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### Abstract

Predators tend to be large and mobile, enabling them to forage in spatially distinct food web compartments (e.g. littoral and pelagic aquatic macrohabitats). This feature can stabilise ecosystems when predators are capable of rapid behavioural response to changing resource conditions in distinct habitat compartments. However, what provides this ability to respond behaviourally has not been quantified. We hypothesised that predators require increased cognitive abilities to occupy their position in a food web, which puts pressure to increase brain size. Consistent with food web theory, we found that fish relative brain size increased with increased ability to forage across macrohabitats and increased relative trophic positions in a lacustrine food web, indicating that larger brains may afford the cognitive capacity to exploit various habitats flexibly, thus contributing to the stability of whole food webs.

### Keywords

Cognitive ability, food webs, habitat coupling, relative brain size, trophic position.

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## INTRODUCTION

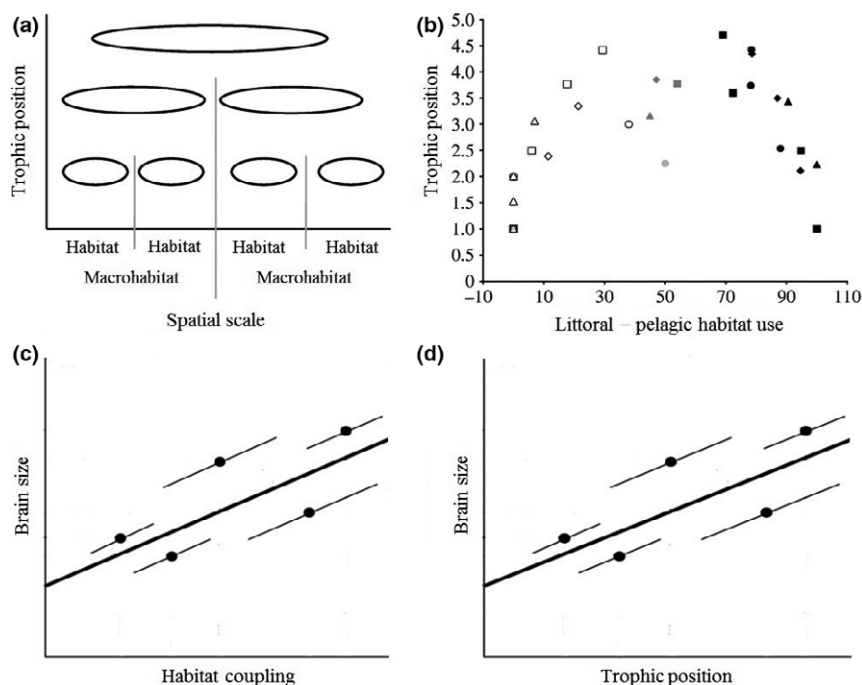
The behaviour of organisms contributes to the structure and stability of food webs. For example research on body size and mobility of organisms in aquatic ecosystems has shown that larger organisms occupying higher trophic positions (i.e. top predators) promote system stability by foraging adaptively upon resources distributed unevenly across spatially distinct habitats (Post *et al.* 2000; Kondoh 2003; Rooney *et al.* 2006; McCann & Rooney 2009). Other research has argued cogently that the inclusion of adaptive foraging not only provides stability to large food web models but also importantly predicts patterns in topology, like connectance and food chain length (Petchey *et al.* 2008; Beckerman *et al.* 2010; Loeuille 2010; Staniczenko *et al.* 2010). It remains unclear if the enhanced mobility conferred by a large body size is enough to explain this adaptive foraging or if additional factors are also important. Cognition is one such factor that would not necessarily vary in tune with body size or mobility and thus, may influence an organism's ability to forage adaptively. Determining the factors underlying adaptive behaviour is critical to understand how stability is naturally maintained in ecosystems.

A breadth of literature in ecology maps body size to ecological traits that collectively combine to form the structure of ecological systems. For example in aquatic ecosystems it is well known that increasing body size is correlated with increased trophic level and mobility (Elton 1927; Rooney *et al.* 2008; Riede *et al.* 2011) (Fig. 1a). Furthermore, organisms that are more mobile tend to be more generalist foragers (those capable of consuming a variety of distinct prey types from different macrohabitats) because, all else equal, their mobility exposes them to a greater number of macrohabitats and prey types (i.e. encounter probability is positively related to mobility) (Pyke *et al.* 1977). Finally, since spatial autocorrelation in resources appears to decrease with increasing spatial scale (Qi & Wu 1996), adaptive foraging across large-scaled habitat divisions by large mobile organisms promises

gains in foraging performance. This patterning in body size and mobility, therefore, leads to some clear predictions on the structure of ecological systems: (1) lower trophic level organisms should tend to be more compartmentalised in a given macrohabitat; and, (2) higher trophic level organisms should increasingly forage (or couple) across macrohabitats (Fig. 1a).

