Canadian Journal of Fisheries and Aquatic Sciences
Journal canadien des sciences halieutiques et aquatiques

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Journal:	<i>Canadian Journal of Fisheries and Aquatic Sciences</i>
Manuscript ID	cjfas-2017-0412.R1
Manuscript Type:	Article
Date Submitted by the Author:	06-Nov-2017
Complete List of Authors:	Edmunds, Nicholas; University of Guelph, Integrative Biology McCann, Kevin; University of Guelph, Laberge, Frédéric; University of Guelph, Integrative Biology
Is the invited manuscript for consideration in a Special Issue? :	N/A
Keyword:	FISHES < Organisms, heart ventricle, PELAGIC < Environment/Habitat, littoral, TROPHIC RELATIONSHIPS < General

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1 **Relative heart size and fish foraging ecology in a lake food web**

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10

11 **Abstract**

12 Organisms occupying high trophic positions in food webs are proposed to exert a stabilizing
13 effect on ecosystems under changing conditions. A possible mechanism underlying this
14 phenomenon is higher mobility of large-bodied predators, which allows broad spatial scale
15 foraging across habitats. If top predators foraging flexibly across habitats display an enhanced
16 capacity for mobility, they should have increased blood circulation requirements compared to
17 other organisms in a food web. Blood circulation capacity can be estimated from the relative size
18 of the heart ventricle; the muscle mass that powers the cardiac pump. We sampled wild teleost
19 fish in a freshwater lake food web to investigate the relationship between relative heart ventricle
20 size and individual measures of trophic position, energy channel use (littoral vs. pelagic), and
21 foraging flexibility (coupling) estimated from N^{15} and C^{13} stable isotope signatures. The results
22 showed that relative ventricle size is associated with increased relative trophic position and
23 decreased use of littoral carbon across species, but not within-species, in this system. The
24 foraging flexibility required for coupling was not associated with heart ventricle size.

25

26 **Keywords:** fish, heart ventricle, myocardium, pelagic, littoral, coupling, trophic position, within-
27 species centering.

28

29 **Dimension cardiaque relative et alimentation des poissons dans un réseau alimentaire**

30 **lacustre**

31 **Resumé**

32 On croit que les organismes occupant les hautes positions trophiques des réseaux alimentaires
33 stabilisent les écosystèmes lors de conditions changeantes. Un mécanisme possible sous-tendant
34 ce phénomène est la mobilité accrue des prédateurs de grande taille qui permettrait l'alimentation
35 sur de grands espaces couvrant différents habitats. Si les grands prédateurs qui s'alimentent de
36 façon flexible dans différents habitats ont une mobilité accrue, ils devraient avoir des besoins en
37 circulation sanguine accrus en comparaison avec les autres organismes d'un réseau alimentaire.
38 La capacité de circulation sanguine peut être estimée par la dimension relative du ventricule
39 cardiaque; la masse musculaire qui propulse la pompe cardiaque. Plusieurs espèces de poissons
40 téléostéens ont été échantillonnées dans un réseau alimentaire lacustre dans le but d'évaluer le
41 rapport entre la dimension relative du ventricule cardiaque et des mesures individuelles de
42 position trophique, de l'usage du canal énergétique littoral, et de la flexibilité de l'alimentation
43 estimée à partir du couplage entre les canaux énergétiques littoral et pélagique. Ces dernières
44 variables ont été estimées à partir de signatures isotopiques de N^{15} and C^{13} . Les résultats
45 démontrent que la dimension relative du ventricule est associée à une augmentation de la
46 position trophique et a une diminution de l'usage du canal énergétique littoral entre les espèces,
47 mais pas au sein des populations, dans ce système lacustre. La flexibilité d'alimentation
48 nécessaire au couplage n'est pas associée à la dimension du ventricule cardiaque.

49 **Mots clés:** Poissons, ventricule cardiaque, myocarde, pélagique, littoral, couplage, position
50 trophique, centrage intra-espèce.

Introduction

Food webs are complex biological systems characterized by a hierarchy of organisms exploiting localized resources. It has been proposed that higher level organisms in food webs, those occupying higher trophic positions, may actually promote system stability through their abilities to adaptively forage on different lower level food web compartments in response to changing environmental conditions (Kondoh 2003; Rooney et al. 2006). Data suggesting that higher level organisms have more developed foraging abilities on average than organisms in lower trophic positions support this hypothesis (Peters 1983; Rooney et al. 2008). Understanding the underlying mechanisms of this stabilizing phenomenon is imperative because current human impacts on food webs are skewed toward organisms occupying high trophic positions, such as top predatory fish, and thus could threaten the stabilizing influence provided by these organisms in nature.

