided by laboratory environments for fish vs. mammals.

Although there are often large differences in brain size between wild and captive-reared animals, the general repertoire of behaviors in domesticated animals appears qualitatively similar to their wild counterparts (reviewed in Price 1999). For instance, the types of movements used by hatchery and wild cod to capture and handle live prey are similar (Steingrund & Ferno 1997). However, the probability or rates at which some behaviors are performed can differ between wild and domesticated fish; for example, it has been reported that domesticated fish have an increased propensity to approach novel objects (trout: Sundstrom et al. 2004), lower courtship rates (salmon: Fleming et al. 1996), and more rapid resumption of behaviors after a predator encounter (salmon: Einum & Fleming 1997) than their wild counterparts. Fish bred in hatcheries typically also show maladaptive responses to predators (e.g. higher activity levels in the presence of a predator) when compared with their wild counterparts (reviewed in Huntingford 2004). Although it is well established that, across species, brain region size is correlated with sensory capabilities (primates: Barton 1998; teleosts: Kotrschal et al. 1998; mammals: Krubitzer 1995) and ecology and behavior (fish: Brandstätter & Kotrschal 1990; Huber et al. 1997; birds: de Kort & Clayton 2006; Lefebvre et al. 1997; Lucas et al. 2004; primates: Reader & Laland 2002), whether brain size differences cause the behavioral differences between wild and captive animals of the same species is not known.

Lab environments are also known to affect the temperament of animals [i.e. consistent individual differences in behavior across time or context (Réale et al. 2007)]. For instance, in studies looking at movement around open areas and exploration of novel objects, animals reared in enriched environments generally show less anxiety and fear-related behavior than individuals reared in un-enriched environments (mice: Benaroya-Milshtein et al. 2004; pigs: Bolhuis et al. 2005; rabbits: Hansen & Berthelsen 2000; chickens: Jones & Waddington 1992).

In this study, we conducted comparisons of both brain size and temperament of wild-caught and lab-reared guppies (*Poecilia reticulata*). Guppies are a model organism for the study of mate choice and anti-predator behavior (Houde 1997; Magurran 2005) and thus it is important to know whether lab-reared fish are representative of their wild counterparts. We first compared telencephalon and optic tectum size and temperament of wild-caught guppies with their first generation offspring. Then, in a separate experiment with guppies from another population, we reared guppies in five types of laboratory environment that varied in size and spatial complexity and again compared telencephalon and optic tecta size and temperament. We studied these two brain regions because they are involved in important ecologically-relevant functions. For instance, the telencephalon is implicated in spatial memory (Salas et al. 1996; Portavella et al. 2002; Broglio et al. 2003) and the optic tectum in visual processing and integration (Kotrschal et al. 1998; Broglio et al. 2003). We also measured exploratory behavior [i.e. how an individual behaves in novel situations (Réale et al. 2007)], a temperament trait that can affect

fitness in the wild (Réale et al. 2007; Smith & Blumstein 2008) and is relevant to how animals behave when subjected to novel experimental procedures and apparatus.

Method

Part 1: Wild vs. Laboratory Rearing Environment

The phenotypes of wild-caught females were compared with their lab-reared daughters. A total of 26 adult female guppies were captured in low-predation sites of the Quare River in the Oropuche River drainage (map grid reference: PS 969 810) in Trinidad in 2004 and 2005 and transported in sealed plastic bags filled with conditioned water [containing Stresscoat (Aquarium Pharmaceuticals, Chalfont, PA, USA), AmQuel (Kordon, Hayward, CA, USA) and Novaqua (Kordon)] and highly oxygenated air to the University of Toronto. The predation regime in the sites used in this study has been well-established by numerous previous studies [reviewed in Magurran (2005)]. The sites are small streams with multiple pools separated by riffles and small waterfalls; they change in volume and current during heavy rainfall. Offspring from these wild-caught mothers were born and reared in the lab. laboratory tank temperatures were maintained at 25°C, within the range of temperatures (24–26°C) typically observed in guppy streams in Trinidad (Reznick & Endler 1982), and were kept on a 12 h:12 h light–dark schedule. Fish were fed flake food once per day every day of the week and live brine shrimp nauplii once per day on weekdays. Females were likely pregnant when captured in the wild, but were initially housed in group tanks with other wild-caught fish from the same population for a few days and may have also mated while in captivity. Broods of wild female guppies are typically sired by multiple males (Houde 1997; Kelly et al. 1999), so it is unknown whether the offspring of mothers in this study are full- or half-sibs.

The offspring were reared in similar spatial environments in the 2004 and 2005 experiments, but in different social environments in the 2 yr (Fig. 1). In 2004, 10 wild-caught females were isolated in small tanks (12.5 cm wide × 30 cm long × 20 cm high, water filled to 15 cm) with light colored gravel substrate. To reduce the stress of isolation, the females were in visual contact with guppies in other tanks. Guppies are live-bearing fish, so offspring were born live in the tank with their mother. In 2004, male offspring were removed from the tank at maturity but female offspring remained in the tank with their

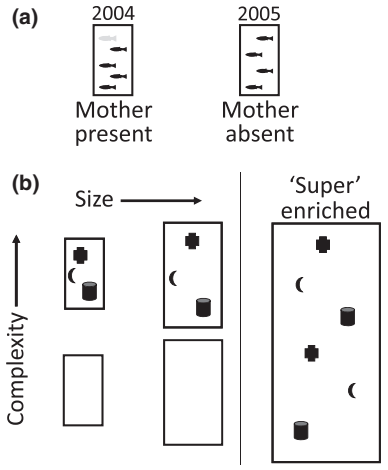

Fig. 1: Schematic of the rearing laboratory rearing environments. (a) In the comparison of wild-caught mothers vs. their lab-reared daughters, the daughters were raised in un-enriched tanks, in 2004 with their mother present (grey symbol) and in 2005 with their mother absent. (b) In the enriched vs. un-enriched environment experiment, guppies were reared in tanks varying in size and spatial complexity. Tanks are drawn to scale.

mother present (guppies do not provide parental care). In 2005, 16 wild-caught females were isolated in small tanks (same size as above), and their offspring were removed from their mother's tank within 24 h of birth and placed in another small tank with their siblings. A maximum of four offspring (range 1–4, average 3.0) were placed in each tank in 2005, depending on the number of offspring produced by a mother. Body length was measured using UTHSCSA IMAGETOOL (version 3) from digital photographs taken of fish anesthetized in MS222 (Canadian Council on Animal Care, 2005). We only looked at females in this experiment to reduce differences due to sex.

