

Foraging ecology of native pumpkinseed (*Lepomis gibbosus*) following the invasion of zebra mussels (*Dreissena polymorpha*)

S.F. Colborne, A.D.M. Clapp, F.J. Longstaffe, and B.D. Neff

Abstract: Invasive species are a major concern both biologically and economically because of the rates at which they spread and their alterations to ecosystems. Zebra mussels (*Dreissena polymorpha*) are of concern in many regions owing to expenses related to industrial equipment damage and control education efforts. However, their greatest cost may be their ecological impact across North America. We tested pumpkinseed (*Lepomis gibbosus*) from three populations in Ontario (Canada) to determine if there was variation across populations or sampling periods in the consumption of zebra mussels. Using a combination of stomach content and stable isotope analyses, we found that pumpkinseed diet consisted of primarily zebra mussels across all populations and sampling periods, including a minimum of 54% in all lakes and sampling periods based on stable isotope mixing models. The current resource use patterns of pumpkinseed in our study populations indicate that there has been a shift from their pre-invasion diet of benthic littoral invertebrates such as gastropods. The large-scale consumption of invasive mussels by native fishes may have important implications for natural biological control of these mussels, decreasing overall mussel abundance and rate of spread. We discuss our results in the context of their implications for the overall health and dynamics of aquatic ecosystems.

Résumé : Les espèces envahissantes constituent une importante préoccupation sur le plan tant biologique qu'économique en raison de la vitesse à laquelle elles se propagent et des modifications qu'elles causent aux écosystèmes. Les moules zébrées (*Dreissena polymorpha*) sont préoccupantes dans de nombreuses régions en raison des dépenses reliées aux dommages au matériel industriel et aux efforts de sensibilisation à leur contrôle. Le coût le plus important qui leur est associé pourrait toutefois être leur impact écologique dans l'ensemble de l'Amérique du Nord. Nous avons examiné des crapets-soleil (*Lepomis gibbosus*) de trois populations en Ontario (Canada) pour déterminer s'il y avait des différences entre populations ou entre périodes d'échantillonnage sur le plan de la consommation de moules zébrées. Sur la base de contenus stomacaux et d'analyses d'isotopes stables, nous avons établi que le régime alimentaire des crapets-soleil consistait principalement de moules zébrées dans toutes les populations et périodes d'échantillonnage, en constituant au moins 54 % dans tous les lacs et durant toutes les périodes d'échantillonnage, selon les modèles de mélange d'isotopes stables. Les habitudes actuelles d'utilisation des ressources des crapets-soleil dans les populations étudiées indiquent que leur alimentation, constituée, avant l'invasion, d'invertébrés côtiers benthiques tels que des gastéropodes, a depuis changé. La consommation à grande échelle de moules envahissantes par des poissons indigènes pourrait avoir d'importantes conséquences pour le contrôle biologique naturel de ces moules, en réduisant l'abondance globale et la vitesse de propagation des moules. Nous discutons de nos résultats en ce qui concerne la santé et la dynamique globales des écosystèmes aquatiques. [Traduit par la Rédaction]

Introduction

Invasive species are among the top threats to global biodiversity and ecosystem stability (Ricciardi et al. 1998; Sousa et al. 2013), particularly in aquatic environments that have been subject to hundreds of human-introduced species (Strayer 2010). Bivalves are among the most frequently studied invasive species because of their wide range of impacts following introduction and because of the number of bivalve species that have become invasive around the world (reviewed by Sousa et al. 2013). In North America, much attention has focused on zebra (*Dreissena polymorpha*) and quagga mussels (*Dreissena rostriformis*), which were introduced to the Great Lakes region in the late 1980s and have since spread through the Mississippi watershed and as far west as California (Strayer 2009). Dreissenids are attributed with causing over US\$270 million damage to the Great Lakes region following their introduction because of their effects on a variety of industrial systems, including piping for drinking water and power genera-

tion plants (Connelly et al. 2007; Strayer 2009). These invasive mussels also have a major ecological impact. For example, once zebra mussels have colonized a lake, they can reach densities between 10 000 to 700 000 individuals per square metre, up to 100-fold higher than in their native range, likely owing to the lack of predators and native competing species (Griffiths et al. 1991; Ricciardi et al. 1997). At these high densities, zebra mussels alter the physical characteristics of littoral habitats simply by their presence (Ricciardi et al. 1997) and alter plankton density and species composition (e.g., Jaeger Miehls et al. 2009; Fishman et al. 2010; Gergs et al. 2011). These changes in the availability and composition of seston (phytoplankton, zooplankton, and suspended matter) in the water column affects overall water clarity and the densities of larger zooplankton, which in turn impacts the food available for zooplanktivorous fish (Zhu et al. 2006; Higgins and Vander Zanden 2010; Pace et al. 2010).

