

# Isolating the effects of ontogenetic niche shift on brain size development using pumpkinseed sunfish ecotypes

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## Funding information

Natural Sciences and Engineering  
Research Council of Canada,  
Grant/Award Numbers:  
RGPIN-2014-06383, RGPIN-2019-04710

## Abstract

A functional relationship between relative brain size and cognitive performance has been hypothesized. However, the influence of ontogenetic niche shifts on cognitive performance is not well understood. Increases in body size can affect niche use but distinguishing nonecologically relevant brain development from effects associated with ecology is difficult. If survival is enhanced by functional changes in ecocognitive performance over ontogeny, then brain size development should track ontogenetic shifts in ecology. We control for nonecologically relevant brain size development by comparing brain growth between two ecotypes of Pumpkinseed sunfish whose ecologies diverge over ontogeny from a shared juvenile niche. Brain size differs between ecotypes from their birth year onwards even though their foraging ecology appears to diverge at age 3. This finding suggests that the eco-cognitive requirements of adult niches shape early life brain growth more than the requirements of juvenile ecology.

## KEYWORDS

brain size, cognitive ecology, development, ontogenetic niche shift, sunfish

## 1 | INTRODUCTION

Shifts in an individual's ecology during development are ubiquitous across the animal kingdom. Such ontogenetic niche shifts (ONS) arise because body size growth generally regulates a plethora of ecological interactions related to sociality, feeding, species interactions, and avoiding predation (Nakazawa, 2015; Werner & Gilliam, 1984; Wilson, 1975). From an evolutionary perspective, selection on traits can also vary over ontogeny and shape adaptive divergence and even speciation based on variation at different life stages (de Roos, Leonardsson, Persson, & Mittelbach, 2002; Ebenman, 1992). ONS is widely observed across the animal kingdom, including in fish (Dahlgren & Eggleston, 2000; Mittelbach, Osenberg, & Leibold, 1988; Sadzikowski & Wallace, 1976), amphibians (Kolarov, Ivanovic, & Kalezić, 2011), reptiles (Subalusky, Fitzgerald, & Smith, 2001), and birds (Carravieri, Weimerskirch, Bustamante, & Chere, 2017). Less well

understood is how ecocognitive requirements may change with ONS and how this affects brain development.

The cognitive demands of an organism's ecology are difficult to directly measure, but brain size appears to relate to the cognitive requirements of particular ecological conditions. Brain size has been linked to cognitive ability (Benson-Amram, Dantzer, Stricker, Swanson, & Holekamp, 2016; Buechel, Boussard, Kotrschal, van der Bijl, & Kolm, 2018; Kotrschal et al., 2013; MacLean et al., 2014), supporting the hypothesis that brain size is related to variation in cognitive requirements likely as a consequence of larger brains having greater amounts of neurons and neuronal connectivity (Herculano-Houzel, 2005). Brain tissue is also energetically costly (Navarrete, van Schaik, & Isler, 2011; Niven & Laughlin, 2008) underscoring Jerison's principle of proper mass (Jerison, 1973), which posits that individuals will not have more brain mass than required for adequate

cognitive performance. Functional relationships between brain size and ecocognitive performance are supported by consistent relationships between brain size and ecological differences between species (Fischer, Bessert-Nettelbeck, Kotrschal, & Taborsky, 2015; Kruska, 1988; Lecchini et al., 2014) as well as between individuals within populations of fish (Axelrod, Laberge, & Robinson, 2018; Evans, Chapman, Mitrofanov, & Bernatchez, 2013; Gonda, Herczeg, & Merila, 2009; Walsh, Royles, Beston, & Munch, 2016). However, it is not clear whether such relationships necessarily also arise as ecological conditions change over ontogeny.