These simple predictions from long-standing organismal traits are met when we investigate the structure of aquatic food webs (Peters 1986; Rooney *et al.* 2006). Here, stable isotopes and/or stomach content data, which operate on the macrohabitat scale (e.g. littoral vs. pelagic), show that energy from the littoral or pelagic environment is isolated lower in the web (Fig. 1b) and that higher trophic level organisms progressively couple more between macrohabitats (Fig. 1a and b; e.g. top predators derive carbon from both littoral and pelagic macrohabitats, whereas lower level organisms have more compartmentalised carbon sources). In summary, data within aquatic ecosystems display an intriguing pattern which scales from small to large habitats, whereby mobile generalists couple across different energetic pathways.

Recent theory argues that this repeated structure (i.e. generalist coupling of isolated lower level compartments) represents an extraordinary property that allows ecosystems to respond to, and buffer against, environmental stochasticity (McCann & Rooney 2009). In the most simplified sense, this theory argues that resources in different habitats (from micro to macro habitats) do not always respond in a synchronous manner with changing environmental conditions. This differential habitat/species response creates a spatial and temporal mosaic of resource productivity on the landscape. Larger mobile organisms, that presumably are capable of rapid behavioural responses to changing conditions, then interact with this landscape of species variability in a way that prevents any single species, or sets of species, from monopolising nutrients and energy. If one habitat is thriving while the other is not, then the mobile predator responds by foraging more in the more productive habitat (called the birdfeeder effect) (Eveleigh *et al.*



**Figure 1** The structure of aquatic food webs predicted by body size and mobility, and the within- and between-species brain size hypotheses. (a) The architecture of an aquatic food web is predicted by body size and mobility. In this figure coupling is hierarchical such that increases in trophic position or body size allows switching from singular habitat use, to habitat coupling, to macrohabitat coupling (graphic obtained from McCann & Rooney 2009). (b) Empirical data (stomach contents or stable isotope data) from four aquatic food webs that display the predicted hump shape pattern of coupling over different spatial scales. Each symbol represents the average trophic position and habitat use value for functionally different food web levels: white circles, Chile detrital channel; white squares, Cantabrian detrital channel; white triangles, Chesapeake phytoplankton channel; white diamonds, Bering detrital channel; black circles, Chile phytoplankton channel; black squares, Cantabrian phytoplankton channel; black triangles, Chesapeake detrital channel; black diamonds, Bering phytoplankton channel; grey circles, Chile couplers; grey squares, Cantabrian couplers; grey triangles, Chesapeake couplers; grey diamonds, Bering couplers (graphic obtained from McCann & Rooney 2009). (c) The expected relationship between brain size and habitat coupling predicted by our behavioural flexibility hypothesis. (d) The expected relationship between brain size and trophic position predicted by the trophic level hypothesis. In both panels (c) and (d), the thicker line depicts the predicted relationship between-species and the smaller lines depict predicted within-species relationships.

2007). If spatially distant habitats are more likely to respond differentially to regional or localised abiotic forcing (Qi & Wu 1996), then we expect that adaptive foraging ought to be most important at larger spatial scales with larger, more mobile organisms. On the other hand, if organisms perceive variation at all scales (Wiens 1989), then the ability to adaptively forage will be selected for independent of trophic level. It is necessary to point out that this presumed ability for rapid behavioural response is critical for this structure to be stabilising, as slower or poorly informed behavioural decisions generate lags that can make such a system highly unstable (Abrams 2007).

