

Interspecific and intraspecific comparisons reveal the importance of evolutionary context in sunfish brain form divergence

Caleb J. Axelrod  | Frédéric Laberge | Beren W. Robinson

Department of Integrative Biology,
University of Guelph, Guelph, ON, Canada

Correspondence

Caleb J. Axelrod, Department of Integrative
Biology, University of Guelph, Guelph, ON
N1G 2W1, Canada.
Email: caxelrod@uoguelph.ca

Funding information

Canadian Network for Research and
Innovation in Machining Technology, Natural
Sciences and Engineering Research Council
of Canada, Grant/Award Number: RGPIN-
2019-04710 and RGPIN-2014-06383

Abstract

Habitats can select for specialized phenotypic characteristics in animals. However, the consistency of evolutionary responses to particular environmental conditions remains difficult to predict. One trait of great ecological importance is brain form, which is expected to vary between habitats that differ in their cognitive requirements. Here, we compared divergence in brain form and oral jaw size across a common littoral–pelagic ecological axis in two sunfishes at both the intraspecific and interspecific levels. Brain form differed between habitats at every level of comparison; however, divergence was inconsistent, despite consistent differences in oral jaw size. Pumpkinseed and bluegill species differed in cerebellum, optic tectum and olfactory bulb size. These differences are consistent with a historical ecological divergence because they did not manifest between littoral and pelagic ecotypes within either species, suggesting constraints on changes to these regions over short evolutionary time scales. There were also differences in brain form between conspecific ecotypes, but they were inconsistent between species. Littoral pumpkinseed had larger brains than their pelagic counterpart, and littoral bluegill had smaller telencephalons than their pelagic counterpart. Inconsistent brain form divergence between conspecific ecotypes of pumpkinseed and bluegill sharing a common littoral–pelagic habitat axis suggests that contemporary ecological conditions and historic evolutionary context interact to influence evolutionary changes in brain form in fishes.

KEYWORDS

adaptive divergence, brain morphology, cognition, evolutionary constraint, parallel evolution, sunfish

1 | INTRODUCTION

Environments that vary tremendously in ecological characteristics should cause trait specialization by animals when selection favours particular morphological and behavioural characteristics that optimize performance. This should be particularly true for traits

that directly regulate the interaction between an animal and its environment. What remains unclear is the degree to which particular habitat types consistently select for the same evolutionary outcomes. When considering populations diverging between a replicated set of alternate ecological conditions, we expect parallel patterns of trait divergence between divergent groups, in the

absence of opposing effects (Losos, 1992; Schluter & Nagel, 1995; Stuart et al., 2017).

However, other factors can also influence trait responses to identical ecological pressures, including intrinsic population genetic factors and the local fitness landscape. Characteristics of population genetic structure, such as limited phenotypic or heritable variation, or tight trait covariance relationships, can limit local adaptation over the short evolutionary term (Bolstad et al., 2015; Conner et al., 2011; Schwenk, 1995). One of several different possible adaptations may also evolve in response to identical ecological pressures when multiple phenotypic optima exist and populations differ slightly in standing phenotypic variation (Whitlock et al., 1995; Wright, 1932). For example, Blows et al. (2003) demonstrated three fitness peaks in colour ornamentation in male guppies, suggesting that multiple effective ways exist for male guppies to attract females. Hence, the occurrence of multiple optimal phenotypes in a particular environment can make it difficult to predict the evolutionary response to that environment based on knowledge of ecological conditions alone. In addition, the evolutionary history of a population can also influence a population's propensity to evolve a particular phenotype.

As the evolutionary response of a population to selection is regulated by the geometry of trait variance and covariance, that geometry can differ among lineages because of historic differences in selection, drift and subsequent trait integration (Haber, 2015). One result is that the covariation of traits can vary between species. Consequently, these historic influences on trait variance and covariance in a population shape evolutionary responses to contemporary selection (Montgomery et al., 2016). For example, the evolutionary capacity of a population of ecological generalists may be more labile than that of an ecologically specialized population if the latter has adaptively evolved functionally codependent traits that contribute to phenotypic integration (Monteiro & Nogueira, 2010) or enhanced phenotypic modularity (Espinosa-Sota & Wagner, 2010). In the absence of a detailed understanding of species' trait variance and covariance, it is difficult to predict short-term responses to selection.

One trait that is ideally suited to testing evolutionary responses to similar ecological conditions is brain form. We use 'brain form' as a general term that encompasses variation in both brain size and morphology. Brain size and morphology (i.e. proportional relationships among brain regions) are important traits that shape cognitive ability and behaviour and have been linked to variation in ecological performance. Cognitive tests (learning and/or problem-solving tests) suggest functional links between relative brain size and cognitive ability (Benson-Amram et al., 2016; Buechel et al., 2018; Kotrschal et al., 2013; MacLean et al., 2014), thought to be the result of greater numbers of neurons and neuronal connections in larger brains (Herculano-Houzel & Lent, 2005; Marhounová et al., 2019). Here, we define cognition broadly as all information processing done by the central nervous system, including processing of sensory, motor and higher integrative functions of the brain. Furthermore, brain size is often related to habitat use, both interspecifically (Fischer et al., 2015; Kruska, 1988; Lecchini et al., 2014; Shumway, 2008) and intraspecifically (Ahmed et al., 2017; Axelrod et al., 2018; Evans

et al., 2013; Gonda et al., 2009; Walsh et al., 2016), with larger brains generally associated with habitats that are expected to present greater cognitive challenges.

Relative brain region size may also functionally shape some aspects of cognition, behaviour and sensory integration in relation to environmental conditions (Kotrschal et al., 1998; Healy & Rowe, 2007; Schellart, 1991). Brain regions appear specialized for particular cognitive or sensory functions (summarized in Table 1). Like relative brain size, relative brain region size has been associated with particular habitats that appear to require specific cognitive abilities (Gonzalez-Voyer & Kolm, 2010; Kruska, 1988; Lecchini et al., 2014; Shumway, 2008; White & Brown, 2014). Brain tissue is understood to be particularly energetically costly (Aiello & Wheeler, 1995; Isler & van Schaik, 2006, 2009; Kotrschal et al., 2013; Navarette et al., 2011; Niven & Laughlin, 2008), and so brain tissues are expected to be no larger than required by local conditions (Jerison, 1973). For example, phylogenetic comparative analysis has revealed that relaxed selection for larger brains results in the evolution of smaller brains in bats (Safi et al., 2005).

Replicated or consistent relationships between habitat and brain form are commonly used to infer brain size and region functions, and these patterns can be evaluated using interspecific or intraspecific comparisons. Each approach has its strengths and limitations (Gonda et al., 2013). Interspecific comparisons may be limited by unknown but important historic evolutionary differences between species that constrain contemporary evolutionary responses to selection in unexpected ways. Additionally, interspecific comparisons would likely not reveal within-generation plastic effects on brain form that could be observed using within-species comparisons. Experimental tests have revealed the potential for plasticity to be an important mechanism that influences diversity in brain form (Crispo & Chapman, 2010; Eifert et al., 2015; Gonda et al., 2009, 2012). Intraspecific comparisons avoid these challenges but may be limited because divergent ecotypes may not reflect evolved responses, or reliably signal long-term evolutionary responses. Combining both approaches may be an effective way to test the consistency of trait responses to environmental conditions over a wider span of evolutionary scale in order to indirectly test for adaptive evolution and elucidate potential mechanisms that could limit adaptive responses (Foster, 1998; Hall & Tropepe, 2020; Riopel et al., 2008). A combined approach requires a shared divergence in ecological niche use by species and by ecotypes nested within species, and subsequently focuses on how evolutionary history in the form of species' effects may influence contemporary responses to selection represented by replicated ecotype differences.

A hierarchical comparative approach, employing both within-species and between-species comparisons, along a consistent axis of ecological divergence can also generate insights about mechanisms that cause different trait states under similar ecological conditions. Mechanistic hypotheses about factors that influence trait change can be developed, and to a limited extent tested, using a hierarchical comparative approach because they generate different predictions for groups that share a common axis of ecological divergence

TABLE 1 Fish brain region, function and their potential ecological relevance

Brain region	Function	Ecological relevance	Reference
Cerebellum	Motor coordination	Foraging	Huber et al. (1997)
		Predator avoidance	Pollen et al. (2007)
			Gonzalez-Voyer and Kolm (2010)
Optic tectum	Vision, orientation	Foraging	Huber et al. (1997)
		Predator detection/avoidance	Pollen et al. (2007)
Olfactory bulb Telencephalon	Olfaction	Foraging	Huber et al. (1997)
		Predator detection	Pollen et al. (2007)
		Laberge and Hara (2001)	
	Navigation, learning, sensory integration	Habitat navigation	Pollen et al. (2007)
		Foraging	Gonzalez-Voyer and Kolm (2010)
	Predator avoidance	Park and Bell (2010)	
		Costa et al. (2011)	
Hypothalamus	Social behaviour, endocrine control	Mating	Pollen et al. (2007)
		Social interactions/competition	Gonzalez-Voyer and Kolm (2010)

but differ in the historical timing of divergence, as we expect different constraints to act over different timescales. Additionally, by including multiple within-species comparisons we can evaluate the possibility of multiple adaptive peaks, as these could result in different patterns of phenotypic divergence among ecotypes of different species exposed to similar ecological pressures (Peiman & Robinson, 2017).

