Sex determination protocol

Pond fish
Genotyping was carried out using a genotyping-by-sequencing protocol (Elshire et al. 2011) using the enzyme PstI. The full details of this protocol are available in Samuk et al. (2017). The protocol and post-processing yielded approximately 30,000 polymorphic markers across the genome. In order to build a panel of informative markers, we focused on the 2289 markers found on chromosome 19 (the sex chromosome). We used discriminant function analysis (R function "dfa") to identify loci that discriminated between 4 physically sexed females and 4 physically sexed males. We then confirmed that the markers that separated known males from known females were homozygous in the females and heterozygous in the males (i.e. the markers were located in the heteromorphic sex-determining region). In the end, this method yielded 142 sex-linked diagnostic markers. These 142 markers were then used to determine the sex of the F3 individuals who had not been physically sexed.

Common garden fish
Fish from the common garden experiment were physically sexed via dissection of the gonads.

References

Elshire, R. J., Glaubitz, J. C., Sun, Q., Poland, J. A., Kawamoto, K., Buckler, E. S., & Mitchell, S. E. (2011). A robust, simple genotyping-by-sequencing (GBS) approach for high diversity species. *PloS one 6*(5): e19379.

Samuk, K., Owens, G.L., Delmore, K.E., Miller, S., Rennison, D.J. & Schluter, D. (2017). Gene flow and selection interact to promote adaptive divergence in regions of low recombination. Mol Ecol 26: 4378-4390.

SUPPLEMENTAL FIGURES & TABLES

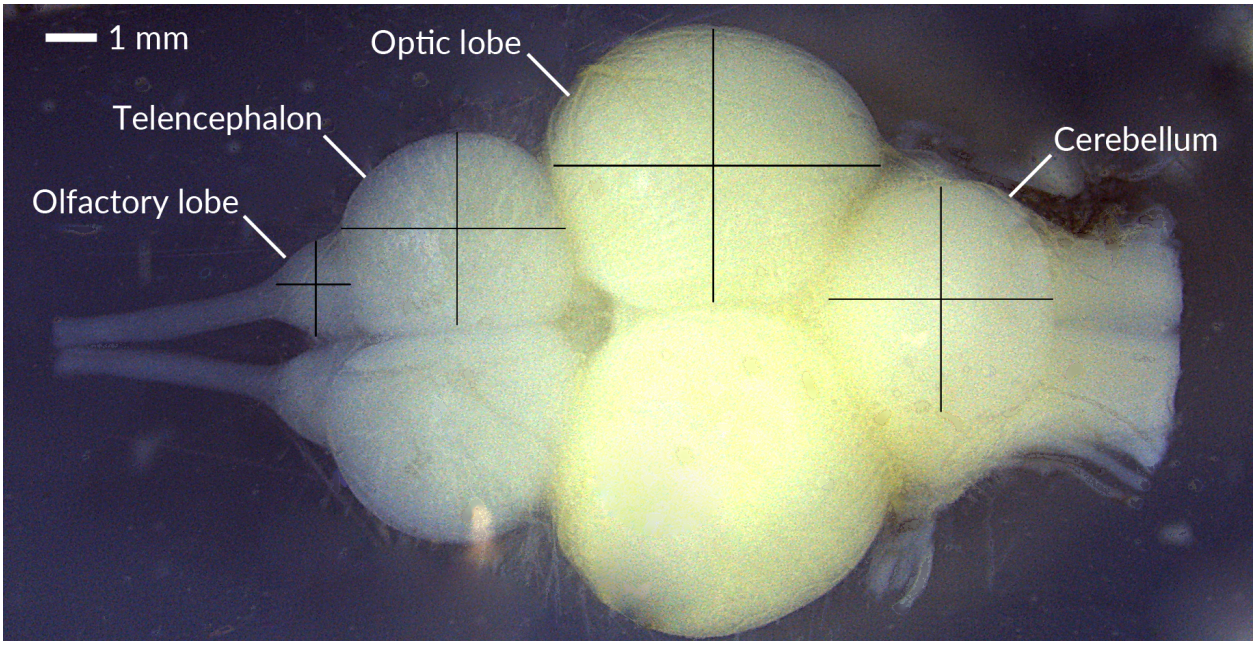

Figure S1 Dorsal view of a threespine stickleback brain magnified under a stereomicroscope. The brain is resting in a triangular divot carved into an agar plate flooded with 40% isopropyl alcohol. Black lines represent maximal length and widths measured to approximate surface area of each lobe (labelled in white). A metal ruler (not shown) was used to normalize lengths among photographs.

