

# Food Web Structure Shapes the Morphology of Teleost Fish Brains

Nicholas B. Edmunds · Kevin S. McCann · Frédéric Laberge

Department of Integrative Biology, University of Guelph, Guelph, Ont., Canada

## Key Words

Fish · Brain anatomy · Food web · Habitat use · Trophic position · Within-species centering · Trade-offs

## Abstract

Previous work showed that teleost fish brain size correlates with the flexible exploitation of habitats and predation abilities in an aquatic food web. Since it is unclear how regional brain changes contribute to these relationships, we quantitatively examined the effects of common food web attributes on the size of five brain regions in teleost fish at both within-species (plasticity or natural variation) and between-species (evolution) scales. Our results indicate that brain morphology is influenced by habitat use and trophic position, but not by the degree of littoral-pelagic habitat coupling, despite the fact that the total brain size was previously shown to increase with habitat coupling in Lake Huron. Intriguingly, the results revealed two potential evolutionary trade-offs: (i) relative olfactory bulb size increased, while relative optic tectum size decreased, across a trophic position gradient, and (ii) the telencephalon was relatively larger in fish using more littoral-based carbon, while the cerebellum was relatively larger in fish using more pelagic-based carbon. Additionally, evidence for a within-species effect on the telencephalon was found, where it increased in size with tro-

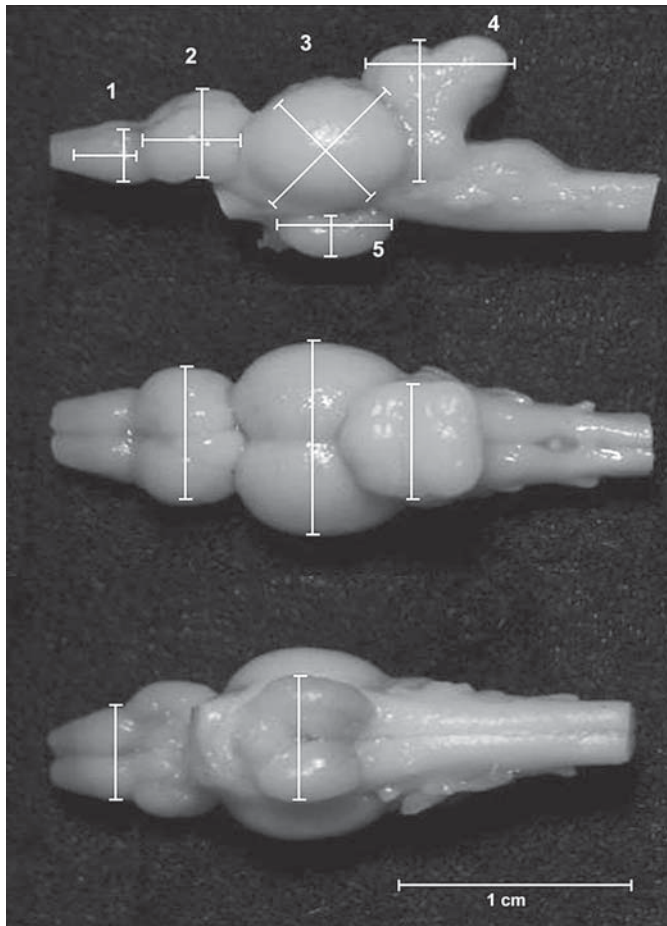
phic position. Collectively, these results suggest that food web structure has fundamentally contributed to the shaping of teleost brain morphology.

© 2016 S. Karger AG, Basel

## Introduction

Cognitive demands resulting from interactions between organisms and their environments are thought to require matching amounts of neural processing by brain tissue dedicated to that function [Jerison, 1973]. Changes to brain size or morphology (region-specific changes) require strong cognitive pressure because of the high energetic costs of neural tissue [Niven and Laughlin, 2008; Navarrete et al., 2011]. Therefore, variation in brain size and morphology could reflect how different organisms have adapted to the specific demands of their environment.

Environmental demands have previously been shown to alter the size and morphology of teleost fish brains [e.g. Kotrschal et al., 1998; Gonzalez-Voyer and Kolm, 2010]. Typically, these changes are studied over evolutionary time in the diversity of species radiations. For example, changes in brain size and morphology have been linked to social structure and the physical environment across



**Fig. 1.** Brain regions examined in this study. From top to bottom: lateral, dorsal and ventral views of a walleye brain. The top image labels show the left olfactory bulb (1), telencephalon (2), optic tectum (3), cerebellum (4) and hypothalamus (5). Lines represent measurements made on each brain region. Volumes were obtained by applying the ellipsoid equation  $V = (L \times W \times H)\pi/6$ . Scale bar = 1 cm.

closely related species of African cichlids [van Staaden et al., 1994; Huber et al., 1997; Pollen et al., 2007], or diet and habitat use between genetically distinct populations of sticklebacks [Gonda et al., 2009; Park and Bell, 2010]. Additionally, recent evidence suggests an ecological role for natural variation in brain size within species, or even brain plasticity in individuals [Gonda et al., 2009, 2011; Kotrschal et al., 2012, 2013]. Evidence for fish brain plasticity has been found when examining brain development in response to environmental simplification [Ebbesson and Braithwaite, 2012; Park et al., 2012], changes in sensory brain morphology during ontogeny [Brandstätter and Kotrschal, 1990; Montgomery et al., 1997; Wagner,

2003; Lisney et al., 2007; Salas et al., 2015] and changes across seasons [Dunlap et al., 2001; McCallum et al., 2014]. The mechanism for such changes could involve neurogenesis, which is a lifelong process in many parts of the brain in fish [Zupanc, 2006; Kaslin et al., 2008].

Total brain size has been examined in an attempt to relate cognitive abilities to ecological patterns. For example, Edmunds [2015] used relative and absolute brain size to relate fish cognitive ability to the structure of food webs, and Kondoh [2010] used brain size as a proxy for learning abilities in fish predator-prey relationships. However, because the brain is modular and regions within the brain have specific functions, it is unclear what changes in total brain size truly represent [Healy and Rowe, 2007]. Furthermore, variation in the size of specific regions that have known functions may point to the ecological and evolutionary processes that have led to this brain variation. For example, changes in brain morphology could result from a concerted increase or decrease in size across all brain regions, or in a mosaic fashion where only one or a few regions change in size while other regions remain unchanged [Finlay and Darlington, 1995; Barton and Harvey, 2000; Gonzalez-Voyer and Kolm, 2010; Noreikiene et al., 2015]. Thus, studies investigating size variation in specific brain regions are important for determining what factors influence patterns of change in brain size.

