

RESEARCH ARTICLE

Evolutionary divergence in phenotypic plasticity shapes brain size variation between coexisting sunfish ecotypes

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Abstract

Mechanisms that generate brain size variation and the consequences of such variation on ecological performance are poorly understood in most natural animal populations. We use a reciprocal-transplant common garden experiment and foraging performance trials to test for brain size plasticity and the functional consequences of brain size variation in Pumpkinseed sunfish (*Lepomis gibbosus*) ecotypes that have diverged between nearshore littoral and offshore pelagic lake habitats. Different age-classes of wild-caught juveniles from both habitats were exposed for 6 months to treatments that mimicked littoral and pelagic foraging. Plastic responses in oral jaw size suggested that treatments mimicked natural habitat-specific foraging conditions. Plastic brain size responses to foraging manipulations differed between ecotypes, as only pelagic sourced fish showed brain size plasticity. Only pelagic juveniles under 1 year-old expressed this plastic response, suggesting that plastic brain size responses decline with age and so may be irreversible. Finally, larger brain size was associated with enhanced foraging performance on live benthic but not pelagic prey, providing the first experimental evidence of a relationship between brain size and prey-specific foraging performance in fishes. The recent post-glacial origin of these ecotypes suggests that brain size plasticity can rapidly evolve and diverge in fish under contrasting ecological conditions.

KEYWORDS

adaptive divergence, brain size, ecological performance, ontogeny, phenotypic plasticity, sunfish

1 | INTRODUCTION

Phenotypic plasticity, the process by which traits change within an individual's lifetime in response to environmental cues, is widespread across taxa (Schlichting & Pigliucci, 1998). One trait of functional and ecological importance in animals that may be phenotypically plastic is brain size. Much remains unknown about the contributions of phenotypic plasticity and heritable variation to overall patterns of brain size variation, the evolutionary potential of plasticity in brain size, and how plastic responses in brain size may change over ontogeny (Striedter, 2005; Gonda et al., 2013; Hall & Tropepe, 2020).

Brain size should be particularly relevant to ecological interactions through causal links with animal behaviour and cognitive ability. We purposely define cognition broadly as all information processing done by the central nervous system, including sensory, motor, and higher integrative functions of the brain (see Shettleworth, 2001 for a related definition), because we want to encompass all aspects of central neural processing that may be influenced by brain size. Brain size has been connected to cognitive ability using learning and problem-solving tests (Benson-Amram et al., 2016; Buechel et al., 2018; Kotrschal et al., 2013a, 2013b; MacLean et al., 2014), although extensions to natural conditions are challenging (Salena et al., 2021). This relationship is thought to

represent the effects of greater numbers of neurons and neuronal connections in larger brains that enhance cognitive processing (Herculano-Houzel & Lent, 2005; Marhounová et al., 2019). The functional effects of brain size on ecological performance are supported by comparative relationships between brain size and habitat use among species (Kruska, 1988; Shumway, 2008; Sol et al., 2010; Lecchini et al., 2014; Fischer et al., 2015), and intraspecifically among individuals of different populations showing consistent differences in ecology (Ahmed et al., 2017; Axelrod et al., 2018; Evans et al., 2013; Gonda et al., 2009; Walsh et al., 2016). In these cases, individuals with larger brains tend to be associated with more complex environments. Smaller brains under reduced cognitive challenges are expected because brain tissue is metabolically costly (Isler & van Schaik, 2006; Navarrete et al., 2011; Niven & Laughlin, 2008). The relationship between brain size and variation in eco-cognitive challenges is expected regardless of the proximate mechanisms that regulate brain size. Comparative studies of brain variation cannot easily distinguish these mechanisms, which instead require experimental study.

Adaptive variation in brain size and cognitive ability requires that these traits influence ecological performance such as foraging, predator avoidance, and mating success. Brain size may influence foraging on cryptic, elusive, or evasive prey if it involves challenges related to habitat navigation, memory, and sensory perception. Links between brain size and foraging performance are supported by comparative studies of populations that differ in foraging complexity (Ahmed et al., 2017; Axelrod et al., 2018; Park & Bell, 2010). Studies also suggest that brain size may similarly influence predator avoidance and mating performance to the extent that these interactions also require cognitive ability (Corral-López et al., 2017; Kotrschal et al., 2015; Walsh et al., 2016). Otherwise, few experimental tests of the effects of brain size on ecological performance under relevant conditions exist.

Both heritable genetic variation and phenotypic plasticity influence brain size. Species differences in relative brain size often reflects genetic differences in brain size (Striedter, 2005). Within populations, genetic variation in brain size has been detected using common garden experiments with Trinidadian killifish (*Rivulus hartii*) (Walsh et al., 2016), and through artificial selection on brain size in guppies (*Poecilia reticulata*) (Kotrschal et al., 2013a, 2013b). Functional plastic responses in brain size might be expected when individuals experience environmental conditions that generate variable cognitive challenges, and when induced response are possible and can be reliably matched to those conditions, such as in amphibians (Gonda et al., 2010) and mammals (Diamond et al., 1966). Experimental tests of brain size plasticity in fish are rare, despite the expectation that it may be quite common because most fishes and amphibians maintain widespread neurogenesis well into adulthood in contrast to birds and mammals (Kaslin et al., 2008; Sorensen et al., 2013; Zupanc, 2006). For example, plastic brain size responses to social and predator cues occur in ninespine stickleback (*Pungitius pungitius*) (Gonda et al., 2011), to light cues in the extremophile short-fin molly (*Poecilia mexicana*) (Eifert et al., 2015), and to experimental

environmental enrichment in a variety of fishes (Burns et al., 2008; DePasquale et al., 2016; Fong et al., 2019; Herczeg et al., 2015).

Additionally, variation in brain size may reflect the evolutionary divergence of brain size plasticity, such as between constant versus variable eco-cognitive conditions. For example, the olfactory region of the brain is more plastic in freshwater compared to marine populations of threespine stickleback (*Gasterosteus aculeatus*) (Gonda et al., 2012), and brain mass plasticity is variable among African cichlids (*Pseudocrenilabrus multicolor*) that vary in exposure to dissolved oxygen levels due to individual differences in dispersal (Crispo & Chapman, 2010). These studies suggest that variation in brain size plasticity in fishes may be a factor influencing variation in brain morphology in the wild.

Ontogenetic changes in plastic brain size responses are also largely a mystery. Minimal neurogenesis in adult mammals and birds suggests that adult brain size plasticity may be limited to brain regions that still exhibit neurogenesis at this life stage (Striedter, 2005). Fish, in contrast, maintain widespread neurogenesis into adulthood possibly permitting extensive brain size plasticity throughout their lifetime (Zupanc, 2006). However, as far as we know, ontogenetic changes in brain size plasticity have not been tested. Changing ecological conditions over ontogeny related to body growth strongly affect foraging, predator avoidance, and social interactions (Nakazawa, 2015; Werner & Gilliam, 1984; Wilson, 1975) and may favour sustained plasticity through life. Conversely, when plasticity in brain size is costly and cognitive environmental conditions are more constant, then genetically canalized brain size development might be favoured (Hensley, 1993; Lehman & Campbell, 2007; Meuthen et al., 2018).