Wild mothers in both 2004 and 2005 were sacrificed approx. Ten months after being captured as adults in the wild. Their age at capture is unknown, but the average age of maturation for guppies from low-predation populations is 6 mo (Reznick et al. 1997), so, given their body lengths (standard length, range: 21.0–31.5 mm), the mothers were likely 15 to 18-mo old when sacrificed. Daughters were sacrificed at 12 to 16-mo old, when they were a similar size to their mothers (size range: 21.9–30.3 mm). Because we were unable to sacrifice the daughters at both the same age and same body length as their mothers (because the daughters grew more quickly than their mothers), we chose size as the variable to control because it was more readily measured. Body length did not differ significantly between mothers

Ethology **115** (2009) 122–133

and daughters (two-tailed paired t-tests: mother mean = 25.3 mm, daughter mean = 24.9 mm, df = 21, t = 0.68, p = 0.50). An additional 18 females were captured in same manner from the same populations in 2006 and sacrificed within 3 wk of transport to the laboratory to compare brain size in recently caught wild fish with wild fish held in captivity for longer periods.

Brain size

The procedure for measurement of the telencephalic lobes and optic tecta (Fig. 2) was as follows. Fish were euthanized by over-anesthetization in buffered MS222 (Holloway et al. 2004; Canadian Council on Animal Care 2005; Barreto et al. 2007) and photographs taken to determine body length. The fish was then decapitated, and the brain removed and fixed in 4% paraformaldehyde for 24 h. Using a microscope-mounted digital camera (Leica DFC280 camera on a Leica MZ6 dissecting microscope; Leica Microsystems, Wetzlar, Germany), a dorsal photograph of the brain was taken and UTHSCSA IMAGETOOL used to measure the length, width, and area of each region from above. The right telencephalon and optic tectum were measured except when they were damaged during dissection, in which case the left structure was measured. Each fish was coded so that the person measuring the brain was blind to treatment. To negate inter-observer variability, all measurements were made by one person (JGB). Two images were taken of a subset of the brains and area measurements were highly repeatable between images (Intraclass correlation: telencephalon; n = 14, r = 0.99, p < 0.001; optic tectum, n = 14, r = 0.99, p < 0.001). Photographs of mother and daughter brains were randomly interspersed to reduce any biases due to order of measurement. Between one and four daughters from each mother were observed and the average taken as the estimate for that maternal line. For a subset of brains, lateral photographs were also taken to allow measurement of the height and area of the region from the side. As volume (calculated as depth × length × width) was highly correlated with the area calculated from the dorsal photographs (n = 31, telencephalon: r = 0.90, p < 0.001; optic tectum: r = 0.77, p < 0.001), dorsal area was used as a surrogate for brain volume.

Activity level and exploratory behavior

An activity level test and three behavioral assays of temperament were run in random order for wild-caught mothers from 2005 and their lab-reared daughters a few days prior to brain removal: open-field test, emergence test, and novel-object test. However, a separate study later showed that, while the open-field test is a reliable test of exploratory behavior, the emergence test and novel-object test used in the present study were not valid tests of boldness in guppies (Burns *in press*). The results from the emergence tests and novel-object test are thus not reported. Behaviors in the open-field test are not correlated with general activity level (Burns *in press*). Tests for mothers and offspring were run concurrently, so wild-caught mothers had been acclimated in the laboratory for multiple months before testing. One day prior to the beginning of testing, each fish was isolated in a small 8 l tank with a white gravel substrate to prevent visual contact with other fish or the experimenter. Each fish was run in the open-field test and activity level test in random order with one day between tests to allow for recovery. Between one and four daughters from each mother were observed and the average taken as the estimate for that maternal line.

The open-field test was used to measure exploratory behavior, which is how an individual responds to novel environments, resources or objects (Réale et al. 2007; Smith & Blumstein 2008). Two variables were measured: the time the animal was motionless and the swimming rate when not frozen. These two variables (log-transformed) were entered in a principal components analysis and the first principal component was extracted. Because this principal component had a positive loading for swimming rate and a negative loading for time frozen (Percent variation explained = 70.4%; eigenvalue = 1.41; loadings, time frozen = −0.839; swimming rate = 0.839), we interpreted it as exploratory behavior.

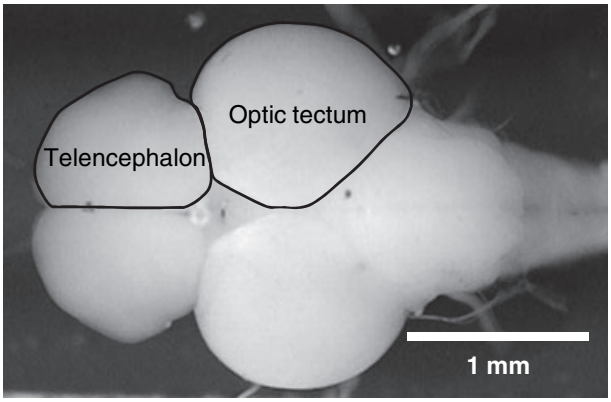

Fig. 2: Dorsal view of the brain of a guppy.

The open-field apparatus was a green, plastic rectangular tub (33 cm × 28 cm, with 12 cm high sides). Lines on the bottom divided it into 24 rectangles, each 5.5 cm × 7 cm. Lighting was provided by two 30 W daylight spectrum fluorescent bulbs 1.5 m above the open-field apparatus. A fish was netted in the home isolation tank and quickly transferred to the center of the open-field apparatus. To avoid disturbing the fish during the test, the fish was viewed on a television screen connected to a video camera (Panasonic PV-GS35, Secamcus, NJ, USA) positioned 1.5 m above the apparatus. After a 60 s acclimation period (to reduce the effects of any differences between fish in the stress associated with netting), time frozen, and swimming rate (number of rectangles entered per time not frozen) were recorded for 180 s.