The present study assessed if the relative size of specific brain regions (both in proportion to brain mass or body mass) relate to fish habitat use and foraging abilities. Five brain regions were chosen for examination: the olfactory bulbs, telencephalon, optic tectum, cerebellum and hypothalamus (walleye brain; fig. 1). These regions were selected because of their significance to ecological interactions and their distinct and measurable morphology. Table 1 highlights studies on the ecological role of each of these regions. The olfactory bulbs are paired structures, located at the rostral end of the brain, that receive chemosensory input from olfactory receptor neurons located in the olfactory rosettes [Hara, 1992; Laberge and Hara, 2001]. Projection neurons of the olfactory bulbs (the mitral cells) then send olfactory information to the telencephalon [Becerra et al., 1994]. The telencephalon is the primary site for higher-order integrative brain functions [Davis et al., 1981; Davis and Kassel, 1983]. It receives abundant input from other brain regions and displays extensive intra-regional local connections. The telencephalon is involved in learning, memory and complex behaviors, such as spatial navigation [Davis and Kassel, 1983; Demski, 1983; Portavella et al., 2002]. The superficial lay-

**Table 1.** Brain regions examined in this study, their known functions, and suggested links between the relative region size and ecology

Brain region	Known functions	Suggested ecological links	References
Olfactory bulb	olfaction	– diet – predator avoidance/prey detection	Huber et al., 1997; Pollen et al., 2007
Telencephalon	learning, sensory integration, spatial navigation	– habitat use – diet – feeding behavior	Huber et al., 1997; Pollen et al., 2007; Gonzalez-Voyer and Kolm, 2010; Park and Bell, 2010
Optic tectum	vision, orienting response	– diet – predator avoidance/prey detection	Huber et al., 1997; Pollen et al., 2007
Cerebellum	motor coordination, motor learning	– habitat use – diet – feeding behavior	Huber et al., 1997; Pollen et al., 2007; Gonzalez-Voyer and Kolm, 2010
Hypothalamus	neuroendocrine control, mating, behavior regulation	– complex mating behaviors – social organization	Pollen et al., 2007; Gonzalez-Voyer and Kolm, 2010

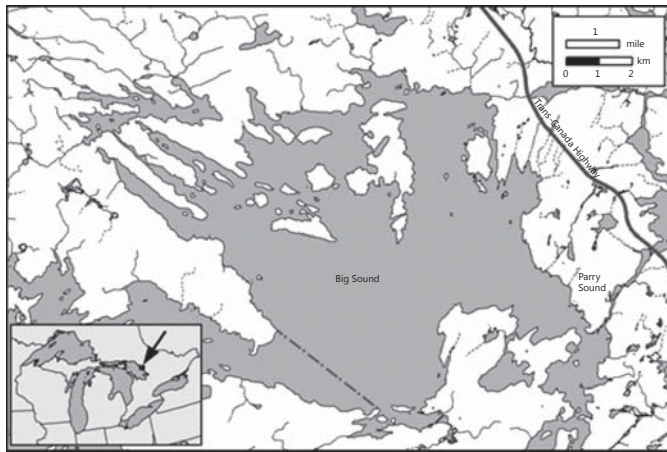
ers of the optic tectum receive visual information directly from the eye retinal ganglion cells via the optic nerve. The deeper layers of the optic tectum receive sensory afferents from multiple sensory modalities. This organization is thought to be involved in orienting responses toward salient sensory stimuli in the environment [Northcutt, 1983]. The cerebellum is responsible for the coordination of motor activity, balance in the water column and motor learning [Demski, 1983; Butler and Hodos, 1996]. The hypothalamus is involved in neuroendocrine and behavior regulation, such as the coordination of hormonal and behavioral responses during stress and reproduction [Peter and Fryer, 1983; Butler and Hodos, 1996].

Trophic relationships in aquatic systems contribute to the structure of food webs [Vander Zanden et al., 2000]. Measuring the trophic position of fish provides a continuous classification of the functional role of individuals (i.e. predator, consumer, omnivore, etc.) [Post, 2002]. The trophic position can also reveal variation in diet that exists within and between species [Vander Zanden and Rasmussen, 1996]. Lower trophic positions are generally smaller nonpiscivorous fish that feed close to the base of the food web on zooplankton, invertebrates and vegetation. Fish at higher trophic positions, on the other hand, are generally piscivorous – feeding on a variety of other fish species. It is thought that fish mobility and behavioral complexity increase with trophic position, and therefore successful foraging at higher trophic positions may require increased cognitive abilities [Warren and Lawton, 1987; Rooney et al., 2008]. Feeding at higher trophic positions may place demands on brain structures such as the telencephalon for enhanced learning abilities (table 1). Additionally, successful predation or avoidance

of predators is dependent upon sensory abilities to detect nearby prey or warn of approaching predators [Johannesen et al., 2012; Kotrschal et al., 2015; van der Bijl et al., 2015]. For this reason, selection for larger sensory brain regions (olfactory bulbs and optic tectum) across a trophic position gradient may also be seen (table 1).

Typically, in freshwater lakes, habitat types can be divided into the pelagic open water and littoral shallow water environments [France, 1995]. These different habitats may place different cognitive pressures on the fish that inhabit them. Littoral near-shore environments have comparably smaller vertical areas to traverse (i.e. are two-dimensional) and are structurally complex due to rocks, plants and dead organic debris, which may provide both refuge for prey and ambush cover for predators. Conversely, pelagic environments are structurally simpler, large open spaces (i.e. are three-dimensional). Thus, foraging behavior is likely to vary between these two habitats and dissimilar cognitive demands in each habitat may require greater capacities in different brain structures for optimal function. For example, since the fish telencephalon has been implicated in spatial cognition by lesion studies [Rodriguez et al., 2002; Portavella and Vargas, 2005], selection for a larger telencephalon may be favored for superior spatial navigation skills in response to the structural complexity of littoral environments, while species residing in open water pelagic zones may favor a larger cerebellum for better three-dimensional movement coordination (table 1). Furthermore, use of both littoral and pelagic habitats (coupling) involving movement from place to place and additional habitat-specific cognitive demands may require morphological attributes needed in both habitats.





**Fig. 2.** Location of the sampling site in Big Sound Bay, Georgian Bay, Parry Sound, Ontario, Canada.

A food web approach and stable isotope techniques were used to assess the relationship between ecological variables and brain morphology in 99 adult individual fish from 16 species sampled from the same lake. Because variation in brain morphology may be present both within and between species, we used the ‘within-group centering’ method described by van de Pol and Wright [2009] to examine these effects independently. Between-species effects represent the product of evolution, whereas within-species effects may result from phenotypic plasticity, influencing brain growth in individuals, or natural variation present within a population. This technique was used in an attempt to elucidate the current and past evolutionary pressures acting to shape the morphology of teleost fish brains in a food web.

## Material and Methods

### Sampling and Preparation

Fish were collected from Big Sound Bay in Lake Huron near Parry Sound, Ontario, Canada (fig. 2). Collection occurred over the periods August 17–24, 2013, and August 23–29, 2014, using a variety of techniques, including angling, minnow traps, seine nets and gill nets. A total of 99 adult fish from 16 species were used in this analysis (table 2). Big Sound Bay is cut off from the rest of Lake Huron’s Georgian Bay by a channel approximately 6 km long. Sampling and procedures were approved by the Ontario Ministry of Natural Resources (permit No. UGLMU2013-06a, UGLMU2014-07) and the University of Guelph animal care committee.