We evaluated the proximate mechanisms contributing to variation in brain size, as well as the link between brain size and ecological performance, in a natural population of Pumpkinseed sunfish (*Lepomis gibbosus*) that are trophically diverging between alternate lake habitats. Pumpkinseed typically inhabit the nearshore (littoral) habitat of lakes. Morphological traits such as larger heads, oral and pharyngeal jaws, and more widely spaced gill rakers enhance feeding on benthic macroinvertebrates, such as snails (reviewed in Robinson et al., 1993; Weese et al., 2012). In certain lakes, pumpkinseed have also colonized the offshore (pelagic) habitat (Robinson et al., 2000). During the summer growing season, pelagic pumpkinseed congregate around submerged rocky shoals where they feed extensively in the water column on large zooplankton prey, such as *Daphnia* spp. (Gillespie & Fox, 2003; Jastrebski & Robinson, 2004; Robinson et al., 1993, 2000; Weese et al., 2012). Pelagic individuals express smaller oral and pharyngeal jaws and more densely packed gill rakers than littoral fish in the same lake (Axelrod et al., 2018; Jarvis et al., 2017, 2020; Jastrebski & Robinson, 2004; Robinson et al., 1993). Divergent ecotype morphologies are related to foraging performance in their respective habitats and prey groups (Parsons & Robinson, 2007; Robinson et al., 1996).

Relative brain size also differs between littoral and pelagic pumpkinseed ecotypes, suggesting that these lake habitats may generate different cognitive requirements that contribute to

ecotype divergence. Adult pumpkinseed from the littoral habitat have on average 8.3% larger brains than pelagic sourced individuals, with no differences in the proportional size of brain regions (Axelrod et al., 2018). A positive relationship between oral jaw width and brain size also occurs within each habitat, further supporting functional links between brain size and trophic ecology. The difference in brain size is apparent among individuals in their birth summer (i.e., only a few months old) and is maintained throughout life, despite diversification in feeding ecology occurring between the ecotypes only once fish are 3 years old (Axelrod et al., 2020). Brain size differences so early in life (before divergence in foraging ecology) suggest that if brain size plasticity is an important mechanism in this system, its potential may decrease with age, as continuous plasticity would lead to a consistent match between changing relative brain size and changing ecology throughout life. A direct test of decreased plasticity in brain size with age has not yet been performed.

Here we evaluated, (i) the proximate mechanisms of brain size variation between littoral and pelagic ecotypes; (ii) the influence of ontogeny on plastic responses; and (iii) the consequences of brain size variation on foraging performance in pumpkinseed sunfish. We focused specifically on variation in brain size, rather than individual brain regions, because littoral and pelagic ecotypes of pumpkinseed do not differ in the proportional size of any brain region (Axelrod et al., 2018), and the goal of our study was to explore proximate mechanisms of this natural trait variation. We used a reciprocal transplant experiment to rear juvenile pumpkinseed from both habitats under different feeding conditions that mimic foraging regimes in natural littoral (benthic feeding on large particles) and pelagic conditions (water column feeding on small particles). Inherited genetic effects predict larger brains in littoral-sourced than pelagic-sourced fish regardless of feeding treatments given differences noted in the field (Axelrod et al., 2018, 2020). Maternal or early life (before collection) plastic effects could also lead to this pattern; however, we cannot evaluate these. Functional plasticity predicts larger brains developing in the benthic feeding treatment that mimics littoral foraging regardless of ecotype. Furthermore, differences in brain responses to feeding treatment between ecotypes would suggest that brain size plasticity has evolutionarily diverged.

In addition, we addressed two secondary questions related to the influences of ontogeny and cognitive challenge on plastic brain size responses. To evaluate how plasticity changes over ontogeny, we used three age categories: age-0 (fish in their birth year), age-1 year-old, and older age-2-3 years-old juvenile pumpkinseed. Loss of plastic brain responses would be indicated by absent or reduced plastic responses in older fish. In addition to foraging complexity, temporal environmental variability has been associated with larger brain size in birds (Schuck-Paim et al., 2008; Sayol et al., 2016). To test this potential effect, we also include a third “variable” feeding treatment where foraging regimes temporally alternate between benthic and water column treatments. If temporal variability in foraging is more cognitively challenging than a more constant foraging regime, then larger brains are expected under the variable foraging treatment.

Finally, we preliminarily test the influence of brain size on foraging performance by measuring individual success at capturing live benthic or planktonic prey using a subset of individuals at the end of the rearing experiment. The larger brain size of wild littoral compared to pelagic pumpkinseed suggests that benthic foraging is more cognitively challenging perhaps because of the cryptic nature of benthic prey against a substrate, complexity of the background visual environment or evasiveness of larger mobile benthic prey. As such, we predict a stronger positive effect of brain size on benthic foraging performance in comparison to foraging on zooplankton prey.

2 | METHODS

2.1 | Fish collection

All fish were collected from Ashby Lake, Ontario, over 3 weeks of sampling in August and September of 2016. Sunfish ecotypes in Ashby Lake have been extensively studied in terms of their foraging ecology and morphology (Jastrebski & Robinson, 2004; Weese et al., 2012), have spatially assortative spawning that occurs in both habitats (Colborne et al., 2016) to provide a local source of recruits, and have been shown to differ in brain size (Axelrod et al., 2018, 2020, 2021). Ashby Lake is one of 12 known populations of divergent pumpkinseed in the postglacial lakes of the Adirondack region of upstate New York, USA, and eastern Ontario, Canada (Weese et al., 2012). It is the most extensively studied of these populations and is representative of the general patterns of ecological and morphological diversification between lake habitats.

Individuals were collected from five littoral and five pelagic sites and then mixed within habitats to incorporate any site variation within habitat level variation before transport to the Hagen Aqualab (University of Guelph; further details in Appendix S1: Supplementary 1). Juveniles of different ages were used in this experiment. In both habitats, age-0 (birth year) individuals were captured using hand nets while snorkel diving, while older juveniles (age 1–3 years) were collected using standard minnow traps (sample sizes in Appendix S1: Supplementary 2). Age was initially estimated in the field based on body size, and subsequently determined more precisely at the termination of the experiment by counting annuli on scales.

2.2 | Experimental design

We use a reciprocal-transplant common garden design to test proximate mechanisms of brain size variation in pumpkinseed ecotypes (Januszkiewicz & Robinson, 2007; Parsons & Robinson, 2006; Robinson & Wilson, 1996). Juvenile pumpkinseed were reared for 6 months, from September 2016 through February 2017, in a fully crossed factorial design involving two habitat sources (littoral or pelagic), three foraging treatments (benthic, water column, variable), and two age groups (age-0 and older juveniles age-1, 2, 3 years)

for a total of 12 source-treatment-age groupings. Foraging treatments mimicked physical feeding conditions in the natural habitats. The water column treatment involved feeding on suspended small particles mimicking pelagic zooplankton prey while in the benthic treatment large food particles were consumed at the benthos. The variable feeding treatment alternated water column and benthic feeding treatments at a two-week interval throughout the experiment. The same food was used in all treatments to keep nutritional quality consistent. Each of the 12 source-treatment-age groupings were assigned to one tank, with fish occupying replicate sections within each tank. Further details of the experimental design can be seen in Appendix S1: Supplementary 2.