This approach has the potential to resolve some aspects of a long-standing uncertainty about the evolutionary lability of brain size and morphology. At issue is whether distinct brain region sizes can evolve independently (often referred to as 'mosaic' brain form evolution), or whether evolutionary change in brain regions is constrained and brain size evolves through 'concerted' change among brain regions (Montgomery et al., 2016; Striedter, 2005). Previous work across animal taxa has found support for both these patterns. Brain region size in primates and bats is predicted by whole brain size (Finlay & Darlington, 1995; Finlay et al., 2001), suggesting concerted evolution of brain regions. A concerted pattern of brain morphology variation has also been observed in lizards (Powell & Leal, 2014) and fish (Axelrod et al., 2018). Contrary to these findings, primates and insectivorous mammals have also been shown to exhibit mosaic change in the size of brain regions (Barton & Harvey, 2000). A mosaic pattern of brain evolution has also been inferred in birds (Iwaniuk et al., 2004), cichlids (Gonzalez-Voyer & Kolm, 2010) and stickleback (Noreikiene et al., 2015). Finally, many studies have found results that support a combination of concerted and mosaic patterns of brain region evolution, including in fish (Gonzalez-Voyer et al., 2009; Sukhum et al., 2018), lizards (Hoops et al., 2017), amphibians (Liao et al., 2015) and birds (Gutiérrez-Ibáñez et al., 2014;

Moore & DeVoogt, 2017). Progress in resolving uncertainty over the degree of constraint in brain region evolution has stagnated perhaps because of a phenomenological focus on pattern in brain morphology evolution that is poorly connected to mechanistic processes. Identifying and then testing specific mechanisms of brain change should facilitate our understanding of how brains evolve and perhaps resolve some inconsistencies in the literature. Here, we demonstrate how a hierarchical comparative approach can be used to evaluate the effects of some of these mechanisms, focusing specifically on the potential of historical constraint, variation in plastic responses and multiple local adaptive peaks to influence variation in brain form.

We evaluated the consistency of the effects of littoral and pelagic lake habitats on brain form between and within species of two congeneric freshwater Centrarchid sunfish species, pumpkinseed (*Lepomis gibbosus*) and bluegill (*L. macrochirus*). Pumpkinseed and bluegill are North American freshwater ray-finned fish that diverged approximately 15 mya (Near et al., 2005). Typically, pumpkinseed and bluegill are ecologically specialized for the littoral and pelagic habitat, respectively (Osenberg et al., 1992; Robinson & Wilson, 1994; Werner & Gilliam, 1984). Pumpkinseed have larger heads, fewer, shorter and more widely spaced gill rakers, larger oral jaws and more robust pharyngeal jaws than bluegill, morphological specializations that enhance feeding on large macroinvertebrates. Conversely, bluegill morphological specializations enhance foraging on small zooplankton present in both habitats but dominating in the pelagic habitat (reviewed in Robinson et al., 1993). These species are ideal for evaluating the consistency of habitat effects on brain form because postglacial lake populations of each species

can also be composed of coexisting ecotypes that have diverged along the same littoral–pelagic axis over the last 12,000 years, forming a nested evolutionary hierarchy of replicated ecological divergence (Figure 1) (Chipps et al., 2004; Ehlinger & Wilson, 1988; Ehlinger, 1990; Gillespie & Fox, 2003; Jastrebski & Robinson, 2004; Robinson et al., 1993; Robinson et al., 2000; Weese et al., 2012; Wilson et al., 1996). Inhabiting these different lake habitats may demand different cognitive requirements of sunfish ecotypes because the brains of littoral pumpkinseed are 8.3% larger than in pelagic individuals, without differences between the sexes or in brain region morphology (Axelrod et al., 2018). The importance of habitat-specific foraging requirements in shaping brain size is further supported by a positive relationship between oral jaw size and brain size in pumpkinseed within each habitat (Axelrod et al., 2018). Although foraging morphology and tactics differ between littoral and pelagic ecotypes of bluegill (e.g. Ehlinger & Wilson, 1988), no data on brain form are currently available in this species.

Our comparative investigation involves a nested hierarchy of replicated divergence between littoral and pelagic habitats of inland lakes that allows us to test the consistency of the relationship between brain form and habitat use in these sunfishes. A consistent relationship between habitat and brain form within and among species would be strong evidence that habitats have particular cognitive requirements that favour adaptive brain form evolution. The complete absence of divergence in brain form between conspecific ecotypes and between species would serve as strong evidence that pelagic and littoral habitats do not differ in their eco-cognitive requirements. Inconsistent differences in brain form between ecotypes of pumpkinseeds and ecotypes of bluegills would suggest either multiple successful cognitive performance strategies or that the evolutionary histories of the species affect contemporary phenotypic responses to habitat divergence. Differences in brain form between species that do not occur between conspecific ecotypes would suggest that evolutionary history constrains contemporary changes in brain form.

2 | METHODS

2.1 | Sample collection

Pumpkinseeds were collected in August of 2016 and 2017 from Ashby Lake, Ontario (45.092N, 77.351W), via angling from four littoral and four pelagic sites in early August of 2016 and 2017 ($N = 131$), euthanized using an overdose of clove oil (100 ppm) and preserved in 10% buffered formalin. Analysis of the pumpkinseed ecotypes in isolation was published in Axelrod et al. (2018). Bluegills were collected in July of 2017 from Holcomb Lake, Michigan (42.507N, 85.434W) ($N = 97$). The small size of Holcomb Lake limited collection to one littoral and one pelagic site, but otherwise sampling procedures were identical to that for pumpkinseed. These lake populations were chosen as they both exhibit within-species ecotype divergence (pumpkinseed, Jastrebski & Robinson, 2004; bluegill, Ehlinger & Wilson, 1988; Wilson et al., 1996). Sampling was performed in accordance with animal use and welfare protocols administered by the animal care committee of the University of Guelph under the guidelines set by the Canadian Council on Animal Care.

2.2 | Sample processing

All individuals were given uninformative labels, so as to conceal identity during processing. Pumpkinseed were processed in 2017 and bluegill in early 2019, so species identity was known during processing. Though processed at different times, samples from both species were held in formalin for less than 2 years, so shrinkage of brain tissue should not generate confounding results. Fish were blotted to remove excess formalin and weighed, and their standard length and oral jaw width were measured using digital callipers. The oral jaw width was measured as the maximum distance between the maxillaries and standard length as the distance between the tip of the nose and the end of the caudal peduncle.

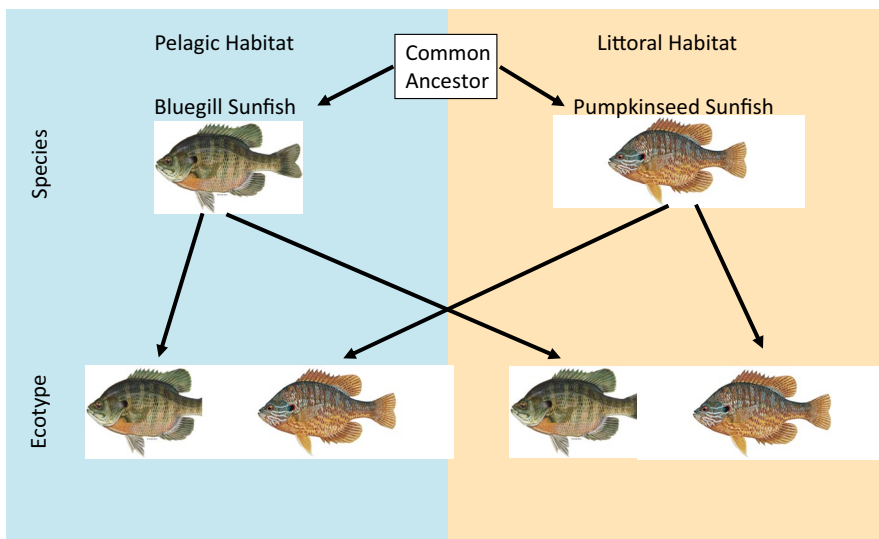


FIGURE 1 Diagram representing the hierarchical pattern of replicated ecological divergence of pumpkinseed (*Lepomis Gibbosus*) and bluegill sunfish (*L. macrochirus*) and their respective ecotypes between littoral (orange) and pelagic (blue) lake habitats. The two species diverged approximately 15 million years ago (Near et al., 2005), whereas ecotypes within each species diverged in postglacial lakes that formed following the Wisconsin glaciation approximately 12,000 years ago (Weese et al., 2012)

All fish were aged by counting annuli on a sample of at least five scales per individual. Brains were removed via dissection, and excess cranial nerves were trimmed. The spinal cord of all brains was cut at the level of the obex to ensure consistency in comparisons. Dissection damaged the brains of 16 pumpkinseed and three bluegill individuals and so these were excluded from analyses, leaving 113 pumpkinseed and 94 bluegill.

Brain mass and brain region volumes were used as estimates of total and regional brain size, respectively. Following removal, the region size and weight of brains were measured. To measure region size, brains were digitally photographed from dorsal, ventral and lateral orientations using a Canon Powershot G10 camera connected to an Olympus SZ61 dissection microscope. Brains were oriented for imaging using a sculpted well of Styrofoam, and consistent lateral orientation was achieved by ensuring that both sizes of bilaterally symmetrical regions were vertically aligned. The linear length, width and depth of each region (cerebellum, optic tectum, telencephalon, olfactory bulbs, and hypothalamus), including both lobes of bilaterally symmetrical regions, were measured using the NeuroLucida (MBF Bioscience) quick line measurement function, and the volume of each region was estimated as $V = (L \times W \times H)\pi/6$ (Ullmann et al., 2010; White & Brown, 2015). Only one lateral view of the brain was photographed, and so the depth of each lobe of bilaterally symmetrical regions was assumed to be the same. Blotted brain weight was measured using an Accu-124D scale (Fisher Scientific) at a resolution of 0.0001 g.

2.3 | Statistical methods

The consistency of the relationship between brain form and habitat was evaluated by comparing traits among four groups of sunfish that encompass two within-species ecotype comparisons as well as one species-level comparison (sites within each habitat were combined for pumpkinseed). We compared oral jaw width, a morphological proxy of habitat use (Axelrod et al., 2018, 2020; Jarvis et al., 2017), between species and ecotypes in order to evaluate their historical and contemporary patterns of ecological divergence. We then tested for species and ecotype effects on brain mass and brain region volume. We use general linear models that included species (pumpkinseed vs. bluegill) and ecotype (littoral vs. pelagic) as main effects (species-ecotype models). Oral jaw width and brain mass models included a body length covariate to account for trait allometry. Body length was preferred over body mass because length is less affected by short-term changes in body condition. Separate species-ecotype models analysed variation in each of the five brain regions and included brain mass as a covariate because we focus on the contribution of each region to the whole brain. All two-way interactions were also included in these models.