Fish were weighed (g) and measured (cm); large fish were weighed with a Rapala Pro Select Digital Scale (50-lb capacity) and small fish were weighed with a balance scale (Mettler Toledo PB1502-S, Columbus, Ohio, USA). Fish body cavities were opened to allow the examination of reproductive organs. They were recorded as ‘male’, ‘female’, or ‘unknown’ if the sex was unidenti-

able (i.e. immature or very small fish). Muscle samples were then taken from the dorsal caudal end for use in stable isotope analysis of  $^{13}\text{C}$  and  $^{15}\text{N}$ . For large fish, the heads were then removed from the body and the lower jaws were detached. The base of the spinal cord was then exposed so that fixative could penetrate the cranial cavity and fix the brain. Fish heads and entire small fish were placed into buffered neutral formalin (10%) for fixation, where they remained until further dissection. In addition to fish, bivalve mussels and littoral snails were collected for use as baselines in stable isotope analysis. These organisms accurately reflect within-lake spatial differences in  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  between shallow water littoral and open water pelagic zones [Post, 2002].

During the months following field sampling (September to December 2013 and 2014), brains were removed from the heads of individual fish. The brains were trimmed of excess cranial nerves and the spinal cord was cut at the level of the obex before blotting to remove excess formalin and weighting using a Fisher Scientific accu-124D scale (with a resolution of 0.0001 g). In addition, the average weight of the eyes was acquired from all 99 fish used in this study and the average weight of the olfactory rosettes was acquired from lake trout, walleye, cisco and whitefish individuals.

### Brain Region Measurements

Digital images of the dorsal, ventral and left sides of the brain were taken through an Olympus SZ61 dissection microscope using a Cannon Powershot G9 digital camera and PSREMOTE v.1.7 software. The length, width and depth was measured (to the nearest 0.01  $\mu\text{m}$ ) on the digital images for each of the 5 brain regions studied (see fig. 1 for measurement illustrations) using the quick measure line tool function in NeuroLucida (MBF Bioscience, Williston, Vt., USA). Regional measurements were then translated into estimates of volume using the ellipsoid formula:  $V = (L \times W \times H)\pi/6$  [van Staaden et al., 1994; Huber et al., 1997; Pollen et al., 2007]. The proportional volume that each region contributes to the brain was obtained by dividing the region volume by the total brain weight, giving an estimate of relative region size ( $\text{mm}^3/\text{g}$ ). The total brain weight was used because it is difficult to measure the total volume of small, complexly shaped brains accurately. In addition, the volume of each brain region was corrected for body mass by taking the residuals from the region volume ( $\text{mm}^3$ ) to body size (g) quadratic relationships. These values were used in supplementary analyses to verify the patterns found using correction for brain size because changes in brain region size would influence brain mass, thus skewing the relative size of other regions even if they do not change in size. This approach also allowed testing for potential concerted change across brain regions [Finlay and Darlington, 1995], which could not be detected using correction for brain size.

### Isotope Analyses

Stable isotope signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were used to acquire measures of habitat use and trophic position. Muscle samples from individual fish and baseline organisms were dried at 70°C for 2 days, ground into powder and sent to the University of Windsor GLIER laboratories for isotopic analysis (Windsor, Ont., Canada).  $\delta^{13}\text{C}$  isotopic values were corrected for fat content using the equation:  $\delta^{13}\text{C}_{(\text{corrected})} = \delta^{13}\text{C} + (-3.32 + 0.99 \times \text{C/N})$  [Post et al., 2007]. Resulting  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were used in the calculations of percent littoral carbon, habitat coupling and trophic position. Random muscle samples analyzed in triplicate showed average coefficients of variation of 0.9% for nitrogen and 1.7% for carbon.

**Table 2.** Total fish per species used in this analysis and body size ranges

Common name	Latin name	n used (M, F)	Size range, cm
Lake trout	<i>Salvelinus namaycush</i>	10 (5, 5)	53–80
Whitefish	<i>Coregonus clupeaformis</i>	2 (2, 0)	52.5–54
Cisco	<i>Coregonus artedii</i>	12 (4, 8)	17.5–29.5
Rainbow smelt	<i>Osmerus mordax</i>	6 (?)	9–14
Walleye	<i>Sander vitreus</i>	9 (2, 7)	45.5–69
Yellow perch	<i>Perca flavescens</i>	6 (6, 0)	16–21
Smallmouth bass	<i>Micropterus dolomieu</i>	8 (5, 3)	19–50
Rock bass	<i>Ambloplites rupestris</i>	3 (?)	17–19.5
Pumpkinseed	<i>Lepomis gibbosus</i>	6 (?)	6.5–9
Northern pike	<i>Esox lucius</i>	4 (3, 1)	56–96
Bluntnose minnow	<i>Pimephales notatus</i>	5 (?)	7.5–8.5
Spottail shiner	<i>Notropis hudsonius</i>	6 (?)	8–10
Alewife	<i>Alosa pseudoharengus</i>	8 (3, 5)	14–19
Round goby	<i>Neogobius melanostomus</i>	4 (?)	8–10.5
Burbot	<i>Lota lota</i>	5 (5, 0)	47–57.5
Trout perch	<i>Percopsis omiscomaycus</i>	5 (0, 5)	8.5–11

Numbers for each sex are indicated in parentheses, when available (? = unidentified). All individuals included were adults to avoid potential ontogenetic changes in brain morphology. Brain size and ecological data are available online in Edmunds [2015].

The carbon ratios of consumers and baselines were calculated to measure the dietary littoral carbon use of the fish, which was used to represent habitat use with the equation:

$$\text{littoral carbon usage} = (\delta^{13}\text{C}_{\text{Fish}} - \delta^{13}\text{C}_{\text{mussel}}) / (\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{mussel}}),$$

where  $C_{\text{Fish}}$ ,  $C_{\text{mussel}}$  and  $C_{\text{snail}}$  are the carbon signatures of consumers, mussels and snails, respectively [Tunney et al., 2012]. The scale of this equation ranges from 0 to 1, where values closer to 0 indicate greater use of pelagic carbon sources and values approaching 1 indicate greater use of littoral carbon sources. This equation was then altered slightly to measure the amount of habitat coupling displayed by organisms with the equation:

$$\text{coupling} = 0.5 - |0.5 - (\delta^{13}\text{C}_{\text{Fish}} - \delta^{13}\text{C}_{\text{mussel}}) / (\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{mussel}})|,$$

where  $C_{\text{Fish}}$ ,  $C_{\text{mussel}}$  and  $C_{\text{snail}}$  are the carbon signatures of consumers, mussels and snails, respectively [Tunney et al., 2012]. The scale of this equation ranges from 0 to 0.5, where values closer to 0.5 indicate greater amounts of coupling between the pelagic and littoral habitats and lower values indicate the predominant use of one habitat type.

Additionally, the nitrogen ratio of consumers and baselines was used to estimate fish trophic position with the equation:

$$\text{trophic position} = [(\delta^{15}\text{N}_{\text{Fish}} - \delta^{15}\text{N}_{\text{mussel}}) / 3.4] + 2,$$

where  $N_{\text{Fish}}$  and  $N_{\text{mussel}}$  are the nitrogen signatures of fish and mussels, respectively. The value of 3.4 is the assumed per trophic level increase in  $\delta^{15}\text{N}$  and +2 is added because we are using primary consumers (i.e. mussels) instead of primary producers [an approach suggested by Vander Zanden et al., 2000].