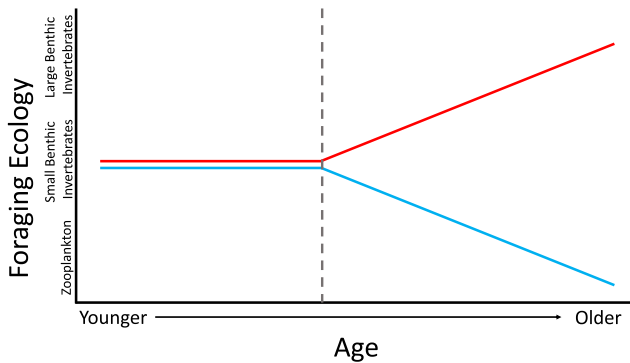
Although ontogenetic shifts in brain size and morphology (i.e., the relative size of brain regions) have been noted, specifically in fish, the link between brain structure and ONS has been largely unexplored. For example, in four species of cyprinid fishes changes in brain morphology over ontogeny reflect a general decrease in the size of the visual processing center with age as well as species-specific increases in the size of other sensory regions that seem related to each species' adult ecological niche (Brandstatter & Kotrschal, 1990). A shift from larger visual brain regions in juveniles to a greater importance of other sensory systems (and associated brain regions) in adults has been observed in many fishes, including Galaxias (*Galaxias vulgaris*; Cadwallader, 1975), Arctic Silverfish (*Pleuragramma actarctica*; Montgomery, Bjorn, & Sutherland, 1997), deep-sea grenadier (*Coryphaenoides armatus*; Wagner, 2003), and elasmobranchs (Lisney, Bennett, & Collin, 2007). Shifts in relative brain size can also occur over maturation associated with the onset of breeding (Buechel et al., 2019). However, whether changes in brain size and morphology over ontogeny functionally reflect shifts in ecology is not known.

The primary challenge to evaluating relationships between brain morphology and ecology over ontogeny is that changes in brain morphology or size caused by changes in ecology are difficult to isolate from "background" developmental changes unrelated to ontogenetic shifts in ecology. The effect of an ecological shift on brain size could be experimentally isolated from nonecological developmental changes by allocating replicate individuals with a shared ontogenetic trajectory to different ecological conditions and observing subsequent brain development. This has the advantage of testing the influence of a particular environmental condition, but at the potential cost of more realistic ecological complexity that likely shapes individual cognitive performance. Alternatively, a natural system can be observed where individuals that share a common developmental trajectory diverge ecologically over ontogeny. This provides the ecological complexity most likely to influence cognitive performance, but at the cost of identifying which specific

ecological conditions are related to cognitive performance and its attendant effects on brain size.

We used a natural population of pumpkinseed sunfish (*Lepomis gibbosus*) that is adaptively diverging between different lake habitats to isolate the effect of ONS on brain development. Pumpkinseed sunfish are freshwater centrarchids native to northeastern North America that in some postglacial lakes have diversified into ecotypes that coexist by inhabiting different habitats. Typically, pumpkinseed sunfish inhabit the shallow in-shore (littoral) lake habitat, where a set of specialized adult traits, such as large heads, shorter and widely spaced gill rakers, large oral jaws and robust pharyngeal jaws enhances feeding on benthic macro-invertebrate prey (reviewed in Robinson, Wilson, Margosian, & Lotito, 1993; Weese, Ferguson, & Robinson, 2012). In oligotrophic postglacial lakes in upstate New York and the Canadian Shield of Ontario, pumpkinseeds also inhabit the deep, offshore (pelagic) habitat, often in close proximity to submerged rocky shoals where adults feed extensively on large pelagic zooplankton (Gillespie & Fox, 2003; Jastrebski & Robinson, 2004; Robinson et al., 1993; Robinson, Wilson, & Margosian, 2000; Weese et al., 2012). Pelagic fish have smaller heads, narrowed gaps among adjacent gill rakers, narrower external jaws and less robust pharyngeal jaws. The adaptive divergence of adult ecotypes is supported by the divergent morphological traits between ecotypes across replicated populations (Jastrebski & Robinson, 2004; Robinson et al., 2000; Weese et al., 2012); trait variation that is related to prey-specific feeding performance in lab experiments (Parsons & Robinson, 2007) and to individual growth and body condition in nature (Robinson, Wilson, & Shea, 1996); and a pattern of trait divergence that parallels different sunfish species adapted to littoral and pelagic lake habitats (Riopel, Robinson, & Parsons, 2008). Despite adult differences in ecology, nonecological ontogenetic differences in brain growth between ecotypes are unlikely. We see no distinction in neutral alleles between the ecotypes (Riopel et al., 2008), suggesting minimal divergence in nonecological characteristics, either by drift or by indirect selection. However, evidence from common garden experiments suggests functional genetic differences between pumpkinseed ecotypes (Parsons & Robinson, 2006; Robinson & Wilson, 1996).