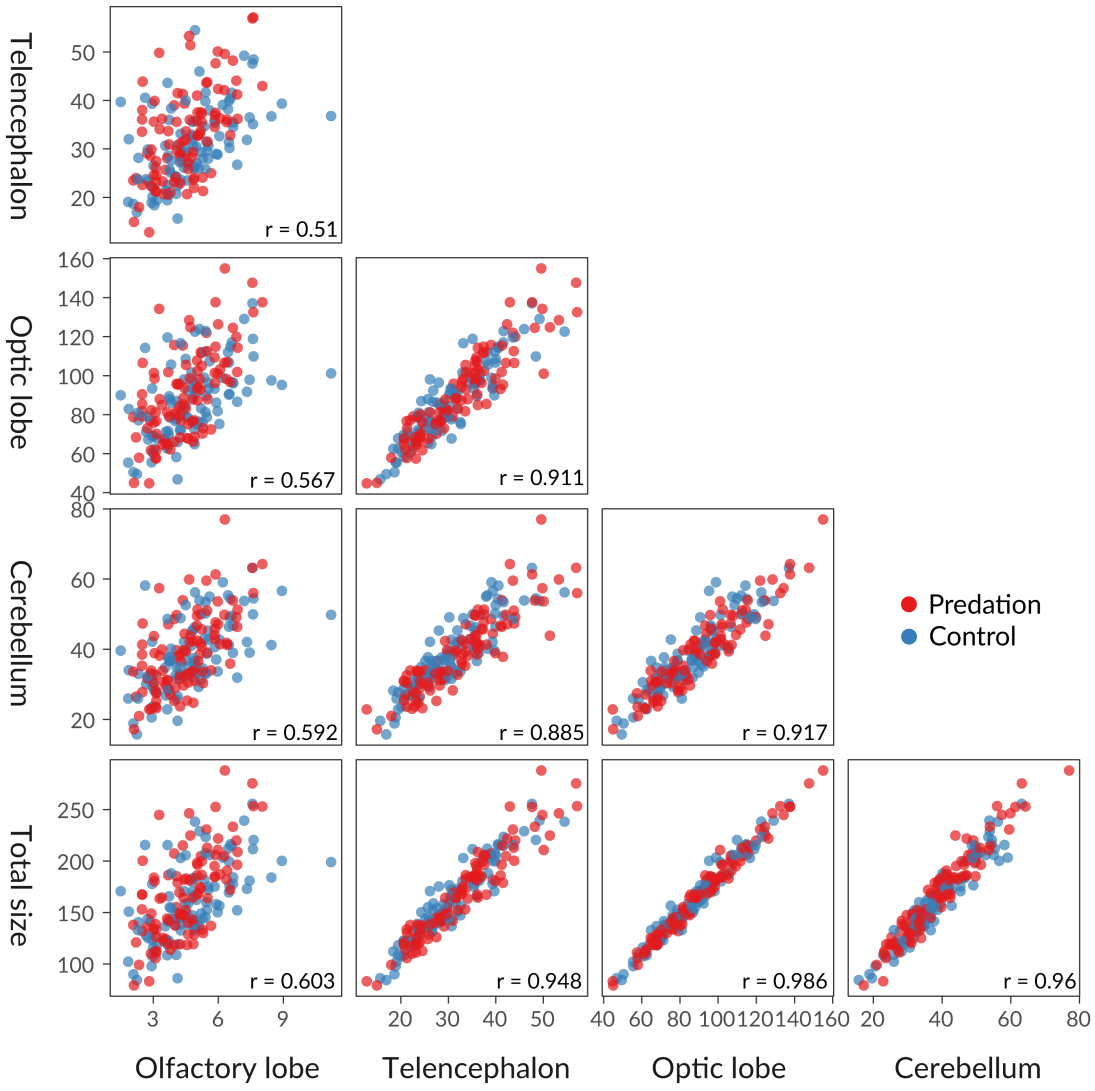

Figure S2 Correlations among brain regions measured in the experimental fish. Each plot shows the correlation between a pair of brain lobes (indicated on the major x and y axes), with each point representing the region measurements within a single individual. All region sizes (ellipsoidal areas) are expressed as in units of mm^2.