#### Data Analysis

The analyses assessed if brain region size is associated with the ecological variables estimated from stable isotope signatures. All analyses used measures of relative brain region size corrected either for brain weight or for body weight. Separate analyses were conducted to compare these size correction methods. The main analytical approach involved linear mixed effects modeling (LMEM) and a mean-centering procedure. LMEM was used to account for the clustered nature of the data from different species, while centering allowed investigation of intraspecific and interspecific variation in brain size simultaneously (see below). The dataset with centered variables was not amenable to an LMEM multivariate approach; therefore, LMEM on mean-centered data was performed for each brain region separately. Shapiro-Wilk tests indicated that the relative size of the olfactory bulbs, telencephalon and cerebellum were normally distributed. However, the relative size of the optic tectum and hypothalamus had to be normalized using square root and  $\log_{10}$  transformations, respectively.

LMEMs were used to account for the clustered nature of our data, where multiple individuals were sampled from each species. Species grouping was modeled as a random effect, allowing for correlations within species clusters. Sex may also influence the brain and ecology of fish, thus fish sex (male, female or unidentified) was also included as a random effect in all LMEMs. Since variation in brain structure and ecology may be present both within and between species, we used the ‘within-subject centering’ method described in van de Pol and Wright [2009] by applying centering to within-species data. This effectively split the ecological variables (i.e. trophic position, littoral carbon use and habitat coupling) into within-species and between-species variation. Within-species variation was calculated for each individual by subtracting the species mean value from each individual’s observa-

tion value (i.e.  $x_{is} - \bar{x}_s$ ). The between-species variation was obtained by replacing each individual value with its species mean value. By including both variables as fixed effects in the models it was possible to evaluate both the within- and between-species effects of ecology on relative brain region size and, ultimately, fish brain morphology.

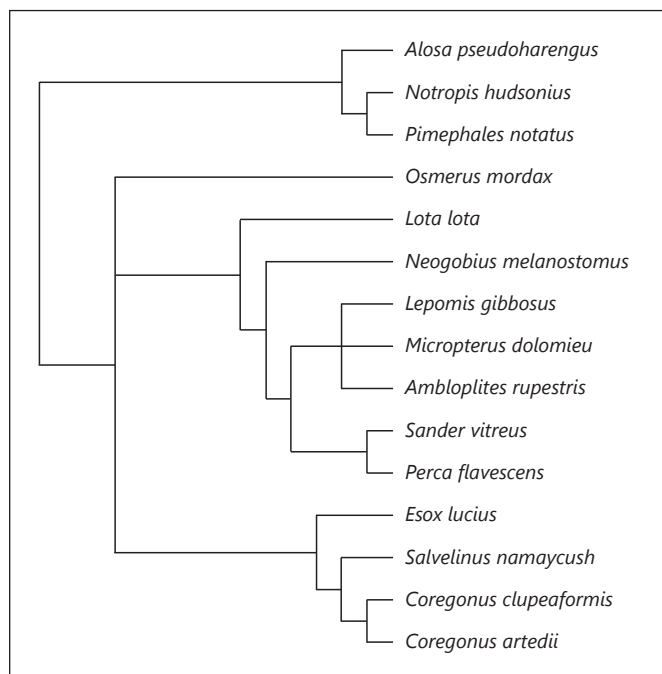
We followed a backwards elimination stepwise procedure to build a model for each brain region [Guyon and Elisseff, 2003]. For each region, we began with a model containing all ecological variables within and between species as well as fish sex as fixed effects. Within- and between-species effects for each ecological variable were treated as grouped pairs for all exclusions. Thus, a variable pair was only excluded if both within- and between-species effects were not statistically significant (i.e.  $p > 0.05$ ). The significance of each fixed effect was assessed using the R package *lmer-Test*, which obtains p values from t tests of the models fixed effects via the Satterthwaite approximation for degrees of freedom [Schaalje et al., 2002]. All significant effects obtained with correction for brain size were verified by completing the above process using region size corrected for body mass (i.e. residual brain region size). In addition, trophic position was also corrected for body size in all verification models.

If any of the between-species ecological variables were found to have a significant effect on the size of a brain region, the relationship was verified with a phylogenetic generalized least squares (PGLS) regression to account for phylogenetic relatedness among species [Freckleton et al., 2002]. PGLS tests were done within the R Studio platform v.2.1. *caper* and *ape* packages using average values of the relative brain region size and ecological variables for each species. A PGLS test also requires an accurate phylogenetic tree to compare physical properties to branch lengths. A tree was made using the tree builder function in the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov/>), which uses a diverse array of phylogenetic resources to build phylogenetic trees [Sayers et al., 2011; Benson et al., 2013]. The resulting tree (fig. 3) was checked for accuracy against a more extensive phylogeny of ray-finned fish [Near et al., 2012].

## Results

The LMEM backwards elimination procedure excluded sex and the within- and between-species effects of habitat coupling from all individual brain region models. Additionally, the within- and between-species effects of littoral carbon usage were excluded from models of the olfactory bulbs and optic tectum data, while the within- and between-species effects of trophic position were excluded from the model of cerebellum and hypothalamus data. The remaining variables had significant effects on the size of brain regions either between species (table 3) or within species (telencephalon only, see below).

Between species, trophic position was significantly associated with an increased relative size of the olfactory bulbs and decreased relative size of the optic tectum (table 3). Since the size of the olfactory bulbs was signifi-



**Fig. 3.** Phylogenetic tree used in all PGLS regression analyses to determine if results were influenced by the phylogenetic relatedness between species. This tree was built using the taxonomy browser in the National Center for Biotechnology Information database (<http://www.ncbi.nlm.nih.gov/>).

cantly associated with the mass of the olfactory rosettes ( $R^2 = 0.65$ , d.f. = 33,  $p < 0.001$ ) and the size of the optic tectum was significantly associated with the mass of the eyes ( $R^2 = 0.64$ , d.f. = 97,  $p < 0.001$ ), it suggests that evolution has favored fish that make greater use of olfaction and a lesser use of vision at high trophic positions in the food web. Between species, littoral carbon usage was significantly associated with an increased relative size of the telencephalon and hypothalamus, and a decrease in relative size of the cerebellum. This suggests that evolution has favored species with a proportionally larger telencephalon and hypothalamus in the littoral habitats, but a proportionally larger cerebellum in species that use more of the pelagic habitat. Table 3 shows estimates and statistical values of the modeled effects as well as results of PGLS regressions to account for phylogenetic relatedness. All of the between-species significant effects obtained by LMEMs were confirmed by PGLS. In addition, all between-species results were supported when using correction for body size (online suppl. table S1; see [www.karger.com/doi/10.1159/000445973](http://www.karger.com/doi/10.1159/000445973) for all online suppl. material).