Pumpkinseed sunfish ecotypes are effective for testing questions about the importance of ecological shifts over ontogeny for shaping brain size development for two reasons. First, juvenile sunfish less than approximately 70 mm standard length in both habitats are often constrained by their small body size to a similar benthic niche where structure provides refuge from visual predators. Release from this predation risk permits the ecological differentiation of larger



**FIGURE 1** Idealized ontogeny of foraging ecology of divergent Pumpkinseed sunfish (*Lepomis gibbosus*) ecotypes (Red: littoral; Blue: pelagic ecotype). Shifts in prey type by each ecotype with age result from the functional effects of increasing body size. The dashed gray line represents the point during development when individuals reach a size large enough to release them from the burden of predation from gape limited fish predators at approximately 70 mm standard length [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

fish (Arendt & Wilson, 1997; Mittelbach, 1984; Mittelbach et al., 1988; Osenberg, Mittelbach, & Wainwright, 1992; Osenberg, Werner, Mittelbach, & Hall, 1988). A graphical representation of our expectation of this divergence in foraging ecology during ontogeny can be seen in Figure 1. Ecotypes of pumpkinseed sunfish can mature at age 3 (Fox, 1994; Gillespie & Fox, 2003), and adults of the two ecotypes spatially segregate by spawning at benthic sites in the inshore littoral habitat or offshore on submerged rocky shoals in the pelagic habitat (Colborne, Garner, Longstaffe, & Neff, 2016; Jastrebski & Robinson, 2004). Age-0 juveniles also reliably recruit to each habitat after a short, approximately, 1-month larval stage (B.W. Robinson, personal observation). Importantly, the divergence into different adult ecological niches can be estimated by comparing a resource-related morphological trait, oral jaw width, between the habitats, where larger jaw width (adjusted for body size) is correlated with greater use of larger benthic invertebrate prey (W. M. C. Jarvis, Comeau, Colborne, & Robinson, 2017). Second, different ecological conditions between lake habitats likely shape the cognitive requirements of adult pumpkinseed sunfish because brain size differs between adult ecotypes. The brains of littoral individuals are on average 8.3% larger than for pelagic individuals, with no differences between the sexes or differences in brain region morphology (Axelrod et al., 2018). Additionally, brain size variation within habitats is related to oral jaw width, suggesting that individuals that make more use of larger benthic invertebrate prey require larger brains than individuals that feed on zooplankton prey in the water column (Axelrod et al., 2018).

We compare brain size development between pumpkinseed ecotypes that diverge in ecology over ontogeny to

isolate the effects of shifting ecological conditions on brain growth from nonecological developmental changes. The hypothesis that changes in brain size over ontogeny are partially caused by ONS predicts that brain size does not diverge between juveniles of the two ecotypes while they share a common benthic niche and only diverges as each ecotype shifts into different adult niches, as signaled by a divergence in oral jaw width.

## 2 | METHODS

### 2.1 | Sampling

Samples of pumpkinseed sunfish were collected from Ashby Lake, Ontario (45.092N, 77.351W). Age-0 sunfish (~13–26 mm) were collected via hand netting from five littoral and five pelagic sites in August of 2016 ( $n = 103$ ). At the same time, larger juvenile sunfish (~30–60 mm) were collected using minnow traps from five littoral and five pelagic sites ( $n = 139$ ). Adult sunfish (~70–125 mm) were collected via angling from four littoral and four pelagic sites in August of 2016 ( $n = 50$ ) and 2017 ( $n = 81$ ). Data on relative brain size of adult sunfish was published in Axelrod et al. (2018). All captured fish were euthanized with an overdose of clove oil (100 ppm) and preserved in 10% buffered formaldehyde. Sampling procedures were approved by the University of Guelph animal care committee under the *Guidelines of the Canadian Council on Animal Care*.

### 2.2 | Processing

All fish were assigned uninformative labels to avoid unconscious bias during processing. They were then blotted dry and weighed. The standard (body) length and oral jaw width (maximum distance between maxillaries) was measured using digital callipers for adult and larger juvenile fish (age-1 and greater). An ocular micrometer (Leica MZ-8 dissection microscope) was used to measure jaw width of age-0 fish. Fish age was estimated from annuli counts on a minimum of four scales per individual mounted on slides and viewed using a dissecting microscope. Since fish are born in the summer, fish sampled in the summer of their birth are classified as age-0. Adult heads were removed, a small incision was made in the brain case and heads were replaced in buffered formaldehyde for an additional 24 hr to allow postfixation of brain tissue. No postfixation was used for juvenile fish. Whole brains of all fish were removed by dissection. The spinal cord of each brain was trimmed at the obex and excess cranial nerves were removed. The brains were then blotted to remove excess formaldehyde

and weighed using an Accu-124D scale (Fisher Scientific) at a resolution of 0.0001 g. Samples preserved in formaldehyde can shrink and so affect estimates of brain mass (F.L. Laberge, personal observation). It seems reasonable to assume a uniform rate of shrinkage for both ecotypes and so this is unlikely to bias our results because samples of both ecotypes were processed at the same time.

