

Eye Size Does Not Change with Artificial Selection on Relative Telencephalon Size in Guppies (*Poecilia reticulata*)

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Keywords

Telencephalon · Eye size · Optomotor response

Abstract

Introduction: Variation in eye size is sometimes closely associated with brain morphology. Visual information, detected by the retina, is transferred to the optic tectum to coordinate eye and body movements towards stimuli and thereafter distributed into other brain regions for further processing. The telencephalon is an important visual processing region in many vertebrate species and a highly developed region in visually dependent species. Yet, the existence of a coevolutionary relationship between telencephalon size and eye size remains relatively unknown. **Methods:** Here, we use male and female guppies artificially selected for small- and large-relative-telencephalon-size to test if artificial selection on telencephalon size results in changes in eye size. In addition, we performed an optomotor test as a proxy for visual acuity. **Results:** We found no evidence that eye size changes with artificial selection on telencephalon size. Eye size was similar in both absolute and relative terms between the two selection regimes but was larger in females. This is most likely because of the larger body size in females, but it could also reflect their greater need for visual capacity due to sex-specific differences in foraging and mating behaviour. Although the optomotor response was stronger in guppies with

a larger telencephalon, we found no evidence for differences in visual acuity between the selection regimes. **Conclusion:** Our study suggests that eye size and visual perception in guppies do not change rapidly with strong artificial selection on telencephalon size.

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Introduction

The vertebrate eye varies greatly in size across taxa [1], reflecting divergent selection pressures and functional adaptations [2–7]. A larger eye consists of more retinal photoreceptive cells that can receive more visual information compared to a smaller eye [8–10]. Eye size evolution has been suggested to be driven by light levels [11–14], predator-prey interactions [2, 15, 16], and habitat complexity [13, 17]. As the visual sensory system of species adapts to meet ecological challenges, their brains may undergo evolutionary changes to accommodate these sensory requirements [2, 18]. The increased visual information that a larger eye can receive may require a greater need for visual processing of neural tissue in the brain [2–5, 9]. Hence, it has been proposed that brain size [5, 18–21], or the size of certain brain regions [2, 22], coevolve with larger eyes in order to cope with such demands.

In vertebrates, visual information is transferred from the retina in the eye and processed by distinct regions in the brain, such as the optic tectum and the telencephalon [23–26]. The optic tectum enables animals to accurately coordinate and orientate eye and body movements towards the source of sensory input [25, 27, 28] and can coevolve with eye size [24, 25]. The telencephalon is a key brain region behind the processing of visual information in vertebrates, such as when animals distinguish an object from the background, when they determine object distance and size, and when they recognize if an object is novel or familiar [25]. Within the telencephalon, the main visual centre is the dorsal telencephalon that in turn consists of several specialized subregions [26]. The number of these subregions varies between species depending on visual capabilities, and sometimes the morphology of these subregions also covary with eye size [26]. There may thus be a link between evolutionary changes in telencephalon size and eye size. Given the visual processing role of the telencephalon, and the selection pressures associated with increased eye size and increased telencephalon size [2, 29–31], it is likely that eye size and telencephalon size coevolve and are developmentally linked. A larger telencephalon provides more neural tissue for processing signals from the retina in the eyes. Furthermore, the retina develops from the central nervous system (i.e., the brain) during ontogeny [32, 33], which provides a substantial link between telencephalon size and eye size. This link has, to our knowledge, only been demonstrated in the Trinidadian killifish (*Anablepsoides hartii*), but the direction of the association is inconsistent and varies between the sexes [18, 22]. Hence, there is currently no consensus regarding if/how telencephalon size is associated with eye size in vertebrates.

Inter- and intraspecific correlative comparative analyses can identify evolutionary patterns [2, 34, 35] but lack the possibility to investigate causal relationships [36]. Artificial selection, on the other hand, can reveal whether behavioural or physiological variation can be attributed to heritable differences in a given trait [37–39]. In this study, we provide an experimental approach to investigate the evolutionary link between changes in telencephalon size and eye size. For this, we use guppies (*Poecilia reticulata*) artificially selected by Fong et al. [40] for small- and large-relative-telencephalon-size over three generations. Since there is a positive association between eye size and visual acuity in fish [13, 41], we tested if potential variation in eye size between the selection lines also generates differences in visual acuity using an optomotor test. Investigating if changes in telencephalon size are interconnected with changes in eye size and divergence in

visual acuity is important for understanding their evolution as well as for determining if behavioural differences between these selection regimes could be caused by differences in aspects of the visual system.