**Table 3.** Model estimates and statistics of the significant LMEM between-species effects of ecological variables on relative brain region size corrected for brain size

Brain region	Variable	Model estimate	Statistic		Result of PGLS		
			t	p	estimate	R <sup>2</sup>	p value
Olfactory bulbs	TP	14.6±3.7	3.9	0.001	14.9±3.8	0.56	0.002
Telencephalon	LC	55.7±18.3	3.0	0.007	70.6±19.2	0.53	0.003
Optic tectum	TP	-2.1±1.0	-2.1	0.05	-2.6±1.0	0.37	0.022
Cerebellum	LC	-82.0±20.2	-4.0	0.001	-82.6±30.5	0.38	0.02
Hypothalamus	LC	0.45±0.09	5.0	<0.001	0.39±0.07	0.60	<0.001

LC = Littoral carbon usage; R<sup>2</sup> = coefficient of determination; TP = trophic position.

Relative telencephalon size increased with trophic position within species. Figure 4 depicts the raw data of the relationships between relative telencephalon size and trophic position for each species, which cumulatively contribute to a significant within-species relationship ( $17.9 \pm 5.6$ ,  $t = 3.2$ ,  $p = 0.002$ ). This within-species effect of trophic position indicates that a proportionally larger telencephalon may favor predation on larger prey. This within-species effect was also found when using residual telencephalon volume and trophic position, but not when using residual telencephalon volume and residual trophic position (online suppl. table S2).

## Discussion

The relative size of the five brain regions examined in this study varied in nonrandom patterns with ecological variables estimated from fish muscle isotopic signatures. Relative region size was significantly associated with either littoral-pelagic habitat use or fish trophic position, but no association was found with littoral-pelagic habitat coupling. Relationships between brain region size and food web variables included effects within and between species groups. Between-species results were supported when taking into account the phylogenetic relatedness of the species sampled.

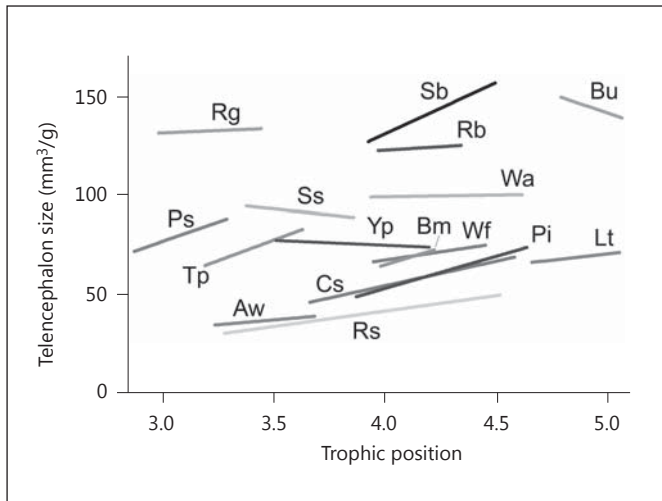
These results contribute to a growing body of literature that examines the effects of the environment and ecology on brain evolution. Although the results presented here cover a wide phylogenetic range of fish, the patterns found are relatively consistent with past studies of cichlid fish. The association between the size of sensory brain regions and trophic position found here is similar to a previous finding in cichlids indicating that the olfactory bulb

and optic tectum vary with feeding type [Huber et al., 1997]. Variation in the size of the telencephalon and the cerebellum with differential habitat use was also found by Gonzalez-Voyer and Kolm [2010] in cichlids. Additionally, we found that telencephalon size varies with trophic position, which is similar to past studies indicating an association of this region with diet and predator-prey interactions [Huber et al., 1997].

### *Brain Regions and Trophic Position*

In aquatic systems, vision and olfaction are important in predator-prey interactions. Our results suggest that trophic position has influenced the evolution of sensory capacities in teleosts. With an increasing trophic position between species, the olfactory bulbs showed a significant increase in relative size, while the optic tectum showed a significant decrease in relative size. This suggests that sensory trade-offs may exist across a trophic position gradient between species. This result is supported by documented behaviors exhibited by the species of predators and prey in this system. Pelagic or benthopelagic predators (i.e. lake trout and burbot) feed in low-light, deep waters, while littoral generalist predators (i.e. walleye and smallmouth bass) feed at dawn and dusk when light penetration in the water column is low [Emery, 1973; Ryder, 1977]. In addition, the littoral predator northern pike feeds in dense vegetation using sit-and-wait ambushing tactics [Savino and Stein, 1989]. These feeding behaviors occur under conditions where vision is not favored. Thus, olfaction could have been selected for as a way to detect the location of prey in conditions when vision is not favored. Conversely, for prey species, vision would be favored to decrease predation risk, as large predators can be seen from afar, allowing for timely avoidance responses [Cerri, 1983]. Predator avoidance by fish schooling is also





**Fig. 4.** Estimated within-species relationships between trophic position and the relative size of the telencephalon corrected for brain size. The lines represent relationships for each species, which cumulatively contribute to a significant within-species relationship between relative telencephalon size and trophic position ( $17.9 \pm 5.6$ ,  $t = 3.2$ ,  $n = 99$ ,  $p = 0.002$ ). Species abbreviations: Aw = alewife; Bm = bluntnose minnow; Bu = burbot; Cs = cisco; Lt = lake trout; Pi = pike; Ps = pumpkinseed; Rb = rock bass; Rg = round goby; Rs = rainbow smelt; Sb = smallmouth bass; Ss = spottail shiner; Tp = trout-perch; Wa = walleye; Wf = whitefish; Yp = yellow perch.

dependent upon vision for success, and thus may be selected for in schooling species occupying lower trophic positions, such as yellow perch, cisco, rainbow smelt and alewife in the system studied here [Shaw, 1978].

Another possible explanation for effects on sensory brain regions may be that fish at higher trophic positions must be able to track their prey over large distances because prey fish are not uniformly distributed throughout the environment. This behavior favors olfaction capabilities, as they act over much larger distances compared to vision [DeBose and Nevitt, 2008]. In addition, at lower trophic positions, fish feed upon organisms that are well camouflaged and difficult to detect in their environments. Small prey, such as zooplankton, are clear bodied and difficult to see in the water column; other prey, such as benthic invertebrates, commonly occupy muddy bottoms or complex habitats with vegetation or dead organic matter where they can hide. Therefore, greater visual capacities may be favored in fish occupying lower trophic positions.