Activity level, which can affect survival in the wild (e.g. Werner & Anholt 1993), was measured in the home isolation tank and videotaped with the video camera 1 m above the tank. A grid of 5 cm × 4 cm rectangles was marked on the clear plastic tank lid and the number of rectangles the fish swam through during a 180 s period was counted. Activity level was log-transformed before statistical analysis.

Statistical analysis

Because of the difficulties associated with comparing the size of a morphological trait between individuals of different body lengths, we chose to analyze differences in brain size between of mothers and daughters in two ways using ANOVA. In the first case, the dependent variable was absolute brain region size, with treatment (mother vs. daughter) and year as main effects, and maternal line as a random factor. In the second case, the independent variables remained the same but the dependent variable was the residual brain region size from a log–log regression of brain region size vs. body length. Exploratory behavior and activity level were also regressed on log body length and the residuals analyzed with ANOVA with treatment as the main effect and maternal line as a random factor.

Although previous research has sometimes used brain region size relative to a control brain region (e.g. Pravosudov et al. 2006), we could not use this technique because an analyses of residual telencephalon size (on body length) and residual optic tectum size (on body length) revealed that fish with relatively large telencephalons also tended to have relatively large optic tectums (Pearson product moment correlation: daughters, $r = 0.52$, $p = 0.019$; mothers, $r = 0.60$, $p = 0.005$). As the area of the telencephalic lobes and optic tecta did not vary independently, optic tectum size could not be used as a control brain structure to compare with telencephalon size. Also, measurements of another potential reference area, the cerebellum, were not repeatable because of difficulties delineating the border of the structure in the photographs. Thus, we used residual telencephalon and residual optic tectum sizes rather than telencephalon size relative to optic tectum size in our analyses.

All of the fish in our study were sexually mature, but we needed to know whether the relationship between brain and body length was linear to confirm that our use of linear statistical models for brain and body length was appropriate. We tested for linearity by comparing Akaike's information criterion (AIC; with lower scores indicating better goodness of fit, considering the complexity of a model against how well it fits the data) for linear vs. second-order polynomial line fitting for telencephalon and optic tectum size vs. body length in wild-caught and lab-reared fish. A linear fit was better in each case (telencephalon: linear fit $R^2 = 0.29$, AIC = −162.3; polynomial fit $R^2 = 0.30$, AIC = −161.0; optic tectum: $R^2 = 0.25$, AIC = −169.7; polynomial fit $R^2 = 0.25$, AIC = 167.9). Thus, while there is often an allometric relationship between brain size and body length in fishes (Brandstätter & Kotrschal 1990) across a species' entire size range, there was a linear relationship over the size range we were analyzing.

In our initial analyses, we evaluated the treatment x year interaction but neither the interaction nor the factor 'year' were ever significant (all $p > 0.17$) in the full model, so year was removed from the models for the analyses presented. Statistical tests were conducted using SPSS 14.0.

Part 2: Enriched vs. Un-Enriched Rearing Environments

In this experiment, the laboratory rearing environment was manipulated and the effects on brain size and temperament were measured. The first four treatments were based upon the independent manipulation of two factors: tank size and spatial complexity. The two tank sizes were: (1) small (12.5 cm wide × 30 cm long × 20 cm high, filled to 15 cm: volume = 5.6 l), the same as in the wild vs. laboratory experiment in Part 1, and (2) large (20 cm wide × 40 cm long ×25 cm high, filled to 20 cm, volume = 16 l). Spatial complexity had two levels: (1)

Ethology 115 (2009) 122–133

'barren', with no objects, and (2) 'enriched' with one artificial plant (~12 cm high and 6 cm wide), one snail shell (~5 cm diameter), and one black PVC plumbing elbow (~4 cm diameter, ~10 cm long). The same numbers of objects were placed in both the small and large enriched tanks, but the objects were spaced further apart (but always in the same relative position) in the large tanks.

A fifth 'super-enriched' treatment had a larger tank size (31 cm wide × 59 cm long tank × 30 cm high filled to 24 cm, volume = 43.9 l), greater spatial complexity (double the number of objects as in enriched treatment), and a learning opportunity. Fish in super-enriched tanks were fed in the morning and afternoon in a specific corner of the tank. The feeding corner for each feeding period was changed every 2 wk. The purpose was to provide an opportunity for the fish to learn an association between time of day and feeding location, then to change the association after 2 wk so that there were new learning opportunities throughout the rearing period. While this super-enriched treatment did not allow isolation of the factors contributing to phenotypic differences between it and the other treatments, the super-enriched treatment expanded the range of levels of each factor and could indicate future research directions.

The experimental subjects were 83 female and 85 male guppy fry taken from their mother's isolation tank on the day of their birth and transferred to one of the five treatment tanks. After the large differences noted between mothers and daughters in the wild vs. laboratory rearing environment experiment, we decided to include males in this analysis. The mothers were wild-caught fish from a high-predation population on the Oropuche River (map grid reference: QS 042 787) in Trinidad. There were nine tanks per treatment and four fish were placed in each tank (36 fish per treatment). Subjects were assigned to treatment tanks randomly, except that offspring from the same mother were never put into the same tank. The identity of the mothers that contributed offspring to each tank was tracked, but the mother of each fish in the tank was unknown (other than being from one of four possible mothers). There were 30 mothers, each of which contributed between 1 and 10 offspring (mean = 5.6 offspring, SD = 2.7); offspring were tested at 4–5 mo of age.

Fish were maintained in the same general conditions as in the wild vs. laboratory experiments (Part 1), but the feeding regime was different. Fish were fed flake food twice per day on weekdays and once per day on weekends. The amount of food was disseminated using measuring spoons specifically designed to provide quantities of flakes appropriate to the age of the guppies (as per Kolluru & Grether 2005). Thus, food amounts did not differ between treatments.