Fish were housed in an indoor water recirculating system located in an isolated room of the Hagen Aqualab that provided exceptional control of air and water temperature, water filtration, aeration and ambient light cycle. Logistics limited assigning each of the 12 source-treatment-age groupings into one long tank, further subdivided into 7 sections separated by mesh screening. This approach relieved concerns about potential mixing of small age-0 fish among sections in a tank, but at the cost that treatment and random tank effects could be confounded. We minimized this confounding risk in two ways. First, by water homogenization among all tanks sharing the single recirculating system. Approximately 75% of the water flowing through all tanks was mixed, filtered, combined with 25% new water, and continuously recirculated back to constantly blend water conditions among all tanks. Second, by systematic allocation of source, feeding treatment and age to equally spread spatial effects due to tank 'rack' and tank height (within rack) equally across all three factors (further details in Appendix S1: Supplementary 3). Nevertheless, we cannot rule out other possible causes of random tank effects with this design that could influence brain and jaw size results.

2.3 | Foraging performance trials

Sunfish from this experiment are good subjects for testing the relationship between brain size and foraging performance because brain size is expected to vary for multiple reasons (source habitat, experimental foraging treatment, age). A subsample of sunfish at the termination of the experiment were used to evaluate foraging performance on two different types of live prey, *Daphnia magna* (typical pelagic prey), and small amphipods (*Echinogammarus ischnus*) (common benthic prey; roughly 2–3 times larger than the *Daphnia*). We used live, novel prey to increase the potential cognitive challenge of foraging on both types of prey compared to learned foraging techniques from non-living food. Seven fish per group (source by treatment by age) for a total of 84 individuals were used in these foraging trials (however some samples became desiccated during storage and were lost). Each individual was tested on both types of prey consecutively in a counterbalanced sequence to control for presentation order differences. Trials took place over 5 min in clean 10-gallon glass aquaria. In the pelagic foraging trial, aquaria without sediment initially held ~100 live *Daphnia magna*. In the benthic foraging trial,

tanks with gravel sediment initially held ~20 live amphipods (further details in Appendix S1: Supplementary 4). Video recordings of trials were used to determine individual participation identified as active prey chasing and bite attempts. Our analysis focuses on foraging success, estimated as the number of each prey item present in an individual's dissected stomach.

2.4 | Sample processing

After 6 months of rearing, all fish were euthanized using an overdose of clove oil (100 ppm) and stored in 10% buffered formalin. Before processing, individuals were assigned a non-identifying label to avoid unconscious bias, though some information about age was known because age-0 and older juveniles were processed at different times. Shrinkage of soft tissue is possible over long-term storage in fixative. To avoid tissue shrinkage affecting comparisons of traits between sources and treatment groups (our primary focus), all samples of an age group were processed within the same month. Two age groups (age-0 and age > 0) were processed within a year of each other (2019 and 2020), so shrinkage may have contributed to trait differences between age groups. Blotted wet weight, standard body length and oral jaw width (maximum distance between the maxillaries) were determined for each individual, and a sample of scales was collected from just above the lateral line behind the pectoral fin for age determination. Fish age (years since birth) was determined by counting annuli on scales under a dissecting microscope with the absence of annuli interpreted as age-0. Brains were removed via dorsal dissection (Axelrod et al., 2018). The spinal cord of each brain was trimmed at the level of the obex and excess cranial nerves were removed. Brain blotted wet weight was used to estimate brain size and taken with an Accu-124D scale (Fisher Scientific) at a resolution of 0.1 mg.

2.5 | Statistical analyses

2.5.1 | Proximate mechanisms of brain size variation

Our basic approach involves linear mixed effects models that partition trait variation (oral jaw width or brain mass) into components related to source habitat (littoral or pelagic) to evaluate potential heritable variation; foraging treatment (benthic, water column, variable) to evaluate phenotypic plasticity; and their interaction to evaluate differences in plastic responses between habitat sources. We also include age (years) in the model, the three-way interaction between age, foraging treatment, and source habitat, as well as all two-way interactions between variables. We interpret a significant interaction between age and foraging treatment as statistical evidence of a change in plastic responses to foraging treatment with age that may also differ between source ecotypes (indicated by the 3-way interaction). Standard length was included in the models as a covariate to account for allometric effects. Length and

mass variables were natural log-transformed to improve residual normality. Standard length was used because it is less subject to short term fluctuations in condition than body mass. All models included tank section as a random effect to account for possible effects of section location or fish density. Akaike-based comparisons of models including or excluding the random section effect generally indicated a better model fit when random section was included. Hence, parameter tests occur while accounting for section effects in the model. When initial models indicated changing plastic responses with age (age-related interactions), then we further explored the effects of age on proximal mechanisms of trait variation using separate models for each age class. Three linear mixed effects models were applied to age-0, age-1, and combined age-2-3 fish because the sample size of age 3 individuals was low ($n = 19$). Trait means compared among treatment groups represent the mean plastic response between foraging treatments, rather than plastic responses of individual genotypes.

We first used these models to test whether foraging treatments induced variation in a trophic trait, oral jaw width, to evaluate whether the experimental treatments mimicked differences in natural foraging conditions between littoral and pelagic habitats. Oral jaw width can be used as a morphological proxy for individual trophic ecology because they are larger in pumpkinseed from littoral compared to pelagic habitats in the wild (Axelrod et al., 2018, 2020; Jarvis et al., 2017). We interpret differences consistent with field observations as evidence that the treatments induced functional oral jaw responses that partially mimic natural trophic ecology. Significant feeding treatment effects were subsequently explored using contrasts among treatment levels.

Similar linear mixed effects models were employed to study variation in brain mass. Any significant interaction between source and foraging treatment in age-separated models were explored further with two additional mixed effects models, one for each source habitat (littoral and pelagic). These separate source models include only standard length and foraging treatment as main effects, tank section as a random effect, and used the parameter transformations above.

Foraging treatments generated differences in body size (see Section 3) that may affect brain growth. We test for possible confounding effects of differential growth among treatments using similar mixed effects models that relate standard length to collection source, feeding treatment, age, the three-way interaction between age, foraging treatment, and source habitat, as well as all two-way interactions. All statistical analyses were conducted using the R program for statistical computing (version 3.6.3; R Core Team, 2020), and we report 2-sided p -values throughout.

2.5.2 | Foraging performance trials

We used separate generalized linear negative-binomial models for each prey type to test whether foraging success (consumed prey count) was related to brain size while statistically accounting

for other factors that can influence foraging success. Negative-binomial models were selected to account for overdispersion in the prey counts because zeros associated with individuals that did not participate in the feeding trial were removed prior to analysis ($n = 10$), and the negative-binomial models had better fit than zero-inflated models based on AIC scores. The number of prey items consumed by an individual was modelled against its size-adjusted brain mass (residuals estimated from a linear model of brain mass against standard length for all fish included in the foraging trials), size-adjusted oral jaw width (residuals estimated from a linear model between oral jaw width and standard length for all fish combined), standard length, foraging treatment, and source habitat as predictor variables. We include oral jaw width and standard length, to account for any effects on foraging success and to test for direct brain size effects. Interactions were not included in the absence of specific predictions and sample size was limited to 49 out of the original 84 individuals tested. Video evidence revealed that 10 individuals (12%) did not attempt feeding on both prey and 25 individuals were unfortunately lost from sample desiccation during storage.