#### *Brain Regions and Habitat Use*

Fish with different foraging styles will have different isotopic carbon muscle contents due to variation between pe-

riphyton (littoral) and phytoplankton (pelagic) carbon sources [France, 1995]. The cognitive demands in these different habitats are expected to have shaped the brain morphology of species over evolutionary time. As expected, our results show that brain morphology varies with littoral-pelagic habitat use between species. The telencephalon tends to be proportionally larger in species that forage on littoral-based carbon sources and the cerebellum tends to be proportionally larger in species with pelagic carbon signatures. The telencephalon, which is involved in spatial navigation and learning [Portavella et al., 2002], may be more important for littoral fish species because of the more complex structure of that habitat and the more extensive breadth of prey it displays. This idea is additionally supported by previous studies showing that fish species occupying complex habitats have a relatively large telencephalon [Bauchot et al., 1989; Huber et al., 1997; Shumway, 2008; Gonzalez-Voyer and Kolm, 2010]. Conversely, the cerebellum, which is responsible for motor control, may be more important for pelagic species because of the three-dimensional nature of large open waters. Predator-prey interactions in the pelagic habitat may also be drawn out because there is nowhere to seek refuge, so swimming abilities for enhanced pursuit or escape response will be needed. This idea is supported by our results and the large relative cerebellum size in pelagic sharks and highly active pelagic marine teleosts [Kruska, 1988; Lisney and Collin, 2006]. On the other hand, research showing an association between large cerebellum size and high habitat complexity in fish is at odds with these findings [Bauchot et al., 1989; Pollen et al., 2007; Shumway, 2008; Gonzalez-Voyer and Kolm, 2010]. This might be due to methodological differences between studies, especially with respect to how habitat use was evaluated (i.e. carbon isotopic signatures vs. qualitative attribution or video image analysis of the preferred habitat of a species based on literature), or other factors resulting from increased species richness in the most complex habitats. Additionally, it is important to note that there is no habitat equivalent in complexity to coral reefs in the lacustrine system studied here. A threshold in habitat complexity might be needed to observe a positive association between this variable and cerebellum size. Life in the most complex habitats, such as coral reefs, might even rely on similar three-dimensional orientation mechanisms used by pelagic fish in open waters, which could explain why different species could benefit from a larger cerebellum in both situations.

Relative hypothalamus size also increased with littoral carbon usage between species. Due to the complexity of the fish hypothalamus and its functional diversity, it is difficult to directly infer why the size of this brain region



varies across habitats. Past studies have suggested that the hypothalamus is associated with mating behaviors and sociality in African cichlids, with more complex mating strategies and greater levels of sociality associated with a larger hypothalamus [Pollen et al., 2007; Gonzalez-Voyer and Kolm, 2010]. This may also explain the relationship between habitat use and hypothalamus size in the present study, as three of the primarily littoral species studied belong to the Centrarchidae family, which display complex mating behaviors such as male nesting and egg guarding [Pflieger, 1966; Keenleyside, 1978].

#### *Within-Species Effect of Trophic Position*

Interestingly, relative telencephalon size increased with increasing trophic position within species. This result must be taken with caution because the effect is not seen when using residual trophic position, which implies that differences in body size within the sampled populations could drive the relationship observed when using uncorrected values of trophic position. Nevertheless, the potential for this within-species effect of trophic position is worthy of discussion because such an effect would implicate phenotypic plasticity or natural variation in telencephalon size as potential mechanisms. Results suggesting a role for fish brain plasticity in ecology have been seen in patterns of whole brain size [Gonda et al., 2009; Kotrschal et al., 2012; Edmunds, 2015], and seasonal plasticity of telencephalon size has been documented in the round goby [McCallum et al., 2014]. It is thus possible that the telencephalon is the main brain region contributing to this phenomenon. Attaining higher trophic positions typically indicates that fish are feeding upon larger, more energetically valuable prey [Pyke et al., 1977]. This can be beneficial as fish may be able to shift their energy allocation to other important functions such as gonadal development or brain growth [Isler and van Schaik, 2009]. However, prey located at higher trophic positions tend to display more complex behaviors, making successful foraging by predators more demanding. Growth of the telencephalon in fish occupying high trophic positions may then enhance learning abilities and sensory integration, enabling fish to be behaviorally flexible and respond to more complex prey behaviors. Interestingly, recent evidence obtained in guppies suggests a relationship between brain size and predator avoidance ability [Kotrschal et al., 2015; van der Bijl et al., 2015]. It is thus possible that both pressures for successful predation and predator avoidance contribute to increased telencephalon size with trophic position in an arms race-like mechanism [Jerison, 1973; Kondoh, 2010].

#### *Habitat Coupling*

The within- and between-species habitat coupling variables were removed from models for each brain region through backwards elimination. This indicates that variation in the size of the brain regions studied was not explained by the amount of littoral-pelagic habitat coupling displayed by individuals or species. This result is unexpected given that Edmunds [2015] found that relative total brain size increases with the amount of habitat coupling within and between species using a superset of 298 individuals that included the 99 fish used in the present study. Post hoc analyses trying to elucidate why no effect of habitat coupling on brain morphology was found in the present study showed that total brain size in the subset of fish used here was not significantly associated with habitat coupling (within species:  $0.084 \pm 0.065$ ,  $t = 1.3$ ,  $p = 0.2$ , or between species:  $0.022 \pm 0.12$ ,  $t = 0.2$ ,  $p = 0.86$ ). Since the subset of fish analyzed here contained only adult individuals, it is possible that the relationship between habitat coupling and brain size is driven primarily by discrepancies between adult and juvenile fish. Alternatively, this effect might be subtle and the sample size used in the present study too small to detect it. In the latter case, achieving higher levels of habitat coupling might require slight changes in size across multiple brain regions that were not detected in the present study. A balanced increase across regions, contributing to a larger total brain size, could be the factor affording fish an enhanced capacity for habitat coupling. Because brain growth is constrained by energetic limitations, small increases in the size of these regions may be all that couplers can achieve. Ultimately, total brain size may be a better determinant of habitat coupling ability because of functional trade-offs between brain regions and energetic constraints.

#### *Mean-Centering Approach – Interpretation of Results*

Through the use of a centering approach to mixed models [van de Pol and Wright, 2009], we were able to differentiate within- and between-species effects of ecology on the size of brain regions. The between-species portion of this approach is easy enough to interpret as the product of evolution over time. However, the within-species variation that was detected in the telencephalon is more difficult to interpret. This effect could be the result of brain size plasticity during the lifetime of individuals, or it could be due to natural variation in telencephalon size within populations. For the case in which natural variation is responsible for telencephalic variation, regional size would be directly responsible for the ecologi-

cal interactions of individuals (ecology via brain size). For the case in which brain phenotypic plasticity is responsible, regional size would be influenced by the ecology of an individual (brain size via ecology). At the present time we are unable to determine whether one or both of these mechanisms are contributing to within-species patterns. Laboratory studies should prove useful to elucidate the mechanism(s) at play.

## Conclusion

Here, we have used novel methodology to examine both within- and between-species relationships between ecology and brain morphology in teleost fish. By using continuous ecological variables obtained in a food web context, we attempted to access a more accurate portrayal of these relationships. We found that the relative size of each of the five regions examined varied between-species and identified potential evolutionary trade-offs between different regions within the brain. Furthermore, we found

evidence to suggest that within-species variation in telencephalon size has a direct impact on the trophic interactions of individual fish. Through this examination we have identified the brain as a critical component that influences how organisms interact with their environment and each other in ecological systems. In addition, this study provides further understanding of the underlying ecological mechanisms that contribute to changes in brain morphology in teleost fish.