One liter of water was replaced in each tank every 2 wk to minimize differences in water quality. Fish in each treatment were thus disturbed to the same extent during water changes. The proportion of water changed was greater in the smaller tanks because they are more likely to build up fish waste products. Water quality was monitored once per month using test strips (Jungle Laboratories Corp., Cibolo, TX, USA) for nitrate, nitrite, total hardness, total alkalinity and pH. Water quality results were tested with repeated measures ANOVA using proc GLM in SAS 9.0, and contrast statements used to delineate which treatments, if any, differed from one another. There were some water quality differences between treatments. Nitrate levels were higher in small tanks (repeated measures ANOVA: full model, $F_{4,40} = 7.25$, $p = 0.001$; contrasts between small tanks and other treatments, all $p < 0.05$; overall means, small tanks = 27.6 ppm, other treatments: 12.5 ppm) and the super-enriched tank had higher pH's than the other treatments (repeated measures ANOVA: full model, $F_{4,40} = 4.55$, $p = 0.004$; contrasts between super-enriched treatment and other treatments, all $p < 0.05$; overall means, super-enriched = 7.6, other treatments = 7.3). No other differences were significant.

Brain size

Telencephalon and optic tectum sizes were measured using the same protocol as in the wild vs. laboratory experiment.

Exploratory behavior

The temperaments of fish in the enriched vs. unenriched experiment were measured in only the open-field test. Fish were netted in their treatment tank and immediately transferred to the open-field apparatus for testing. The details of the test are otherwise identical to the wild vs. laboratory experiment. Time frozen and swimming rate were entered in a principal components analysis and the first principal component was extracted. As above, this principal component scored positively for swimming rate and negatively for time frozen (Males: percent variation explained = 58.8%; eigenvalue = 1.18; loadings, time frozen = −0.766; swimming rate = 0.766;

Females percent variation explained = 62.6%; eigenvalue = 1.25; loadings, time frozen = −0.791; swimming rate = 0.791), we interpreted it as exploratory behavior.

Statistical analysis

The effects of tank size and spatial complexity on telencephalon size, optic tectum size, and exploratory behavior were analyzed using ANOVA with treatment (small-barren, small-enriched, large-barren, large-enriched, super-enriched) as the main factor and tank (nested within treatment) as the other factor. Brain region sizes were residuals of log brain regions size to log body length regression. Absolute brain regions sizes were not used because of differences in body size between treatments (Males: ANOVA, treatment, $F_{4,84} = 5.39$, $p = 0.001$, tank(treatment), $F_{37,84} = 2.31$, $p = 0.004$; Females, ANOVA, treatment, $F_{4,81} = 29.0$, $p < 0.001$, tank(treatment), $F_{34,811} = 1.86$, $p = 0.027$). In *post hoc* Tukey's tests, males and females from super-enriched tanks were larger than fish from the other treatments. Also, males from the large tanks were larger than males from the small tanks and females from large-barren tanks were larger than females from small tanks. Analyses were run in SPSS 14.0.

The sexes were analyzed separately because the range of overlap between male and female body lengths was narrow. The regression of log brain region size against log body size on both sexes combined may not accurately represent the slope within each sex, and ANOVA has limited power to compare slopes when there is little overlap in range because as you move further from their means, the confidence intervals of the slopes become larger. Thus any interactions between sex and body length would be difficult to detect, necessitating that the sexes be analyzed separately.

Results

Part 1: Wild vs. Laboratory Rearing Environment

Brain size

In both types of analyses (i.e. with absolute brain region area and log–log regression of brain area on body length), mothers had larger telencephalic lobes than their daughters [means (SE): mothers = 0.81 (0.02) mm^2, daughters = 0.65 (0.01) mm^2; ANOVA-absolute size: treatment, $F_{1,19} = 43.76$, $p < 0.001$; maternal line, $F_{19,19} = 1.24$, $p = 0.321$; ANOVA-log–log residuals: treatment, $F_{1,19} = 66.29$, $p < 0.001$; maternal line, $F_{19,19} = 2.12$, $p = 0.055$] (Fig. 3a). This pattern also held for optic tecta [means (SE): mothers = 1.19 (0.03) mm^2, daughters = 0.99 (0.02) mm^2; ANOVA-absolute size: treatment, $F_{1,19} = 52.35$, $p < 0.001$; maternal line, $F_{19,19} = 1.63$, $p = 0.148$; ANOVA-log–log residuals: treatment, $F_{1,19} = 93.86$, $p < 0.001$; maternal line, $F_{19,19} = 3.3$, $p = 0.006$) (Fig. 3b). There were positive relationships between mothers' brain region sizes and daughters' brain region sizes (log-log residuals, Pearson product-moment correlation: telencephalon, $n = 20$, $r = 0.455$, $p = 0.044$; optic tectum, $n = 20$, $r = 0.536$, $p = 0.015$). To determine whether the range and variation of sizes of brain regions was greater for wild-caught than lab-reared fish, we compared coefficients of variation. The variances were not significantly different for optic tectum size for mothers and daughters (coefficients of variation: mothers = 7.7%, daughters = 8.7%; F-test for unequal variance: $F_{19,19} = 1.06$, $p = 0.882$), but there was greater variance in the mother's telencephalon sizes than in the daughter's telencephalon sizes (coefficients of variation: mothers = 11.0%, daughters = 5.8%; F-test for unequal variance: $F_{19,19} = 5.17$, $p = 0.001$).

Because teleosts have continuous brain growth throughout life (Leyhausen et al. 1987; Brandstätter

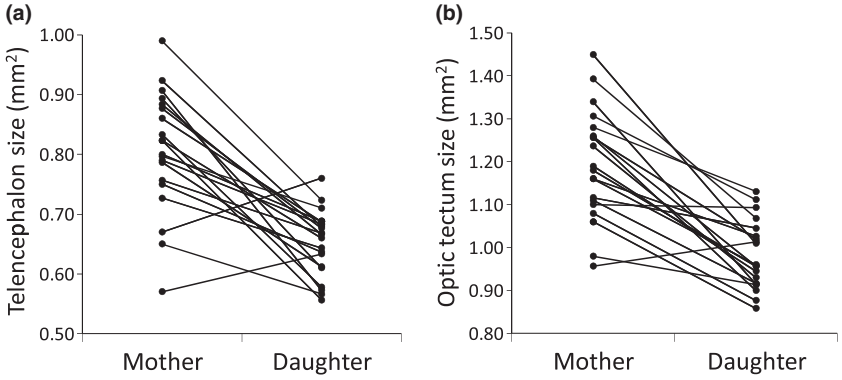

Fig. 3: Wild-caught mothers have relatively larger telencephalic lobes and optic tecta than their lab-reared daughters. Lines connect mothers to the average phenotype of their daughters.