While the above represents an enticing set of theoretical and empirical ideas, one unresolved and critical empirical issue is whether mobile organisms are indeed capable of rapid, informed behavioural responses. The ability to rapidly modify one's foraging behaviour requires flexibility which is difficult to quantify without enormous quantities of fine-grained behavioural data. Here, we attempt to bridge this behavioural mechanistic gap using brain size as a proxy for cognitive ability (e.g. Kondoh 2010). The use of brain size as a proxy for cognitive abilities has a long history. Jerison's (1973) principle of proper mass sums up the main underlying idea – that the mass of neural tissue dedicated to a particular function needs to be matched to the amount of neural processing required

for that function. This idea was later refined based upon the observation that nervous tissue is energetically expensive to maintain, and thus could only be increased under strong selective pressure (Niven & Laughlin 2008; Navarrete *et al.* 2011). Under relaxed pressure, brain tissue can decrease in size (Safi *et al.* 2005). In addition to the intergenerational effects of natural selection, ecological pressure acting on long-lived organisms like fish could also alter brain size through phenotypic plasticity (i.e. the ability of individuals to invest in brain tissue when needed) (McCallum *et al.* 2014).

An organism's decision making is directly related to its cognitive capacity: the ability to acquire and process information in the nervous system (Real 1993). This association raises questions about how cognitive capacity may influence the behaviour of organisms in large ecological systems. Thus, the exploration of the stability theory mentioned above within this cognitive framework leads to some simple predictions of how brain size ought to change with food web structure. First, if organisms capable of coupling across habitats require more advanced cognitive abilities, then increased coupling should correlate with increased brain size (Fig. 1c thick solid line; Behavioural Flexibility Hypothesis). Second, if ability to couple across habitats increases with increased trophic position and attaining higher trophic positions requires advanced

cognitive abilities, then brain size should increase with increasing trophic position (Fig. 1d thick solid line; Trophic Level Hypothesis). Note that the two hypotheses are not mutually exclusive. While these patterns are presumed to occur on the 'average individual' or the species level, it has also been speculated that intraspecific trait variability may influence these types of ecological dynamics. For example variation may promote stability in populations through mechanisms such as the 'portfolio effect' by protecting populations from extreme temporal fluctuations through response diversity (Bolnick *et al.* 2011). Thus, the examination of the above hypotheses simultaneously at both within-species as well as between-species levels could reveal a role for intraspecific brain size variation (i.e. plasticity or natural variation) in the stability of aquatic food webs (Fig. 1c and d, thin solid lines).

Most previous comparative studies of the brain have used species or higher taxonomic levels and average values of brain size (Kotrschal *et al.* 1998). Investigations of within-population variation in brain size have been valuable in answering specific questions about the relationship between brain features and ecology or behaviour (e.g. Gonda *et al.* 2013). Many such studies were done in fish, taking advantage of a wide range of variation in ecology, body size, behaviour and brain types to choose from, as well as lifelong brain growth (Kaslin *et al.* 2008). For example active foraging in young brook charr correlates with increased telencephalon size, which is consistent with the proposed role of this brain structure in spatial cognition (Wilson & McLaughlin 2010). Here, we accounted for both within- and between-species variation in brain size using a linear mixed effects model (LMEM) approach (see: Bolker *et al.* 2009; van de Pol & Wright 2009). Finally, stable isotope methods allowed the use of quantitative variables of habitat use and trophic position for each individual, unlike previous studies that attributed qualitative ecology or behaviour variables to a taxonomic unit.

An empirical test of the behavioural flexibility and trophic level hypotheses was conducted using stable isotope signatures and brains of 298 individuals from 16 species of fish sampled from the same food web. The hypotheses were tested at both within- and between-species levels to determine if patterns exist across multiple organisational scales. The results show that relative brain size increased with macrohabitat use (coupling) and higher relative trophic level within- and between-species in this food web. These results suggest a role for brain size and cognitive function in shaping and maintaining food web structure.

## MATERIAL AND METHODS

### Sampling

Fish were collected from Big Sound Bay in Lake Huron near Parry Sound, Ontario, Canada during August 17–24, 2013 and August 23–29, 2014 (see Fig. S1). Fish were sampled at the same time of year to minimise the potential effects of environmental or seasonal plasticity (McCallum *et al.* 2014). Fish were caught using a variety of techniques including angling, minnow traps, seine nets and gill nets. A total of 298 fish from 16 species were caught and used in this analysis (see

Table S1). Big Sound Bay is cut off from the rest of Georgian Bay by a ~ 6 km channel. Sampling and procedures were approved by the Ontario Ministry of Natural Resources (permit # UGLMU2013-06a, UGLMU2014-07) and the University of Guelph animal care committee.