3 | RESULTS

3.1 | Jaw width effects

Plastic responses by oral jaws appeared to decrease with age and were replaced by source effects that may represent either heritable differences, maternal effects, or early plastic responses subsequently made irreversible with age (combined-age model Table 1: Age - Treatment interaction, $p < 0.005$). Separate-age models indicated that oral jaw width responded to foraging treatment in age-0 and age-1 but not in older juveniles (Table 2). Responses were qualitatively similar to differences observed between habitats in the field. In age-0 fish, mean oral jaw width was greater in the benthic and variable foraging treatments in comparison to the column treatment (Benthic - Column = 0.16 ± 0.04 , $t_9 = 4.4$, $p = 0.0005$; Variable - Column = 0.11 ± 0.04 , $t_9 = 3.2$, $p = 0.007$; Variable - Benthic = -0.05 ± 0.03 , $t_9 = -1.4$, $p = 0.2$) (Figure 1a). In age-1 individuals, mean oral jaw width was also greater in the benthic than in the other two feeding treatments (Benthic - Column = 0.08 ± 0.03 , $t_{19} = 2.4$, $p = 0.03$; Variable - Column = -0.03 ± 0.03 , $t_{19} = -0.9$, $p = 0.37$; Variable - Benthic = -0.1 ± 0.03 , $t_{19} = -3.5$, $p = 0.005$) (Figure 1b, Table 2). These patterns support our expectation that the benthic and water column treatments mimicked physical aspects of foraging under littoral and pelagic conditions respectively (Axelrod et al., 2018). Consistent with previous findings, there also was evidence that source habitat influenced oral jaw width in age-1 and age-2-3 pumpkinseed because littoral sourced individuals had significantly wider jaws than pelagic sourced individuals in older juveniles (Table 2) (Figure 1b, c). There was no indication that foraging-related plasticity in oral jaw width differed between ecotypes at any age (no source by treatment interaction effects; Table 2).

Predictor variable	Sum Square	F	Num df	p
Oral Jaw Width (N = 311). Conditional R ² = 0.94				
SL	6.2	1127	1	<0.0001
Source	0.02	3.9	1	0.054
Treatment	0.03	2.7	2	0.068
Age	0.22	13.3	3	<0.0001
Treatment × Age	0.11	3.3	6	0.0047
Source × Age	0.03	1.5	3	0.21
Source × Treatment	0.03	2.3	2	0.11
Source × Treatment × Age	0.03	0.8	6	0.6
Brain Mass (N = 311). Conditional R ² = 0.96				
SL	13.2	1560	1	<0.0001
Source	0.03	3.9	1	0.051
Treatment	0.01	0.8	2	0.46
Age	1.3	52.2	3	<0.0001
Treatment × Age	0.1	2.1	6	0.058
Source × Age	0.03	1.4	3	0.26
Source × Treatment	0.002	0.1	2	0.9
Source × Treatment × Age	0.1	2.0	6	0.075

Note: Tank section was included as a random effect. Conditional R² of whole models, calculated using the MuMIn R package, are included.

Bolded p values indicate significant effects.

Predictor variable	Sum Square	F	Num df	p
Age 0 (N = 117). Conditional R ² = 0.91				
SL	5.2	118	1	<0.0001
Source	0.0003	0.06	1	0.8
Treatment	0.2	17.4	2	0.0004
Source × Treatment	0.008	0.7	2	0.5
Age 1 (N = 71). Conditional R ² = 0.65				
SL	0.5	104	1	<0.0001
Source	0.04	7.9	1	0.01
Treatment	0.06	6.2	2	0.008
Source × Treatment	0.006	0.7	2	0.5
Age 2–3 (N = 123). Conditional R ² = 0.77				
SL	1.8	339	1	<0.0001
Source	0.005	8.6	1	0.007
Treatment	0.002	0.2	2	0.9
Source × Treatment	0.02	2.2	2	0.1

Note: Tank section was included as a random effect in all models. Conditional R² of whole models, calculated using the MuMIn R package, are included.

Bolded p values indicate significant effects.

3.2 | Brain mass effects

Foraging treatments induced different responses in mean brain mass between the littoral and pelagic sourced pumpkinseed, and brain mass plasticity decreased with age. Reduced plasticity in brain size with age was not as strongly supported as for oral jaw width

(combined-age model [Table 1](#): Age – Treatment interaction, $p = 0.058$; 3-way interaction including source, $p = 0.075$). Models separated by age indicate that age-0 individuals differed in brain size responses to feeding treatment between ecotypes ([Table 3](#): Source – Treatment interaction, $p = 0.008$). Pelagic sourced fish developed larger brains in the benthic and variable foraging treatments and smaller brains

TABLE 1 Summary of analysis of variance of mixed-effects models predicting oral jaw width and brain mass with standard length (SL: Log-transformed), source (littoral, pelagic), treatment (column, benthic, variable), age (0, 1, 2, 3 years), and their interactions

TABLE 2 Summary of analysis of variance of mixed-effects models predicting oral jaw width for each age group with standard length (SL: Log-transformed), source (littoral, pelagic), treatment (column, benthic, variable) and the interaction between source and treatment as predictors