Ethology **115** (2009) 122–133

& Kotrschal 1990), it could be argued that the brain sizes of the wild-caught fish were greater than their lab-reared daughters' because of age differences rather than developmental environment differences. If this were the case, we would expect older wild fish to have larger brains than younger wild fish, when controlling for body length. We tested the effect of age on brain size by comparing the wild females, used as mothers in the experiments described above, to 18 females caught from the same populations in 2006 that were sacrificed within 3 wk of being caught. After regressing log brain regions size on log body length, we used analyzed the effect of age when sacrificed [time in lab: 3 wk (2006) vs. 10 mo (2004 and 2005)]. There was no significant difference in telencephalon or optic tecta size [telencephalon: mean (SE), younger = 0.77 (0.02) mm^2, older = 0.75 (0.02) mm^2; ANOVA: age: $F_{1,36} = 0.25$, $p = 0.876$; optic tecta: (SE), younger = 1.20 (0.03) mm^2, older = 1.11 (0.03) mm^2 ANOVA: age: $F_{1,35} = 0.821$, $p = 0.371$]. The 10 mo in captivity appears to have had minimal effect on the relative size of adult fishes' telencephalic lobes. Therefore, environmental conditions early in life appear to have a larger effect on brain region size than experiences later in life.

Exploratory behavior

There were no significant differences between wild-caught mothers and their lab-reared daughters in exploratory behavior [means PCA scores (SE): mothers = −0.37 (0.41), daughters = −0.16 (0.16); ANOVA: treatment, $F_{1,10} = 0.60$, $p = 0.457$; maternal line, $F_{10,10} = 1.10$, $p = 0.441$] or in activity level [means (SE): mothers = 65.6 (17.8), daughters = 55.0 (10.6); ANOVA: treatment, $F_{1,11,} = 0.81$, $p = 0.387$; maternal line, $F_{11,11} = 1.15$, $p = 0.409$].

Part 2: Enriched vs. Un-Enriched Rearing Environments

Brain size

There were no significant differences between treatments in telencephalon size [Males: treatment, $F_{4,84} = 1.84$, $p = 0.138$, tank(treatment), $F_{37,84} = 0.88$, $p = 0.652$; Females: treatment, $F_{4,81} = 0.37$, $p = 0.829$, tank(treatment), $F_{37,81} = 1.32$, $p = 0.192$; Fig. 4] or optic tectum size for either males or females [Males: treatment, $F_{4,84} = 1.30$, $p = 0.284$, tank(treatment), $F_{37,84} = 1.17$, $p = 0.304$; Females: treatment, $F_{4,81} = 1.14$, $p = 0.351$, tank(treatment), $F_{37,81} = 0.92$, $p = 0.595$; Fig. 4].

Exploratory behavior

There were no significant differences between treatments in exploratory behavior for either males or females [Males, ANOVA, treatment, $F_{4,84} = 0.37$, $p = 0.829$, tank(treatment), $F_{37,84} = 0.60$, $p = 0.942$; Females, treatment, $F_{4,81} = 0.85$, $p = 0.501$, tank (treatment), $F_{37,81} = 1.30$, $p = 0.201$; Fig. 4].

Discussion

We found that lab-reared offspring, compared with their wild-caught mothers, showed a considerable reduction in both telencephalon and optic tectum size. In a separate experiment, laboratory rearing conditions were manipulated in an attempt to identify environmental factors that might have caused these changes in brain size and temperament, but neither brain region size nor temperament were observed to respond to the size or spatial complexity of the laboratory rearing environment. Although the first goal of the present study was to investigate the effects of environment on brain size, our ultimate goal is to reproduce natural temperaments in conjunction with natural brain sizes in lab-reared animals. Unfortunately, it appears we are only at the start of a long road towards attaining that goal. The dramatic declines in the sizes of brain structures that we found (telencephalon = 19.2%, optic tectum = 17.8%) after only one generation in the laboratory – levels comparable with the genetically-based reductions in brain size observed in domesticated animals and their counterparts – implies that aspects of the environment that are critical to normal brain development are missing in the laboratory. We do not yet know the cognitive consequences of smaller brains in guppies, but factors such as reduced brain size and dendritic branching observed in laboratory rodents reared in un-enriched environments are associated with impaired problem-solving ability (Rosenzweig & Bennett 1996).

There are some alternative explanations for our results that should be considered. A potential problem with comparing traits of wild to lab-reared animals is that selective mortality in the wild may reduce the range of phenotypes observed in wild fish. If there was brain size-selective mortality in the field, then variation in brain size would be smaller in the wild-caught sample than in the lab-reared sample. However, there was no significant difference in the variances in optic tectum size of mothers and daughters, and the mothers actually had a greater variance in telencephalon size than their daughters.