Top predators are generally characterized by large body sizes and high mobility (Peters 1983; Rooney et al. 2008), characteristics that challenge blood circulation for adequate supply of oxygen and nutrients to the tissues. The fish blood vascular system is a closed re-circulation system that relies on contraction of heart muscle – the myocardium – to provide the driving force moving the blood (Kardong 2009). The fish heart is a circulatory pump made of 4 compartments (sinus venosus, atrium, ventricle, conus/bulbus) designed to pump blood in one direction from the venous system to the ventral aorta, then on to the gills for re-oxygenation. The ventricle is by far the most muscular of the heart compartments and it generates the force needed to circulate blood. A large ventricular mass increases maximal power output, which is important to accommodate a large cardiac stroke volume or high blood pressure in fish and amphibians (Farrell and Jones 1992; Clark and Rodnick 1999; Kluthe and Hillman 2013).

Different fish species display two main types of heart ventricles: the spongy and mixed types, reflecting two different solutions to the problem of pumping blood to supply body tissues (Agnisola and Tota 1994). The mixed type ventricle displays an additional outer layer of compact myocardium – the compacta – encircling a core network of sponge-like muscle trabeculae – the spongiosa (e.g. Pieperhoff et al. 2009). Cardiac output in fish is primarily modulated by changes in stroke rate and volume. Comparison of the two ventricle types shows that the spongy ventricle type functions as a volume pump moving blood at high stroke volume, but low stroke rate and low pressure, and the mixed ventricle type functions as a pressure pump moving blood at small stroke volume, but high stroke rate and high pressure (Mendonça et al. 2007). Some fish can compensate a decrease in stroke rate with an increased stroke volume, as shown in Pacific Bluefin tunas exposed to low temperature (Blank et al. 2004). Overall, pressure pumping appears better suited for active fish, which demand sustained aerobic muscle activity. Volume pumping might be better suited for less active fish, which only undergo occasional bursts of intense swimming activity.

Looking at heart ventricle features could thus provide a good indication of the pressures for enhanced mobility faced by fishes in their natural environment. The more so because, in addition to potential evolutionary change in the heart across generations, cardiac remodelling over a short period of time has been demonstrated in fish under laboratory conditions (Gamperl and Farrell 2004). For example, cold temperature acclimation can increase ventricle mass in rainbow trout, a fish that remains active at low seasonal temperatures (Farrell and Jones 1992; Klaiman et al. 2011). Further, swimming performance in hatchery-raised rainbow trout correlates with heart ventricle shape, with poor swimmers displaying more rounded ventricles than good swimmers (Claireaux et al. 2005). Interestingly, wild salmonid heart ventricles are much more

elongated compared to hatchery-raised conspecifics (Poppe et al. 2003), suggesting that ecological pressures faced by wild fish could strongly influence their heart morphology.

In the present study, we explored potential relationships between foraging ecology and heart ventricle mass in teleost fishes sampled from a lake food web with distinct pelagic and littoral habitats. Other heart features, such as ventricle shape, compacta thickness, stiffness due to differential collagen content, or cardiac muscle biochemistry could also contribute to differences in heart function, but are not explored here. A food web approach allowed us to obtain quantitative values of trophic position, energy channel use (littoral vs. pelagic), and foraging flexibility for each fish sampled. It was hypothesized that both evolutionary and within-population processes shape heart ventricle morphology in the face of ecological pressures in wild fish. Within-population changes could happen through cardiac remodelling during the lifetime of individuals, but we limited our investigation of this possibility to current ecological pressures by focusing our sampling efforts spatially and seasonally. Since top predators are generally more mobile, thus potentially facing increased swimming requirements, we predicted that fish heart ventricle size corrected for body size should increase with increasing trophic position within a food web. We further predicted that heart ventricle size should increase with pelagic habitat use due to the increased swimming requirements in open water compared to the shallower waters of the littoral habitat. Finally, we predicted that increased foraging flexibility (or coupling) is associated with an increase in mobility that would also require larger heart ventricles.