The interactions between each main effect and either body length or brain mass (depending on the species-ecotype model above) we used to evaluate possible allometric differences between species and between ecotypes. Interaction between the

species and ecotype main effects permitted a preliminary test of the degree to which ecotypes diverged in a similar fashion between habitats. However, we are cautious of an inference of parallel ecotypic divergence solely on the basis of a nonsignificant species by ecotype interaction because this test treats consistent ecotype divergence as the null hypothesis. By focusing on limiting an erroneous conclusion when groups differ by chance alone in order to avoid a type-1 error, this test is biased towards finding evidence of consistent ecotype divergence between habitats. To avoid an inference biased towards concluding parallel ecotypic divergence, we compared any differences between conspecific littoral and pelagic ecotypes for the two species using an additional linear model including either standard length or brain mass as a scaling covariate and a grouping factor that separated each species-ecotype combination (PSLittoral, PSPelagic, BGLittoral, BGPelagic), followed by Tukey HSD pairwise tests among these four groupings (multcomp function in R). Here, the consistency of any differences between littoral and pelagic ecotypes can be directly compared between the two species. We infer parallel patterns of brain form divergence only when conspecific ecotypes diverge in the same way for the two species and in concert with a nonsignificant species and ecotype interaction effect in the species-ecotype models above.

The prediction that brain form divergence between species is consistent with brain form divergence within species predicts significant species and ecotype main effects in our species-ecotype models, acting in the same direction of trait change across habitats. Specifically, this means that species differences in brain form should differ consistently for ecotypes that share the same habitat, meaning that littoral pumpkinseed should differ from littoral bluegill in brain form, and pelagic pumpkinseed should differ from pelagic bluegill in a similar way. We use the Tukey HSD test on the second four-group model described above to additionally test this prediction. As above, we infer similar species and ecotype differences only when the results of both models align with our predictions.

Oral jaw width, brain mass, brain region size and standard length were all natural-log transformed before the analyses to improve linearity and residual normality. All statistical analyses were conducted using the R program for statistical computing (version 3.5.2), and the statistical threshold was set at 0.05.

3 | RESULTS

We found evidence that divergence in oral jaw width, a key feeding-related morphological trait functionally related to consumption of macroinvertebrate prey primarily in the littoral habitat, was hierarchically replicated here, consistent with adaptive divergence processes. Pumpkinseed sunfish, typically a littoral habitat specialist, have larger oral jaws than bluegill who are more specialized for the pelagic habitat. Sunfish ecotypes within species consistently expressed a similar divergence between lake habitats, with littoral ecotypes having larger oral jaws than conspecific pelagic

TABLE 2 Summary of species-ecotype general linear models predicting oral jaw width, brain mass and five brain region volumes

Response variable	Predictor variable	$F_{1,200}$	p
Oral jaw width	SL	662.33	<.0001
	Species	490.01	<.0001
	Ecotype	51.90	<.0001
	SL × Species	1.54	.22
	SL × Ecotype	6.44	.012
	Species × Ecotype	1.88	.17
Brain mass	SL	2,371.83	<.0001
	Species	13.91	.0003
	Ecotype	49.86	<.0001
	SL × Species	0.63	.43
	SL × Ecotype	18.14	<.0001
	Species × Ecotype	2.64	.11
Cerebellum volume	BM	404.89	<.0001
	Species	143.30	<.0001
	Ecotype	2.14	.15
	BM × Species	0.27	.60
	BM × Ecotype	1.24	.27
	Species × Ecotype	0.21	.65
Optic tectum volume	BM	2,811.16	<.0001
	Species	40.36	<.0001
	Ecotype	0.31	.58
	BM × Species	0.012	.91
	BM × Ecotype	0.051	.82
	Species × Ecotype	0.44	.51
Telencephalon volume	BM	1,588.68	<.0001
	Species	23.72	<.0001
	Ecotype	13.36	.00033
	BM × Species	0.98	.32
	BM × Ecotype	6.48	.012
	Species × Ecotype	4.10	.044
Olfactory bulb volume	BM	8.10	.0049
	Species	217.90	<.0001
	Ecotype	0.006	.94
	BM × Species	0.73	.40
	BM × Ecotype	0.43	.51
	Species × Ecotype	0.095	.76
Hypothalamus volume	BM	742.03	<.0001
	Species	1.38	.24
	Ecotype	0.28	.60
	BM × Species	0.47	.50
	BM × Ecotype	0.023	.88
	Species × Ecotype	1.42	.24

Note: The p -values for significant effects are bolded. F subscript reflects numerator and denominator degrees of freedom.

Abbreviations: BM, brain mass covariates; SL, standard length.

ecotypes (Tables 2 and 3, Figure 2), although statistical evidence of this ecotype difference in bluegill was weaker (pelagic–littoral estimate \pm SE = -0.037 ± 0.015 , $t = -2.4$, 2-sided $p = .076$).

Unlike oral jaws, divergence of brain form traits was not hierarchically replicated in these sunfish. Variation arose between species that was not apparent between ecotypes. Volumes of cerebellum, optic tectum and olfactory bulb adjusted for standard length all differed between bluegill and pumpkinseed species, but not between ecotypes within these species. Cerebellum and olfactory bulb volumes were greater in pumpkinseed (Tables 2 and 3, Figure 4a,d), and optic tectum volume was greater in the bluegill. Hypothalamus volume consistently did not differ between species or ecotypes (Tables 2 and 3, Figure 4e).

Furthermore, divergent brain form between conspecific ecotypes was not replicated in the two sunfish species. Pumpkinseed ecotypes differed in relative brain mass, whereas bluegill ecotypes differed in relative telencephalon volume. Species and habitat both predicted whole brain mass after accounting for standard length, and there was no evidence of an interaction between species and ecotype (Table 2), potentially suggesting a consistent change in brain mass between species and ecotypes within species in response to habitat. However, post hoc comparisons from the model that combined species and ecotype into a single factor revealed that brain mass was greater in littoral compared with pelagic ecotypes in pumpkinseed, but not between ecotypes in bluegill (Table 3, Figure 3), indicating that brain mass of ecotypes did not consistently diverged ecotypes of the two species. The effect of standard length on brain mass was also influenced by ecotype in this model (standard length–ecotype interaction, Table 2), suggesting that smaller sunfish differ more in brain size between ecotypes than larger sunfish. However, we interpret this interaction effect as a statistical artefact resulting from pumpkinseed being generally smaller than bluegill (mean and standard error of standard length pumpkinseed = $88.9 \text{ mm} \pm 13.5 \text{ mm}$; bluegill = $117.4 \text{ mm} \pm 19.3 \text{ mm}$) in combination with difference in brain mass between pumpkinseed but not between bluegill ecotypes. A further post hoc analysis of covariance applied separately to each species allowed us to directly test for differences in brain size allometry between ecotypes while avoiding any effects of size differences between species. We found no significant interaction effect between standard length and ecotype on brain mass in either species (Table S1), supporting our interpretation above that body size does not influence variation in brain size.

With respect to brain region size, our analyses also suggest inconsistent within-species habitat effects on telencephalon volume. Telencephalon volume was influenced by species, ecotype and their interaction in our species-ecotype model (Table 2), the latter suggesting that differences between ecotypes were inconsistent between these two species. Post hoc analysis of ecotype groups revealed that littoral bluegill had smaller telencephalons than pelagic bluegill, but no such difference existed between the pumpkinseed ecotypes (Table 3, Figure 4c). Like brain mass above, a significant brain mass by ecotype interaction effect on telencephalon volume likely resulted from the larger body size of bluegill relative to pumpkinseed, and the difference in telencephalon volume between

TABLE 3 Summary of Tukey HSD post hoc tests resulting from ANCOVA models considering four species-ecotype groups

Response variable	Species and ecotype comparison	Estimate ± SE	t	p
Adjusted jaw width	BGP-BGL	-0.037 ± 0.015	-2.4	.076
	PSL-BGL	0.32 ± 0.015	21.7	<.0001
	PSP-BGL	0.22 ± 0.014	15.5	<.0001
	PSL-BGP	0.36 ± 0.018	19.4	<.0001
	PSP-BGP	0.26 ± 0.018	14.8	<.0001
	PSP-PSL	-0.096 ± 0.013	-7.6	<.0001
Adjusted brain mass	BGP-BGL	-0.023 ± 0.016	-1.5	.46
	PSL-BGL	0.099 ± 0.015	6.6	<.0001
	PSP-BGL	-0.0065 ± 0.015	-0.44	.97
	PSL-BGP	0.12 ± 0.019	6.4	<.0001
	PSP-BGP	0.016 ± 0.018	0.90	.80
	PSP-PSL	-1.1 ± 0.013	-8.1	<.0001
Adjusted cerebellum volume	BGP-BGL	0.017 ± 0.030	0.57	.94
	PSL-BGL	0.27 ± 0.028	9.6	<.0001
	PSP-BGL	0.30 ± 0.029	10.4	<.0001
	PSL-BGP	0.25 ± 0.032	7.8	<.0001
	PSP-BGP	0.29 ± 0.034	8.4	<.0001
	PSP-PSL	0.036 ± 0.026	1.4	.49
Adjusted optic tectum volume	BGP-BGL	-0.0008 ± 0.015	-0.052	.99
	PSL-BGL	-0.071 ± 0.014	-5.1	<.0001
	PSP-BGL	-0.080 ± 0.015	-5.4	<.0001
	PSL-BGP	-0.070 ± 0.016	-4.3	.00014
	PSP-BGP	-0.079 ± 0.017	-4.6	<.0001
	PSP-PSL	-0.0089 ± 0.013	-0.68	.90
Adjusted telencephalon volume	BGP-BGL	0.10 ± 0.021	4.9	<.0001
	PSL-BGL	0.11 ± 0.019	5.7	<.0001
	PSP-BGL	0.12 ± 0.020	5.9	<.0001
	PSL-BGP	0.0054 ± 0.022	0.29	.99
	PSP-BGP	0.016 ± 0.024	0.66	.91
	PSP-PSL	0.0093 ± 0.018	0.52	.95
Adjusted olfactory bulb volume	BGP-BGL	-0.013 ± 0.05	-0.26	.99
	PSL-BGL	0.56 ± 0.046	12.1	<.0001
	PSP-BGL	0.57 ± 0.048	11.8	<.0001
	PSL-BGP	0.57 ± 0.054	10.7	<.0001
	PSP-BGP	0.58 ± 0.057	10.3	<.0001
	PSP-PSL	0.014 ± 0.043	0.32	.99
Adjusted hypothalamus volume	BGP-BGL	-0.0046 ± 0.032	-0.14	.99
	PSL-BGL	-0.040 ± 0.030	-1.35	.53
	PSP-BGL	-0.018 ± 0.031	-0.58	.94
	PSL-BGP	-0.036 ± 0.035	-1.03	.73
	PSP-BGP	-0.013 ± 0.037	-0.36	.98
	PSP-PSL	0.022 ± 0.028	0.81	.85

Note: The p-values for significant effects are bolded. Estimates indicate the direction of effect of the first minus the second listed comparison.