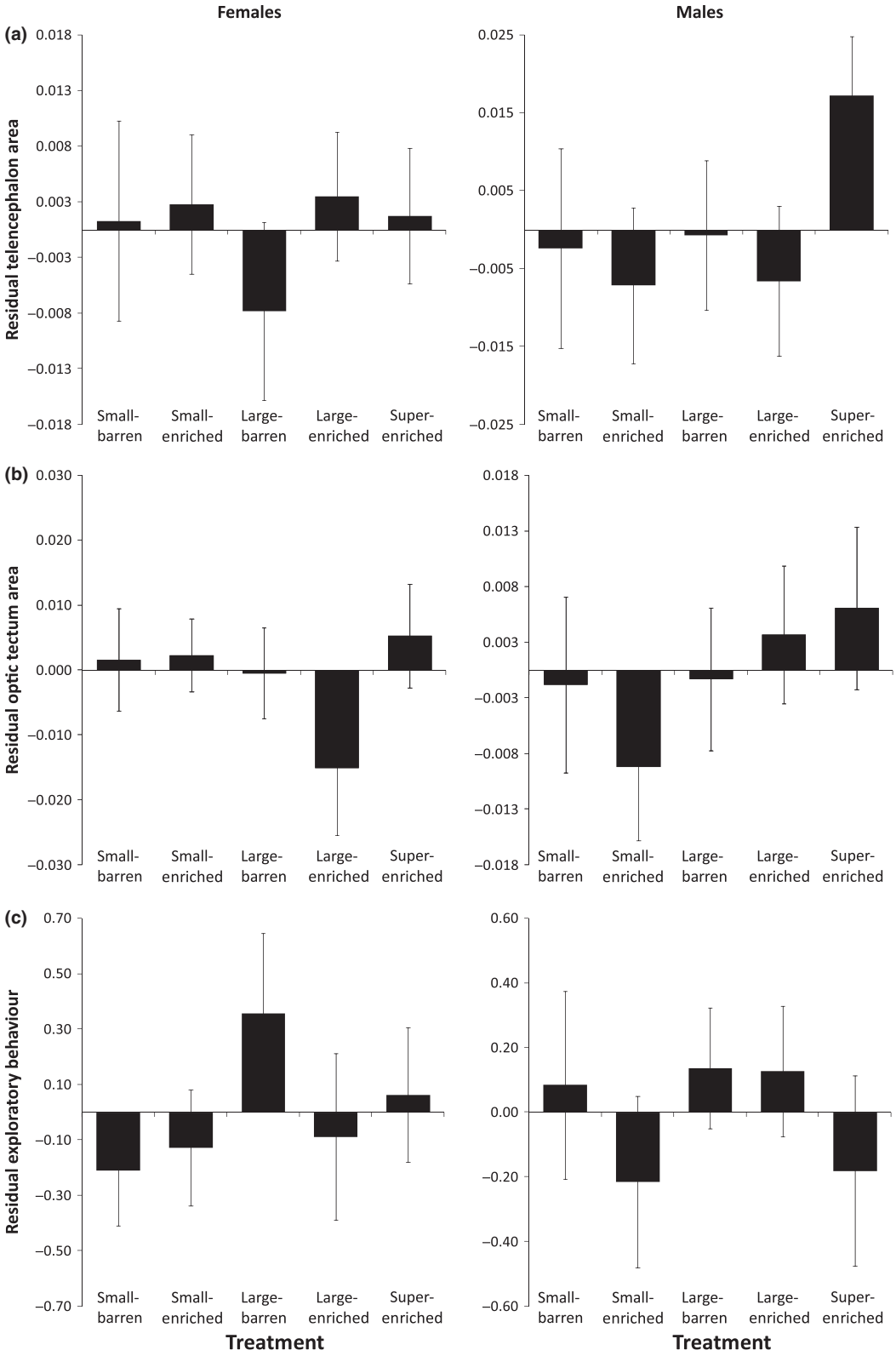

Fig. 4: There were no significant differences between treatments in the enriched vs. un-enriched experiments for (a) telencephalon size, (b) optic tectum size, or (c) exploratory behavior. Residual values are from regressions on log body length.

Ethology **115** (2009) 122–133

Another potential methodologic issue is that we were unable to control for both age and body length in wild-caught mothers and their lab-reared daughters because of the differences in growth rate between guppies in the laboratory and the field. Therefore, we chose to observe brain size when mothers and daughters had similar body lengths rather than when they were of similar age. Guppies in the lab, unless they are on restricted diets, grow more quickly than wild fish. Because teleost fish brains continue to grow throughout life (Leyhausen et al. 1987; Brandstätter & Kotrschal 1990; Ekstrom et al. 2001), it is possible that mothers' brains were larger than their daughters' because they were older when sacrificed. However, it is likely, for the following reasons that most of the differences in relative brain size were caused by the rearing environment. First, we showed that the wild mothers, who had spent 10 mo in the lab, did not have larger brains for their body length than wild female guppies that had spent only 3 wk in captivity, and were, therefore, on average about 9 mo younger. Second, the lab-reared fish probably received better nutrition than their wild mothers. The wild-caught mothers matured in small streams with low resource availability and very slow growth rates (Grether et al. 2001). The mothers may have even suffered malnutrition at some points during development or at least food deprivation. Malnutrition has well known detrimental effects on mammalian neurologic development and cognitive performance (Smart 1993; Gordon 1997).

A potential criticism of our study is that we measured whole brain regions (Healy & Rowe 2007). We could isolate specific brain areas that have defined functions, such as the dorsolateral telencephalon which is heavily responsible for spatial memory in fish, in our photographs. We thus cannot specifically denote whether, for instance, spatial learning is likely to be affected by the smaller brain sizes of lab-reared fish because we did not isolate that functional area. However, the large effects of rearing environment on both telencephalons and optic tecta suggest that many functional areas may be affected. As for data collection, although our photographs of the dorsal area of the brain are probably not as precise a measurement tool as calculation of volume with serial sections, we did find that dorsal area was highly correlated with area from lateral photographs. Therefore, it is highly unlikely that the large differences that we observed between wild-caught and lab-reared fish are the result of imprecision.

The plasticity of brain area size that we observed has broad implications. There is increasing concern for the survivorship of hatchery fish when released to the wild, and also for the welfare of fish in captive settings and their capacity for suffering (Braithwaite & Huntingford 2004; Huntingford et al. 2006). As well, fish are being used as a model system for the study of senescence (Reznick 1997; Reznick et al. 2002; Kishi et al. 2003; Genade et al. 2005). Because all of these areas of study are concerned with cognitive difficulties, it is important that we know whether the fish studied are cognitively representative of wild fish. Therefore, there are multiple reasons to identify appropriate laboratory housing conditions to allow full neurologic and behavioral development. This will clearly not be an easy task, as, thus far, only cerebellum size has responded to enriched habitats in fish (Kihslinger & Nevitt 2006). Any deficiencies in brain size of lab-reared fish may hinder our ability to understand the basic mechanisms of cognition and how it has been shaped by natural selection. Species-specific research programs will be required to identify the factors or combination of factors that drive the differences in brain size and behavior observed when animals are reared in captivity or the wild.