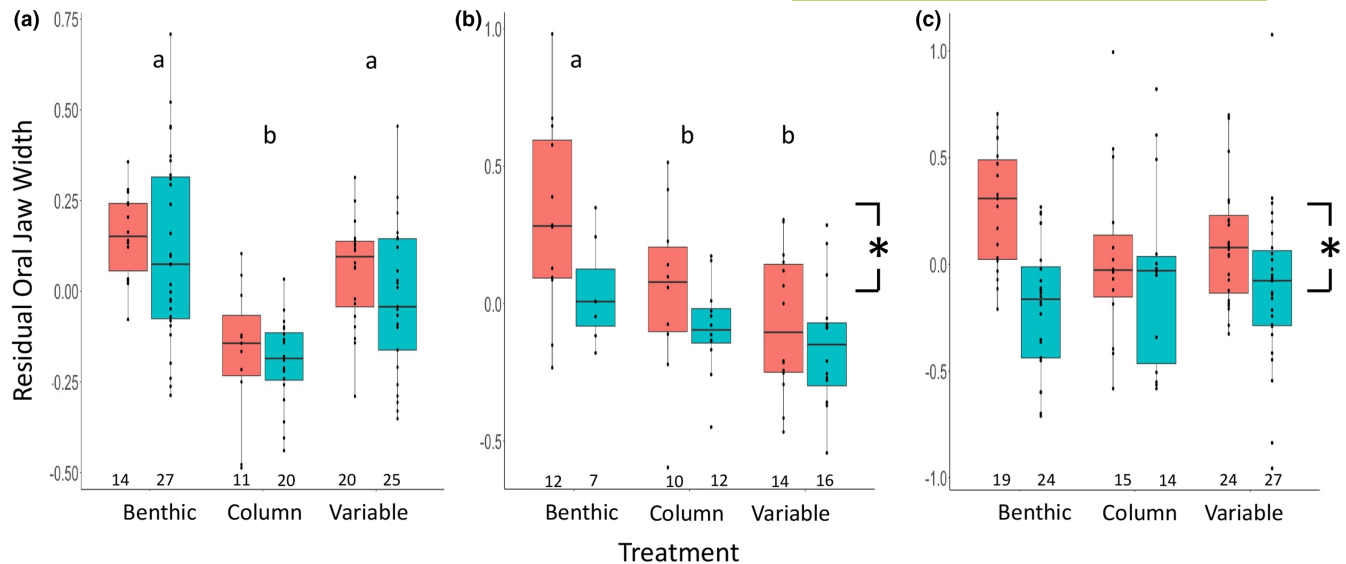


FIGURE 1 Box plots of residual oral jaw width across three foraging treatments. (a) Age-0, (b) age-1, and (c) age-2-3 fish. Habitat source is represented by colours (littoral = red, pelagic = blue). Residual oral jaw width was calculated using linear models of oral jaw width regressed against standard length separately in each age group. The lower-case letters in panels a and b indicate significant differences between levels of foraging treatments obtained from difference contrasts of the relevant age-specific mixed effects models (groups with different letters are significantly different from each other). The asterisks in panels b and c indicates a significant difference between source habitats. Boxes show median and interquartile range (25th to 75th), and whiskers show the data range. Dots show individual data points. Numbers below each box indicate the sample size for that group

in the water column treatment (Benthic - Column = 0.06 ± 0.02 , $t_2 = 2.5$, $p = 0.02$; Variable - Column = 0.07 ± 0.02 , $t_2 = 3.3$, $p = 0.002$; Variable - Benthic = 0.02 ± 0.02 , $t_2 = 0.9$, $p = 0.37$) (Figure 2a). Littoral sourced age-0 fish showed no significant change in brain size between treatments (Table 3) (Figure 2a). Age-1 and Age-2-3 individuals also did not show any plastic response in brain mass to foraging treatments (Table 3) (Figure 2b, c). The mean brain mass of littoral sourced pumpkinseed was greater than that of pelagic individuals in age-2-3 individuals (Table 3) (Figure 2c), confirming field observations on Ashby Lake pumpkinseeds (Axelrod et al., 2018), and that ecotypic divergence in brain size becomes increasingly fixed with age (Axelrod et al., 2020).

3.3 | Foraging success

As expected, larger brain mass was positively associated with increased foraging success on live amphipod prey after accounting for a minor negative effect of oral jaw width (Table 4; Figure 3a; Appendix S1: Supplementary 5). The factors that are related to successful feeding on pelagic *Daphnia* prey are not clear since no model parameters were significant (Table 4; Figure 3b).

3.4 | Mortality and growth

Mean survival over the course of the six-month experiment was 51% (mean of % survival among the 12 treatment groupings), which is typical for long-term rearing experiments like this that require the

collection, handling, and transport of wild juvenile fish of this size (Robinson & Wilson, 1996). Mortality was higher on average in the column foraging treatment than the other two treatments, and slightly higher in age-0 juveniles than older individuals (Appendix S1: Supplementary 6). We found evidence of greater final mean standard length in fish sourced from the pelagic habitat (consistent with field observations; Axelrod et al., 2020), but that standard length was not related to foraging treatment (Appendix S1: Supplementary 7 and 8). However, growth variation likely had limited direct influence on relative brain size because standard length in age-0 fish did not vary significantly among treatments (Appendix S1: Supplementary 8A) and variation in body size of age-1 fish was not accompanied by relative brain size differences (compare Appendix S1: Supplementary 8B and Figure 2b).

4 | DISCUSSION

Using a reciprocal transplant experimental design, we found evidence that a variety of proximal mechanisms likely contribute to brain size variation in these pumpkinseed sunfish. First, we found evidence of divergent plastic responses to feeding treatment early in ontogeny. Mean brain size (adjusted for body size) was larger in fish exposed to benthic foraging conditions compared to pelagic conditions, but only in age-0 fish originating from the pelagic habitat. No changes in brain size were consistently expressed by littoral source age-0 or older juveniles from either habitat to these treatments. This divergence in plasticity is similar to prior evidence of ecotype differences in plastic morphological responses

Predictor variable	Sum Square	F	Num df	p
Age 0 (N = 117). Conditional R ² = 0.91				
SL	11.8	1067	1	<0.0001
Source	0.004	0.3	1	0.6
Treatment	0.005	0.2	2	0.8
Source × Treatment	0.1	5.0	2	0.008
Age 0 Pelagic (N = 72). Conditional R ² = 0.94				
SL	5.0	978	1	<0.0001
Treatment	0.06	5.7	2	0.005
Age 0 Littoral (N = 45). Conditional R ² = 0.91				
SL	6.9	419	1	<0.0001
Treatment	0.08	2.5	2	0.09
Age 1 (N = 71). Conditional R ² = 0.60				
SL	0.6	85	1	<0.0001
Source	0.02	2.1	1	0.2
Treatment	0.007	0.5	2	0.6
Source × Treatment	0.02	1.5	2	0.2
Age 2–3 (N = 123). Conditional R ² = 0.85				
SL	3.3	617	1	<0.0001
Source	0.04	8.1	1	0.01
Treatment	0.02	1.8	2	0.2
Source × Treatment	0.006	0.6	2	0.6

Note: Significant source by treatment interaction effects were further explored using separate models for each source habitat including only standard length and treatment as predictors. Tank section was included as a random effect in all models. Conditional R² of whole models, calculated using the MuMIn R package, are included.

Bolded *p* values indicate significant effects.

to feeding treatments (Parsons & Robinson, 2006), predatory cues (Januskiewicz & Robinson, 2007), and differences in predator-induced anti-predator behaviours in these sunfish (Robinson et al., 2000). Second, source ecotypes differed in brain size regardless of feeding treatment. Mean brain size was consistently larger in littoral compared to pelagic sourced pumpkinseed of age-2-3 regardless of treatment, similar to prior field observations (Axelrod et al., 2020). This could be due to plastic responses to field conditions either by juveniles prior to collection or maternal effects on juveniles, or to genetic differences between pumpkinseed ecotypes expressed later in development. Lastly, we found preliminary evidence that brain size may functionally influence feeding behaviour but only on more difficult to capture prey. Brain size had a positive influence on foraging performance on live mobile benthic macroinvertebrate prey, but not on smaller zooplankton prey suspended in the water column. A functional relationship between brain size and prey-specific foraging performance suggests that selection on brain size may be prey specific.

Foraging treatments simulated aspects of ecology that distinguish littoral from pelagic conditions because cues related to feeding mode (food location and size) induced predictable changes in oral jaw size in age-0 and age-1 pumpkinseed. Plasticity in oral jaw size also seems irreversible in age-2 and older fish. Adult oral jaw

size is greater in littoral pumpkinseed (Axelrod et al., 2018; Jarvis et al., 2020), indicating that jaw size is related to foraging performance especially on larger prey commonly encountered there (Parsons & Robinson, 2007). As with brain size, larger oral jaw widths were induced by both the continuous and variable benthic feeding treatments in age-0 pumpkinseed, suggesting that the benthic foraging imposed here differed from the mode of feeding on suspended small particles.