Abbreviations: BGL, bluegill-littoral; BGP, bluegill-pelagic; PSL, pumpkinseed-littoral; PSP, pumpkinseed-pelagic.

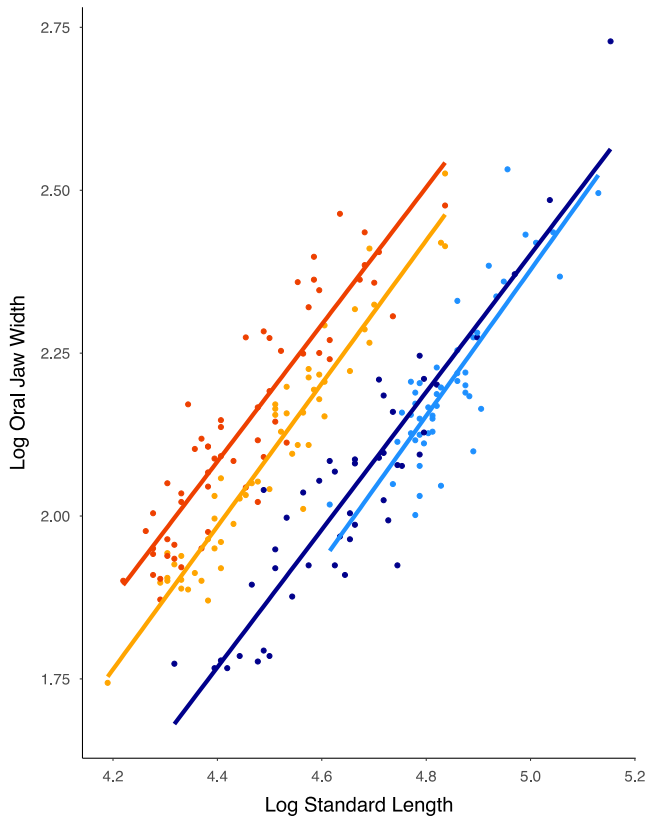


FIGURE 2 Relationships between natural-log oral jaw width (mm) and natural-log standard length (mm) of pumpkinseed sunfish from Ashby Lake (orange) and bluegill sunfish from Holcomb Lake (blue) collected from the littoral (dark colouration) and pelagic (light coloration) habitats in each lake

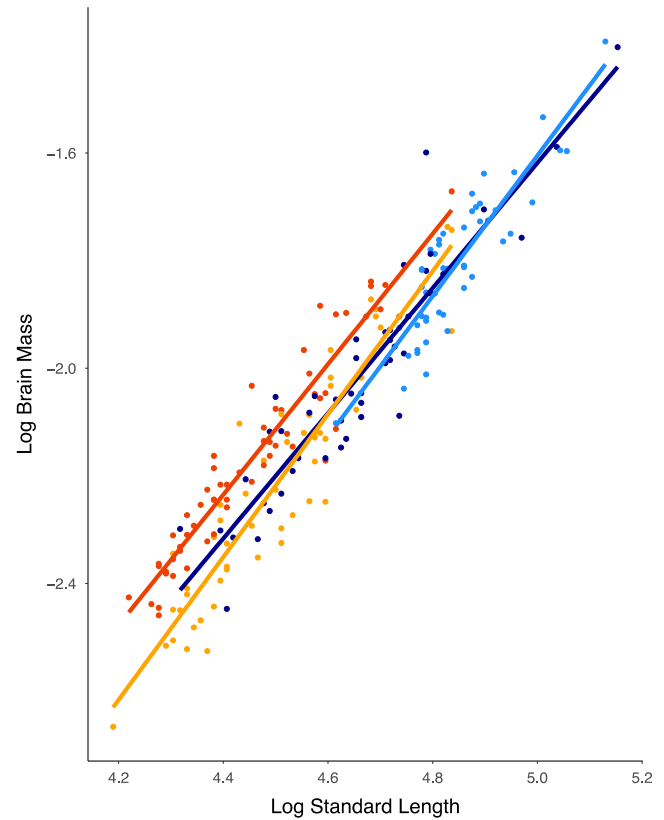


FIGURE 3 Relationships between natural-log brain mass (g) and natural-log standard length (mm) of pumpkinseed sunfish from Ashby Lake (orange) and bluegill sunfish from Holcomb Lake (blue) collected from the littoral (dark colouration) and pelagic (light coloration) habitats in each lake

ecotypes being present only within bluegill. Similar to brain mass above, this inference is supported by ANCOVA models applied separately to each species that showed that no brain regions were influenced by significant interactions between ecotype and brain mass (Table S1).

4 | DISCUSSION

Prior studies have shown effects of habitat on fish brain size and morphology. Here, we investigated the consistency of relationships between divergence in brain form and ecological divergence across the littoral–pelagic ecological axis using a hierarchy of comparisons: among ecotypes within species that are evolutionarily recent and between pumpkinseed and bluegill sunfish species that diverged in the more distant evolutionary past. First, we find strong evidence of a replicated pattern of ecological and adaptive divergence across the littoral–pelagic axis in oral jaw size, a key feeding trait. Patterns of brain form variation can now be interpreted against this hierarchically replicated pattern of ecological divergence. Second, we find habitat effects on brain form across every comparison we examine for all but one brain trait, although none of these effects were

consistently replicated among ecotypes and species. Brain size and telencephalon size diverged between ecotypes of pumpkinseed and bluegill, respectively, but neither trait diverged between conspecific ecotypes in both species. Cerebellum, optic tectum and olfactory bulb size all diverged between the two species, but not between conspecific ecotypes within species. Hypothalamus size was the only trait that did not diverge between habitats in any comparison. Our results generally support our hypothesis that habitats vary in their cognitive requirements and that this manifests in variation in brain form. The inconsistency of these effects, however, suggests that the specific evolutionary characteristics of a species, such as life history and trait variation and covariation, influence how fish brains respond to the different cognitive requirements of the littoral and pelagic habitats.

4.1 | Ecological and brain form divergence

The difference in oral jaw width between species, as well as between ecotypes within species, is strong evidence in support of a replicated pattern of ecological divergence across the littoral–pelagic axis and supports our understanding of habitat-specific foraging ecology

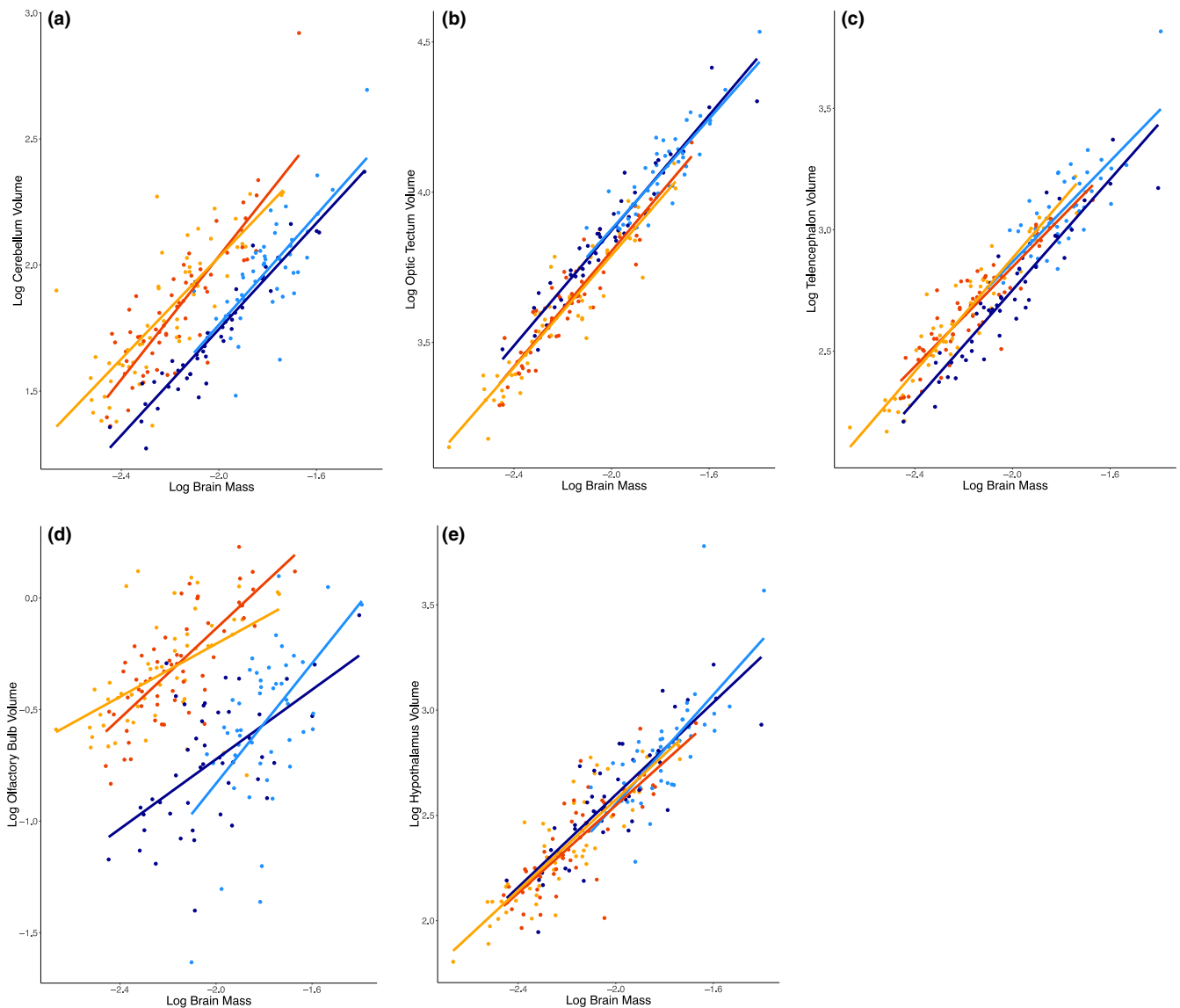


FIGURE 4 Relationship between natural-log brain region volumes (mm^3) and natural-log brain mass (g) of pumpkinseed sunfish from Ashby Lake (orange) and bluegill sunfish from Holcomb Lake (blue) collected from the littoral (dark colouration) and pelagic (light coloration) habitats in each lake. Brain region volumes are for: (a) cerebellum, (b) optic tectum, (c) telencephalon, (d) olfactory bulb and (e) hypothalamus