It is thought that one of the primary biological mechanisms contributing to the spread and high densities of zebra mussels in

Received 12 August 2014. Accepted 24 February 2015.

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North America is the release from natural predation that exists in their native Ponto-Caspian ranges (Molloy et al. 1997; Watzin et al. 2008). Native predators of zebra mussels include roach (*Rutilus rutilus*) and round goby (*Neogobius melanostomus*) (Molloy et al. 1997), but these predators are not native in all regions that now contain the mussels. In lakes that have been invaded, however, there is evidence of native birds (e.g., Petrie and Knapton 1999; Werner et al. 2005), turtles (e.g., Bulté and Blouin-Demers 2008), and fish (e.g., Magoulick and Lewis 2002; Watzin et al. 2008) consuming zebra mussels as a major portion of their diet.

Here we focus on pumpkinseed (*Lepomis gibbosus*), an abundant native fish and one of the few sunfishes capable of crushing hard-shelled invertebrates, particularly snails (Gastropoda) and juvenile native bivalves (Unionoida) (Mittelbach 1984; Wainwright 1996; Mittelbach et al. 1999). In addition to their diet of hard-shelled native invertebrates, pumpkinseed have been shown to consume zebra mussels in Lake Champlain (44°32'N, 73°20'W; Watzin et al. 2008), Lake Erie (42°08'N, 80°05'W; Andraso 2005), and Opinicon Lake, a small temperate lake that is part of the Rideau Canal system (44°34'N, 76°19'W; Locke et al. 2014). As a result of these previous studies, it has been established that wild populations are capable of consuming zebra mussels in substantial quantities. To assess whether or not pumpkinseed represent a general predator to zebra mussels, however, it must be determined if pumpkinseed consume these mussels across multiple populations and over extended periods of time. To our knowledge, temporal patterns of mussel consumption by pumpkinseed has only been examined previously in Lake Champlain, where across 3 years considerable variability in zebra mussel consumption by pumpkinseed was reported (Watzin et al. 2008). Indeed, the spatial and temporal relationships between pumpkinseed and zebra mussels have not been established for the smaller lakes that characterize the Canadian Shield region. This is a relationship that must be examined to understand how a native fish may impact an invasive species of general concern in that region.

We estimated pumpkinseed diet across three lakes and time periods (i.e., spring and summer) using two common methods of assessing fish foraging ecology that provide different temporal scales of diet: stomach content and stable isotope analyses. Stomach contents provide detailed quantitative measurements of short-term diet (i.e., within hours to a day or two; Pinnegar and Polunin 1999; MacNeil et al. 2005) and can be used to identify the common prey types found in fish diets. To infer diet over longer time periods, we used stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). We used these isotopes because carbon isotope ratios remain relatively constant (changing <1%) between a consumer and its resources (DeNiro and Epstein 1981; Peterson and Fry 1987), as compared with the predictable increases in $\delta^{15}\text{N}$ (3%–5%) with each trophic level (Peterson and Fry 1987; Post 2002). We expected that if pumpkinseed have undergone a general resource shift as a result of the zebra mussel invasion, then pumpkinseed diets should contain large quantities of mussels across multiple populations (i.e., lakes). Zebra mussel populations have been reported to reach densities of approximately 3000 individuals·m⁻² (Bulté and Blouin-Demers 2008) as compared with pre-mussel maximum snail densities of approximately 40 individuals·m⁻² (Keast 1978) in the same lake. Given the high abundance of zebra mussels in the lakes, we also expected that a shift to consume zebra mussels would be temporally consistent across the primary foraging season for pumpkinseed (i.e., spring and summer).