Methods

Model System

We conducted this study at Stockholm University's freshwater aquarium facilities in October 2019. The artificial selection procedure for relative telencephalon size (relative to the rest of the brain) was performed by Fong et al. [40] by using laboratory-bred descendants of wild-caught guppies from high-predation areas in the Quare River, Trinidad. From three breeding stocks (hereafter replicates), one up-selected line and one down-selected line per replicate were created, resulting in three up-selected lines and three down-selected lines. The offspring of 75 breeding pairs per replicate with the 20% smallest and 20% largest relative-telencephalon-volume were used for the next generation. After three generations of this selection procedure, a difference of approximately 5% in telencephalon volume was established [40, 42]. For details on the selection procedure, see ref. [40].

We housed sexually mature fish in sex-specific stock tanks in groups of 8–9 females and 40 males, sorted by selection regime and replicate. We determined sexual maturity as the visible development of gonopodium in males and gravid spot in females. The laboratory was maintained at a 12:12 h dark: light scheme and $24 \pm 1^\circ\text{C}$ water temperature. The tanks were enriched with 2 cm gravel, biological filter, snails (*Planorbis* sp.), java moss (*Taxiphyllum* sp.), and/or artificial plants for the well-being of the fish. The fish were fed 6 days per week with flake food and *Artemia* hatchlings interchangeably.

Experimental Procedure

Eye Size

We examined eye size in 234 guppies, with 119 individuals (59 females and 60 males) from the up-selected regime and 115 individuals (55 females and 60 males) individuals from the down-selected regime. These individuals were equally distributed among the three independent replicates. To measure eye size, we individually anaesthetized the guppies with 0.2 mg/L benzocaine and placed them on a glass surface placed over graph paper, which was used to calibrate the measurements. Eye size was measured from photographs taken of the left side of the body using a fixed Canon EOS 1,200 camera – eye size was thus measured only for the left eye of each individual. We measured eye diameter and body length to the nearest 0.001 mm using ImageJ software [43]. From these measurements, we calculated both absolute eye area and relative eye area in mm^2 (relative eye area = absolute eye area/body length). The experimenter (MG-O) was unaware of telencephalon selection regime and replicate, since all tanks were coded by running numbers.

Optomotor Response

Visual acuity is defined as the ability of the visual sensory system to perceive spatial details and distinguish shapes and details of an object, i.e., the sharpness of vision [15, 41]. In fish, visual acuity is often estimated behaviourally by using the optomotor

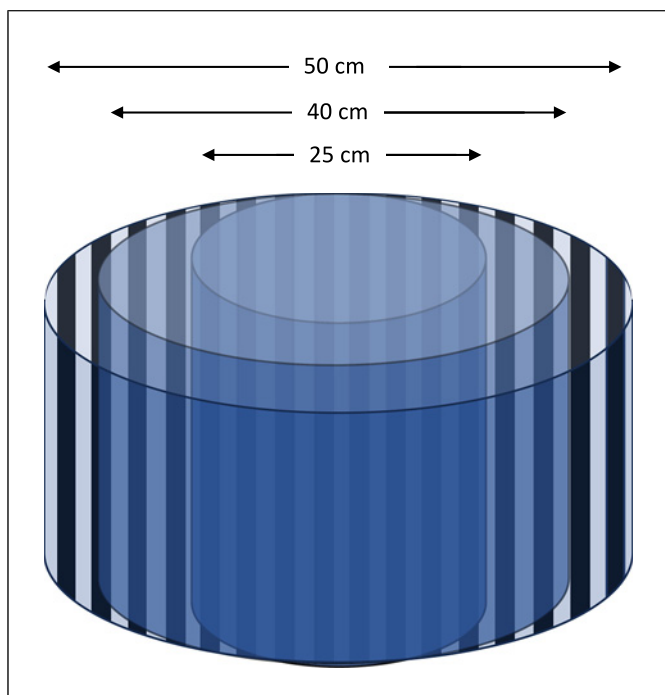


Fig. 1. Optomotor apparatus. Female and male guppies from large- and small-telencephalon-size-selection regimes were placed between the inner (25 cm) and middle (40 cm) walls to measure their optomotor response towards different stripe widths in black and white projected from above on the outer (50 cm) wall.

response [15, 41, 44]. The optomotor response is a stereotyped behaviour that can be induced by placing the fish inside a cylinder-shaped experimental chamber and projecting a rotating pattern with vertical black and white stripes on the outer chamber wall [15]. Fish have an innate tendency to remain stationary in flowing water to avoid being transferred downstream [15]. Therefore, fish tend to follow the rotation of a rotating pattern to keep its position in the environment. The width of the stripes is successively made smaller, until the fish can no longer distinguish the rotating pattern, i.e., the limit of visual acuity is reached when the optomotor response ceases [41].