## 2.3 | Statistical methods

To test the hypothesis that brain size development is related to ONS, we first estimate the age when feeding ecology diverges between the sunfish ecotypes using oral jaw width as a morphological proxy (Axelrod et al., 2018; W. M. C. Jarvis et al., 2017), and then test whether brain size, estimated using brain mass, diverges at the same developmental point. The relationship between oral jaw width and habitat, as well as relationships between brain mass and predictor variables, were assessed using linear mixed-effects models. Standard length was included as a covariate in all jaw size and brain mass models to correct for body size. We use standard length as a measure of body size because it is unaffected by short term changes in body condition that affect body mass. Brain mass, jaw size, and body length were log-transformed to normalize model residuals. We assess the developmental points at which ecology and brain mass diverge between the habitats with a separate model for each age class, other than ages 4, 5, and 6, which were combined as these age classes share common ecological conditions within each habitat. Sunfish can reach maturity as early as age 3 (Fox, 1994; Gillespie & Fox, 2003) and so we include a model for age 3 fish to increase the specificity of our analysis of ontogenetic events. The final jaw size and brain mass models for each age group included standard length and capture habitat as fixed effects, as well as collection site within each habitat as a random effect. An interaction between habitat and standard length was initially included in all models but was subsequently excluded when it was not statistically significant ( $\alpha > 5\%$ ; Table 1).

## 3 | RESULTS

Juvenile sunfish of both ecotypes appear to share a common juvenile niche because oral jaw width only diverges later in ontogeny at age 3. Mean relative oral jaw width (adjusted for standard length) was similar between sunfish sampled from the two habitats until fish were at least 3 years old, when littoral fish expressed on average

larger oral jaws than those from the pelagic habitat (Table 1, Figure 2). Divergence in this resource-related trait occurred at the body size of approximately 70 mm standard length (Figure 3), which is the size expected to release sunfish from most forms of fish predation. The standard length of all age classes within each habitat can be seen in Figure 3. Within each age class, oral jaw width allometry did not differ between habitats, except for age-0 fish where there was some evidence that the width of oral jaws of pelagic individuals increased more quickly with body size than for age-0 littoral individuals (body length by habitat interaction, Table 1).

There was no evidence that a divergence in brain mass between sunfish from different habitats corresponded with the divergence in oral jaw width between ecotypes at age 3. Littoral sunfish aged 1 and older all exhibited larger mean brain mass compared to pelagic fish, with no difference between habitats in the slope of the body length-brain mass regressions (Figure 4b-e, Table 1). Brain growth was faster in the natal summer in littoral sunfish, indicated by a steeper allometric relationship between standard length and brain mass in littoral age-0 compared to pelagic age-0 sunfish (Figure 4a, Table 1).

## 4 | DISCUSSION

Prior work has shown that adult pumpkinseed sunfish in the littoral habitat of Ashby Lake have brains approximately 8.3% larger than sunfish inhabiting the pelagic habitat (Axelrod et al., 2018). Here, we investigated the ontogeny of this brain size difference to better understand how it may be related to adult niche divergence. Littoral pumpkinseeds had consistently larger relative brain size through all ages and faster brain growth at age-0 than pumpkinseeds sampled from the pelagic habitat. This is despite evidence that an important trophic trait, oral jaw width, only diverged between ecotypes at age-3, when at a larger body size fish can access divergent adult niches. Hence, brain size divergence between ecotypes of pumpkinseed sunfish does not appear to be directly linked to changing eco-cognitive performance demands related to divergent ontogenetic niche shifts. Instead, we suggest that the cognitive ecology of the adult stage is more important to shaping life-long brain size variation than the ecology of juvenile stages for these sunfish ecotypes.