## Acknowledgements

We thank everybody that helped to collect samples in the field and Joe Scott for his generous financial contribution to the 2014 field season. We would also like to acknowledge the efforts of A. Fisk and the University of Windsor GLIER Stable Isotope Lab. Helpful discussion of this work included members of the McCann lab, R. McLaughlin, A. McAdam and K. Cottenie. Two anonymous reviewers provided helpful comments to improve the manuscript. All data used in this study can be accessed in the appendices of Edmunds [2015]. This research was supported by NSERC discovery grants to K.S.M and F.L.

## References

- Barton RA, Harvey PH (2000): Mosaic evolution of brain structure in mammals. *Nature* 405: 1055–1058.
- Bauchot R, Ridet JM, Bauchot ML (1989): The brain organization of butterflyfishes. *Env Biol Fish* 25:205–219.
- Becerra M, Manso MJ, Rodriguez-Moldes I, Anadón R (1994): Primary olfactory fibres project to the ventral telencephalon and preoptic region in trout (*Salmo trutta*): a developmental immunocytochemical study. *J Comp Neurol* 342:131–143.
- Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Lipman DJ, Ostell J, Sayers EW (2013): GenBank. *Nucleic Acids Res* 41:D36–D42.
- Brandstätter R, Kotrschal K (1990): Brain growth patterns in four European cyprinid fish species (Cyprinidae, Teleostei): roach (*Rutilus rutilus*), bream (*Abramis brama*), common carp (*Cyprinus carpio*) and sabre carp (*Pelecus cultratus*). *Brain Behav Evol* 35:195–211.
- Butler AB, Hodos W (1996): The visceral brain: the hypothalamus and the autonomic nervous system; in Butler AB, Hodos W (eds): *Comparative Vertebrate Neuroanatomy: Evolution and Adaptation*. New York, Wiley-Liss, pp 328–345.
- Cerri RD (1983): The effect of light intensity on predator and prey behaviour in cyprinid fish: factors that influence prey risk. *Anim Behav* 31:736–742.
- Davis RE, Chase R, Morris J, Kaufman B (1981): Telencephalon of the teleost *Macropodus*: experimental localization of secondary olfactory areas and of components of the lateral fore-brain bundle. *Behav Neural Biol* 33:257–279.
- Davis RE, Kassel J (1983): Behavioral functions of the teleostean telencephalon; in Davis RE, Northcutt RG (eds): *Fish Neurobiology*, vol 2. Ann Arbor, University of Michigan Press, pp 237–263.
- DeBose JL, Nevitt GA (2008): The use of odors at different spatial scales: comparing birds with fish. *J Chem Ecol* 34:867–881.
- Demski LS (1983): Behavioral effects of electrical stimulation of the brain; in Davis RE, Northcutt RG (eds): *Fish Neurobiology*, vol 2. Ann Arbor, University of Michigan Press, pp 317–359.
- Dunlap KD, Silva AC, Chung M (2001): Environmental complexity, seasonality and brain cell proliferation in a weakly electric fish, *Brachyhypopomus gauderio*. *J Exp Biol* 214:794–805.
- Ebbesson LOE, Braithwaite VA (2012): Environmental effects on fish neural plasticity and cognition. *J Fish Biol* 81:2151–2174.
- Edmunds N (2015): The Effect of Food Web Structure on Teleost Fish Brain Size and Morphology in an Aquatic Ecosystem; MSc Thesis, Department of Integrative Biology, University of Guelph, Guelph. <http://hdl.handle.net/10214/9118>.
- Emery AR (1973): Preliminary comparisons of day and night habits of freshwater fish in Ontario lakes. *J Fish Res Board Can* 30:761–774.
- Finlay BL, Darlington RB (1995): Linked regularities in the development and evolution of mammalian brains. *Science* 268:1578–1584.
- France RL (1995): Differentiation between littoral and pelagic food webs in lakes using stable carbon isotopes. *Limnol Oceanogr* 40:1310–1313.
- Freckleton RP, Harvey PH, Pagel M (2002): Phylogenetic analysis and comparative data: a test and review of evidence. *Am Nat* 160:712–726.
- Gonda A, Herczeg G, Merilä J (2009): Habitat-dependent and-independent plastic responses to social environment in the nine-spined stickleback (*Pungitius pungitius*) brain. *Proc R Soc B* 276:2085–2092.
- Gonda A, Herczeg G, Merilä J (2011): Population variation in brain size of nine-spined sticklebacks (*Pungitius pungitius*) – local adaptation or environmentally induced variation? *BMC Evol Biol* 11:75.
- Gonzalez-Voyer A, Kolm N (2010): Sex, ecology and the brain: evolutionary correlates of brain structure volumes in *Tanganyikan cichlids*. *PLoS One* 5:e14355.
- Guyon I, Elisseeff A (2003): An introduction to variable and feature selection. *J Mach Learn Res* 3:1157–1182.
- Hara TJ (1992): Mechanisms of olfaction; in Hara TJ (ed): *Fish Chemoreception*. London, Chapman & Hall, pp 150–170.
- Healy SD, Rowe C (2007): A critique of comparative studies of brain size. *Proc R Soc B* 274: 453–464.
- Huber R, van Staaden MJ, Kaufman LS, Liem KF (1997): Microhabitat use, trophic patterns, and the evolution of brain structure in African cichlids. *Brain Behav Evol* 50:167–182.