In this study, we measured the individual optomotor response of 238 guppies (120 small- and 118 large-telencephalon-size-selected), divided equally among sex and the three independent replicates. We used the experimental setup previously used [38, 39]. We placed each guppy in a circular tank with inner, middle, and outer walls (inner and middle area diameter, 25 cm and 40 cm, respectively) and left it to acclimatize for 120 s (shown in Fig. 1). The guppies only had access to the area between the inner and the middle walls. The middle wall was transparent to allow the fish to see the outer wall, but the inner wall was non-transparent. The distance between the middle and the outer wall was 10 cm. We projected the visual stimuli, consisting of rotating vertical black and white stripes, on the outer wall at a constant speed of 2.62 $\text{cm}\cdot\text{s}^{-1}$ by using an Infocus IN114 projector. The visual acuity of guppies is approximately four cycles per degree (cpd) [45, 46]. Since the guppies in this study could vary their distance from the

stimuli (see Fig. 1), we also provide the range of cycles per degree for each stimulus within brackets. The stimuli had six different widths, and these were 0.20 cm (3.1–4.4 cpd representing the lower limit of guppy visual acuity [39, 45, 46]), 0.22 cm (2.8–4.0 cpd), 0.24 cm (2.6–3.6 cpd), 0.27 cm (2.3–3.3 cpd), 0.30 cm (2.0–2.9 cpd), and 0.34 cm (1.8–2.6 cpd). We presented each stimulus width for 60 s at a time. To account for any preferences in swimming direction, each rotating stimulus was projected both clockwise and counterclockwise, i.e., 2 times 60 s per stimulus. Each stimulus width run was preceded by a static image of the same stimulus presented for 60 s, resulting in 2 times 60 s total observation time per stimulus since each rotating stimulus was projected both clockwise and counterclockwise. We randomized the order of stimulus presentation, such that the stimulus was not consistently presented from smallest to largest stripe width or vice versa. All stimulus widths were presented during one experimental trial, i.e., fish were only transferred to the experimental arena and tested once. We defined optomotor response as the time the guppies spent following the rotational stimulus, i.e., circling in the same direction. We recorded the trials by using an EPSON EB-S31 projector and a Sony Handycam HDR-DR11E video camera. The observer (MG-O) scored the behaviour from the videos using BORIS v 2.72 software [47], unaware of the selection regime, replicate, stimulus rotation, speed, and stripe width. Each individual had a running number for identification, and the stimulus was covered on the computer screen so that only the fish was visible.

Statistical Analyses

All analyses and figures were performed in R statistical software [v 4.0.1, <http://R-project.org/>]. Statistical significance of the explanatory variables was computed by using the ANOVA function, specifying Type III Wald χ^2 tests, in the *car* package [48].

Absolute and Relative Eye Size

We asked if eye size in absolute and relative terms is dependent on telencephalon size selection regime, while also testing for sex-specific effects of artificial selection for telencephalon size on eye size. Hence, we ran two independent linear models (LMs) for the dependent variables absolute eye size and relative eye size. We modelled the dependent variables as a function of the explanatory variables selection regime_{small vs. large telencephalon}, sex, their interaction, and body length as a covariate. We standardized body length by mean centring and divided by 1 standard deviation, as this improves the numerical optimization process. We used a log transformation for both absolute and relative eye area to better meet the model assumptions (lm syntax for the two independent full models: $\text{eye size}_{\text{absolute or relative}} \sim \text{selection regime} + \text{sex} + \text{body length} + \text{selection regime}:\text{sex}$). To account for any underlying differences between the three replicates, this variable should be fitted as a random effect nested in selection regime [49]. However, since random effects require at least five levels to accurately estimate among-group variance in the data [49], and the artificial selection procedure was replicated 3 times (see Methods), we fitted replicate as a fixed effect and retained it in the model if the effect was significant. Model validation was performed on all full models by plotting Pearson's residuals versus fitted values and visually inspecting for heteroscedasticity. We confirmed the normality of residuals by visual inspection. Neither of the two models showed any signs of heteroscedasticity and the residuals were roughly

Table 1. Results from two independent LMs testing for the effect of relative telencephalon size on absolute eye size and relative eye size (eye area/body length)

Absolute eye size (LM)	Sum. Sq	df	F-value	<i>p</i> value
(Intercept)	43.175	1	7569.642	<0.001
Selection regime	0.001	1	0.139	0.709
Sex	0.247	1	43.329	<0.001
Body length	0.499	1	87.528	<0.001
Replicate	0.043	2	3.736	0.025
Residuals	1.300	228		
Relative eye size (LM)				
(Intercept)	32.032	1	5786.399	<0.001
Selection regime	<0.000	1	0.050	0.824
Sex	0.227	1	41.002	<0.001
Body length	0.013	1	2.300	0.131
Replicate	0.049	2	4.452	0.013
Residuals	1.262	228		

The columns provide the sum of squares (Sum. Sq.), degrees of freedom (df), F-values, and associated significance values (*p*). Significant (*p* < 0.05) effects are highlighted in bold.

normally distributed. Following Bolker [50], we excluded the nonsignificant (i.e., *p* > 0.1) interactions from the models (lm syntax for the two independent final models: eye size_{absolute or relative} ~ selection regime + sex + body length + replicate).