### Field

Captured fish were processed daily on shore. Each fish was weighed and measured; large fish were weighed with a Rapala Pro Select Digital Scale and small fish were weighed with a laboratory balance (Mettler Toledo PB1502-S, Columbus, OH, USA). Fish body cavities were opened to examine their reproductive organs. Sex was recorded as 'male', 'female' or 'unidentified' for immature or very small fish. Samples were then taken from the dorsal caudal musculature (skin was cut out) of each fish for stable isotope analysis (see Fig. S2). In very small fish, a whole muscle fillet was taken from one side to obtain enough tissue for analysis. The muscle samples were frozen at  $-20^{\circ}\text{C}$  immediately after sampling and remained frozen until processing for stable isotope analysis (see Isotope analysis section). For large fish, the top half of the head was dissected and the base of the spinal cord was exposed before immersion in fixative (10% buffered formalin). The small fish were placed into fixative whole. Samples remained in fixative until further dissection (see Lab section). In addition to fish, bivalve mussels and littoral snails were collected for use as baselines in stable isotope analysis. These were used because they 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).

### Lab

During the months following field sampling (September–December 2013 and 2014), brains were dissected out of each individual fish. The brains were trimmed of excess cranial nerves and the spinal cord was cut at the level of the obex. The brains were then blotted using Kimwipes (Kimberly-Clark, Roswell, GA, USA) to remove excess formalin before weighing using an Accu-124D scale (Fisher Scientific, Waltham, MA, USA) at a resolution of 0.0001 g. The comparisons between fish body weight and brain weight were thus done between 'wet body weight' and 'post-fixation' brain weights.

### Data analysis

Because brain scaling displays a negative allometric relationship (i.e. brain size gets proportionally smaller with larger body size), the effects of individuals and species that differed in body size had to be accounted for before comparisons were made. Linear and Gompertz sigmoid models were fitted to a log-log brain-to-body size growth curve and compared using Akaike information criteria (AIC). The nonlinear Gompertz sigmoid model was found to have the best fit ( $\Delta\text{AIC} = 120.9$ ) (see Fig. S3,  $R^2 = 0.976$ ), which is in line with the nonlinear relationship across fish species observed by Bauchot *et al.* (1988). The Gompertz model is thought to provide an accurate representation of the developmental causes of allometry (Nijhout & German 2012). Then, the

residual values representing the distance away from this relationship were taken and used in all further analyses as representations of relative brain size. Positive values indicate larger than expected brain mass and negative values indicate smaller than expected brain mass corrected for body size. These residuals were not significantly correlated with log transformed body mass ( $R^2 = 0$ ,  $P = 0.974$ ). A separate analysis of uncorrected, absolute brain size was also conducted (see Table S2).

#### Isotope analysis

Stable isotopes of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  were used to acquire measures of habitat coupling 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, ON, Canada).  $\delta^{13}\text{C}$  isotopic values were corrected for fat content using the equation  $\delta^{13}\text{C}_{(corr)} = \delta^{13}\text{C} + (-3.32 + 0.99 * \text{C:N})$  (Post *et al.* 2007). Resulting  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values were used in the calculations of habitat coupling and trophic position. Random muscle samples analysed in triplicate showed that carbon standard error was 0.03 and nitrogen standard error was 0.04.

We used carbon ratios of consumers and baselines to measure the dietary littoral carbon use to represent the habitat use displayed by organisms with the equation:

$$\text{Percent Littoral Carbon Usage} = \frac{(\delta^{13}\text{C}_{\text{Fish}} - \delta^{13}\text{C}_{\text{mussel}})}{(\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{mussel}})}$$

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:

$$\text{Habitat Coupling} = 0.5 - |0.05 - \frac{(\delta^{13}\text{C}_{\text{Fish}} - \delta^{13}\text{C}_{\text{mussel}})}{(\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{mussel}})|}$$

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 macrohabitats and lower values indicate predominant use of one habitat type. In addition, we used nitrogen ratios from consumers and baselines to estimate a consumer's trophic position with the equation:

$$\text{Trophic Position} = \frac{(\delta^{15}\text{N}_{\text{Fish}} - \delta^{15}\text{N}_{\text{mussel}})}{3.4} + 2$$