- Isler K, van Schaik CP (2009): The expensive brain: a framework for explaining evolutionary changes in brain size. *J Hum Evol* 57:392–400.
- Jerison HJ (1973): *Evolution of the Brain and Intelligence*. New York, Academic Press, pp 55–81.
- Johannessen A, Dunn AM, Morrell LJ (2012): Olfactory cue use by three-spined sticklebacks foraging in turbid water: prey detection or prey location? *Anim Behav* 84:151–158.
- Kaslin J, Ganz J, Brand M (2008): Proliferation, neurogenesis and regeneration in the non-mammalian vertebrate brain. *Phil Trans R Soc B* 363:101–122.
- Keenleyside MH (1978): Reproductive isolation between pumpkinseed (*Lepomis gibbosus*) and longear sunfish (*L. megalotis*) (Centrarchidae) in the Thames River, southwestern Ontario. *J Fish Res Board Can* 35:131–135.
- Kondoh M (2010): Linking learning adaptation to trophic interactions: a brain size-based approach. *Funct Ecol* 24:35–43.
- Kotrschal A, Buechel SD, Zala SM, Corral A, Penn DJ, Kolm N (2015): Brain size affects female but not male survival under predation threat. *Ecol Lett* 18:646–652.
- Kotrschal A, Rogell B, Bundsen A, Svensson B, Zajitschek S, Brännström I, Immler S, Maklakov AA, Kolm N (2013): Artificial selection on relative brain size in the guppy reveals costs and benefits of evolving a larger brain. *Curr Biol* 23:168–171.
- Kotrschal A, Rogell B, Maklakov AA, Kolm N (2012): Sex-specific plasticity in brain morphology depends on social environment of the guppy, *Poecilia reticulata*. *Behav Ecol Sociobiol* 66:1485–1492.
- Kotrschal K, van Staaden MJ, Huber R (1998): Fish brains: evolution and environmental relationships. *Rev Fish Biol Fish* 8:373–408.
- Kruska DCT (1988): The brain of the basking shark (*Cetorhinus maximus*). *Brain Behav Evol* 32:353–363.
- Laberge F, Hara TJ (2001): Neurobiology of fish olfaction: a review. *Brain Res Rev* 36:46–59.
- Lisney TJ, Bennett MB, Collin SP (2007): Volumetric analysis of sensory brain areas indicates ontogenetic shifts in the relative importance of sensory systems in elasmobranchs. *Raff Bull Zool* 14:7–15.
- Lisney TJ, Collin SP (2006): Brain morphology in large pelagic fishes: a comparison between sharks and teleosts. *J Fish Biol* 68:532–554.
- McCallum ES, Capelle PM, Balshine S (2014): Seasonal plasticity in telencephalon mass of a benthic fish. *J Fish Biol* 85:1785–1792.
- Montgomery JC, Björn K, Sutherland W (1997): Sensory development of the Antarctic silverfish *Pleuragramma antarcticum*: a test for the ontogenetic shift hypothesis. *Polar Biol* 18:112–115.
- Navarrete A, van Schaik CP, Isler K (2011): Energetics and the evolution of human brain size. *Nature* 480:91–93.
- Near TJ, Eytan RI, Dornburg A, Kuhn KL, Moore JA, Davis MP, Wainwright PC, Friedman M, Smith WL (2012): Resolution of ray-finned fish phylogeny and timing of diversification. *Proc Natl Acad Sci USA* 109:13698–13703.
- Niven JE, Laughlin SB (2008): Energy limitation as a selective pressure on the evolution of sensory systems. *J Exp Biol* 211:1792–1804.
- Noreikiene K, Herczeg G, Gonda A, Balázs G, Husby A, Merilä J (2015): Quantitative genetic analysis of brain size variation in sticklebacks: support for the mosaic model of brain evolution. *Proc Biol Sci* 282 DOI: 10.1098/rspb.2015.1008.
- Northcutt RG (1983): Evolution of the optic tectum in ray-finned fishes; in Davis RE, Northcutt RG (eds): *Fish Neurobiology*, vol 2. Ann Arbor, University of Michigan Press, pp 1–42.
- Park PJ, Bell MA (2010): Variation of telencephalon morphology of the threespine stickleback (*Gasterosteus aculeatus*) in relation to inferred ecology. *J Evol Biol* 23:1261–1277.
- Park PJ, Chase I, Bell MA (2012): Phenotypic plasticity of the threespine stickleback *Gasterosteus aculeatus* telencephalon in response to experience in captivity. *Curr Zool* 58:189–210.
- Peter RE, Fryer JN (1983): Endocrine functions of the hypothalamus of actinopterygians; in Davis RE, Northcutt RG (eds): *Fish Neurobiology*, vol 2. Ann Arbor, University of Michigan Press, pp 165–201.
- Pflieger WL (1966): Reproduction of the small-mouth bass (*Micropterus dolomieu*) in a small Ozark stream. *Am Midl Nat* 76:410–418.
- Pollen AA, Dobberfuhl AP, Scace J, Igulu MM, Renn SC, Shumway CA, Hofmann HA (2007): Environmental complexity and social organization sculpt the brain in Lake Tanganyikan cichlid fish. *Brain Behav Evol* 70:21–39.
- Portavella M, Vargas JP (2005): Emotional and spatial learning in goldfish is dependent on different telencephalic pallial systems. *Eur J Neurosci* 21:2800–2806.
- Portavella M, Vargas JP, Torres B, Salas C (2002): The effects of telencephalic pallial lesions on spatial, temporal, and emotional learning in goldfish. *Brain Res Bull* 57:397–399.
- Post DM (2002): Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* 83:703–718.
- Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montana CG (2007): Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152:179–189.
- Pyke GH, Pulliam HR, Charnov EL (1977): Optimal foraging: a selective review of theory and tests. *Q Rev Biol* 52:137–154.
- Rodriguez F, López JC, Vargas P, Gómez Y, Broglio C, Salas C (2002): Conservation of spatial memory function in the pallial forebrain of reptiles and ray-finned fishes. *J Neurosci* 22:2894–2903.
- Rooney N, McCann KS, Moore JC (2008): A landscape theory for food web architecture. *Ecol Lett* 11:867–881.
- Ryder RA (1977): Effects of ambient light variations on behavior of yearling, subadult, and adult walleyes (*Stizostedion vitreum vitreum*). *J Fish Res Board Can* 34:1481–1491.
- Salas CA, Yopak KE, Warrington RE, Hart NS, Potter IC, Collin SP (2015): Ontogenetic shifts in brain scaling reflect behavioral changes in the life cycle of the pouched lamprey *Geotria australis*. *Front Neurosci* 9:251.
- Savino JF, Stein RA (1989): Behavioural interactions between fish predators and their prey: effects of plant density. *Anim Behav* 37:311–321.
- Sayers EW, Barrett T, Benson DA, Bolton E, Bryant SH, Canese K, et al (2011): Database resources of the national center for biotechnology information. *Nucleic Acids Res* 39:D38–D51.
- Schaalje GB, McBride JB, Fellingham GW (2002): Adequacy of approximations to distributions of test statistics in complex mixed linear models. *J Agric Biol Environ Stat* 7:512–524.
- Shaw E (1978): Schooling fishes: the school, a truly egalitarian form of organization in which all members of the group are alike in influence, offers substantial benefits to its participants. *Am Sci* 66:166–175.
- Shumway CA (2008): Habitat complexity, brain, and behavior. *Brain Behav Evol* 72:123–134.
- Tunney TD, McCann KS, Lester NP, Shuter BJ (2012): Food web expansion and contraction in response to changing environmental conditions. *Nat Comm* 3:1105.
- van de Pol M, Wright J (2009): A simple method for distinguishing within- versus between-subject effects using mixed models. *Anim Behav* 77:753–758.
- van der Bijl W, Thyseus M, Kotrschal A, Kolm N (2015): Brain size affects the behavioural response to predators in female guppies (*Poecilia reticulata*). *Proc Biol Sci* 282:20151132.
- van Staaden MJ, Huber R, Kaufman LS, Liem KF (1994): Brain evolution in cichlids of the African Great Lakes: brain and body size, general patterns, and evolutionary trends. *Zoology* 98:165–178.
- Vander Zanden MJ, Rasmussen JB (1996): A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. *Ecol Monogr* 66:451–477.
- Vander Zanden MJ, Shuter BJ, Lester NP, Rasmussen JB (2000): Within- and among-population variation in the trophic position of a pelagic predator, lake trout (*Salvelinus namaycush*). *Can J Fish Aquat Sci* 57:725–731.
- Wagner HJ (2003): Volumetric analysis of brain areas indicates a shift in sensory orientation during development in the deep-sea grenadier *Coryphaenoides armatus*. *Mar Biol* 142:791–797.
- Warren PH, Lawton JH (1987): Invertebrate predator-prey body size relationships: an explanation for upper triangular food webs and patterns in food web structure? *Oecologia* 74:231–235.
- Zupanc GKH (2006): Neurogenesis and neuronal regeneration in the adult fish brain. *J Comp Physiol A* 192:649–670.