Optomotor Response

We analysed the optomotor response using linear mixed-effect models (LMMs), as implemented in the *lme4* package [51]. First, following Corral-López et al. [39], we compared the general optomotor response of each individual between the static and rotating stimuli for all six stripe widths. We examined the overall time that individual fish spent performing an optomotor reflex, i.e., circling in the same direction as the stimuli. We modelled optomotor response as a function of stimuli (six static and six rotating stimuli) as the explanatory variable and included a random intercept for fish ID to account for repeated measurements. We used a power transformation on optomotor response to better meet the assumptions of the model (lmer syntax for the full and final model: optomotor response ~ stimuli + [1|fish ID]). The residual distribution was roughly normal with no signs of heteroscedasticity.

We then tested the effect of telencephalon size selection regime on optomotor response, as well as for sex-specific effects of artificial selection for telencephalon size. We modelled the optomotor response for every rotating stripe width as a function of the explanatory variables' selection regime_{small vs. large telencephalon}, sex, and their interaction, stimuli were included as covariates, and a random intercept was used for fish ID to account for repeated measurements. We included the interaction between the selection regime and stimulus to investigate if the optomotor response differed between the selection regimes depending on stripe width. We used a power transformation on optomotor response to better meet the model assumptions (lmer syntax for the full model: optomotor response ~ selection regime + sex + stimuli + replicate + selection regime:sex + selection regime:stimuli + [1|fish ID]). Since the residual distri-

bution was roughly normal with no signs of heteroscedasticity, we proceeded with model selection. This was done backwards based on Akaike's information criterion, only non-significant interactions were subject to any exclusion [50] (lmer syntax for the final model: optomotor response ~ selection regime + sex + stimuli + [1|fish ID]).

Finally, in order to test for differences between the selection regimes in activity (defined as any swimming behaviour other than the optomotor response), that could potentially influence the results, we ran two additional LMMs. We modelled the dependent variable activity for static and rotating stimuli, respectively, as a function of selection regime_{small vs. large telencephalon}, a random intercept for fish ID accounted for repeated measurements (lmer syntax for the full and final models: activity_{during static or rotating stimuli} ~ selection regime + [1|fish ID]). The residual distributions were roughly normal with no signs of heteroscedasticity in either model.

Results

Absolute and Relative Eye Size

We asked if eye size, in absolute or relative terms, differed between guppies from the small- and large-telencephalon-size-selection regimes and if there was a sex-specific effect of artificial selection for telencephalon size on eye size. We found no significant effect of telencephalon size selection regime on absolute eye size (Table 1, Fig. 2a). Instead, absolute eye size was strongly dependent on sex and body length (Table 1). On average, females had larger eyes than males (mean mm² ± SE: 6.07 ± 0.05_{females} vs. 3.67 ± 0.03_{males}; Fig. 2a), and larger guppies had larger absolute eye size than smaller guppies (Fig. 3a). We found no significant sex-specific effect of telencephalon size selection on absolute eye size as the interaction between selection regime and sex was non-significant ($F_{1, 227} = 0.852$, *p* = 0.852, Fig. 2a).

We found a similar pattern for relative eye size as for absolute eye size. The telencephalon size selection regimes did not differ in relative eye size (Table 1; Fig. 2b). Females had significantly larger eyes in relative terms compared to males (Table 1; Fig. 2b). We found no relationship between body length and relative eye size (Table 1; Fig. 3b). This means that larger guppies do not have relatively larger eyes, i.e., eye size scales proportionally with body size in guppies from the telencephalon selection size lines tested here. Similar to absolute eye size, we found no evidence for a sex-specific effect of artificial selection for telencephalon size on relative eye size, as the selection regime × sex interaction was nonsignificant ($F_{1, 227} = 0.072$, *p* = 0.789, Fig. 2b).

Optomotor Response

First, we asked whether the optomotor response changed between static and rotating images. As previously reported [38, 39], general optomotor response