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 increase in  $\delta^{15}\text{N}$  per trophic level and a value of 2 is added because we are using primary consumers instead of primary producers (i.e. mussels and snails instead of algae and phytoplankton) (Vander Zanden *et al.* 2000). The relationship between trophic position and relative brain size was first

assessed in a simple LMEM (see Table S3). However, because trophic position is correlated with body size (Warren & Lawton 1987), body size effects were also removed from trophic position by taking the residuals from a trophic position – log body size Gompertz relationship (see Fig. S4,  $R^2 = 0.663$ ). The Gompertz relationship was used because it provided the best fit ( $\Delta\text{AIC} = 15.9$ ) and for consistency with the size correction method used to obtain relative brain size. The residual values, representing the distance away from this relationship, were taken and used as a representation of relative trophic position in all further analyses. Positive values indicate higher than expected trophic position and negative values indicate lower than expected trophic position for a particular body size. A separate analysis showed that these residuals were not significantly correlated with log transformed body mass ( $R^2 = 0$ ,  $P = 0.96$ ). Using relative trophic position removes any species grouping based upon body size and examines variation in trophic position across all size classes. Significant results obtained using this variable would indicate relationships with changes in trophic position, not necessarily trophic position itself.

#### Analysis

##### *Within-population and between-species effects*

We used the 'within-subject centring' method described in van de Pol & Wright (2009) by applying centring to within-species data. As a result, the ecological variables (i.e. percent littoral carbon, habitat coupling and relative trophic position) were separated 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 observation 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 size.

##### *LMEMs*

Linear mixed effects models (LMEMs) were used to account for the clustered nature of our data, where multiple individuals were sampled from each species. Species grouping was initially modelled as a random effect intercept which allowed for individual species correlations. 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. In addition to this, the effect of elongated species was modelled because this body type has been shown to skew estimates of relative brain size (Bauchot *et al.* 1988). The inclusion of each ecological variable (within- and between-species effects together for littoral carbon use, habitat coupling and trophic position), fish sex and body type fixed effects were assessed using AIC techniques. Models including these variables were chosen over simpler models containing only within- and between-species ecological effects if  $\Delta\text{AIC} > 2$ . AIC was chosen over other information criteria because it is better suited for handling more complex 'infinite-dimensional' systems with 'tapering effects' (i.e. few major effects, more

intermediate effects, many small effects, etc.) such as those typically studied in ecology (Burnham & Anderson 2004; Yang 2005). Fixed effects for percent littoral carbon and fish sex were excluded from all subsequent models because their inclusion did not meet  $\Delta AIC > 2$ .

Four models were built in sequence from highest complexity to lowest complexity to determine how within- and between-species effects change under varying model assumptions (Table 1). The first model was built following prior methodology described by van de Pol & Wright (2009), where species groups were assumed to be commonly correlated with account for individual similarities within-species (Table 1, model 1). However, we realised that this model may be suppressing the between-species effects through the assumptions of common correlation, so we relaxed this assumption with the inclusion of a zero correlation term for within-species effects (Table 1, model 2). This permitted within-species relationships to vary independently of one another in an attempt to accurately depict the strength of within-species effects and reveal any between-species effects that may be present. Finally, in light of the correlated nature of coupling and relative trophic position it was important to examine these variables separately. Thus, we created two additional models to test for these effects. The habitat coupling (Table 1, model 3) and relative trophic position (Table 1, model 4) models assessed the within- and between-species effects on relative brain size, while allowing within-species relationships to vary independently. For ease of interpretation the significance of each fixed effect was then assessed using the R package *lmerTest* which obtains *P*-values from the *t*-tests of the models' fixed effects via the Satterthwaite approximation for degrees of freedom (Schalje *et al.* 2002).

Finally, an analysis of high and low ecological performers was conducted in an attempt to disentangle the two hypotheses tested in this study. For this purpose, models 3 and 4 (Table 1) were run again with the data divided into low and high relative trophic position and coupling values respectively. Trophic position values were based on residual values taken

from the body size – trophic position curve shown in Fig. S4, with negative residual values representing low relative trophic positions and positive residual values representing high relative trophic positions. Low and high habitat coupling values were arbitrarily set at below and above a value of 0.2.