We expected and found evidence that trophic ecology changes over ontogeny in both the littoral and pelagic ecotypes of pumpkinseed sunfish. In northern populations, sunfish less than approximately 70 mm standard length are limited to occupying benthic

**TABLE 1** Summary of linear mixed-effects least square model results predicting oral jaw width and brain mass

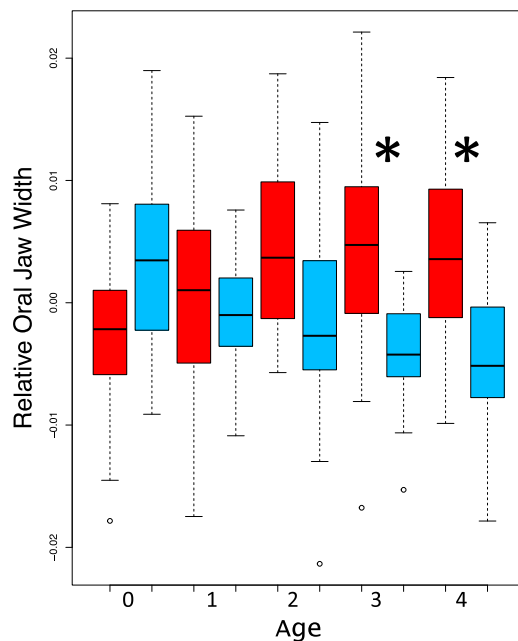
Response variable	Predictor variable	Estimate + SE	t-Value	Error DF	p-Value
Age 0					
Jaw width	SL	0.94 ± 0.055	17.3	89	<.0001
	Habitat	-0.45 ± 0.24	-1.88	8	.10
	SL*Habitat	0.17 ± 0.081	2.09	89	<b>.039</b>
Brain mass	SL	2.12 ± 0.31	19.71	89	<.0001
	Habitat	0.95 ± 0.11	2.1	8	.071
	SL*Habitat	-0.34 ± 0.15	-2.2	89	<b>.03</b>
Age 1					
Jaw width	SL	1.11 ± 0.12	9.13	36	<.0001
	Habitat	-0.036 ± 0.035	-1.03	8	.33
Brain mass	SL	1.5 ± 0.089	16.8	36	<.0001
	Habitat	-0.052 ± 0.022	-2.33	8	<b>.048</b>
Age 2					
Jaw width	SL	0.72 ± 0.11	6.27	42	<.0001
	Habitat	-0.059 ± 0.025	-2.34	7	.052
Brain mass	SL	1.58 ± 0.095	16.71	42	<.0001
	Habitat	-0.11 ± 0.021	-4.95	7	<b>.0016</b>
Age 3					
Jaw width	SL	1. ± 0.054	21.18	36	<.0001
	Habitat	-0.088 ± 0.022	-4.08	7	<b>.0047</b>
Brain mass	SL	1.69 ± 0.064	26.51	36	<.0001
	Habitat	-0.11 ± 0.024	-4.66	7	<b>.0023</b>
Age 4+					
Jaw width	SL	1.03 ± 0.046	22.34	75	<.0001
	Habitat	-0.09 ± 0.015	-6.19	6	<b>.0008</b>
Brain mass	SL	1.32 ± 0.052	25.32	75	<.0001
	Habitat	-0.091 ± 0.016	-5.53	6	<b>.0015</b>

Note: Models included standard body length as a covariate (SL), source habitat (littoral vs. pelagic), and when it is significant, an interaction term between SL and habitat. A positive estimated effect for habitat indicates a larger response value in the pelagic habitat; and for the SL × Habitat interaction term, a higher slope in the pelagic habitat. All  $p < .05$  are bolded.

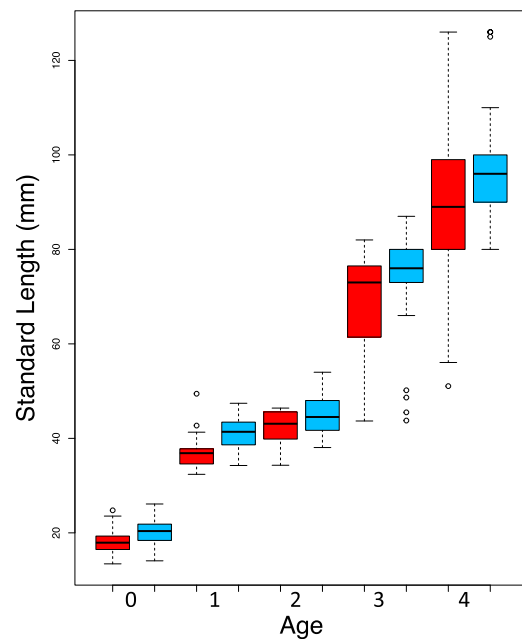
conditions where structure provides refuge from visual predators but where competition over benthic invertebrate resources can be intense (Mittelbach, 1984; Mittelbach et al., 1988, Osenberg et al., 1988). Our body size results further support the hypothesis of a shared juvenile niche as we see an apparent drop in growth rate between the ages of 1 and 2 years old in both ecotypes, consistent with a shared competitive benthic habitat until age 3 (see Figure 3). Adult niche space is partitioned only above this size threshold. In Ashby Lake, both habitats provide refuge structure to small fish to escape from predation. Coarse woody debris, macrophytes, and large cobble are present in the littoral habitat. In the offshore pelagic habitat, a variety of boulder sizes create many cracks and crevices used by small sunfish on submerged shoals that function like