Methods

Sampling

119 A total of 313 fish from 16 species of teleosts were used in this analysis (Table 1). They were
120 sampled during August 17-24, 2013 and August 23-29, 2014 in Big Sound Bay (Figure S2),
121 which is in the Georgian Bay part of Lake Huron near Parry Sound, Ontario, Canada. Fish were
122 caught using angling, minnow traps, seine nets and gill nets. Sampling procedures were approved
123 by the Ontario Ministry of Natural Resources (permit Nos. UGLMU2013-06a, UGLMU2014-07)
124 and the University of Guelph Animal Care Committee. Each fish was measured (total length)
125 and weighed on the day of capture using a Rapala Pro Select Digital Scale (large fish) or a
126 Mettler Toledo PB1502-S balance (small fish). Fish were opened to record sex, which was
127 scored as 'unidentified' for immature or very small fish. Dorsal caudal muscle samples (without
128 skin) were additionally taken from each fish for stable isotope analysis. In very small fish, a
129 whole muscle fillet was taken from one side to obtain enough tissue for analysis. The muscle
130 samples were frozen at -20°C immediately after sampling and remained frozen until further
131 processing. To improve fixation of the interior of heart ventricles of large fish, ventricles were
132 extracted, pumped manually in 0.9% saline to clear excess blood, and a small volume of 10%
133 buffered formalin was injected directly into the ventricle using a blunt needle inserted at the
134 atrio-ventricular junction. Small fish were placed into formalin whole and dissected later in the
135 laboratory. The samples were stored in the formalin solution for a period of 1-3 months before
136 processing in the laboratory, where ventricles were trimmed of excess tissue, blotted using
137 Kimwipes (Kimberly-Clark) to remove excess formalin, and weighted using a Fisher Scientific
138 Accu-124D scale at a resolution of 0.0001g. The values reported are thus obtained from wet
139 body masses and post-fixation ventricle masses.

140

141 *Heart ventricle histology*

Because of the paucity of reports on internal heart ventricle morphology in freshwater fishes, ventricle type had to be assessed for each species using histology (Table 1). Structural differences between heart ventricle types are illustrated by sections of the spongy ventricle of a walleye (*Sander vitreus*) and the mixed ventricle of a lake whitefish (*Coregonus clupeaformis*) (Figure 1). In spongy ventricles, muscle trabeculae reach all the way to the epicardium on the surface of the heart, while in mixed ventricles there is an intervening layer of concentrically-oriented muscle, the compacta, between the spongy muscle trabeculae and the epicardium. Transverse ventricle sections 50 µm thick were cut on a Leica VT1200S vibrating microtome, mounted on gelatinized glass slides, and dried. At least one specimen of every species was stained with the Picrosirius Red method by incubating sections in a solution of 0.1% Sirius Red in saturated picric acid (Electron Microscopy Sciences, Hatfield, PA) for 18 h before differentiation in 0.5% acidified water for 15 min, dehydration in ascending ethanol concentrations, clearing in xylene, and mounting with Cytoseal (ThermoFisher Scientific, Waltham, MA). A modified Masson's trichrome method (Weigert's nucleus stain omitted) was also used to confirm the muscular nature of the thin compacta in some species (e.g. pike and alewife). Briefly, sections obtained as described above were re-fixed in Bouin's fixative (Ricca Chemical Company, Arlington, TX) overnight and briefly washed under tap water before a 10 min incubation in 0.5% Acid Fuchsin (BDH, UK) and 0.5% Xylidine Ponceau (Hartman-Leddon, USA) dissolved in 1% glacial acetic acid. After short washes in distilled water, the sections were then incubated for 5 min in 1% phosphotungstic acid (Fisher), briefly rinsed with distilled water, and then incubated for 10 min in 2% Fast Green FCF (BDH) dissolved in 2% glacial acetic acid. Sections were then washed 3 times for 1 min in distilled water, dehydrated, cleared and mounted as described above.

165

166 *Isotope analyses*

167 In addition to fish, bivalve mussels and littoral snails were collected for use as baselines in stable
 168 isotope analyses. These organisms accurately reflect within lake spatial differences in isotope
 169 signatures between shallow water littoral and open water pelagic zones (Post 2002). Baseline
 170 organisms and fish were collected during the same time period. Mussels were collected by diving
 171 to a depth of approximately 2.5 m over sandy bottoms adjacent to the open water of the study
 172 site. Snails were hand-picked in shallow waters (<50cm) off of rocks, woody material, or out of
 173 the substrate in areas proximate to locations where littoral fishes were obtained. Stable isotopes
 174 ratios of ^{13}C and ^{15}N were used to acquire three distinct quantitative measures of foraging
 175 ecology for the fish under study: energy channel use, coupling, and trophic position. Fish muscle
 176 samples and baseline organisms were dried at 70°C for 2 days, ground into powder, and sent to
 177 the University of Windsor Great Lakes Institute for Environmental Research laboratories for
 178 isotopic analysis (Windsor, ON, Canada). $\delta^{13}\text{C}$ isotopic values were corrected for fat content
 179 using the equation $\delta^{13}\text{C} = -3.32 + 0.99 * \text{C:N}$ (Post et al. 2007). Random muscle samples
 180 analyzed in triplicate showed that carbon and nitrogen standard errors were 0.03 and 0.04,
 181 respectively.