Acknowledgements

The research was supported by an Ontario Graduate Scholarship to JGB, a Natural Science and Engineering Council of Canada grant to FHR and James D. Thomson, Government of Ontario PREA grant to FHR, and the USA government National Science Foundation grants (DEB-0128455 and DEB-0128820) to FHR, A. Houde and K. Hughes. The Government of Trinidad and Tobago kindly gave permission for collection of guppies. Laboratory assistance was provided by John Brett, Seraphina Chung, Joseph Domenichiello, Ann Leung, Aneta Krakowski and Mariska Raj. Anna Price, Sara Shettleworth, Locke Rowe, James Thomson and anonymous reviewers made valuable comments on earlier versions of the manuscript. All experiments were approved by the University of Toronto Animal Care Committee (protocol # 20006260).

Literature Cited

Barreto, R. E., Gontijo, A., Alves-de-Lima, R. O., Raymundi, V. C., Pinhal, D., Reyes, V. A. V., Volpato, G. L. & Salvadori, D. M. F. 2007: MS222 does not induce primary DNA damage in fish. Aquac. Int. **15**, 163—168.

Barton, R. A. 1998: Visual specialization and brain evolution in primates. Proc. R. Soc. Lond., B, Biol. Sci. **265**, 1933—1937.

Benaroya-Milshtein, N., Hollander, N., Apter, A., Kukulansky, T., Raz, N., Wilf, A., Yaniv, I. & Pick, C. G. 2004: Environmental enrichment in mice decreases anxiety, attenuates stress responses and enhances natural killer cell activity. Eur. J. Neurosci. **20**, 1341—1347.

Bolhuis, J. E., Schouten, W. G. P., Schrama, J. W. & Wiegant, V. M. 2005: Behavioural development of pigs with different coping characteristics in barren and substrate-enriched housing conditions. Appl. Anim. Behav. Sci. **93**, 213—228.

Braithwaite, V. A. & Huntingford, F. A. 2004: Fish and welfare: do fish have the capacity for pain perception and suffering? Anim. Welf. **13**, S87—S92.

Brandstätter, R. & Kotrschal, K. 1990: Brain growth patterns in four European cyprinid fish species (Cyprinidae, Teleostei) - roach (*Rutilus rutilus*), bream (*Abramis brama*), common carp (*Cyprinus carpio*) and sabre carp (*Pelecus cultratus*). Brain Behav. Evol. **35**, 195—211.

Broglio, C., Rodriguez, F. & Salas, C. 2003: Spatial cognition and its neural basis in teleost fishes. Fish. Fish. **4**, 247—255.

Burns, J. G. 2008: The validity of three tests of temperament in guppies (*Poecilia reticulata*). J. Comp. Psychol. **122**, 344—356.

Canadian Council on Animal Care. 2005: Guidelines on: The Care and Use of Fish in Research, Teaching and Testing. Canadian Council on Animal Care, Ottawa, Canada, pp. 87.

Einum, S. & Fleming, I. A. 1997: Genetic divergence and interactions in the wild among native, farmed and hybrid Atlantic salmon. J. Fish Biol. **50**, 634—651.

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.

Fleming, I. A., Jonsson, B., Gross, M. R. & Lamberg, A. 1996: An experimental study of the reproductive behaviour and success of farmed and wild Atlantic salmon (*Salmo salar*). J. Appl. Ecol. **33**, 893—905.

Genade, T., Benedetti, M., Terzibasi, E., Roncaglia, P., Valenzano, D. R., Cattaneo, A. & Cellerino, A. 2005: Annual fishes of the genus *Nothobranchius* as a model system for aging research. Aging Cell **4**, 223—233.

Gordon, N. 1997: Nutrition and cognitive function. Brain Dev. **19**, 165—170.

Grether, G. F., Millie, D. F., Bryant, M. J., Reznick, D. N. & Mayea, W. 2001: Rain forest canopy cover, resource availability, and life history evolution in guppies. Ecology **82**, 1546—1559.

Hansen, L. T. & Berthelsen, H. 2000: The effect of environmental enrichment on the behaviour of caged rabbits (*Oryctolagus cuniculus*). Appl. Anim. Behav. Sci. **68**, 163—178.

Healy, S. D. & Rowe, C. 2007: A critique of comparative studies of brain size. Proc. Biol. Sci. **274**, 453—464.

Holloway, A. C., Keene, J. L., Noakes, D. G. & Moccia, R. D. 2004: Effects of clove oil and MS-222 on blood hormone profiles in rainbow trout *Oncorhynchus mykiss*, Walbaum. Aquacult. Res. **35**, 1025—1030.

Houde, A. 1997: Sexual Selection and Mate Choice in Guppies. Princeton University Press, Princeton.

Huber, R., van Staaden, M. J., Kaufman, L. S. & Liem, K. F. 1997: Microhabitat use, trophic patterns, and the evolution of brain structure in African cichlids. Brain Behav. Evol. **50**, 167—182.

Huntingford, F. A. 2004: Implications of domestication and rearing conditions for the behaviour of cultivated fishes. J. Fish Biol. **65**, 122—142.

Huntingford, F. A., Adams, C., Braithwaite, V. A., Kadri, S., Pottinger, T. G., Sandoe, P. & Turnbull, J. F. 2006: Current issues in fish welfare. J. Fish Biol. **68**, 332—372.

Jones, R. B. & Waddington, D. 1992: Modification of fear in domestic chicks, *Gallus gallus domesticus*, via regular handling and early environmental enrichment. Anim. Behav. **43**, 1021—1033.

Kelly, C. D., Godin, J. G. J. & Wright, J. M. 1999: Geographical variation in multiple paternity within natural populations of the guppy (*Poecilia reticulata*). Proc. R. Soc. Lond., B, Biol. Sci. **266**, 2403—2408.

Kihslinger, R. L. & Nevitt, G. A. 2006: Early rearing environment impacts cerebellar growth in juvenile salmon. J. Exp. Biol. **209**, 504—509.