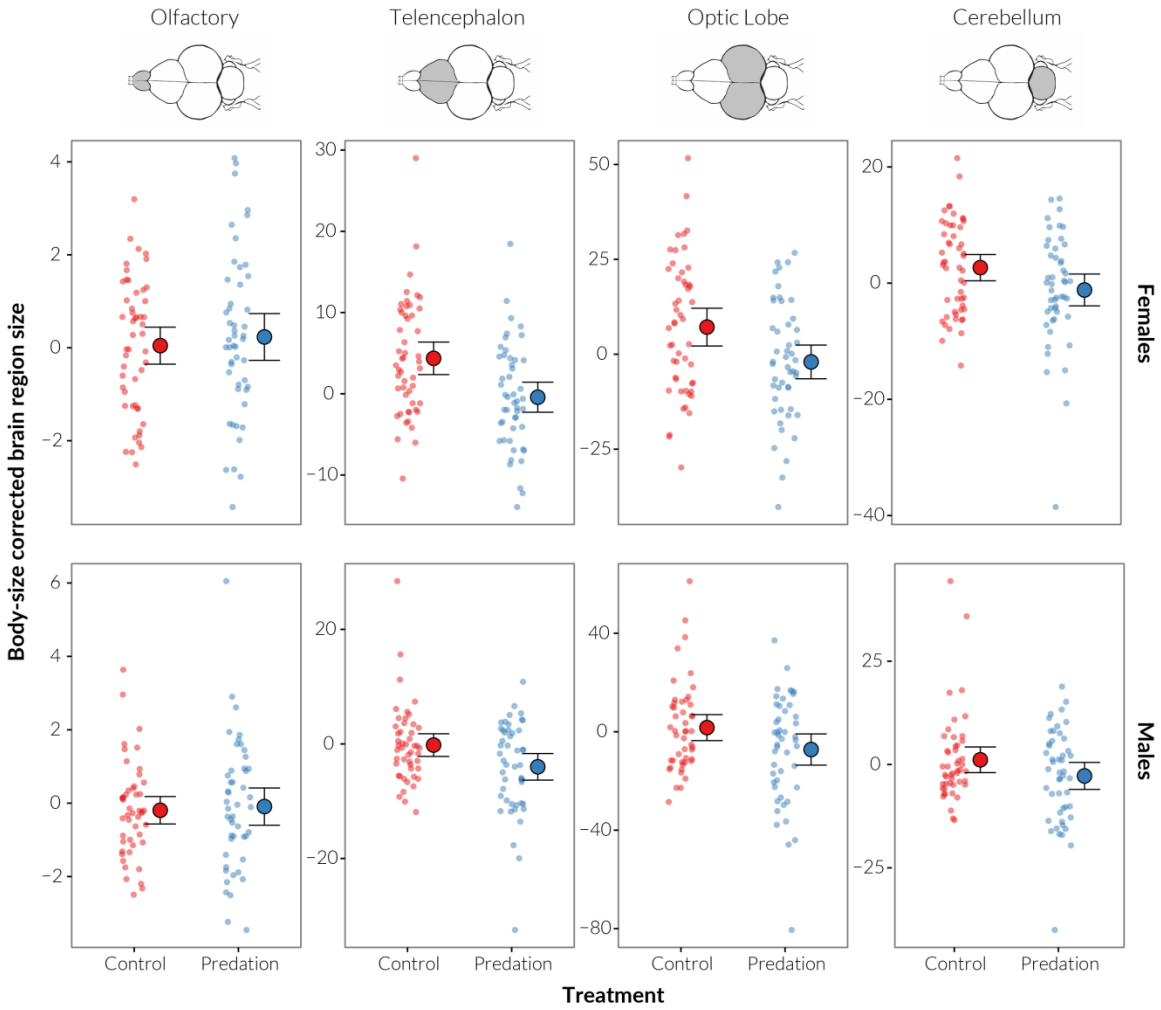

Figure S3 Individuals exposed to predation in experimental ponds evolved smaller telencephala, optic lobes, and cerebellums compared to individuals from the control treatment (n=196). Panels depict the size of each brain region in the predation (red) and control (blue) treatments. Each small, transparent point represents the brain region size (after body size correction) of a single individual. Large, solid points represent means; error bars represent 95% confidence intervals. Females are shown along the top row, and males along the bottom.