182 We used carbon ratios of consumers and baselines to measure littoral carbon use and the
 183 amount of coupling displayed by organisms with the following equations:

$$184 \quad \text{Littoral carbon use} = (\delta^{13}\text{C}_{\text{fish}} - \delta^{13}\text{C}_{\text{mussel}}) / (\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{mussel}})$$

$$185 \quad \text{Coupling} = 0.5 - |0.5 - (\delta^{13}\text{C}_{\text{fish}} - \delta^{13}\text{C}_{\text{mussel}}) / (\delta^{13}\text{C}_{\text{snail}} - \delta^{13}\text{C}_{\text{mussel}})|,$$

where C_{fish} , C_{mussel} and C_{snail} are the carbon signatures of fish, mussels and snails, respectively. The littoral carbon use equation gives a proportion of littoral carbon in the diet ranging from 0 (pelagic specialists) to 1 (littoral specialists). Individual fish $\delta^{13}C$ values that fell beyond end member baseline values in the littoral carbon use equation were set to 0 and 1, as done previously (Vander Zanden and Vadeboncoeur 2002). The scale of the coupling equation ranges from 0 – 0.5, where values closer to 0.5 indicate greater amounts of coupling between the pelagic and littoral energy channels and lower values indicate predominant use of one resource type. We used mean baseline values in the analysis presented here (n=15 mussel samples and n=6 snail samples), but verified that our results remained qualitatively the same when using the maximal range of baseline values and when rejecting the fish that fell beyond end member baseline values (see below).

Additionally, we used nitrogen ratios from consumers and baselines to estimate fish trophic position with the following equation:

$$\text{Trophic Position} = [(\text{LC}(\delta^{15}N_{\text{fish}} - \delta^{15}N_{\text{snail}})/3.4) + (1 - \text{LC}(\delta^{15}N_{\text{fish}} - \delta^{15}N_{\text{mussel}})/3.4)] + 2,$$

where LC is littoral carbon use as above, and N_{fish} , N_{snail} and N_{mussel} are the nitrogen signatures of fish, snails and mussels, respectively. We also used mean baseline values to obtain trophic position. The value of 3.4 is the assumed increase in $\delta^{15}N$ per trophic level and a value of 2 is added because we are using primary consumers instead of primary producers in the equation (i.e. mussels and snails instead of algae and phytoplankton) (Vander Zanden et al. 2000). As trophic position is correlated with fish size (Warren and Lawton 1987), the effect of body size was removed by taking the residuals from a trophic position – $\log_{10}$ body size linear relationship. The residual values, representing the distance away from this relationship, were taken and used in all

further analyses as a representation of relative trophic position. Positive values indicate higher than expected trophic position and negative values indicate lower than expected trophic position for a particular body size.

Data analysis

The $\log_{10}$ of heart ventricle mass scaled nearly isometrically with $\log_{10}$ body mass, so linear regression was used to obtain residual values of ventricle size to correct for body size (Figure 2). The resulting variable was termed ‘relative ventricle size’ and was used in all further analyses. A linear mixed effect modelling (LMEM) approach was used to account for the clustered nature of the data, where multiple individuals were sampled from each species. We modified the “within-subject centering” method described in van de Pol and Wright (2009) by applying centering to within-species data. As a result, the ecological variables were separated into within-species variation, obtained from the equations for littoral carbon use, coupling and trophic position shown above, and between-species variation, obtained by replacing each individual value with its species mean value. By including both variables as fixed effects in a model it was possible to evaluate both the within- and between-species effects of the ecological variables under investigation on relative ventricle size. Between-species effects represent the product of evolution, whereas within-species effects may result from phenotypic plasticity, influencing heart growth in individuals, or natural variation present within a population. This approach was used in an attempt to investigate the current and past evolutionary pressures acting on heart ventricle size in a food web. In order to focus our investigation of within-species effects on potential intraspecific associations between current foraging ecology and relative ventricle size,

we limited our sampling efforts to the same season over two consecutive years in one location. This was done because seasonality and spatial habitat differences could introduce variation in ecological pressures faced by fish not due to foraging ecology.