reefs (Jastrebski & Robinson, 2004). Smallmouth bass (*Micropterus dolomieu*) predators are abundant in both habitats (C.J. Axelrod, personal observation). The divergence in oral jaw width between age-3 ecotypes, when fish reach approximately 70 mm standard length, is consistent with a reduction in predation risk with size and an expansion of resource use by adults and larger sub-adults beyond benthic refugia (Mittelbach, 1984). We suggest that this allows pelagic adults to use abundant zooplankton prey while littoral adults can use increasingly larger benthic invertebrates such as snails (Gillespie & Fox, 2003; Jastrebski & Robinson, 2004; Robinson et al., 1993; Weese et al., 2012). Our hypothesis that brain size divergence between ecotypes would be causally linked to this ontogenetically based trophic divergence is not supported.





**FIGURE 2** Boxplots of relative oral jaw width (jaw width, mm/standard length, mm) of each age class 0–4+-years-old of sunfish in the littoral (red) and pelagic (blue) habitats of Ashby Lake. The asterisks show ages for which the mixed-effects models revealed a significant effect of habitat on oral jaw width. Values are normalized around a mean of 0 in each age class. Boxes show interquartile ranges (25th to 75th) separated by a black line representing the median, with whiskers expressing minimum and maximum values excluding outliers shown as circles [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]



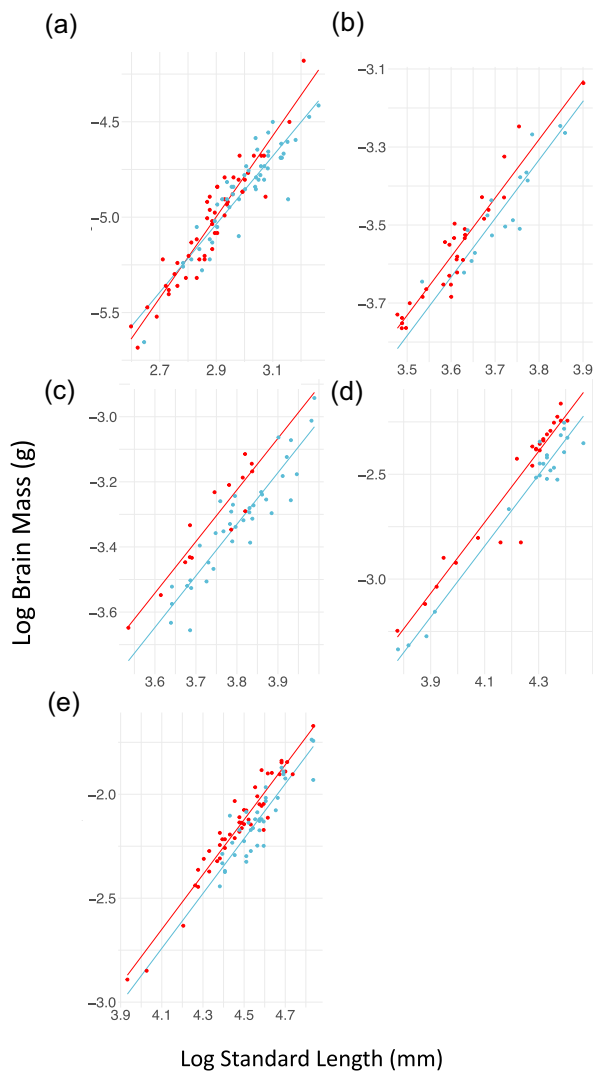
**FIGURE 3** Boxplots of standard length of each age class 0–4+-years-old of sunfish in the littoral (red) and pelagic (blue) habitats of Ashby Lake. Boxes show interquartile ranges (25th to 75th) separated by a black line representing the median, with whiskers expressing minimum and maximum values excluding outliers shown as circles [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