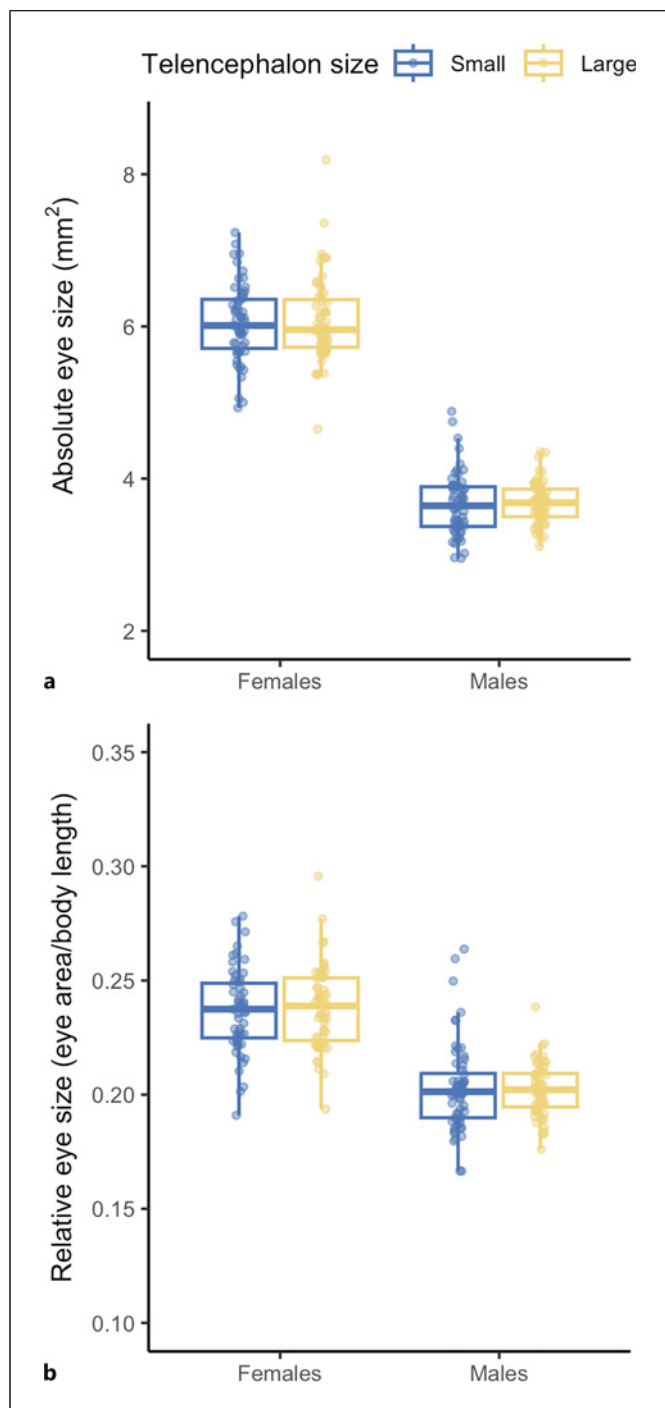


Fig. 2. Eye size in female and male guppies artificially selected for small- (blue) and large (yellow)-relative-telencephalon-size. Shown are boxplots of absolute (a) and relative (b) (eye area/body length). LMs controlling for sex and body length suggest that there is no difference between the selection regimes in neither absolute ($p = 0.71$) nor relative eye size ($p = 0.82$). Horizontal lines indicate medians, boxes indicate the interquartile, and whiskers indicate all points within 1.5 times the interquartile range. Blue and yellow markers show individual data points from 234 individuals.

differed significantly between stimuli for all six static and rotating stripe widths (LMM; $\chi^2 = 272.05$, $df = 11$, $p < 0.001$). This suggests that guppies have a stronger optomotor response towards rotating than static black and white stripes of different widths.

Second, we asked if visual acuity differed between the telencephalon selection regimes. Differences in visual acuity occur when the optomotor response decreases at different rates with thinner stripe widths or if the optomotor response is interrupted at different stripe widths, i.e., when the animal can no longer distinguish the rotating pattern [15, 41, 44]. We found no evidence of a difference in visual acuity between the selection regimes in the optomotor response test used here. The optomotor response did not decrease in relation to stripe width at different rates between the selection regimes (shown in Fig. 4). Nor did it peak at different stripe widths, as would be expected if the selection regimes differed in visual acuity (shown in Fig. 4). The nonsignificant interaction between the selection regime and stimuli corroborates this statement (LMM; $\chi^2 = 1.516$, $df = 5$, $p = 0.911$, Fig. 4). This suggests that the proportion of time performing an optomotor response does not change between the selection regimes as stripe width decreases. Instead, we found a constant difference in optomotor response between the selection regimes across the six stripe widths. Individuals from the large-telencephalon-size-selection regime spent on average 22% more time performing an optomotor response towards the rotating stimuli than individuals from the small-telencephalon-size-selection regime (mean \pm SE: $30.5\% \pm 0.01_{\text{large telencephalon}}$ vs. $25.0\% \pm 0.01_{\text{small telencephalon}}$). This suggests that the optomotor response was stronger in large-compared to small-telencephalon-size-selected guppies. This was confirmed by the significant effect of selection regime (LMM; $\chi^2 = 4.326$, $df = 1$, $p = 0.038$, Fig. 4). We found no evidence for a sex-specific effect of artificial selection on optomotor response (LMM; $\chi^2 = 0.002$, $df = 1$, $p = 0.965$), or any effect of sex on optomotor response (LMM; $\chi^2 = 0.003$, $df = 1$, $p = 0.955$). We found that optomotor response depended on stripe width in this test, i.e., optomotor response decreased significantly with decreasing stripe width (LMM; $\chi^2 = 23.650$, $df = 5$, $p < 0.001$; Fig. 4).