Species grouping was included in all models as a random effect. While prior methodology described by van de Pol and Wright (2009) assumes groups to be commonly correlated (e.g. 1|Spp term in R for species grouping in the present study), this method potentially constrains between-species effects through the over-estimation of within-species effects. Thus, we included a zero correlation term (i.e. 0+within-species effect|Spp term in R), which permitted within-species relationships to vary independently of one another, thereby releasing any between-species effects that may be present. Other potential factors influencing relative heart ventricle size, such as body type (normal or elongated), ventricle type (spongy or mixed), and sex (male, female or unidentified) were assessed as fixed effects and fixed effect interaction terms with ecological variables. Interaction terms were limited to combinations including within-species effects because species of particular types were low in number (e.g. elongated body, mixed ventricles) or did not have sex information, which did not allow enough statistical power to examine interactions at the between-species level.

We used forward exclusion model selection, first by assessing the effects of pairs of ecological variables on their own, then in all combinations possible (Table S1). The potential effect of body type was then assessed by addition to the best fit model obtained in the first step, followed by ventricle type, then sex. Inclusion of these effects in the best-fit model was based on Akaike's information criterion (AIC). More complex models were selected only if inclusion of effects met the criterion of $\Delta AIC > 2$. 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 and Anderson 2004; Yang 2005). The statistical significance of fixed effects was assessed using the R package *lmerTest*, which obtains p-values from *t* tests of the modelled fixed effects via the Satterthwaite approximation for degrees of freedom (Schaalje et al. 2002). LMEM relies on normal distribution of the model residuals and low sensitivity of model fit to unusual observations (West et al. 2014). Visual inspection of the distribution of model residuals and Q-Q plot revealed no important deviation from normality. To assess sensitivity of model fit, an analysis omitting the four greatest outliers (two ciscos and two trout-perch) was performed. Removal of the four outliers from the analysis produced similar results (not shown); therefore, in the following we present results pertaining to the analysis of the whole dataset.

Phylogenetic Generalized Least Squares

Significant between-species effects were verified using phylogenetic generalized least squares (PGLS) to determine if they persisted when accounting for phylogenetic relatedness among species (Freckelton et al. 2002). PGLS tests were done within the R Studio platform v2.1. *caper* and *ape* packages using average values of relative ventricle size and ecological variables for each species. The phylogenetic tree supporting PGLS analyses 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 was checked for

accuracy against a more extensive phylogeny of ray-finned fishes (Near et al. 2012) and is published elsewhere (Edmunds et al. 2016a).

Results

The fish of the Big Sound Bay system occupy a wide range of carbon and nitrogen isotopic space (Figure S1). Species with the most negative carbon signatures such as rainbow smelt, alewife and cisco represent the pelagic end of carbon isotopic space, while species with less negative carbon signatures such as round goby, rock bass and bluntnose minnow represent the littoral end of carbon isotopic space. The sample includes more littoral species compared to pelagic ones, which is in line with an overall lower diversity of fishes in the pelagic habitat of lakes (Benson and Magnuson 1992). The most predatory fish (lake trout, burbot, walleye, pike) occupy a central isotopic space at high nitrogen signatures, suggestive of habitat coupling, but overlap in nitrogen space with smaller species like cisco and rainbow smelt. This hump shape pattern in the carbon-nitrogen isotopic space is a common feature of aquatic food webs (Vander Zanden and Vadeboncoeur 2002; Rooney et al. 2008). The smaller fish species trout-perch and spottail shiner also occupy a central isotopic space, but at lower nitrogen signatures compared to top predators, suggesting that some fish species at lower trophic levels display habitat coupling in this system. Such coupling could be the result of diurnal migration between pelagic and littoral habitats, at least in trout-perch (Emery 1973).

The model of best fit obtained by the forward selection procedure includes body type and the ecological variable pairs trophic position and littoral carbon use (Table S1). This model implies that the combination of these variables best explains the variation in relative heart size

that is not due to variation in body size in the sample of fish under study. No obvious pattern in the data explains the effect of body type, so it might be included in the model of best fit because two of the three elongated species under study (rainbow smelt and burbot) have high values of relative trophic position, which could have produced a spurious correlation in the model. The inclusion of the coupling variable pair in the models did not improve AIC and thus, coupling does not associate with relative size of the heart ventricle in fish of the Big Sound Bay system. Similarly, ventricle type and sex did not improve model fit and were not included in the final model to explain variation in relative heart ventricle size in these fish. Tests of fixed effects based on the model of best fit showed that relative ventricle size is positively associated with increasing trophic position between-species ($p < 0.001$) and negatively associated with increasing littoral carbon use ($p < 0.001$) between-species (Table 2). The within-species relationships were not statistically significant. Analyses of $\delta^{13}C$ isotopic variables obtained using the maximal range of baseline values (Table S2) or rejecting the fish that fell beyond end member baseline values (Table S3) yielded similar results. PGLS analyses showed a positive trend for trophic position, albeit not statistically significant ($p = 0.09$), and a significant negative relationship for littoral carbon use ($p = 0.01$).