(outlined in Figure 1). Pumpkinseed are well adapted to foraging on larger benthic prey, including snails in the littoral habitat, whereas bluegill are better adapted to feed extensively on small zooplankton prey particularly in the pelagic lake habitat (Osenberg et al., 1992; Robinson & Wilson, 1994; Werner & Gilliam, 1984). The oral jaws of both pumpkinseed ecotypes are wider than those of either bluegill ecotype here, consistent with the known species-level ecological divergence. The oral jaws of littoral ecotypes within both species are larger than those of pelagic ecotypes, although evidence of this is weaker in bluegill. Intraspecific variation in oral jaw width has been shown to be a reliable indicator of habitat use in pumpkinseed elsewhere (Axelrod et al., 2018, 2020; Jarvis et al., 2017). These prevailing trends support our understanding that the ecological divergence between pumpkinseed and bluegill is replicated between littoral and

pelagic ecotypes within each species and demonstrate the usefulness of our hierarchical comparison in showing replicated trait divergence across habitats.

Ecological divergence across the littoral–pelagic axis was associated with significant changes in brain form at all levels of comparison, suggesting that habitats likely differ in their eco-cognitive requirements and that this manifests in brain form responses. However, unlike oral jaw width above, the patterns of divergence were inconsistent, and so we have to reject the idea of a simple or single consistent effect of habitat on brain form in these sunfish. Littoral and pelagic diversification was associated with brain form variation between ecotypes within both species, as well as between species; however, ecotype diversification was not the same across species. In pumpkinseed, the whole brain size (adjusted for body size) is larger

in littoral than in pelagic individuals (Axelrod et al., 2018), suggesting that conditions in the littoral habitat are more broadly cognitively challenging. The larger relative telencephalon size in pelagic relative to littoral bluegill suggests that sensory integration, navigation and spatial learning (Table 1) may influence performance in the pelagic habitat. Pelagic foraging is sometimes associated with a larger telencephalon in fish (Gonda et al., 2011; Park & Bell, 2010; Wilson & McLaughlin, 2010), perhaps because of the cognitive requirements of navigating a large volume and deep, three-dimensional habitat. Alternatively, pelagic bluegill may require greater spatial learning and memory because they have to move between the pelagic habitat where they primarily feed and the littoral habitat where they spawn (Colgan et al., 1979). Movement between habitats may also increase the importance of other aspects of cognitive ability such as behavioural flexibility (Healy & Rowe, 2007), which may be related to telencephalon size.

Differences between bluegill and pumpkinseed species in optic tectum, olfactory bulb and cerebellum size reveal a more historic divergence in aspects of brain performance related to the littoral–pelagic divergence than found between conspecific ecotypes. For example, foraging for small zooplankton prey in the pelagic habitat requires more visual acuity, which involves the optic tectum where visual information is processed and integrated (Table 1). Bluegill feeding on small pelagic zooplankton prey may have favoured the evolution of larger optic tecta. Pumpkinseed feeding on cryptic macroinvertebrate prey such as snails and insect larvae in the littoral habitat may use olfaction to enhance finding prey, and so favour the evolution of larger olfactory bulbs. The larger relative cerebellum size in pumpkinseed suggests that performance in the littoral habitat may require greater motor control and function (Table 1). There is precedent for such a relationship since Pollen et al. (2007) and Gonzalez-Voyer and Kolm (2010) found that greater habitat complexity was associated with larger cerebellum size in cichlids. Movement in a more structurally complex littoral habitat might have favoured greater cerebellum size in pumpkinseed. The observed differences in relative brain region size between sunfish species support a general hypothesis of differences in specific cognitive requirements between littoral and pelagic habitats.

4.2 | Mechanisms of brain form variation

Three mechanisms can generate brain form variation between ecotypes in the different habitats; habitat choice, phenotypic plasticity and diversifying selection. First, if individuals are able to switch habitats, then they could select habitats based on a brain phenotype that provides an optimal performance fit (Edelear et al., 2019). Second, brain size and morphology could respond to habitat conditions over an individual's lifetime if phenotypic plasticity allows phenotype to match local conditions (Eifert et al., 2015; Gonda et al., 2009; McCallum et al., 2014). Finally, selection could favour divergent optimum brain forms between habitats if the functional effects of brain form on cognitive

and ecological performance differ between habitats. Kotschal et al. (2013) showed that guppies (*Poecilia reticulata*) could be artificially selected for larger and smaller brains, demonstrating that heritable variation in brain size can evolve under selection. As such, diversifying selection could lead to evolutionary change in brain size, particularly when selection is sustained. Comparative results like ours cannot distinguish among these mechanisms (or a combination of them). Reciprocal transplant or common garden experiments, such as those performed by Walsh et al. (2016), are required to further distinguish these mechanisms.

4.3 | Implications of inconsistencies in brain form divergence

Differences in brain morphology between species that did not manifest between conspecific ecotypes suggest that species-level differences may constrain contemporary evolutionary responses to ecological divergence over the short term. We first consider two possible mechanisms of constraint that could generate this pattern: limited heritable variation and functional or genetic trait covariation. Heritable variation straightforwardly constrains evolutionary responses to selection and can only be increased slowly by mutation or more rapidly by gene flow into a population. However, rapid adaptive evolution suggests that limitations to heritable variation may be uncommon in most natural populations (Barrett & Schluter, 2007). Trait covariation constrains evolutionary responses by allowing opposing forces of selection to simultaneously act on a trait. Noreikiene et al. (2015) suggested some covariation among brain regions in fish, but few if any other studies have measured quantitative estimates of brain trait variance and covariance in fishes and so this will likely be a fruitful direction of future study. Strong sustained selection is required to break tight genetic trait covariation relationships (Bolstad et al., 2015; Conner et al., 2011). The differences in optic tectum, olfactory bulb and cerebellum size between sunfish species here did not manifest between ecotypes in either species, and so could reflect historic effects on the variance–covariance architecture of brain form in each species that limits divergence between conspecific ecotypes. A similar species effect may also account for the divergence in brain size and telencephalon size that were, respectively, unique to pumpkinseed and bluegill ecotypes.

However, at least three other mechanisms can contribute to the unique responses of bluegill and pumpkinseed ecotypes to littoral and pelagic habitats. First, large differences in life history could alter the cognitive challenges faced by each species. For example, during the summer growing and breeding season, pumpkinseed express very strong site fidelity (Jarvis et al., 2020; McCairns & Fox, 2004), and as a result forage and breed in one or the other habitat but rarely both. Bluegill ecotypes also segregate by habitat to feed (Ehlinger & Wilson, 1988), but all breed in the littoral habitat (Colgan et al., 1979). This generates different cognitive challenges related to habitat switching for pelagic but not for littoral bluegill. Furthermore, the cognitive challenges associated with

littoral foraging that favours larger brains may be offset by greater pelagic productivity and hence a relaxation of energy limitation in pelagic bluegill individuals. Brain tissue is energetically expensive to grow and maintain (Aiello & Wheeler, 1995; Isler & van Schaik, 2006, 2009; Kotrschal et al., 2013; Navarette et al., 2011; Niven & Laughlin, 2008; Safi et al., 2005) and so selection should favour smaller brains wherever energy is limited and cognitive challenges are relaxed. Greater available energy in the pelagic habitat could reduce the cost of a larger brain there because pelagic bluegill may be less energy limited than littoral individuals. In summary, life-history differences interacting with ecological differences in resource availability could shape brain size responses.

A second explanation for the differences in brain and telencephalon responses to habitat between pumpkinseed and bluegill ecotypes may reflect evolved differences in the plasticity of brain characteristics. Fish brain size is expected to have a lifelong potential for plasticity as fish maintain widespread neurogenesis through adulthood (Sorensen, et al., 2013; Zupanc, 2006). Brain size plasticity has been demonstrated experimentally in ninespine stickleback (Gonda et al., 2011) and shortfin molly (Eifert et al., 2015). Trait plasticity can evolve under selection (Scheiner, 1993; Schlichting & Pigliucci, 1998). Morphological plasticity has diverged between conspecific pumpkinseed ecotypes (Januszkiwicz & Robinson, 2007; Parsons & Robinson, 2007), and brain morphology plasticity has diverged in other fish (Crispo & Chapman, 2010; Gonda et al., 2012). For example, this may occur between conspecific ecotypes if colonization of the novel habitat favoured phenotypically plastic genotypes over more developmentally canalized types. Additionally, selection on brain form plasticity may differ between species if their life histories or habitat specializations generate differences in exposure to environmental variability. We are currently engaged in reciprocal transplant–common garden studies in order to further evaluate variation in brain form plasticity.