Finally, it is unlikely that the stronger optomotor response in large-telencephalon-size-selected guppies was caused by a higher activity level in this selection regime. The model indicates that there is a negative, nearly significant, effect of telencephalon size on activity level when presented with both the static and the rotating stimuli. Individuals from the large-telencephalon-size-selection regime thus had slightly lower activity levels compared to individuals from the

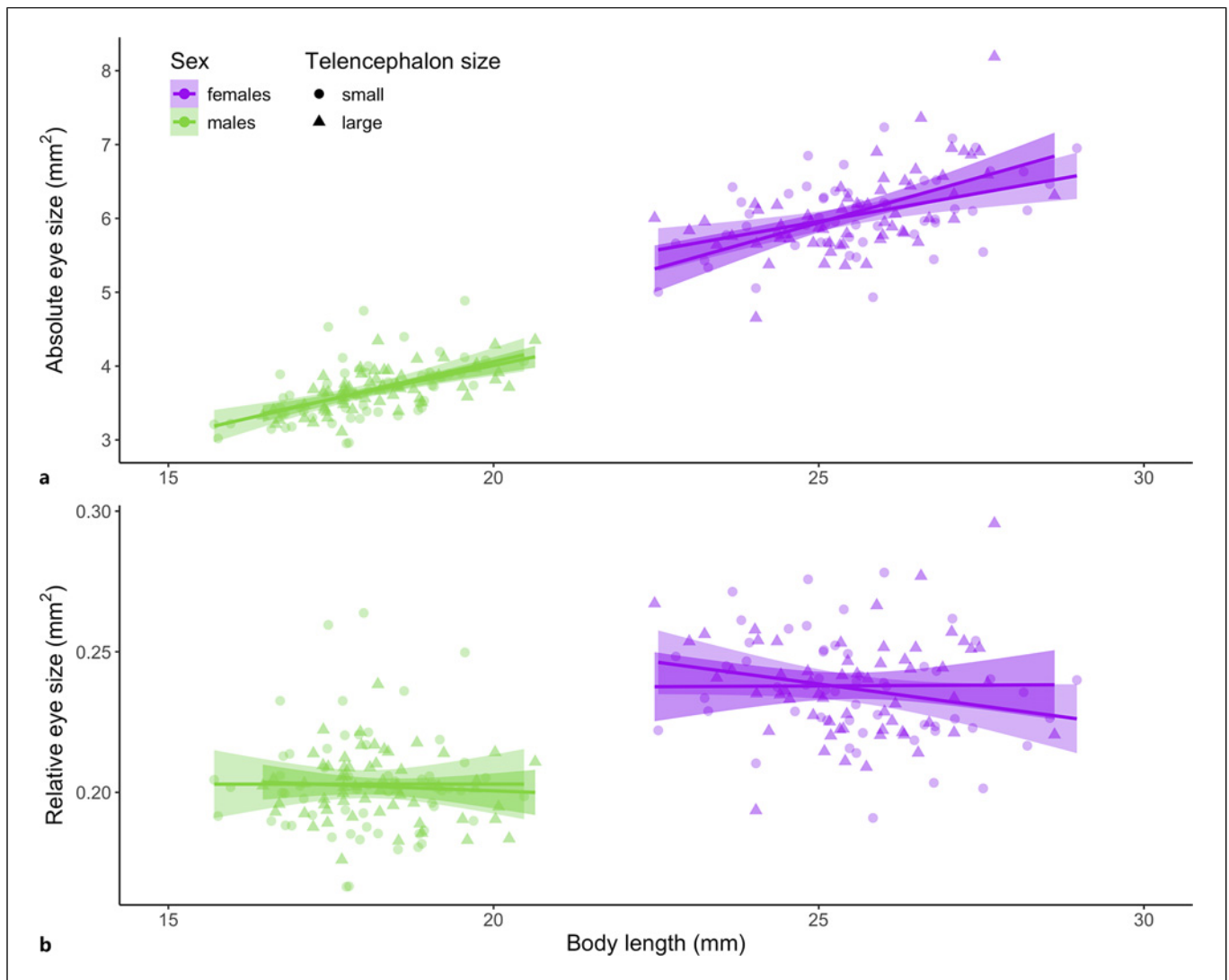


Fig. 3. Relationship between absolute (a) and relative (b) (eye area/body length) eye size and body size. Measurements in female (purple) and male (green) guppies artificially selected for small- (circles) and large (triangles)-relative-telencephalon-size ($N = 234$). Linear regression slopes \pm SE of small- (light green shading) versus large-telencephalon-size (dark green shading) males, and small- (light purple shading) versus large-telencephalon-size (dark purple shading) females.

small-telencephalon-size-selection regime (LMM_{static} : estimate \pm SE = $\chi^2 = 3.79$, $df = 1$, $p = 0.052$; LMM_{rotating} : $\chi^2 = 3.578$, $df = 1$, $p = 0.060$).