## RESULTS

Through the estimation of fixed effects in model 1 we found that within-species relative brain size increased with increased habitat coupling (Estimate  $\pm$  SE =  $0.15 \pm 0.027$ ,  $t = 5.5$ ,  $P < 0.001$ ) and relative trophic position ( $0.064 \pm 0.015$ ,  $t = 4.2$ ,  $P < 0.001$ ), but neither had an effect between-species (habitat coupling:  $0.12 \pm 0.091$ ,  $t = 1.3$ ,  $P = 0.19$ ; relative trophic position:  $-0.03 \pm 0.04$ ,  $t = -0.75$ ,  $P = 0.46$ ). Once species groups were allowed to vary independently (zero correlation: Table 1, model 2) we found that relative brain size increased with habitat coupling within-species ( $0.13 \pm 0.038$ ,  $t = 3.6$ ,  $P = 0.014$ ) and with relative trophic position within- ( $0.06 \pm 0.023$ ,  $t = 2.7$ ,  $P = 0.026$ ) and between-species ( $0.036 \pm 0.017$ ,  $t = 2.2$ ,  $P = 0.029$ ). This model also revealed the expected positive relationship between relative trophic position and habitat coupling between species ( $r = 0.35$ ), which may explain why between-species habitat coupling had little effect in this model ( $0.06 \pm 0.03$ ,  $t = 1.6$ ,  $P = 0.11$ ). When examined independently in models 3 and 4, we found that relative brain size increased with habitat coupling at both within-species ( $0.12 \pm 0.04$ ,  $t = 2.8$ ,  $P = 0.027$ ) and between-species levels ( $0.082 \pm 0.033$ ,  $t = 2.4$ ,  $P = 0.015$ ), and that relative brain size also increased with relative trophic position both within-species ( $0.053 \pm 0.022$ ,  $t = 2.3$ ,  $P = 0.043$ ) and between-species ( $0.047 \pm 0.016$ ,  $t = 2.9$ ,  $P = 0.004$ ). Interestingly, a separate analysis of absolute brain size only showed significant increases with food web variables at the between-species level (see Table S2). This could suggest that forces driving changes (or that can drive change) in absolute brain size are distinct from those acting on relative brain size.

**Table 1** Model equations and fixed effects slope estimates for the effects on relative brain size

Model	Equation	Variables	Estimate	<i>t</i>	<i>P</i>
1	RBS=WTP+BTP+WCOUP+BCOUP+BT+(1 Spp)+(1 Sex)	WTP	$0.064 \pm 0.015$	4.3	< 0.001
		BTP	$-0.03 \pm 0.04$	-0.7	0.46
		WCOUP	$0.15 \pm 0.027$	5.5	< 0.001
		BCOUP	$0.12 \pm 0.09$	1.4	0.19
2	RBS=WTP+BTP+WCOUP+BCOUP+BT+(0 + WTP Spp)+(0 + WCOUP Spp)+(1 Sex)	WTP	$0.061 \pm 0.023$	2.7	0.026
		BTP	$0.036 \pm 0.016$	2.2	0.029
		WCOUP	$0.135 \pm 0.04$	3.6	0.014
		BCOUP	$0.056 \pm 0.035$	1.6	0.11*
3	RBS=WCOUP+BCOUP+BT+(0 + WCOUP Spp)+(1 Sex)	WCOUP	$0.12 \pm 0.04$	2.8	0.027
		BCOUP	$0.082 \pm 0.034$	2.4	0.015
4	RBS=WTP+BTP+BT+(0 + WTP Spp)+(1 Sex)	WTP	$0.053 \pm 0.022$	2.3	0.042
		BTP	$0.047 \pm 0.016$	2.9	0.004
Classic LMEM	RBS = RTP + HC + BT + (1 Spp) + (1 Sex)	RTP	$0.053 \pm 0.014$	3.7	< 0.001
		HC	$0.14 \pm 0.025$	5.5	< 0.001

The classic LMEM without the use of van de Pol & Wright (2009) centring approach is also included to demonstrate that our results are robust and remain consistent with and without the use of this technique. (RBS: relative brain size, Spp: fish species, Sex: fish sex, WTP: within-species relative trophic position, BTP: between-species relative trophic position, WCOUP: within-species habitat coupling, BT: Body type, BCOUP: between-species habitat coupling, RTP: calculated relative trophic position, HC: calculated littoral-pelagic habitat coupling).

\*In model 2 BCOUP and BTP are correlated with an  $r = 0.35$ , which may explain why BCOUP had no significant effect.