A final explanation for differences between species in the pattern of ecotype divergence is the presence of multiple adaptive peaks within one or the other lake habitat (Blows et al., 2003; Peiman & Robinson, 2017). The hypothesis of a consistent relationship between brain form and habitat makes a restrictive assumption that a single phenotypic optimum exists for each habitat. This need not be the case with regard to brain form. If multiple cognitive strategies provide similar functional performance in a given habitat, then local adaptation within a habitat may not be consistent, and adaptive divergence between habitats may not be replicated. Testing this explanation is difficult since it requires quantifying relationships between different brain forms and eco-cognitive performance in particular habitats and then comparing performance and fitness of individuals with different brain phenotypes under natural conditions.

4.4 | Implications for evolutionary mechanisms influencing brain morphology

More generally, the results of our hierarchical analysis may explain some of the lack of consistency in tests of ‘concerted’ versus

‘mosaic’ brain morphology evolution in the literature. Indeed, the patterns of divergence in brain form within and between two sunfish species here are consistent with both concerted and mosaic patterns of brain change. Pumpkinseed ecotypes diverged with respect to whole brain size, roughly suggesting a concerted pattern of brain change. Bluegill ecotypes on the other hand diverged with respect to a single brain region, the telencephalon, interpretable as a mosaic pattern of brain change. In addition, the relative sizes of certain brain regions (cerebellum, optic tectum, olfactory bulb) can evolve independently given enough time (15 million years between sunfish species here consistent with mosaic change), but not over shorter evolutionary scales (~12,000 years between conspecific ecotypes). Our findings show how dichotomizing brain form evolution between two phenomenological patterns, mosaic versus concerted, ignores a crucial evolutionary idea. Concerted brain form evolution very likely results from strong functional, developmental or genetic patterns of brain region covariance and low trait variance. Mosaic trait evolution, in contrast, likely reflects the opposite, weak trait covariation and high trait variance. Populations will vary along a continuum with respect to this underlying genetic architecture of brain form traits. There has been limited work testing the degree of functional covariation between brain regions, for example in mice (Hager et al., 2012), stickleback (Noreikiene et al., 2015) and guppies (Kotrschal, Zeng, et al., 2017; Kotrschal, Deacon, et al., 2017), highlighting the need for research that furthers our understanding of genetic and functional links between brain regions in order to better predict how this will shape evolutionary change in brain form. This approach represents a shift from phenomenological hypotheses about brain form evolution to more explicit mechanistic hypotheses. Our hierarchical test of brain form variation, in addition to highlighting the effect of the eco-cognitive requirements of different habitats on brain form, demonstrates the importance of ecological and evolutionary context in interpreting factors that regulate brain form evolution.

ACKNOWLEDGMENTS

This work would not have been possible without the support of the Ashby Lake Protective Association, particularly R. and C. Gautier, as well as the residents of Holcomb Lake, Michigan. We thank N. Sakich, C. Nolan and W. Jarvis for field assistance. We also acknowledge funding for this research provided by the Natural Sciences and Engineering Research Council of Canada in the form of Discovery grants (RGPIN-2019-04710, RGPIN-2014-06383).

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

AUTHOR CONTRIBUTIONS

CJA participated in all aspects of study design, field collections, sample processing, statistical analysis and drafted the manuscript. FL and BWR contributed to study design, data analysis and manuscript preparation.

PEER REVIEW

The peer review history for this article is available at <https://publons.com/publon/10.1111/jeb.13763>.

DATA AVAILABILITY STATEMENT

Data deposited at Dryad: <https://doi.org/10.5061/dryad.547d7wm7d>

ORCID

Caleb J. Axelrod  <https://orcid.org/0000-0002-6130-0168>

REFERENCES

- Ahmed, N. I., Thompson, C., Bolnick, D. I., & Stuart, Y. E. (2017). Brain morphology of the threespine stickleback (*Gasterosteus aculeatus*) varies inconsistently with respect to habitat complexity: A test of the clever foraging hypothesis. *Ecology and Evolution*, 7, 3372–3380. <https://doi.org/10.1002/ece3.2918>
- Aiello, L. C., & Wheeler, P. (1995). The expensive-tissue hypothesis: The brain and the digestive system in human and primate evolution. *Current Anthropology*, 36, 199–221.
- Axelrod, C. J., Laberge, F., & Robinson, B. W. (2018). Intraspecific brain size variation between coexisting sunfish ecotypes. *Proceedings of the Royal Society. B, Biological Sciences*, 285, 20181971. <https://doi.org/10.1098/rspb.2018.1971>
- Axelrod, C. J., Laberge, F., & Robinson, B. W. (2020). Isolating the effects of ontogenetic niche shift on brain size development using pumpkinseed sunfish ecotypes. *Evolution and Development*, 22(4), 312–322. <https://doi.org/10.1111/ede.12333>
- Barrett, R. D. H., & Schluter, D. (2007). Adaptation from standing genetic variation. *TREE*, 23, 38–44. <https://doi.org/10.1016/j.tree.2007.09.008>
- Barton, R. A., & Harvey, P. H. (2000). Mosaic evolution of brain structure in mammals. *Nature*, 405, 1055–1058. <https://doi.org/10.1038/35016580>
- Benson-Amram, S., Dantzer, B., Stricker, G., Swanson, E. M., & Holekamp, K. E. (2016). Brain size predicts problem-solving ability in mammalian carnivores. *Proceedings of the National Academy of Sciences of the United States of America*, 113, 2532–2537. <https://doi.org/10.1073/pnas.1505913113>
- Blows, M. W., Brooks, R., & Kraft, P. G. (2003). Exploring complex fitness surfaces: Multiple ornamentation and polymorphism in male guppies. *Evolution*, 57, 1622–1630. <https://doi.org/10.1111/j.0014-3820.2003.tb00369.x>
- Bolstad, G. H., Cassara, J. A., Márquez, E., Hansen, T. F., van der Linde, K., Houle, D., & Pélabon, C. (2015). Complex constraints on allometry revealed by artificial selection on the wing of *Drosophila melanogaster*. *Proceedings of the National Academy of Sciences of the United States of America*, 112, 13284–13289. <https://doi.org/10.1073/pnas.1505357112>
- Buechel, S. D., Boussard, A., Kotschal, A., van der Bijl, W., & Kolm, N. (2018). Brain size affects performance in a reversal-learning test. *Proceedings of the Royal Society. B, Biological Sciences*, 285, 20172031. <https://doi.org/10.1098/rspb.2017.2031>
- Chippis, S. R., Dunbar, J. A., & Wahl, D. H. (2004). Phenotypic variation and vulnerability to predation in juvenile bluegill sunfish (*Lepomis macrochirus*). *Oecologia*, 138, 32–38. <https://doi.org/10.1007/s00442-003-1396-z>
- Colgan, P. W., Nowell, W. A., Gross, M. R., & Grant, J. W. A. (1979). Aggressing habituation and rim circling in the social organization of bluegill sunfish (*Lepomis macrochirus*). *Environmental Biology of Fishes*, 4, 29–36.
- Conner, J. K., Karoly, K., Stewart, C., Koelling, V. A., Sahli, H. F., & Shaw, F. H. (2011). Rapid independent trait evolution despite a strong pleiotropic genetic correlation. *The American Naturalist*, 178, 429–441. <https://doi.org/10.1086/661907>
- Costa, S. S., Andrade, R., Carneiro, L. A., Gonçalves, E. J., Kotschal, K., & Oliveira, R. F. (2011). Sex Differences in the dorsolateral telencephalon correlate with home range size in blennioid fish. *Brain, Behavior and Evolution*, 77, 55–64. <https://doi.org/10.1159/000323668>
- Crispo, E., & Chapman, L. J. (2010). Geographic variation in phenotypic plasticity in response to dissolved oxygen in an African cichlid fish. *Journal of Evolutionary Biology*, 23, 2091–2103. <https://doi.org/10.1111/j.1420-9101>
- Edelaar, P., Baños-Villalba, A., Quevedo, D. P., Escudero, G., Bolnick, D. I., & Jordán-Andrade, A. (2019). Biased movement drives local cryptic coloration on distinct urban pavements. *Proceedings of the Royal Society. B, Biological Sciences*, 286, 20191343. <https://doi.org/10.1098/rspb.2019.1343>
- Ehlinger, T. J. (1990). Habitat choice and phenotype-limited feeding efficiency in bluegill: Individual differences and trophic polymorphism. *Ecology*, 71, 886–896. <https://doi.org/10.2307/1937360>
- Ehlinger, T. J., & Wilson, D. S. (1988). Complex foraging polymorphism in bluegill sunfish. *Proceedings of the National Academy of Sciences of the United States of America*, 85, 1878–1882. <https://doi.org/10.1073/pnas.85.6.1878>
- Eifert, C., Farnworth, M., Schulz-Mirbach, T., Riesch, R., Bierbach, D., Klaus, S., Wurster, A., Tobler, M., Streit, B., Indy, J. R., Arias-Rodriguez, L., & Plath, M. (2015). Brain size variation in extremophile fish: Local adaptation versus phenotypic plasticity: Brain size variation in extremophile fish. *Journal of Zoology*, 295, 143–153. <https://doi.org/10.1111/jzo.12190>
- Espinosa-Soto, C., & Wagner, A. (2010). Specialization can drive the evolution of modularity. *PLoS Computational Biology*, 6, e1000719. <https://doi.org/10.1371/journal.pcbi.1000719>
- Evans, M. L., Chapman, L. J., Mitrofanov, I., & Bernatchez, L. (2013). Variable extent of parallelism in respiratory, circulatory, and neurological traits across lake whitefish species pairs. *Ecology and Evolution*, 3, 546–557. <https://doi.org/10.1002/ece3.469>
- Finlay, B., & Darlington, R. B. (1995). Linked regularities in the development and evolution of mammalian brains. *Science*, 268, 1578–1584. <https://doi.org/10.1126/science.7777856>
- Finlay, B. L., Darlington, R. B., & Nicastro, N. (2001). Developmental structure in brain evolution. *The Behavioral and Brain Sciences*, 24, 263–278.
- Fischer, S., Bessert-Nettelbeck, M., Kotschal, A., & Taborsky, B. (2015). Rearing-group size determines social competence and brain structure in a cooperatively breeding cichlid. *The American Naturalist*, 186, 123–140. <https://doi.org/10.1086/681636>
- Foster, S. A. (1998). Nested biological variation and speciation. *Philosophical Transactions of the Royal Society of London. Series B*, 353, 207–218. <https://doi.org/10.1098/rstb.1998.0203>
- Gillespie, G. J., & Fox, M. G. (2003). Morphological and life-history differentiation between littoral and pelagic forms of pumpkinseed. *Journal of Fish Biology*, 62, 1099–1115. <https://doi.org/10.1046/j.1095-8649>
- Gonda, A., Herczeg, G., & Merila, J. (2009). Adaptive brain size divergence in nine-spined sticklebacks (*Pungitius pungitius*)? *Journal of Evolutionary Biology*, 22, 1721–1726. <https://doi.org/10.1111/j.1420-9101>
- Gonda, A., Herczeg, G., & Merilä, J. (2011). Population variation in brain size of nine-spined sticklebacks (*Pungitius pungitius*) – Local adaptation or environmentally induced variation? *BMC Evolutionary Biology*, 11, 75. <https://doi.org/10.1186/1471-2148-11-75>
- Gonda, A., Herczeg, G., & Merilä, J. (2013). Evolutionary ecology of intra-specific brain size variation: A review. *Ecology and Evolution*, 3, 2751–2764. <https://doi.org/10.1002/ece3.627>
- Gonda, A., Välimäki, K., Herczeg, G., & Merilä, J. (2012). Brain development and predation: Plastic responses depend on evolutionary history. *Biology Letters*, 8, 249–252. <https://doi.org/10.1098/rsbl.2011.0837>