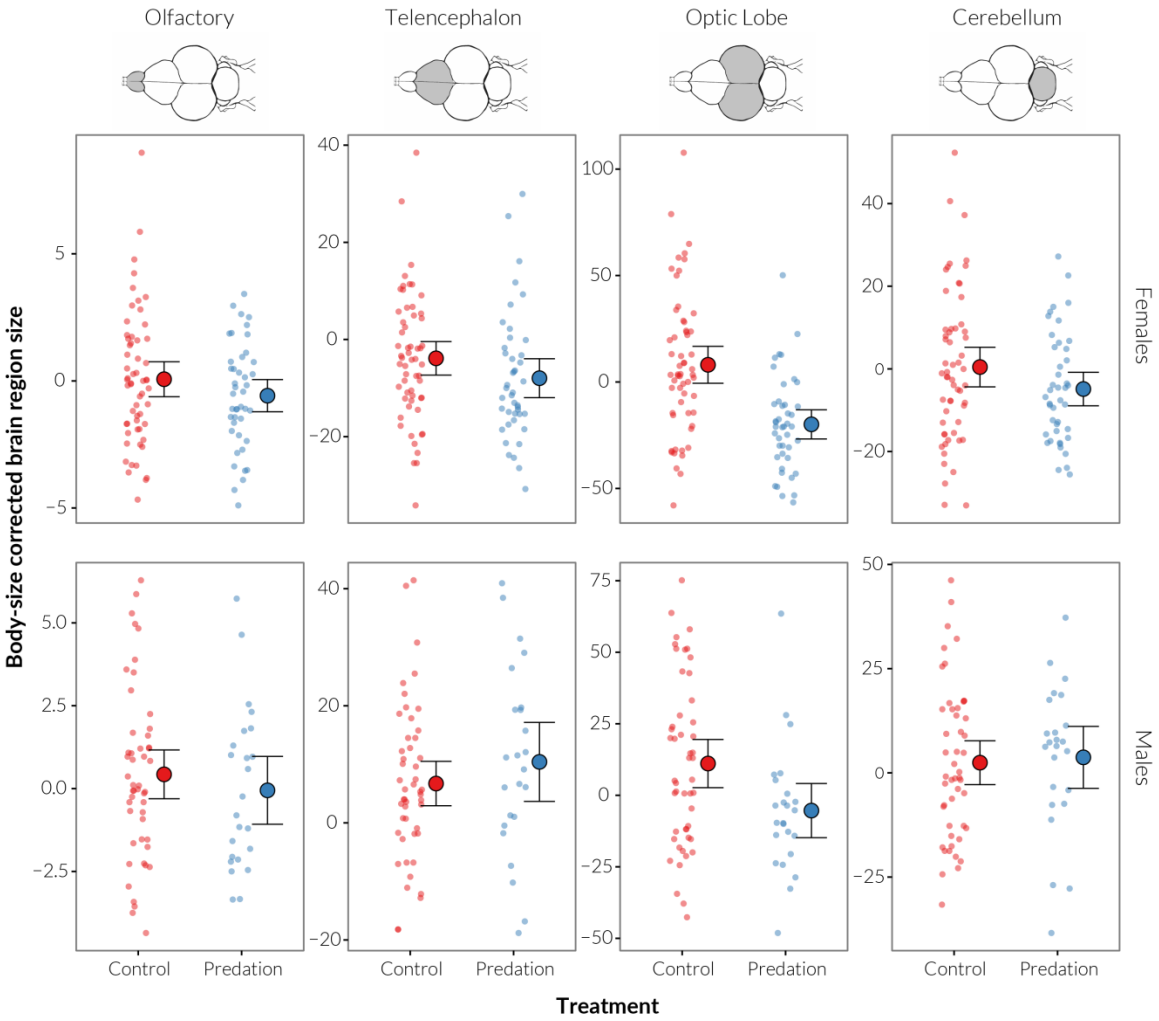

Figure S4 When reared in a common environment, offspring of experimental fish showed a reduction in brain size, but it was limited to the optic lobe (n=183). Panels depict the size of each brain region from a lab-reared offspring of a cross from the predation (red) and control (blue) treatments. Each small, transparent point represents the brain region size (after body size correction) of a single individual. Large, solid points represent means; error bars represent 95% confidence intervals. Females are shown along the top row, and males along the bottom.