## DISCUSSION

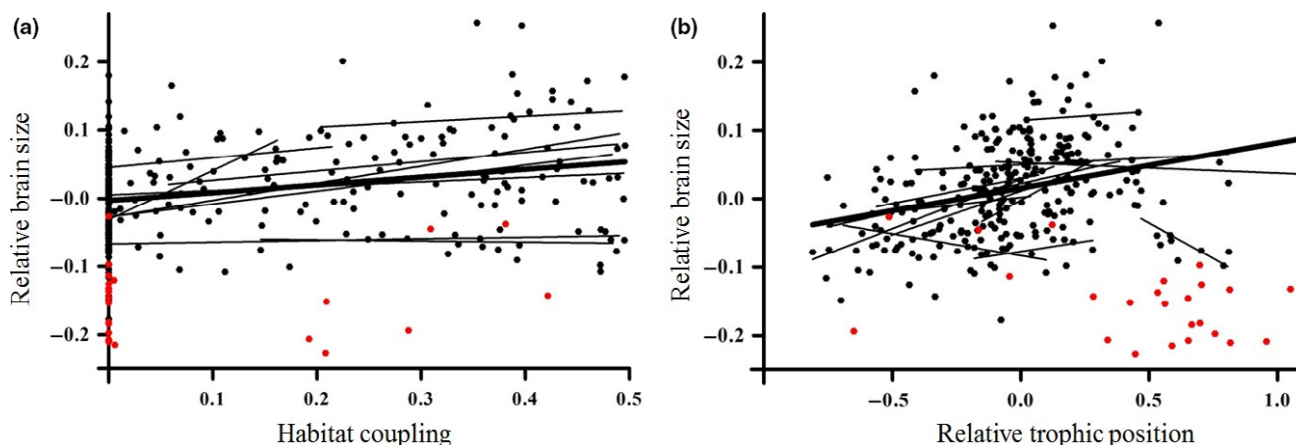
The results for habitat coupling support the Behavioural Flexibility Hypothesis and demonstrate that relative brain size tends to increase with littoral-pelagic habitat coupling within- and between-species in this system (Fig. 2a; Table 1). This suggests that fish cognitive ability may facilitate the flexible use of distinct food web compartments. The use of multiple habitats may require an increased cognitive capacity due to differential learning, sensory and/or motor requirements in pelagic and littoral zones. Interestingly, this pattern has been found at both within- and between-species levels, indicating that variation in habitat coupling both within populations and across different species may contribute to food web stabilisation.

There is evidence in other animals suggesting that flexible, innovative foraging strategies are associated with more elaborate brains (Lefebvre *et al.* 1997; Reader & Laland 2002; Ratcliffe *et al.* 2006). Interestingly, feeding innovation capacity does not correlate with diet breadth, but it correlates with habitat use flexibility (Overington *et al.* 2011). If these relationships represent general properties of brains and behaviour, habitat coupling, as measured here, is a measure of habitat use flexibility that could relate conceptually to feeding innovation, but it would not reflect ecological generalism (diet breadth). However, it is also possible that the relationship between ecological generalism and elaborate brains is taxon-specific, as suggested by results in insects (Farris & Roberts 2005). More work is needed to establish the precise aspects of fish habitat coupling that would require increased cognitive capacity.

The results found for relative trophic position support the Trophic Level Hypothesis and demonstrate that relative brain size tends to increase with trophic position within- and between-species in this system (Fig. 2b; Table 1). This result

is further partially supported by a supplementary analysis which showed that relative brain size increased with between-species trophic position not adjusted for body size (see Table S3). This suggests that attaining higher trophic positions both within- and between-species by feeding on relatively larger, more mobile organisms may require enhanced cognitive abilities to be successful. Higher cognitive capacity could allow individuals to respond adequately and more quickly to their prey, thus contributing to successful predation and higher than expected trophic positions. These results further tie cognition and brain size into the structure of food webs and suggest that the brain may be involved in the maintenance of important top-down regulatory processes.