Kihslinger, R. L., Lema, S. C. & Nevitt, G. A. 2006: Environmental rearing conditions produce forebrain differences in wild Chinook salmon *Oncorhynchus tshawytscha*. Comp. Biochem. Physiol., Part A Mol. Integr. Physiol. **145**, 145—151.

Kishi, S., Uchiyama, J., Baughman, A. M., Goto, T., Lin, M. C. & Tsai, S. B. 2003: The zebrafish as a vertebrate model of functional aging and very gradual senescence. Exp. Gerontol. **38**, 777—786.

Kolluru, G. R. & Grether, G. F. 2005: The effects of resource availability on alternative mating tactics in guppies (*Poecilia reticulata*). Behav. Ecol. **16**, 294—300.

de Kort, S. R. & Clayton, N. S. 2006: An evolutionary perspective on caching by corvids. Proc. Biol. Sci. **273**, 417—423.

Kotrschal, K., Van Staaden, M. J. & Huber, R. 1998: Fish brains: evolution and environmental relationships. Rev. Fish Biol. Fisheries **8**, 373—408.

Krubitzer, L. 1995: The organization of neocortex in mammals: are species differences really so different? Trends Neurosci. **18**, 408—417.

Kruska, D. 1988: Mammalian domestication and its effect on brain structure and behavior. In: Intelligence and Evolutionary Biology (Jerison, H. J. & Jerison, I., eds). Springer-Verlag, Berlin, pp. 211—250.

Kruska, D. C. T. 2005: On the evolutionary significance of encephalization in some eutherian mammals: effects of adaptive radiation, domestication, and feralization. Brain Behav. Evol. **65**, 73—108.

Lefebvre, L., Whittle, P., Lascaris, E. & Finkelstein, A. 1997: Feeding innovations and forebrain size in birds. Anim. Behav. **53**, 549—560.

Leyhausen, C., Kirschbaum, F., Szabo, T. & Erdelen, M. 1987: Differential growth in the brain of the weakly electric fish, *Apteronotus leptorhynchus* (Gymnotiformes), during ontogeny. Brain Behav. Evol. **30**, 230—248.

Lucas, J. R., Brodin, A., de Kort, S. R. & Clayton, N. S. 2004: Does hippocampal size correlate with the degree of caching specialization? Proc. R. Soc. Lond., B, Biol. Sci. **271**, 2423—2429.

Magurran, A. E. 2005: Evolutionary Ecology: The Trinidadian Guppy. Oxford University Press, Oxford.

Newberry, R. C. 1995: Environmental enrichment: increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. **44**, 229—243.

O'Regan, H. J. & Kitchener, A. C. 2005: The effects of captivity on the morphology of captive, domesticated and feral mammals. Mamm. Rev. **35**, 215—230.

Portavella, M., Vargas, J. P., Torres, B. & Salas, C. 2002: The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish. Brain Res. Bull. **57**, 397—399.

van Praag, H., Kempermann, G. & Gage, F. H. 2000: Neural consequences of environmental enrichment. Nat. Rev. Neurosci. **1**, 191—198.

Pravosudov, V. V., Kitaysky, A. S. & Omanska, A. 2006: The relationship between migratory behaviour, memory and the hippocampus: an intraspecific comparison. Proc. Biol. Sci. **273**, 2641—2649.

Price, E. O. 1999: Behavioral development in animals undergoing domestication. Appl. Anim. Behav. Sci. **65**, 245—271.

Reader, S. M. & Laland, K. N. 2002: Social intelligence, innovation, and enhanced brain size in primates. Proc. Natl Acad. Sci. USA **99**, 4436—4441.

Réale, D., Reader, S. M., Sol, D., McDougall, P. T. & Dingemanse, N. J. 2007: Integrating animal temperament within ecology and evolution. Biol. Rev. **82**, 291—318.

Reznick, D. N. 1997: Life history evolution in guppies (*Poecila reticulata*): guppies as a model for studying the evolutionary biology of aging. Exp. Gerontol. **32**, 245—258.

Reznick, D. & Endler, J. A. 1982: The impact of predation on life-history evolution in Trinidadian guppies (*Poecilia reticulata*). Evolution **36**, 160—177.

Reznick, D. N., Shaw, F. H., Rodd, F. H. & Shaw, R. G. 1997: Evaluation of the rate of evolution in natural populations of guppies (*Poecilia reticulata*). Science **275**, 1934—1937.

Reznick, D., Ghalambor, C. & Nunney, L. 2002: The evolution of senescence in fish. Mech. Ageing Dev. **123**, 773—789.

Rosenzweig, M. R. & Bennett, E. L. 1996: Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav. Brain Res. **78**, 57—65.

Salas, C., Rodriguez, F., Vargas, J. P., Duran, E. & Torres, B. 1996: Spatial learning and memory deficits after telencephalic ablation in goldfish trained in place and turn maze procedures. Behav. Neurosci. **110**, 965—980.

Smart, J. L. 1993: Malnutrition, learning and behavior: 25 years on from the MIT symposium. Proc. Nutr. Soc. **52**, 189—199.

Smith, B. R. & Blumstein, D. T. 2008: Fitness consequences of personality: a meta-analysis. Behav. Ecol. **19**, 448—455.

Steingrund, P. & Ferno, A. 1997: Feeding behaviour of reared and wild cod and the effect of learning: two strategics of feeding on the two-spotted goby. J. Fish Biol. **51**, 334—348.

Stuermer, I. W. & Wetzel, W. 2006: Early experience and domestication affect auditory discrimination learning, open field behaviour and brain size in wild Mongolian gerbils and domesiticated Laboratory gerbils (*Meriones unguiculatus* forma domestica). Behav. Brain Res. **173**, 11—21.

Sundstrom, L. F., Petersson, E., Hojesjo, J., Johnsson, J. I. & Jarvi, T. 2004: Hatchery selection promotes boldness in newly hatched brown trout (*Salmo trutta*): implications for dominance. Behav. Ecol. **15**, 192—198.

Werner, E. E. & Anholt, B. R. 1993: Ecological consequences of the trade-off between growth and mortality rates mediated by foraging activity. Am. Nat. **142**, 242—272.