Discussion

This is the first study examining the relationship between telencephalon size and eye size in animals from artificial selection regimes with different telencephalon size. We found no support for the hypothesis that relative telencephalon size

and eye size are developmentally linked. This pattern was consistent across males and females from the small- and large-telencephalon-size-selection regimes, i.e., we found no sex-specific effect of selection on telencephalon size on eye size. Although optomotor response was stronger in large compared to small-telencephalon-size-selected guppies, we found no differences between the selection regimes in visual acuity.

Relative telencephalon size did not predict eye size in either absolute or relative terms in this study. This suggests that, although brain size and eye size can coevolve [2, 18, 21, 39], telencephalon size evolution may be decoupled from

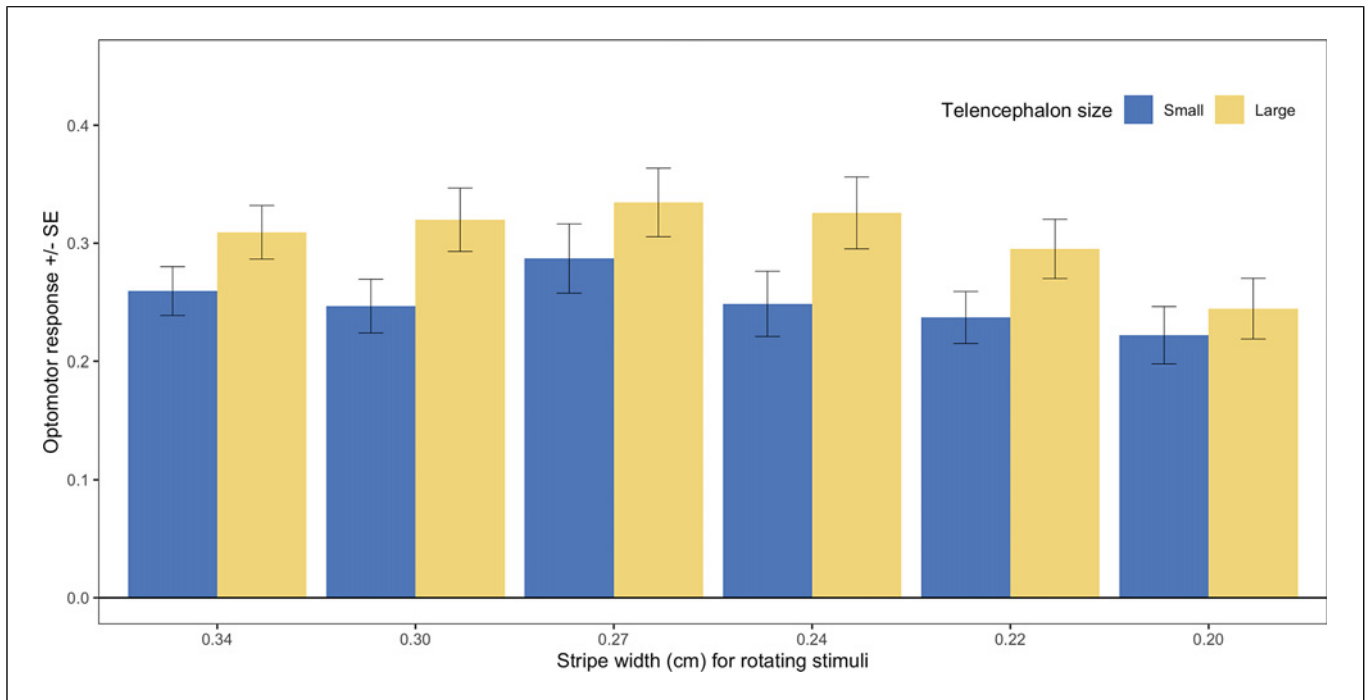


Fig. 4. Effect of artificial selection on relative telencephalon size on optomotor response. Error bars show the average optomotor response (i.e., proportion time spent circling) towards rotating black and white stimuli with different stripes in small- (blue) and large (yellow)-telencephalon-size-selected guppies. An LMM indicated a relatively constant difference in optomotor response between the selection regimes ($p = 0.038$). Data from 238 individuals.