The mixed modelling approach presents difficulties for illustration of relationships between variables because of the multidimensional nature of the models. As an alternative, graphs of non-modelled species average values for each ecological variable against species averages of relative heart ventricle size are shown to summarize the between-species relationships evaluated in this study (Figure 3). The lines of best fit corresponding to significant relationships in Table 2 are only included for illustration purpose.

Discussion

We hypothesised that evolutionary (i.e. between-species) and within-population processes influence relationships between foraging ecology and heart morphology in wild fish. Our hypothesis received only partial support from the present analysis, as significant between-species, but not within-species, relationships involving relative ventricle size and ecological variables were found. The lack of within-species relationships linking relative heart ventricle size and the ecological variables examined in the present study suggest that remodelling of the heart does not contribute to individual foraging abilities in teleosts. Relative size of the heart ventricle in fish of the Big Sound Bay system increases with increasing relative trophic position and decreases with increasing use of the littoral habitat across species. The lack of PGLS support for the effect of trophic position indicates that phylogenetic relatedness could have contributed to the observed relationship. This may be due to the small sample of species available for this test. Thus, we consider that this relationship is probably not due solely to phylogenetic relatedness among the species analysed; however, this result will require future confirmation with a larger number of species from this system, if possible. Despite the latter caveat, these results are in accordance with our predictions that relative heart ventricle size should be shaped by evolutionary processes to deal with increased swimming requirements associated with enhanced predation abilities and life in the open waters of the pelagic habitat. Our other prediction that larger relative ventricle size would associate with increased coupling was not met. Thus, it appears that coupling ability, which we presume is associated with foraging flexibility, does not require enhancement of swimming capacity through increased heart muscle mass.

The association between relative ventricle size and trophic position across species may relate to the evolution of enhanced swimming abilities for predators feeding upon larger prey.

Fish that achieve relatively high trophic positions do so by capturing larger prey, which reduces the number of foraging events required to meet energetic needs (Kaufman et al. 2006; Jensen et al. 2012). Although this strategy could reduce the frequency of foraging events, the swimming requirements for foraging events on more mobile prey should increase in intensity. Thus, predation at higher trophic levels could challenge the circulatory system during less frequent but more intense bouts of foraging. Additionally, the capture of larger prey brings about greater demands on the digestive system for breakdown and assimilation of large meals. Since digestion in fish is associated with increased cardiac output, achieved by larger stroke volume and increased heart rate (Seth and Axelsson 2009), it is conceivable that a larger heart ventricle could contribute to enhance blood circulation and reduce total time needed for digestion of large meals in predators. It would be interesting to distinguish how locomotor and digestive requirements associated with feeding on larger prey could promote increased heart ventricle size. One could study how the relative size of digestive organs and the heart vary among species on a trophic position gradient to evaluate if digestive constraints due to trophic position contribute to increased heart size in animals.

Although we assumed that blood circulation capacity is the main need driving change in heart ventricle size, it is possible that temporary modulation of peripheral arterial resistance would be sufficient to sustain predation bouts or digestion of large meals without the need for increased blood circulation that would depend on an enlarged ventricle. Regulation of blood flow distribution occurs through local changes in peripheral arterial resistance in relation to metabolic demands (Sandblom and Gräns 2017). Fish at high trophic positions that would only utilize an arterial pressure modulation strategy would need a longer time to regenerate systemic blood pressure after a predation event, which could be accomplished by a sustained higher cardiac

output during recovery or a longer interval between predation events. Different species might use different strategies. It is also possible that fish at high trophic positions increase mean arterial pressure to meet their foraging needs and that ventricle hypertrophy follows as an indirect consequence of this pressure overload, as seen in the pathological ventricle hypertrophy that accompanies human hypertension (Dorn 2007). For now, the above ideas are speculative since arterial pressure is difficult to measure in wild fish.

The large size and three-dimensional nature of pelagic open waters could promote an increased need for sustained swimming, especially with respect to foraging behaviour. Lower prey densities typical of the pelagic environment should increase the cost of foraging activity by promoting more active prey search and pursuit (Giacomini et al. 2013). By comparison, less swimming capacity might be required for benthic search or sit-and-wait strategies characterizing fish in littoral habitats. These differences in fish foraging behaviour are supported by evidence of habitat-specific activity costs in the top predator, Northern pike (*Esox lucius*); a species that makes opportunistic use of the pelagic zones of lakes at the cost of increased locomotion and foraging activity in comparison to the preferred littoral habitat (Eklöv and Diehl 1994; Kobler et al. 2009). The above considerations suggest that the association observed here between relative heart ventricle size and littoral carbon use across species is likely related to the evolution of enhanced swimming abilities for life in the pelagic environment. This observation is supported by previous work showing a positive relationship between relative ventricle size and level of mobility in different fish species (reviewed in Santer 1985).