The smaller brain size of wild adult pelagic pumpkinseed (Axelrod et al., 2018) arises from slower brain growth starting at age-0 in pelagic compared to littoral fish (Axelrod et al., 2020). We propose that brain size differences among pumpkinseed ecotypes result from genetic differences in plastic developmental responses to foraging cues that are also habitat-specific. An earlier reduction in brain growth is initiated by an irreversible plastic response to foraging cues in age-0 pelagic individuals in contrast to more canalized rapid brain growth in littoral fish.

Divergent morphological responses to foraging and to predator cues have been replicated in a number of pumpkinseed populations (Januskiewicz & Robinson, 2007; Parsons & Robinson, 2006) but this is the first evidence that brain size plasticity may have diverged between ecotypes. An enhanced plastic brain size response may have been favoured during the colonization of the pelagic

TABLE 3 Summary of analysis of variance of mixed-effects models predicting brain mass for each age group with standard length (SL: Log-transformed), source (littoral, pelagic), treatment (column, benthic, variable) and the interaction between source and treatment as predictors

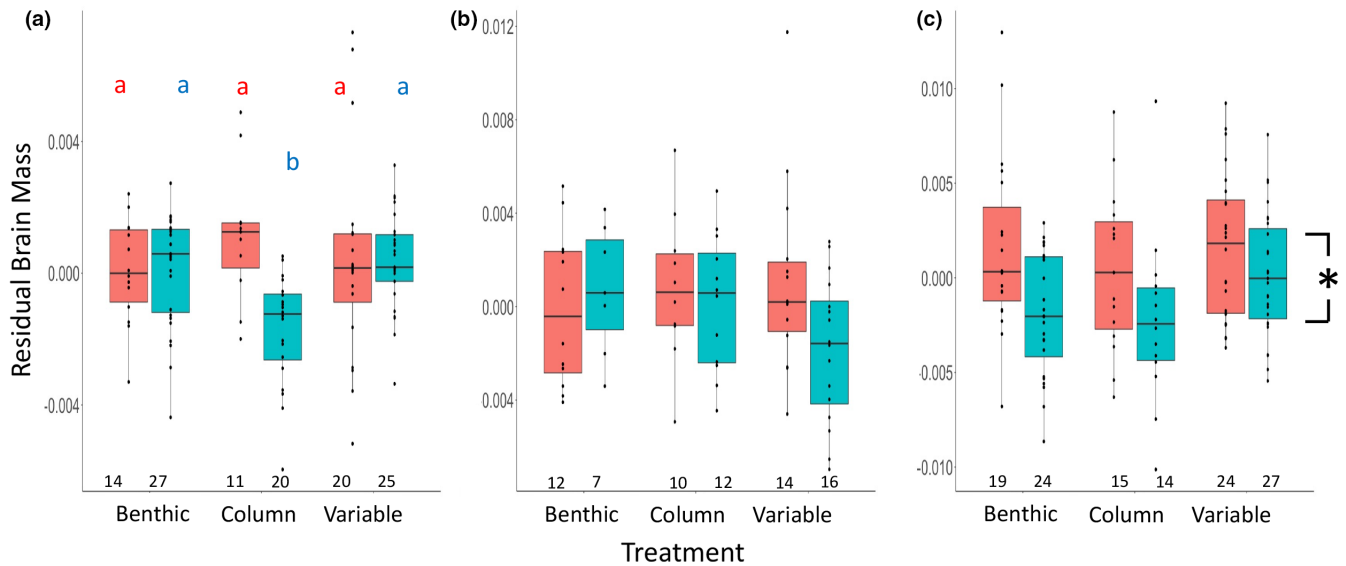


FIGURE 2 Boxplots of residual brain mass across three foraging treatments. (a) Age-0, (b) age-1, and (c) age-2-3 fish. Habitat source is represented by colours (littoral = red, pelagic = blue). Residual brain mass was calculated using linear models of brain mass regressed against standard length separately in each age group. The lower-case letters in panels A indicate significant differences between levels of foraging treatments within each source habitat (littoral = red, pelagic = blue) obtained from difference contrasts of the relevant age- and habitat-specific mixed effects models (groups with different letters are significantly different from each other). The asterisk in panel C indicates a significant difference between source habitats. Boxes show median and interquartile range (25th to 75th), and whiskers show the data range. Dots show individual data points. Numbers below each box indicate the sample size for that group

TABLE 4 Summary of negative-binomial generalized linear models predicting pumpkinseed foraging success on either live *daphnia* cladocerans or *Echinogammarus* amphipods with adjusted brain mass, adjusted oral jaw width, standard length (mm), foraging treatment and collection source as predictors

Predictor comparison	Estimate ± SE	z	p
Daphnia (N = 49). Conditional R ² = 0.15			
Adjusted brain mass	-42.56 ± 59.84	-0.71	0.48
Adjusted oral jaw width	-0.06 ± 0.56	-0.11	0.91
Standard Length	-0.021 ± 0.014	-1.52	0.13
Foraging treatment (column-benthic)	0.1 ± 0.44	0.22	0.82
Foraging treatment (generalist-benthic)	0.13 ± 0.40	0.33	0.74
Foraging treatment (column-generalist)	-0.04 ± 0.43	-0.081	0.94
Collection habitat (pelagic-littoral)	0.45 ± 0.36	1.25	0.21
Amphipods (N = 49). Conditional R ² = 0.55			
Adjusted brain mass	304.67 ± 104.4	2.92	0.004
Adjusted oral jaw width	-2.38 ± 0.92	-2.58	0.01
Standard Length	-0.03 ± 0.021	-1.42	0.15
Foraging treatment (column-benthic)	0.35 ± 0.66	0.52	0.6
Foraging treatment (generalist-benthic)	0.38 ± 0.6	0.64	0.52
Foraging treatment (column-generalist)	-0.04 ± 0.6	-0.06	0.95
Collection habitat (pelagic-littoral)	0.41 ± 0.51	0.79	0.43

Note: Conditional R² of whole models, calculated using the MuMIn R package, are included. Bolded p values indicate significant effects.

habitat from the ancestral littoral habitat (Robinson, 2013; Rohner & Moczek, 2020; Yeh et al., 2004) because individuals capable of developing smaller relative brain size in the pelagic habitat would have reduced metabolic costs that could be allocated elsewhere. Alternatively, greater variability in the eco-cognitive requirements of inhabiting the pelagic habitat compared to the littoral habitat

may have favoured increased plasticity in brain size (Buchanan et al., 2013). Combined with other examples of divergent brain form plasticity (Crispo & Chapman, 2010; Gonda et al., 2012), these results demonstrate evolutionary lability of brain size plasticity in fish. Whether this trend is true in other vertebrate groups is not yet known.