eye size evolution, at least in the guppy populations tested here. From this, we conclude that other factors, such as diverse ecological pressures, play a more important role in shaping the evolution of eye size than the evolution of telencephalon size. Eye size can for instance not only evolve as a consequence of variation in foraging strategy, predator pressure, habitat, and offspring production [2, 6, 21, 52, 53] but also abiotic factors such as light conditions [11, 12]. It could also be other aspects of brain morphology, such as neuron density or connectivity between different brain regions, that change with eye size. For instance, neuron density in D17, a small substructure within the visual centre in the telencephalon, is lower in gobiiform fishes with small eyes [26]. Alternatively, intraspecific variation in sensory organs and brain regions can evolve in different directions under certain ecological conditions. For instance, increased predator pressure can lead to the evolution of a larger telencephalon [54, 55], and the evolution of smaller eye size [6, 53] in prey fishes. These different mechanisms can explain why there may be no clear genetic correlation between overall telencephalon size and eye size.

The optomotor response did not change with stripe width between the telencephalon size selection regimes in

our set-up, neither in terms of optomotor response decrease rate nor in terms of optomotor response interruption; the nonsignificant interaction between selection regime and stimuli corroborates this. Furthermore, although the optomotor response was stronger in guppies from the large-telencephalon-size-selection regime, this difference remained relatively constant across stripe width. Together with the lack of differentiation between the telencephalon size selection regimes in eye size, this suggests that visual acuity is not different between the two selection regimes. However, it is worth noting that in this study, we quantified eye size and optomotor responses, two relatively crude proxies of visual acuity [41, 56]. In species with camera-type eyes, like the guppy [41], visual acuity can also be quantified anatomically by measuring the inter-photoreceptor distance and the focal length (i.e., the distance from the inter-photoreceptors to the centre of the pupil) and then calculating the minimum resolvable angle [41]. This approach could potentially identify smaller differences in visual acuity than eye size and behavioural approaches [57, 58]. Hence, we cannot completely rule out that differences in visual acuity between the selection regimes exist. However, these are in

that case most likely of minor degree and of little importance for the behavioural differences previously reported between these selection regimes [42, 59, 60]. We propose that future studies investigate eye anatomy in more detail in these telencephalon size selection regimes for a deeper understanding of the relationship between visual acuity and telencephalon size.

The overall optomotor response was stronger in the large compared to the small selection regime. A possible explanation to this difference could be a higher motivation to follow a moving pattern in the large-telencephalon-size-selected guppies. We can only speculate over possible explanations to this difference. It is possible that there is a difference in the integration of sensory information in the large-telencephalon-size-selection regime. The telencephalon is responsible for integrating sensory information [25], and it is possible that guppies from the large-telencephalon-size-selection regime are better at integrating moving visual cues with other sensory inputs. This could lead to a higher optomotor response in large telencephalon guppies. Given the role of the telencephalon as the cognitive centre in fish [61–64], it is possible that a larger telencephalon facilitates faster processing of moving stimuli and thereby the ability of fish to keep its position in the environment and not be transferred downstream (as described earlier).

Female guppies had larger eyes in both absolute and relative terms than male guppies in this study, a pattern previously reported by Corral-López et al. [39]. The absolute larger eyes in females are most likely explained by differences in body size between the sexes. Female guppies are larger than male guppies, as female reproductive outcome is dependent on body size for maximal production of offspring, whereas male reproductive outcome is dependent on access to females [65]. Body size is a strong predictor of eye size, mainly since a small head has limited space for large eyes [34, 35]. Females in this study were also found to have larger relative eye size in relation to their male counterparts. Relatively larger eyes have previously been associated with better visual abilities in guppies [39]. This is most likely explained by a selection for better visual capacity in females than males due to sex differences in foraging and mating behaviour in Poeciliid fishes [56, 65].

Conclusions

To conclude, our results suggest that there is no developmental relationship between changes in relative telencephalon size and eye size in either absolute or

relative terms. The larger eye size in females can most likely be attributed to differences in body size, foraging, and mating behaviour between the sexes. Despite the stronger optomotor response in guppies with large telencephalons, visual acuity is not different between the selection regimes. We propose that future studies focus in more detail on eye anatomy to test the relationship between visual acuity and telencephalon size.

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Statement of Ethics

The experiment was performed in accordance with ethical applications approved by the Stockholm Ethical Board (Dnr: N8/17 and 17362-2019).

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

A.B. analysed and visualized the data and drafted the initial manuscript. M.G.-O. collected the data. M.G.-O., S.F., and N.K. designed the study. S.F. and N.K. generated the telencephalon size selection regimes. N.K. provided contributions to revisions of the manuscript. All authors contributed to finalizing the paper.

Data Availability Statement

All data and R code are available at <https://doi.org/10.6084/m9.figshare.25213355> and <https://figshare.com/s/bffdddda733100be93b6>.

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