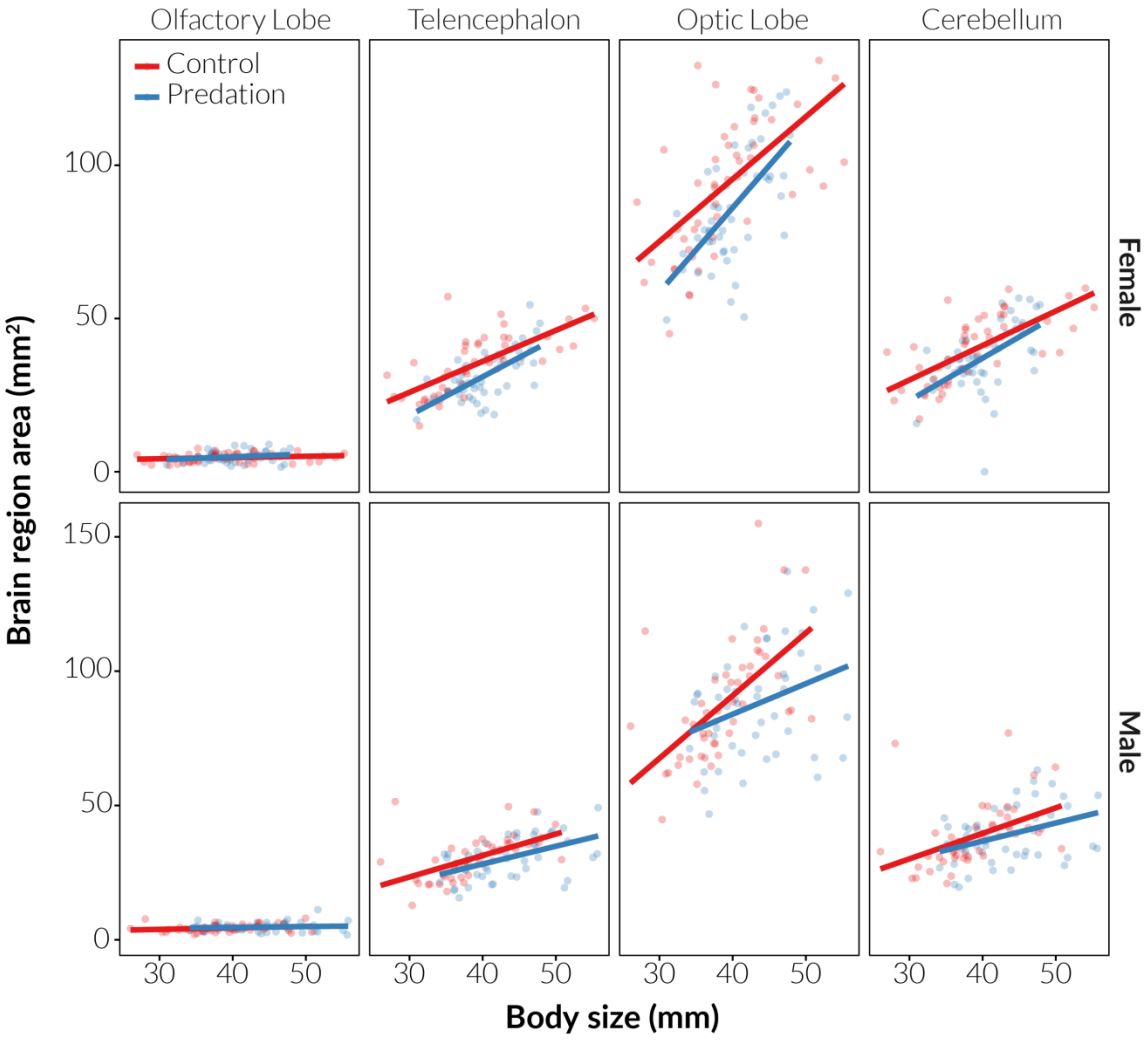

Figure S5 Individuals exposed to the predation pressure treatment show a decrease in telencephalon, optic lobe, and cerebellum size and no decrease in olfactory lobe size compared to individuals from the control treatment (n=196). Each point represents the body size and brain region size of a single individual, and lines represent lines of best fit. Data points and lines are color-coded according to treatment. Separate plots are provided for each sex (rows) and brain region (columns).