- Gonzalez-Voyer, A., & Kolm, N. (2010). Sex, ecology and the brain: Evolutionary correlates of brain structure volumes in tanganyikan cichlids. *PLoS One*, 5, e14355. <https://doi.org/10.1371/journal.pone.0014355>
- Gonzalez-Voyer, A., Winberg, S., & Kolm, N. (2009). Brain structure evolution in a basal vertebrate clade: Evidence from phylogenetic comparative analysis of cichlid fishes. *BMC Evolutionary Biology*, 9, 238. <https://doi.org/10.1186/1471-2148-9-238>
- Gutiérrez-Ibáñez, C., Iwaniuk, A. N., Moore, B. A., Fernández-Juricic, E., Corfield, J. R., Krilow, J. M., Kolominsky, J., & Wylie, D. R. (2014). Mosaic and concerted evolution in the visual system of birds. *PLoS One*, 9, e90102. <https://doi.org/10.1371/journal.pone.0090102>
- Haber, A. (2015). The evolution of morphological integration in the ruminant skull. *Evolutionary Biology*, 42, 99–114. <https://doi.org/10.1007/s11692-014-9302-7>
- Hager, R., Lu, L., Rosen, G. D., & Williams, R. W. (2012). Genetic architecture supports mosaic brain evolution and independent brain-body size regulation. *Nature Communications*, 3, 1079. <https://doi.org/10.1038/ncomms2086>
- Hall, Z. J., & Troppe, V. (2020). Using teleost fish to discern developmental signatures of evolutionary adaptation from phenotypic plasticity in brain structure. *Frontiers in Neuroanatomy*, 14, 10. <https://doi.org/10.3389/fnana.2020.00010>
- Healy, S. D., & Rowe, C. (2007). A critique of comparative studies of brain size. *Proceedings of the Royal Society. B, Biological Sciences*, 274, 453–464. <https://doi.org/10.1098/rspb.2006.3748>
- Herculano-Houzel, S., & Lent, R. (2005). Isotropic fractionator: A simple, rapid method for the quantification of total cell and neuron numbers in the brain. *Journal of Neuroscience*, 25, 2518–2521.
- Hoops, D., Vidal-García, M., Ullmann, J. F. P., Janke, A. L., Stait-Gardner, T., Duchêne, D. A., Price, W. S., Whiting, M. J., & Keogh, J. S. (2017). Evidence for concerted and mosaic brain evolution in dragon lizards. *Brain, Behavior and Evolution*, 90, 211–223. <https://doi.org/10.1159/000478738>
- 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, Behavior and Evolution*, 50, 167–182. <https://doi.org/10.1159/000113330>
- Isler, K., & van Schaik, C. P. (2006). Metabolic costs of brain size evolution. *Biology Letters*, 2, 557–560. <https://doi.org/10.1098/rsbl.2006.0538>
- Isler, K., & van Schaik, C. P. (2009). The expensive brain: A framework for explaining evolutionary changes in brain size. *Journal of Human Evolution*, 57, 392–400. <https://doi.org/10.1016/j.jhev.2009.04.009>
- Iwaniuk, A. N., Dean, K. M., & Nelson, J. E. (2004). A mosaic pattern characterizes the evolution of the avian brain. *Proceedings of the Royal Society. B, Biological Sciences*, 271, S148–S151. <https://doi.org/10.1098/rsbl.2003.0127>
- Januszkiewicz, A. J., & Robinson, B. W. (2007). Divergent walleye (*Sander vitreus*)-mediated inducible defenses in the centrarchid pumpkinseed sunfish (*Lepomis gibbosus*): Induced morphological defenses in sunfish. *Biological Journal of the Linnean Society*, 90, 25–36. <https://doi.org/10.1111/j.1095-8312.2007.00708.x>
- Jarvis, W. M. C., Comeau, S. M., Colborne, S. F., & Robinson, B. W. (2017). Flexible mate choice may contribute to ecotype assortative mating in pumpkinseed sunfish (*Lepomis gibbosus*). *Journal of Evolutionary Biology*, 30, 1810–1820. <https://doi.org/10.1111/jeb.13127>
- Jarvis, W. M. C., Peiman, K. S., Cooke, S. J., & Robinson, B. W. (2020). Low connectivity between sympatric populations of sunfish ecotypes suggests ecological opportunity contributes to diversification. *Evolutionary Ecology*, 34, 391–410. <https://doi.org/10.1007/s10682-020-10042-4>
- Jastrebski, C. J., & Robinson, B. W. (2004). Natural selection and the evolution of replicated trophic polymorphisms in pumpkinseed sunfish (*Lepomis gibbosus*). *Evolutionary Ecology Research*, 6, 285–305.
- Jerison, H. J. (1973). *Evolution of the brain and intelligence*. Academic Press.
- Kotrschal, A., Deacon, A. E., Magurran, A. E., & Kolm, N. (2017). Predation pressure shapes brain anatomy in the wild. *Evolutionary Ecology*, 31, 619–633. <https://doi.org/10.1007/s10682-017-9901-8>
- Kotrschal, A., Rogell, B., Bundsen, A., Svensson, B., Zajitschek, S., Brännström, I., Immler, S., Maklakov, A. A., & Kolm, N. (2013). Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Current Biology*, 23, 168–171. <https://doi.org/10.1016/j.cub.2012.11.058>
- Kotrschal, K., Van Staaden, M. J., & Huber, R. (1998). Fish brains: evolution and environmental relationships. *Reviews in Fish Biology and Fisheries*, 8, 373–408.
- Kotrschal, A., Zeng, H. L., van der Bijl, W., Öhman-Mägi, C., Kotrschal, K., Pelckmans, K., & Kolm, N. (2017). Evolution of brain region volumes during artificial selection for relative brain size. *Evolution*, 71, 2942–2951. <https://doi.org/10.1111/evo.13373>
- Kruska, D. C. T. (1988). The brain of the basking shark (*Cetorhinus maximus*). *Brain, Behavior and Evolution*, 32, 353–363. <https://doi.org/10.1159/000116562>
- Laberge, F., & Hara, T. J. (2001). Neurobiology of fish olfaction: A review. *Brain Research Reviews*, 36, 46–59. [https://doi.org/10.1016/s0165-0173\(01\)00064-9](https://doi.org/10.1016/s0165-0173(01)00064-9)
- Lecchini, D., Lecellier, G., Lanyon, R. G., Holles, S., Poucet, B., & Duran, E. (2014). Variation in brain organization of coral reef fish larvae according to life history traits. *Brain, Behavior and Evolution*, 83, 17–30. <https://doi.org/10.1159/000356787>
- Liao, W. B., Lou, S. L., Zeng, Y., & Merilä, J. (2015). Evolution of anuran brains: Disentangling ecological and phylogenetic sources of variation. *Journal of Evolutionary Biology*, 28, 1986–1996. <https://doi.org/10.1111/jeb.12714>
- Losos, J. B. (1992). The evolution of convergent structure in *Caribbean anolis* communities. *Systematic Biology*, 41, 403–420. <https://doi.org/10.1093/sysbio/41.4.403>
- MacLean, E. L., Hare, B., Nunn, C. L., Addessi, E., Amici, F., Anderson, R. C., Aureli, F., Baker, J. M., Bania, A. E., Barnard, A. M., Boogert, N. J., Brannon, E. M., Bray, E. E., Bray, J., Brent, L. J., Burkart, J. M., Call, J., Cantlon, J. F., Cheke, L. G., ... Zhao, Y. (2014). The evolution of self-control. *Proceedings of the National Academy of Sciences of the United States of America*, 111, E2140–E2148. <https://doi.org/10.1073/pnas.1323533111>
- Marhounová, L., Kotrschal, A., Kverková, K., Kolm, N., & Němec, P. (2019). Artificial selection on brain size leads to matching changes in overall number of neurons. *Evolution*, 73, 2003–2012. <https://doi.org/10.1111/evo.13805>
- McCairns, R., & Fox, M. (2004). Habitat and home range fidelity in a trophically dimorphic pumpkinseed sunfish (*Lepomis gibbosus*) population. *Oecologia*, 140, 271–279.
- McCallum, E. S., Capelle, P. M., & Balshine, S. (2014). Seasonal plasticity in telencephalon mass of a benthic fish: Seasonal plasticity in telencephalon mass. *Journal of Fish Biology*, 85, 1785–1792. <https://doi.org/10.1111/jfb.12507>
- Monteiro, L. R., & Nogueira, M. R. (2010). Adaptive radiations, ecological specialization, and the evolutionary integration of complex morphological structures. *Evolution*, 64, 724–744. <https://doi.org/10.1111/j.1558-5646.2009.00857.x>
- Montgomery, S. H., Mundy, N. I., & Barton, R. A. (2016). Brain evolution and development: Adaptation, allometry and constraint. *Proceedings of the Royal Society. B, Biological Sciences*, 283, 20160433. <https://doi.org/10.1098/rspb.2016.0433>
- Moore, J. M., & deVoogd, T. J. (2017). Concerted and mosaic evolution of functional modules in songbird brains. *Proceedings of the Royal Society. B, Biological Sciences*, 284, 20170469. <https://doi.org/10.1098/rspb.2017.0469>
- Navarrete, A., van Schaik, C. P., & Isler, K. (2011). Energetics and the evolution of human brain size. *Nature*, 480, 91–93. <https://doi.org/10.1038/nature10629>
- Near, T. J., Bolnick, D. I., & Wainwright, P. C. (2005). Fossil calibrations and molecular divergence time in centrarchid fishes (*Teleostei: Centrarchidae*). *Evolution*, 59, 1768–1782. <https://doi.org/10.1111/j.0014-3820.2005.tb01825.x>