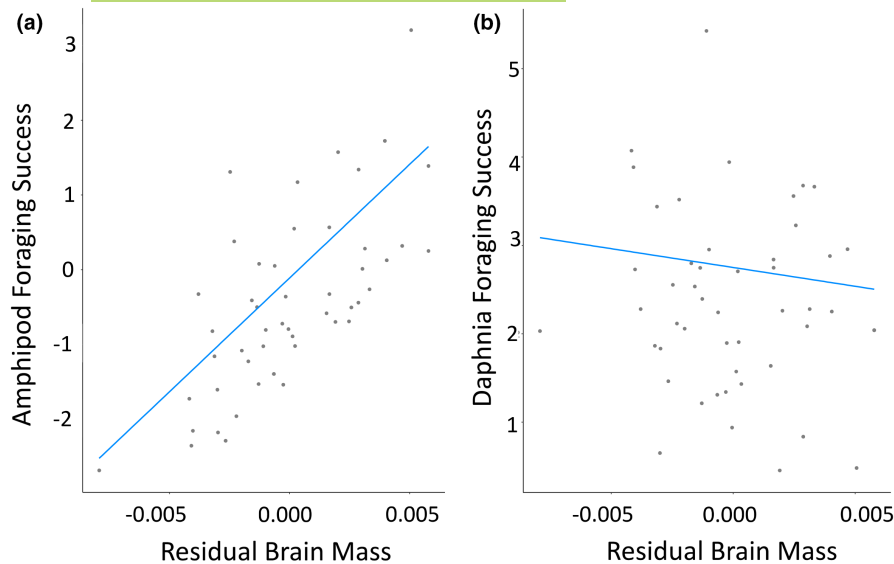


FIGURE 3 Linear relationships between residual brain mass (estimated from linear regression of brain mass against standard length) and foraging success (A: Amphipods, B: *Daphnia*). Foraging success is represented as partial residuals after accounting for other variables in the negative-binomial generalized linear models (see Table 4). Lines represent the linear relationships between residual brain mass and foraging success

4.1 | Functional variation in brain size

Variation in brain size may reflect differences in cognitive ability that functionally influences ecological performance, but also differences in energy availability, differential mortality, or patterns of genetic covariation between brain size and other traits. We interpret variation in brain size here as primarily a response to differences in the cognitive requirements of different foraging modes because we have evidence to reject alternative explanations. Differences in energy availability are unlikely to explain our result because food amount and quality were held constant among our experimental treatments and energetic limitations do not explain brain size variation in the field (Axelrod et al., 2020). Nevertheless, fish may allocate energy differently to somatic versus brain tissue, leading to differences in both body size and relative brain size. Such differential energy allocation could explain opposite patterns in somatic and brain size in age-0 fish of the column treatment as well as all age-2-3 fish (compare Figure 2 and Appendix S1: Supplementary 8), but inconsistent patterns in age-1 fish do not support this explanation.

If mortality is not random with respect to brain size, then this difference in mortality could confound the observed treatment effect. However, we believe that differential mortality is unlikely to have contributed to variation in brain size among feeding treatments because reduced survival occurred in the column foraging treatment for all age and source groups, but we only observe brain size differences in age-0 pelagic sourced fish. Ultimately, we cannot reject that mortality may reflect selection under experimental conditions because we did not make measurements on fish that died prior to the end of the experiment. Although unlikely, if selection under experimental conditions played a role, it may reduce the direct relevance of these results for natural conditions.

It is more difficult to rule out whether other traits correlated with brain size account for treatment effects in age-0 pelagic fish in the absence of detailed quantitative genetic analyses (Walker, 2010), but we consider this explanation unlikely. Head or

cranial size might seem to be a plausible correlated trait to explain differences in brain size. However, brain size is not constrained by cranium size in fish. Brains of pumpkinseed do not completely fill the braincase and instead are surrounded by abundant lipid tissue within the braincase as observed in other fish (Kotrschal et al., 1998). Oral jaw and brain sizes covary in wild adults (Axelrod et al., 2018) and in age-2+ fish here (compare Figures 1c and 2c) but do not covary in age-0 sunfish (compare Column treatments in Figures 1a and 2a). The contrasting effects of foraging treatment on age-0 oral jaws and brain size are also not consistent with a strong genetic correlation. Jaws covary with other ventral head traits in the buccal region, but the cranial (dorsal head) and buccal head regions of pumpkinseed are largely independent traits that do not covary strongly at the intraspecific scale (Jastrebski & Robinson, 2004; Robinson et al., 2000; Weese et al., 2012). In the absence of compelling evidence for other traits correlated with brain size, or of differences in energy availability among foraging treatments or mortality among source groups, we propose that differences in relative brain size more likely reflect differences in cognitive conditions between foraging treatments.

Complex foraging tasks may require greater cognitive ability achieved through larger brain size (Ahmed et al., 2017; Park & Bell, 2010). Feeding benthically on mobile or cryptic prey in a more complex littoral habitat plausibly presents greater cognitive challenges than feeding on abundant zooplankton in the water column of the pelagic habitat because benthic feeding in fishes requires greater search effort (Crowder & Cooper, 1982; Ehlinger, 1989; Ehlinger & Wilson, 1988; Warfe & Barmuta, 2004) and precise body positioning for attack (Higham, 2007). Two lines of evidence further support a functional link between brain size and cognitive performance during foraging. In pelagic age-0 fish, benthic feeding resulted in larger brains, consistent with our expectation that benthic foraging imposes additional cognitive challenges compared to feeding on small water column prey (Axelrod et al., 2020). Furthermore, our foraging performance tests revealed that brain

size was related to foraging success on mobile benthic amphipods after statistically accounting for effects of oral jaw size and body size, but was unrelated to foraging success on pelagic zooplankton prey. We did not directly measure cognitive performance related to feeding here, but we think it likely that benthic versus water column feeding modes require different cognitive responses by pumpkinseed. The induced differences in oral jaw size that replicate differences between wild pumpkinseed ecotypes coupled with habitat-specific behavioural foraging differences (e.g. Ehlinger & Wilson, 1988) strongly suggest different modes of foraging are required to find and consume benthic and pelagic prey groups. Furthermore, foraging performance can be increased by fine-tuning the control of relevant musculature which is also a component of general cognitive ability (Wainwright, 1986). Directly assessing cognition is difficult (Salena et al., 2021), but experimental approaches need to be devised that better discriminate the influence of cognitive ability from morphological effects on foraging performance. Regardless, our results demonstrate that foraging mode can induce plastic responses in brain size in pumpkinseed.

We found no evidence that temporal variability in foraging mode required greater cognitive ability. In age-0 pelagic sourced individuals, the variable treatment increased brain size to the same level as in the benthic treatment, suggesting that the cognitive challenge of the variable treatment is primarily associated with benthic feeding. A similar correspondence in body form responses occurred for fish reared in exclusively benthic and variable feeding treatments in a prior study (Parsons & Robinson, 2007). If similar responses in brain size occur under natural conditions, then the energetic costs of a larger brain used by periodic foraging on benthic prey (ie., by a trophic generalist) may be equivalent to individuals that specialize exclusively on benthic prey. Hence, only individuals that specialize on pelagic zooplankton prey may benefit from the lower energetic costs of a smaller brain (Isler & van Schaik, 2006; Kotschal et al., 2013a, 2013b; Navarrete et al., 2011; Niven & Laughlin, 2008), demonstrating a benefit to pelagic specialization that could promote ecotypic divergence (Parsons & Robinson, 2007).