Relative size of the heart ventricle was not associated with coupling in this system. We assumed that high coupling values represent a relatively equal incorporation of carbon obtained from different prey that forage preferentially on the littoral or pelagic energy channels and

indicate the ability to forage flexibly on different prey. However, predators could theoretically obtain a high coupling value by specializing on a prey that displays high coupling. Figure S1 shows that two potential prey species from Big Sound Bay show such high average coupling values (i.e. trout-perch and spottail shiner). We consider the possibility of specialization on high coupling prey unlikely in light of current knowledge of foraging behaviour in the predator species under study. First, predators such as lake trout and walleye are known to consume a broad diversity of fish when they are available (Martin 1970; Scott and Crossman 1973; Vander Zanden and Rasmussen 1996; Vander Zanden et al. 1997; Bur et al. 2008). Second, while coupling in these fish predators varies with littoral habitat size, water temperature and water transparency, the habitat use of their main prey species remains relatively constant in a preferred macrohabitat (Dolson et al. 2009; Tunney et al. 2012; Tunney et al. in press). It is interesting to note that the present finding of no association between relative heart size and coupling contrasts with results obtained on brain size using the same sample of fish, where relative brain size was associated positively with increased coupling and trophic position both at between- and within-species scales (Edmunds et al. 2016b). The relationship between brain size and coupling was taken as evidence suggesting that larger brains may afford the cognitive capacity to exploit various habitats flexibly, thereby promoting system stability (Rooney et al. 2006; 2008). When taking the present results into account, it appears that behavioural flexibility might be a more important feature of organisms for system stability than the enhanced mobility afforded by large body sizes or large heart ventricles.

Effects on ventricle size were only observed at the between-species scale, suggesting that evolution is the main force driving change in heart morphology in the face of ecological pressures. When contrasted with the results on brain size, it suggests that the heart might not be

as plastic as the brain when it comes to the effects of ecology on organ morphology, or that environmental factors (e.g. temperature and seasonality) are the main drivers of heart plasticity instead of ecological factors. Indeed, the fish heart is very plastic, as important changes in relative ventricle size and other heart variables, including the Ca^{2+} sensitivity of force generation, have been demonstrated following temperature acclimation (Klaiman et al. 2014; Keen et al. 2017). For example, it is thought that temperate fish that remain active throughout the year have to deal with a large increase in blood viscosity at low winter temperatures (Graham and Fletcher 1983). Ventricle hypertrophy associated with low temperature acclimation could allow the extra pumping capacity needed to circulate more viscous blood as well as help compensate for the influence of lower temperature on myofilament function. This environmentally-induced plasticity could differ from ecologically-induced plasticity, not only in the cues triggering it, but also in the mechanisms engaged to exert change in target organs. A topic of interest for future studies could be more subtle functional aspects of the heart that could be influenced by foraging ecology, such as connective tissue content or biochemical regulation of myocardium contraction. Additionally, individual variation in environmentally-induced plasticity of ventricle size across seasons could influence patterns of variation in foraging performance within populations. Thus, an interesting question for the future is whether relationships between foraging ecology and heart ventricle size emerge in populations of active fish at the seasonal peak of remodelling.

In conclusion, attributes of the freshwater lake system under study suggest that foraging ecology can influence the evolution of relative heart ventricle size in fish, but it does not appear to induce cardiac remodelling of heart size. The results additionally suggest that increased blood circulation capacity and mobility afforded by larger heart ventricles could benefit fish by enhancing capture of larger prey and life in the open waters of pelagic environments. However,

increased fish mobility does not appear to enhance the flexible exploitation of different energy channels and habitats, which has been proposed to underlie the ecosystem stabilization effects of top predators (Kondoh 2003; Rooney et al. 2006; 2008). Enhanced predator features other than mobility, such as behavioural flexibility, could influence ecosystem stability.

Acknowledgements

We thank everybody who helped collect samples in the field, Sophy Ding for help in the laboratory, and Joe Scott for his generous financial contribution to the 2014 field season. We would also like to acknowledge the efforts of Aaron Fisk and the University of Windsor GLIER Stable Isotope Lab. Todd Gillis provided helpful comments on the manuscript. This research was supported by the NSERC Discovery Grant program (K.S.M and F.L.) and the Canada First Research Excellence Fund (K.S.M.).