The pressure for enhanced cognition in trophic interactions might critically depend on abilities of prey to evade predation because relatively large brain size is associated with enhanced anti-predator behaviour (birds: Møller & Erritzøe 2014; female guppies: Kotrschal *et al.* 2015; van der Bijl *et al.* 2015). This is likely why predators normally show a positive bias towards small-brained prey (Shultz & Finlayson 2010). Achieving a higher relative trophic position in a food web might require feeding on large-brained prey, which would require enhanced cognition in the predator. Smaller brained predators would be limited in the prey types they can consume, limiting the trophic position they can achieve. This is in line with the observation of Kondoh (2010) that larger-brained fish predators tend to prey on larger brained prey. However, our data does not allow us to decide if larger brains are needed to achieve high trophic position or coupling values, or rather if large brains are the result of high trophic or coupling activities. There are examples for both evolution of local adaptations and experience-dependent plasticity in brain-behaviour relationships. For example enhanced food-caching activity in chickadees is thought to be a result of evolution of local adaptations for large hippocampus size (Croston *et al.* 2015),



**Figure 2** The raw data representing within- and between-species effects of ecological variables on relative brain size. (a) The raw data of the relationship between relative brain size and habitat coupling. The large bold line represents the between-species relationship and each of the smaller lines represents within-species relationships. The within-species lines of five species are missing due to unanimous zero coupling values within the species. (b) The raw data of the relationship between relative brain size and relative trophic position. The large bold line represents the between-species relationship and each of the smaller lines represents within-species relationships. *Note:* these graphs show linear regressions based upon raw data to directly relate to the hypotheses shown in Fig. 1c and d. Thus, these depictions do not take into account model structure or the relationships that may exist between ecological variables. Data points for elongated species are depicted in red and within-species relationships for these species are excluded for clarity. Elongated species have much lower relative brain sizes (Bauchot *et al.* 1988) and fish body type had a significant effect in all models computed in this study despite a low count of elongated species (24 individuals from 3 species).

while mushroom body size in bees increases with foraging experience (Withers *et al.* 2008). An obvious question of interest for future investigations will be to evaluate the mechanisms determining relationships between brain size and ecological variables. The presence of significant within- and between- species relationships between brain size and food web variables suggest that both brain size evolution and within-population processes (i.e. plasticity or natural variation) influence changes in brain size. However, the brain is a modular organ with different regions supporting different functions. Thus, it is unclear how changes in the size of specific brain regions may contribute to these results (Healy & Rowe 2007). Future studies should also examine how brain regions vary in response to food web variables to help us to better understand the functional associations between ecology and the nervous system.

Our results support both the Behavioral Flexibility and Trophic Level hypotheses, which suggest that coupling and brain size patterns happen throughout the food web (i.e. brain size increases for couplers at all trophic levels both within and between species). This may imply that the association between cognitive ability and flexible resource use in fish is a feature that is ubiquitous throughout the food web. Nonetheless, we also found a tendency for this increase in relative brain size with coupling to be heightened with increasing trophic position (Table S4A), suggesting there may be an even greater benefit to enhanced brain size with increased trophic position. This result is consistent with the argument that smaller grain sizes (i.e. foraging scales) may not respond as strongly to the need for flexible behaviour. This result may be related to the empirical argument that spatial autocorrelation in resources tend to be more positive at smaller scales (i.e. resource variation in space is lower) thus weakening the benefits of adaptive foraging. An additional analysis of subsets of low and high couplers (Table S4B) suggest a general effect of trophic position on relative brain size across all fish, with the possible exception of an effect within-species for high couplers. Further work needs to be done on the mechanisms behind our results. If this is true, it suggests further evidence that more mobile, larger organisms may be the most potent stabilisers in whole food webs.

The association between cognitive ability and continuous habitat and behavioural variables were examined for the first time in a food web context. The results indicate that both within-population and between-species processes may be exerting effects on relative brain size or vice versa. This work provides additional evidence for a relationship between ecology and the nervous system. Furthermore, and importantly, the consistency of these results, both within- and between-species, suggests that this may be a fundamental pattern in nature that weaves the cognitive ability of organisms into food webs at a variety of scales (i.e. within-species and between species; see Bolnick *et al.* 2011 for discussion of the role within-species can play in food webs). Furthermore, from existing food web theory, this patterning in coupling, trophic position and cognitive ability suggest that this neuro-ecological relationship ought to play a fundamental role in the stability of food webs by providing key organisms (i.e. couplers) the cognitive ability to respond rapidly to changing resource

conditions (McCann 2007). Incorporating cognition into how we think about food webs can help us to understand how individuals and populations will respond in the face of changing environmental conditions in the future.

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#### AUTHOR CONTRIBUTIONS

N.B.E. completed all brain dissections and weighing. All authors contributed to the design of the study and the sampling and processing of fish in the field. N.B.E. analysed the results and wrote the initial draft of the manuscript with input from all authors. All authors contributed to revisions of the manuscript.

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