#### 4.1 | Potential causes of variation in juvenile brain growth

Three explanations could account for the observed differences in brain size between sunfish ecotypes: habitat-specific differences in available energy, ecological differences between habitats that are not associated with resource use, and selection for early life brain growth arising from diverging adult ecological conditions. The available energy hypothesis predicts faster brain growth and larger brains in the habitat with the greater available energy (Navarrete et al., 2011; Niven & Laughlin, 2008). Our evidence suggests that energy is more available in the pelagic compared with the littoral habitat throughout life, including age-0 fish (Figure 3). Larval and age-0 juvenile sunfish feed on zooplankton in both habitats but large zooplankton prey are approximately three fold more abundant in the pelagic habitat and make up a larger part of age-0 pelagic sunfish diet (B.W. Robinson unpublished results). Age-0 juveniles from the pelagic habitat also have a mean body size approximately 11% larger than samples from littoral habitats, congruent

with superior growth in the pelagic habitat that results from more abundant zooplankton resources (Figure 3). These patterns of superior growth indicate greater energy availability in the pelagic rather than littoral habitat, and so cannot explain why sunfish from the littoral habitat allocate more energy early on to develop larger brains.

Second, juvenile ecology could differ between the habitats in ways unrelated to foraging, such as predation pressure or sociality. Predator presence has been shown to influence brain size in killifish (Walsh et al., 2016), with predator-rich environments selecting for smaller brains than predator-free environments, potentially due to greater energetic investment in somatic tissue. Larger brains have also been shown to improve behavioral performance in the presence of predators in guppies artificially selected for larger or smaller brains (van der Bijl, Thyselius, Kotrschal, & Kolm, 2015). Smallmouth bass seem equally abundant in littoral and pelagic habitats in Ashby Lake suggesting that bass predation risk could be quite similar across habitats. Nevertheless, predator avoidance strategies may differ between habitats because of differences in water depth (shallower in the littoral habitat) or refuge structure (requiring more active predator alertness in the structurally more complex littoral habitat). These differences in the ecocognitive impacts of habitat-specific predation risk, unrelated to resource use, could influence brain size.



**FIGURE 4** Linear fits of the relationship between brain mass and standard length (both natural log-transformed) after adjusting for other factors in the mixed-effects models for sunfish sampled from littoral (red) and pelagic (blue) habitats. Panel A shows age 0 individuals, B age 1, C age 2, D age 3, and E age 4-6 years. Data in panels D-E was published in Axelrod et al. (2018) [Color figure can be viewed at [wileyonlinelibrary.com](http://wileyonlinelibrary.com)]

Brain size also can be influenced by sociality (deMeester, Huyghe, & van Damme, 2019), with larger brains generally being associated with more social environments because social environments are thought to be more cognitively challenging. Sunfish are social fish and have been used in studies of dominance (e.g., Beacham & Newman, 1987). Mark-recapture studies of sunfish in Ashby Lake show that sunfish density is approximately eightfold greater in the pelagic shoal habitat than in the littoral habitat due to the latter habitat's greater volume (Jarvis, 2018), suggesting that social interactions could be more prevalent in the pelagic habitat. For example, male sunfish nesting on pelagic shoals appear to experience greater social interaction during

spawning than in the littoral habitat based on in situ remote video (B.W. Robinson, unpublished results). However, the greater opportunity for sociality on pelagic shoals would typically predict larger brains in the pelagic habitat for all ages, which we did not find. Although we believe that foraging ecology is likely the main determinant of brain size differences between ecotypes, we cannot rule out that other ecological factors unrelated to foraging may differ between habitats to influence brain size.

Third, differences in brain size between the ecotypes as early as age 0 may suggest that selection could have shaped divergent brain growth trajectories in this system, favoring individuals with quicker brain growth in the littoral habitat because of differences in adult cognitive requirements. Previous work has suggested that foraging performance in the littoral and pelagic habitats is affected by ecologically relevant traits (Ehlinger & Wilson, 1988; Parsons & Robinson, 2007; Robinson et al., 1996) that likely include oral jaw width (W. M. C. Jarvis et al., 2017), indicating both that habitat use is different between adult fish and that success as adults is influenced by functional traits related to trophic divergence. The brain size differences between adult fish occupying these habitats found by Axelrod et al. (2018) show that these ecological conditions likely involve different cognitive requirements. These results suggest that brain growth trajectories in early life have diverged between the ecotypes as a result of selection for adult ecological requirements. This hypothesis requires that there is a limit on the ability of pumpkinseeds to shift their brains to match changes in current ecological conditions. One possibility is that adult differences in brain size and cognitive performance requirements are large enough that it would take a long time to fully develop these differences. Another possibility is that there are constraints that limit the ability of adult sunfish to change their brain in accordance with their ecological requirements, potentially as a result of reduced neurogenesis rates in adulthood (Edelmann et al., 2013; Tozzini, Baumgart, Bettistoni, & Cellerino, 2012). In either case, early brain growth differences between the ecotypes could benefit the lifetime fitness of individuals by easing their transition into the eco-cognitive requirements of adulthood.