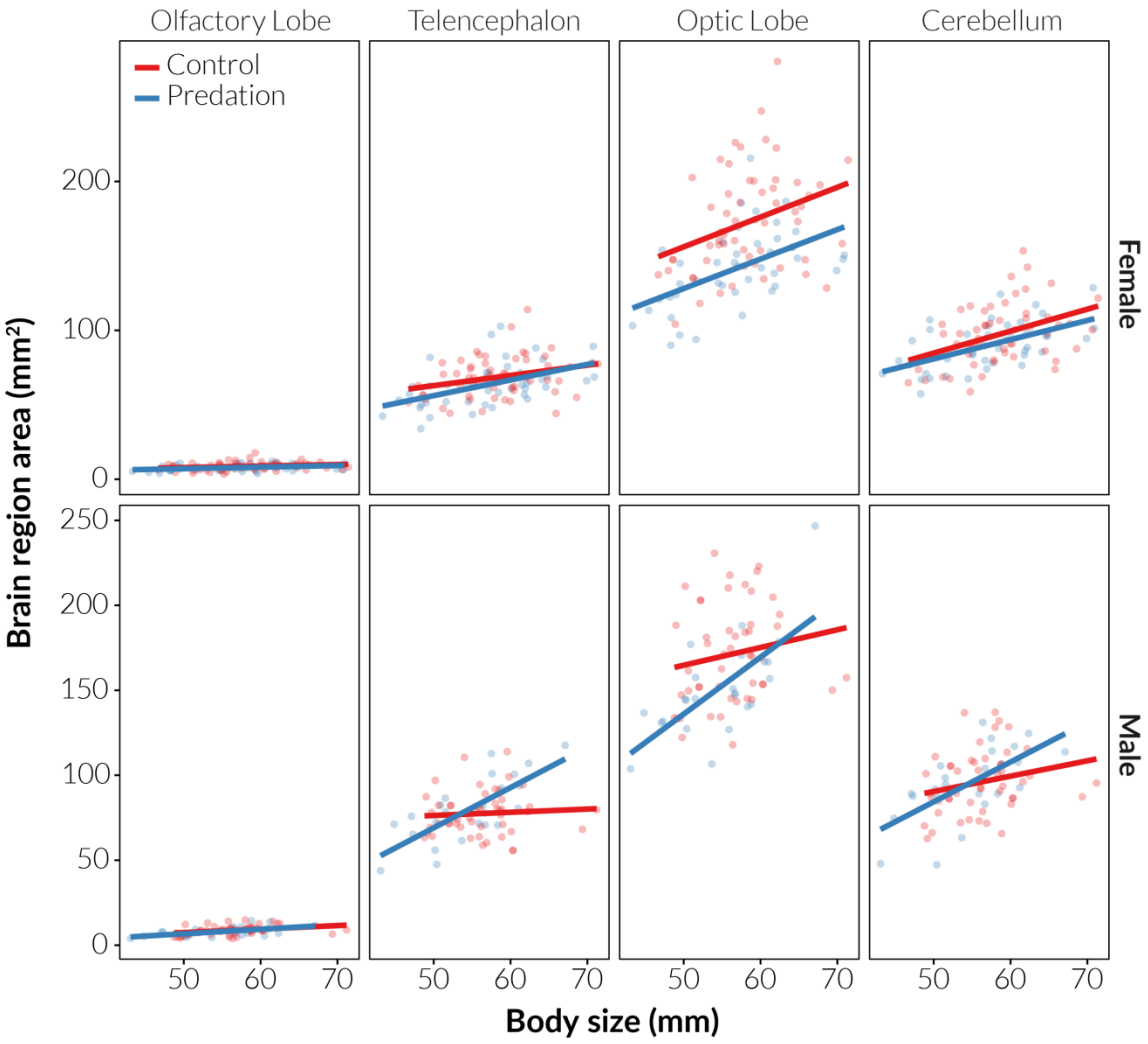

Figure S6 Common garden individuals show a decrease in optic lobe size only and no decrease in olfactory, optic lobe, or cerebellum size when compared to individuals from the control treatment with no predation exposure (n=183). Each point represents the body size and brain region size of a single individual, and lines represent lines of best fit. Data points and lines are color-coded according to treatment. Separate plots are provided for each sex (rows) and brain region (columns).

Table S1 Summary of total number of individual sticklebacks sampled in the pond and common garden experiments. Totals are partitioned by pond within cross. NP = no predation, P = predation.

Treatment	Cross ID	Pond ID	*n* Pond	*n* Common
NP	1	5	20	44
P	1	11	20	17
NP	2	14	19	-
P	2	12	19	10
NP	4	8	21	23
P	4	10	19	6
NP	6	6	19	13
P	6	7	19	31
NP	7	9	20	30
P	7	13	20	9