Data accessibility

All data used in this study can be accessed in supplementary Table S4.

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- 578

579 Table 1. Species sampled, sample sizes, and heart ventricle types.

Common Name	Latin Name	Sample Size	Ventricle Type
Alewife	<i>Alosa pseudoharengus</i>	33	Mixed
Bluntnose minnow	<i>Primephales notatus</i>	6	Spongy
Burbot*	<i>Lota lota</i>	5	Spongy
Cisco	<i>Coregonus artedii</i>	36	Mixed
Lake trout	<i>Salvelinus namaycush</i>	48	Mixed
Lake whitefish	<i>Coregonus clupeaformis</i>	2	Mixed
Northern pike*	<i>Esox lucius</i>	4	Mixed
Pumpkinseed	<i>Lepomis gibbosus</i>	9	Spongy
Rock bass	<i>Ambloplites rupestris</i>	10	Spongy
Rainbow smelt*	<i>Osmerus mordax</i>	15	Spongy
Round goby	<i>Neogobius melanostomus</i>	20	Spongy
Smallmouth bass	<i>Micropterus dolomieu</i>	58	Spongy
Spottail shiner	<i>Notropis hudsonius</i>	10	Spongy
Trout-perch	<i>Percopsis omiscomaycus</i>	8	Spongy
Walleye	<i>Sander vitreus</i>	19	Spongy
Yellow perch	<i>Perca flavescens</i>	30	Spongy

580 *Elongated species

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Table 2. Parameter estimates and statistics for the effects on relative heart ventricle size. The model of best fit equation is $rHeart = WTP + BTP + WLC + BLC + BT + (0+WTP|SPP) + (0+WLC|SPP)$. Model selection procedure is detailed in Table S1. Note that species average trophic position did not significantly correlate with average littoral carbon use ($r = -0.36$, $p = 0.17$, $R^2 = 0.13$) or coupling ($r = 0.2$, $p = 0.47$, $R^2 = 0.04$).

Variable	Estimate	t value	p value	Result of PGLS (estimate, R^2 , p value)
Intercept	0.14 ± 0.03	4.49	<0.001*	
BTP	0.09 ± 0.02	3.64	<0.001*	0.12 ± 0.07 , 0.22, $p=0.09$
WTP	-0.005 ± 0.06	-0.08	0.94	
BLC	-0.15 ± 0.02	8.35	<0.001*	-0.19 ± 0.06 , 0.41, $p=0.01^*$
WLC	-0.003 ± 0.04	-0.09	0.93	
BT	-0.06 ± 0.03	2.19	0.03*	

Abbreviations: AIC: Akaike information criterion, BLC: between-species littoral carbon use, BT: body type, BTP: between-species trophic position, PGLS: phylogenetic generalized least squares regression, R^2 : coefficient of determination, rHeart: relative heart size, SPP: species, WLC: within-species littoral carbon use, WTP: within-species trophic position.

Figure 1. Examples of spongy and mixed fish heart ventricles. Sections stained by the Picrosirius Red method show the typical internal morphology of a spongy (A: walleye) and a mixed heart ventricle (B: lake whitefish); the latter with an outer layer of compact myocardium (within arrow limits). Scale bar is 5 mm.

Figure 2. Relationship between $\log_{10}$ body mass and $\log_{10}$ heart ventricle mass for the 313 fish sampled in this study. The regression is significant ($F(1, 311) = 17340$, $p < 0.0001$), with an R^2 of 0.98. The relationship equation is $y = -0.166 \pm 0.012 + x0.939 \pm 0.007$. The distance away from the linear regression curve (residual values) was used as an individual measure of relative ventricle size in analyses. Symbol legend is AW: alewife, BM: bluntnose minnow, BU: burbot, CI: cisco, GO: round goby, LT: lake trout, PI: northern pike, PS: pumpkinseed sunfish, RB: rock bass, RS: rainbow smelt, SM: smallmouth bass, SS: spottail shiner, TP: trout-perch, WA: walleye, WF: lake whitefish, YP: yellow perch.

A

B

C

Figure 3. Summary of the between-species relationships evaluated in this study. A: relative trophic position, B: littoral carbon use, and C: coupling. The graphs show non-modelled species

617 average values for each ecological variable against species averages of relative heart ventricle
618 size. Lines of best fit obtained through linear regressions are shown for illustration purpose for
619 the significant relationships obtained by the linear mixed effect modelling approach. Symbols for
620 each species are as in Figure 2.

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