- Niven, J. E., & Laughlin, S. B. (2008). Energy limitation as a selective pressure on the evolution of sensory systems. *Journal of Experimental Biology*, 211, 1792–1804. <https://doi.org/10.1242/jeb.017574>
- Noreikiene, K., Herczeg, G., Gonda, A., Balázs, G., Husby, A., & Merilä, J. (2015). Quantitative genetic analysis of brain size variation in sticklebacks: Support for the mosaic model of brain evolution. *Proceedings of the Royal Society. B, Biological Sciences*, 282, 20151008. <https://doi.org/10.1098/rspb.2015.1008>
- Osenberg, C. W., Mittelbach, G. G., & Wainwright, P. C. (1992). Two-stage life histories in fish: The interaction between juvenile competition and adult performance. *Ecology*, 73, 255–267. <https://doi.org/10.2307/1938737>
- Park, P. J., & Bell, M. A. (2010). Variation of telencephalon morphology of the threespine stickleback (*Gasterosteus aculeatus*) in relation to inferred ecology. *Journal of Evolutionary Biology*, 23, 1261–1277. <https://doi.org/10.1111/j.1420-9101>
- Parsons, K. J., & Robinson, B. W. (2007). Foraging performance of diet-induced morphotypes in pumpkinseed sunfish (*Lepomis gibbosus*) favours resource polymorphism. *Journal of Evolutionary Biology*, 20, 673–684. <https://doi.org/10.1111/j.1420-9101>
- Peiman, K. S., & Robinson, B. W. (2017). Comparative analyses of phenotypic trait covariance within and among populations. *The American Naturalist*, 190, 451–468. <https://doi.org/10.1086/693482>
- Pollen, A. A., Dobberfuhl, A. P., Scace, J., Igulu, M. M., Renn, S. C. P., Shumway, C. A., & Hofmann, H. A. (2007). Environmental complexity and social organization sculpt the brain in lake Tanganyikan cichlid fish. *Brain, Behavior and Evolution*, 70, 21–39.
- Powell, B. J., & Leal, M. (2014). Brain organization and habitat complexity in Anolis lizards. *Brain, Behavior and Evolution*, 84, 8–18. <https://doi.org/10.1159/000362197>
- Riopel, C., Robinson, B. W., & Parsons, K. J. (2008). Analyzing nested variation in the body form of Lepomid sunfishes. *Environmental Biology of Fishes*, 82, 409–420. <https://doi.org/10.1007/s10641-00709303-9>
- Robinson, B. W., & Wilson, D. S. (1994). Character release and displacement in fishes: A neglected literature. *The American Naturalist*, 144, 596–627.
- Robinson, B. W., Wilson, D. S., & Margosian, A. S. (2000). A pluralistic analysis of character release in pumpkinseed sunfish (*Lepomis gibbosus*). *Ecology*, 81, 2799–2812. <https://doi.org/10.1890/0012-9658>
- Robinson, B. W., Wilson, D. S., Margosian, A. S., & Lotito, P. T. (1993). Ecological and morphological differentiation of pumpkinseed sunfish in lakes without bluegill sunfish. *Evolutionary Ecology*, 7, 451–464. <https://doi.org/10.1007/BF01237641>
- Safi, K., Seid, M. A., & Dechmann, D. K. N. (2005). Bigger is not always better: When brains get smaller. *Biology Letters*, 1, 283–286. <https://doi.org/10.1098/rsbl.2005.0333>
- Scheiner, S. M. (1993). Genetics and evolution of phenotypic plasticity. *Annual Review of Ecology and Systematics*, 24, 35–68. <https://doi.org/10.1146/annurev.es.24.110193.000343>
- Schellart, N. (1991). Interrelations between the auditory, the visual and the lateral line systems of teleosts; a mini-review of modelling sensory capabilities. *Netherlands Journal of Zoology*, 42, 459–477. <https://doi.org/10.1163/156854291X00450>
- Schlichting, C. D., & Pigliucci, M. (1998). *Phenotypic evolution: A reaction norm perspective*. <https://www.cabdirect.org/cabdirect/abstract/19980108896>
- Schluter, D., & Nagel, L. M. (1995). Parallel speciation by natural selection. *The American Naturalist*, 146, 292–301.
- Schwenk, K. (1995). A utilitarian approach to evolutionary constraint. *Zoology*, 98, 251–262.
- Shumway, C. A. (2008). Habitat complexity, brain, and behavior. *Brain, Behavior and Evolution*, 72, 123–134.
- Sorensen, C., Johansen, I. B., & Overli, O. (2013). Neural plasticity and stress coping in teleost fishes. *Gen. Comp. Endocrinol.*, 181, 25–34. <https://doi.org/10.1016/j.ygcen.2012.12.003>
- Striedter, G. F. (2005). *Principles of brain evolution*. Sinauer.
- Stuart, Y. E., Veen, T., Weber, J. N., Hanson, D., Ravinet, M., Lohman, B. K., Thompson, C. J., Tasneem, T., Doggett, A., Izen, R., Ahmed, N., Barrett, R. D. H., Hendry, A. P., Peichel, C. L., & Bolnick, D. I. (2017). Contrasting effects of environment and genetics generate a continuum of parallel evolution. *Nature Ecology and Evolution*, 22, 158.
- Sukhum, K. V., Shen, J., & Carlson, B. A. (2018). Extreme enlargement of the cerebellum in a clade of teleost fishes that evolved a novel active sensory system. *Current Biology*, 28, 3857–3863.e3. <https://doi.org/10.1016/j.cub.2018.10.038>
- Ullmann, J. F. P., Cowin, G., & Collin, S. P. (2010). Quantitative assessment of brain volumes in fish: Comparison of methodologies. *Brain, Behavior and Evolution*, 76, 261–270. <https://doi.org/10.1159/000321467>
- Walsh, M. R., Broyles, W., Beston, S. M., & Munch, S. B. (2016). Predator-driven brain size evolution in natural populations of Trinidadian killifish (*Rivulus hartii*). *Proceedings of the Royal Society. B, Biological Sciences*, 283, 20161075. <https://doi.org/10.1098/rspb.2016.1075>
- Weese, D. J., Ferguson, M. M., & Robinson, B. W. (2012). Contemporary and historical evolutionary processes interact to shape patterns of within-lake phenotypic divergences in polyphenic pumpkinseed sunfish, *Lepomis gibbosus*: Historical effects on pumpkinseed divergence. *Ecology and Evolution*, 21, 574–592. <https://doi.org/10.1002/ece3.72>
- Werner, E. E., & Gilliam, J. F. (1984). The ontogenetic niche and species interactions in size-structured populations. *Annual Review of Ecology, Evolution, and Systematics*, 15, 393–425. <https://doi.org/10.1146/annurev.es.15.110184.002141>
- White, G., & Brown, C. (2014). A comparison of spatial learning and memory capabilities in intertidal gobies. *Behavioral Ecology and Sociobiology*, 68, 1393–1401. <https://doi.org/10.1007/s00265-014-1747-2>
- White, G. E., & Brown, C. (2015). Variation in brain morphology of intertidal gobies: A comparison of methodologies used to quantitatively assess brain volumes in fish. *Brain, Behavior and Evolution*, 85, 245–256. <https://doi.org/10.1159/000398781>
- Whitlock, M. C., Phillips, P. C., Moore, F. B. G., & Tonsor, S. J. (1995). Multiple fitness peaks and epistasis. *Annual Review of Ecology and Systematics*, 26, 601–629. <https://doi.org/10.1146/annurev.es.26.110195.003125>
- Wilson, A. D. M., & McLaughlin, R. L. (2010). Foraging behaviour and brain morphology in recently emerged brook charr, *Salvelinus fontinalis*. *Behavioral Ecology and Sociobiology*, 64, 1905–1914. <https://doi.org/10.1007/s00265-010-1002-4>
- Wilson, D. S., Muzzall, P. M., & Ehlinger, T. J. (1996). Parasites, morphology, and habitat use in a bluegill sunfish (*Lepomis macrochirus*) population. *Copeia*, 1996, 348.
- Wright, S. (1932). The roles of mutation, inbreeding, crossbreeding and selection in evolution. *Proceedings of the Sixth International Congress of Genetics*, 1, 356–366.
- Zupanc, G. K. H. (2006). Neurogenesis and neuronal regeneration in the adult fish brain. *J. Comp. Phys. A*, 192, 649–70. <https://doi.org/10.1007/s00359-006-0104>

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

How to cite this article: Axelrod CJ, Laberge F, Robinson BW.

Interspecific and intraspecific comparisons reveal the importance of evolutionary context in sunfish brain form divergence. *J Evol Biol*. 2021;34:639–652. <https://doi.org/10.1111/jeb.13763>