Several uncertainties exist about the relationships between brain size, cognitive ability and feeding performance. For example, we cannot distinguish whether treatment cues related to foraging mode or to differences in social conditions induced brain size responses. Multiple individuals regularly interacted when feeding from a single chunk of food in the benthic treatment contributing to direct interference interactions, while such close feeding interactions were not observed in the column treatment. Among species, brain size generally increases with sociality (de Meester et al., 2019; Triki et al., 2019). Plastic brain size responses also occur in response to social environment in ninespine stickleback (Gonda et al., 2009), common frogs (*Rana temporaria*) (Gonda et al., 2010), and guppies (Kotschal et al., 2012). Such social environment effects could be removed by isolating individuals to better resolve foraging-related cues that influence brain size.

With respect to influences on foraging performance, it is unclear why smaller oral jaws were weakly associated with increased foraging performance on live amphipods. This runs contrary to previous findings of larger oral jaws in littoral fish which are more likely to feed on large benthic invertebrates (Axelrod et al., 2020, 2021; Jarvis et al., 2020). Smaller jaws may be more effective at capturing small prey from gaps in the gravel especially because the amphipod prey used here were mostly small (<4 mm) in comparison to greater prey size variation in the field (e.g. Osenberg & Mittelbach, 1989). These uncertainties demonstrate additional challenges to discerning and interpreting functional morphological effects on foraging performance.

Finally, we recognize that we did not directly measure aspects of cognition in relation to brain size. Nor were we able to evaluate whether treatment-induced changes in brain size directly influenced foraging success. Foraging performance was assessed over a short interval under conditions that are unlikely to fully replicate performance under more natural conditions, and so relationships between brain size and more realistic prey-specific feeding performance will require more careful study (e.g., Ehlinger, 1989; Ehlinger & Wilson, 1988). Nevertheless, these are the first results to demonstrate a link between brain size and prey-specific foraging performance in fish, supporting an assumption made in many comparative studies of brain size (Gonda et al., 2013).

4.2 | Ontogenetic effects on brain size plasticity

Brain size plasticity not only differed between pumpkinseed ecotypes but there was weak evidence in the model that combined all ages that it was also absent in older juveniles, indicating that brain size responses to foraging cues waned with age. Maintenance of widespread adult neurogenesis in fish (Zupanc, 2006) suggests that brain size could be plastic throughout life, but the lack of observable plasticity in brain size in sunfish age-1 and older here suggests that it has been disfavoured in these pumpkinseed. Alternatively, neurogenesis in adult fish may not be sufficient to generate plastic brain size responses throughout ontogeny. Though observable into adulthood, rates of neurogenesis have been observed to be reduced in adult fish compared to juveniles (Edelmann et al., 2013; Tozzini et al., 2012). Declining neurogenesis could reduce the scope of plastic responses in brain size. If this is the case, then the lack of life-long brain size plasticity in pumpkinseed is not an evolutionary response to changes in trophic ecology but represents a developmental constraint that may be more widespread. Although brain size plasticity has been widely observed in fish (reviewed in Gonda et al., 2013), our study is the first to demonstrate that it is not maintained over ontogeny. Testing for a similar loss of brain size plasticity with age across other populations and species will determine the generality of this phenomenon.

Plastic responses in oral jaw morphology also appeared to decline over ontogeny, suggesting that loss of plasticity in traits that

influence foraging performance with age may be a general phenomenon in these sunfish. We observed plasticity in oral jaw width in age-0 and age-1 individuals collected from both habitats, but not in older juveniles. As with brain size, this could be the result of constraints on the ability for jaws to alter their rate of growth after a certain age due to molecular or physiological limitations. Broadly, these results suggest that irreversible morphological plastic responses in these sunfish might limit the flexibility of foraging ecology in adults (Gabriel et al., 2005) and promote ecotypic divergence (Parsons & Robinson, 2007; Yeh et al., 2004).

4.3 | Study caveats

It is important to acknowledge various uncertainties about our inferences due to study design. The possibility of age-0 fish moving among tank sections motivated us to replicate source-treatment groups in a single tank using partitions. Hence, treatment differences could represent a random tank effect arising from diverging abiotic or biotic water-borne conditions. We believe that this is unlikely given the extensive pooling and rapid recirculation of water from and to all tanks in our system (see Section 2). Random effects could also reflect a room position effect that governs exposure to human activity and influence stress (Speare et al., 1995). A single purpose room housed the recirculating tank system where all tanks were adjacent to the walkway in that space, effectively homogenizing tank exposure to human activity. However, tanks were vertically arrayed in groups of three that likely reduced exposure to human activity for the top compared to middle and bottom tanks with similar higher exposure. Pelagic age-0 fish feeding from the column (bottom tank) had smaller brains than age-0 pelagic fish feeding benthically (top tank) or in the variable feeding treatment (middle tank). For age-0 littoral fish, no brain size differences occurred between treatments and the vertical tank locations were changed (ie., treatment position: benthic - bottom, column - middle, variable - top). Vertical tank position effects seem unlikely given the similarity of responses observed in the middle and top tanks in both source groups. However, we cannot exclude that other unknown random tank effects may have influenced our results here.

An additional uncertainty in our study derives from the use of wild caught fish. Pumpkinseed sunfish are difficult to rear from birth in lab conditions (B. W. Robinson, *personal observation*), which is why we opted to use wild caught individuals. Time spent in natural conditions prior to capture could have influenced trait variation among source populations due to maternal effects and/or early life plasticity. We consider this unlikely to explain divergent patterns of age-0 brain size plasticity because we would expect these effects to produce differences in mean brain size between source populations regardless of foraging treatment, which we did not observe in age-0 fish. However, prior environmental effects could explain divergence in oral jaw size and brain size between source populations in older juveniles.

5 | CONCLUSION

We evaluated the contributions of three proximal mechanisms to variation in brain size in pumpkinseed ecotypes that are functionally diverging between lake habitats. We found evidence of divergent plastic responses to feeding cues by juveniles of the ecotypes, suggesting that plastic developmental responses that shape brain size may have evolutionarily diverged between pumpkinseed ecotypes in the 12000 years since the Wisconsin glacial maximum. We also show, for the first time in fish, that the opportunity for plastic brain size responses appears to decrease over ontogeny despite the capacity for adult neurogenesis. Elucidating these proximate mechanisms is important both for our understanding of brain size evolution as well as adaptive diversification. Variation in functional plastic responses can influence adaptive diversification (Muschick et al., 2011; Robinson, 2013; Rohner & Moczek, 2020; Yeh et al., 2004). The evolutionary consequences of plasticity in brain size should be particularly relevant should this regulate an individual's ecological and social interactions through a range of sensory processes, higher integrative functions, and behavioural outputs. By linking brain size to foraging success after accounting for trophic morphology, we also provide novel evidence of an effect of brain size variation on feeding performance on more difficult to ingest benthic prey. These results suggest that the evolution of plasticity may be an important cause of variation in brain size among populations, and so encourage further studies of constraints on plastic brain size responses, and on the cognitive and ecological consequences of plasticity in brain form.

AUTHOR CONTRIBUTIONS

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

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data used in this manuscript is available at on dryad at <https://doi.org/10.5061/dryad.3tx95x6jz>

PEER REVIEW

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