Table S2 Tests of allometric scaling between brain lobe size and square standard length for sticklebacks sampled from experimental ponds. Each row reports the result of an *F*-test, against a null hypothesis of a slope of 1 (isometry) along with slopes for each region, sex and treatment are provided (rightmost columns), along with 95% confidence intervals.

		F-test (H0: slope = 1)		Treatment-specific slope (95% CIs)	
Region	Sex	Statistic	*p*-value	Control	Predation
Olfactory lobe	M	$F_{1,92} = 1.379$	0.604	0.849 (0.643-1.121)	1.081 (0.804-1.453)
	F	$F_{1,98} = 9.662$	0.003	0.785 (0.598-1.032)	1.541 (1.159-2.049)
Telencephalon	M	$F_{1,92} = 0.388$	0.904	0.938 (0.765-1.151)	0.984 (0.758-1.278)
	F	$F_{1,98} = 2.4$	0.128	0.821 (0.677-0.996)	1.19 (0.95-1.49)
Optic lobe	M	$F_{1,92} = 1.649$	0.365	0.873 (0.706-1.079)	0.879 (0.664-1.164)
	F	$F_{1,98} = 0.228$	0.635	0.727 (0.588-0.899)	1.057 (0.838-1.335)
Cerebellum	M	$F_{1,92} = 0.17$	0.812	0.956 (0.769-1.189)	1.034 (0.785-1.361)
	F	$F_{1,97} = 3.336$	0.074	0.827 (0.679-1.009)	1.242 (0.979-1.576)
Total size	M	$F_{1,92} = 1.345$	0.542	0.887 (0.721-1.091)	0.918 (0.697-1.21)
	F	$F_{1,98} = 0.767$	0.386	0.76 (0.621-0.931)	1.106 (0.879-1.391)