## 4.2 | Implications of brain size variation

The difference in early life brain size between the ecotypes suggests that brain size variation in this population is determined either by inherited genetic differences, or by early-life environmental programming. The proximate mechanism that generates trait variation in intraspecific systems is important to understand because it can alter the ecological and evolutionary consequences

of changing environmental conditions (Donelson et al., 2019). Inherited genetic differences and phenotypic plasticity have both been shown to play a role in shaping brain size variation in fish populations. For example, using a common garden experiment, Walsh et al. (2016) found that predator regime-based differences in brain size of male killifish are primarily genetically based. Additionally, Kotrschal et al. (2013) demonstrated a genetic basis for brain size variation by artificially selecting for large and small-brained guppies. Other studies have shown that fish brains can plastically change in response to environmental conditions. For example, Gonda, Herczeg, and Merilä (2011) found that that brain morphology variation between marine and freshwater populations of ninespine stickleback (*Pungitius pungitius*) largely reflected plastic responses to the environment. Additionally, Eifert et al. (2015) found plastic effects of light environment on brain morphology in neotropical extremophile fish (*Poecilia mexicana*). Environmental effects on brain size could occur through early life sensory cues, epigenetic inheritance (Bludau, Royer, Meister, Neumann, & Menon, 2019), or maternal effects (Jasarevic, Rodgers, & Bale, 2015). The proximate mechanism generating the life-long differences in brain size between sunfish ecotypes remains unclear and further research may clarify this issue.

Finally, our data also suggest that brain tissue growth might not be as constrained by energetic costs as has been previously expected, at least during certain life stages. Brain tissue is understood to be energetically costly (Navarrete et al., 2011; Niven & Laughlin, 2008), and relaxed selection for greater cognitive ability has been shown to result in the evolution of smaller brains (Safi, Seid, & Dechmann, 2005). Our results show that sunfish living in the littoral habitat in their first 2 years of life have larger brains than pelagic individuals, without an apparent concurrent ecological benefit, contrary to Jerison's principle of proper mass. As reviewed by Stearns (1989) and Schluter, Price, and Rowe (1991), trade-offs due to conflicting selection pressures at different stages of life can be important for shaping trait evolution. Our results suggest that the energetic cost associated with a large brain size might be tolerated in early life stages if it is offset by benefits in the adult life stage.

## 5 | CONCLUSION

It is difficult to test the influence of ecological shifts across ontogeny on brain size development because it may be confounded with background brain developmental processes unrelated to niche shifts. Our comparison of wild-caught pumpkinseed sunfish ecotypes that

share a common benthic niche as small juveniles and diverge into distinct littoral and pelagic adult niches revealed that brain size development is not temporally related to the divergence into adult niches. Despite a similar benthic ecology as small juveniles, sunfish living in the littoral habitat exhibited faster brain growth in their natal summer season and maintained a larger relative brain size as they matured compared to pelagic individuals. This suggests that the ecocognitive performance of adults may be more important in shaping brain size development than ecocognitive performance of small juveniles. Confirming this observation in other evolutionarily independent populations would strengthen this preliminary conclusion. These findings demonstrate the importance of a developmental perspective on studying brain size diversity to explore the links between brain size, cognition, and ecology.

## ACKNOWLEDGMENTS

This work would not have been possible without the support of the Ashby Lake Protective Association, particularly R. C. Gautier. The authors thank Nick Sakich, Will Jarvis, Colby Nolan, and Helmi Hess for field assistance. The authors also acknowledge funding for this research from the Natural Sciences and Engineering Research Council of Canada in the form of Discovery grants to each of Beren W. Robinson (RGPIN-2019-04710) and Frédéric Laberge (RGPIN-2014-06383).

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**How to cite this article:** Axelrod CJ, Laberge F, Robinson BW. Isolating the effects of ontogenetic niche shift on brain size development using pumpkinseed sunfish ecotypes. *Evolution & Development*. 2020;22:312–322. <https://doi.org/10.1111/ede.12333>