Materials and methods

Fish collection

Pumpkinseed were collected from three lakes over multiple sampling periods during 2011 and 2012 ($n = 203$). A total of 78 pumpkinseed were collected from Opinicon Lake (Ontario, Canada, 44°34'N, 76°19'W) over two sampling periods: spring 2011

(25–30 May 2011, $n = 50$) and summer 2011 (17–21 August 2011, $n = 28$). Seventy-five fish were sampled from Lower Beverley Lake (Ontario, Canada, 44°36'N, 76°08'W) in spring 2011 (24–28 June 2011, $n = 50$) and summer 2011 (18–24 August 2011, $n = 25$). The following year, pumpkinseed were collected from Indian Lake (Ontario, Canada, 44°36'N, 76°20'W, $n = 50$) during spring 2012 (19 May – 3 June 2012). Fish were collected from multiple sites distributed around each lake using a combination of dip-netting by swimmers and angling using a small (2–3 cm) piece of earthworm suspended directly from the side of a research boat. Only fish greater than 100 mm in total body length were retained for our analysis to reduce the likelihood of collecting individuals that were undergoing an ontogenetic niche shift from their juvenile to adult diet (see Mittelbach 1984; Osenberg et al. 1988; Rezsú and Specziár 2006). All fish were immediately euthanized with clove oil and stored on ice for transport to the Queen's University Biological Station for dissection after measuring the total body length (mm) and wet mass (g) of each fish.

Dissections were used to collect the stomach contents of each fish, as well as tissue samples of white muscle and liver for isotopic analysis. The stomach was removed from the body cavity, and the contents were removed and placed in a 1.5 mL microcentrifuge tube (multiple tubes were used as required) and stored at –20 °C for later sorting. A sample of white muscle was collected from the right side of each fish underneath the posterior portion of the dorsal fin, as well as the entire liver. Muscle and liver samples were stored in separate 1.5 mL microcentrifuge tubes at –20 °C for stable isotope analysis. We also determined the sex of each individual through visual examination of the gonads. Males were identified by having cream-coloured gonads that were bifurcated into left and right sections. Females were identified by ovaries that did not show distinct division into sides and were filled with numerous eggs visible through the ovary wall. Juveniles were identified by having small, translucent gonads (Deacon and Keast 1987).

Stomach content analysis

Of the 203 fish collected, 187 had contents in their stomachs. The contents were thawed and sorted under a dissecting microscope and assigned to one of five prey types: (i) snails; (ii) zebra mussels; (iii) other littoral benthic invertebrates (e.g., native mussels, isopods, amphipods, larval Ephemeroptera); (iv) zooplankton (Cladocera and Copepoda; e.g., *Daphnia* spp.); and (v) “other” (terrestrial insects, fish eggs, plant matter, and unidentifiable prey items). Shell fragments were removed from snail and zebra mussel samples after identification to avoid biases in the mass measures of these prey types (i.e., by including material that would not be digested by the fish). Once sorted, the stomach contents were dried for 24 h at 50 °C, after which the dry mass of each prey type was measured to determine the proportion that it contributed to the total dry mass.

Stable isotope analysis

Resource baselines for stable isotope analysis of fish diets are commonly established using snails (Gastropoda) and mussels (Bivalvia) to represent the littoral and pelagic habitats, respectively, because these relatively long-lived invertebrates have isotopic compositions that reflect similar temporal periods to the tissues collected from fish (e.g., Post 2002; Matthews et al. 2010; Correa et al. 2012). Here we collected snails and zebra mussels from the littoral habitat using D-net sweeps of the macrophytes and upper 1–2 cm of sediment and then froze these at –20 °C. Snails and mussels were manually removed from their shells prior to further preparation for stable isotope analysis. Studies of North American lakes typically use native bivalves to establish pelagic resource baselines; here we used zebra mussels because they were found to have similar isotopic compositions to zooplankton collected from pelagic sites in each lake using vertical tows (80 μm mesh size)

through the upper 2–3 m of the water column (see online supplementary material for details¹).

Fish tissue and reference prey samples were prepared for stable isotope analysis by freeze-drying at $-50\text{ }^{\circ}\text{C}$ for 24 h, followed by manually grinding them using a mortar and pestle. Stable isotope ratios of carbon ($^{13}\text{C}:^{12}\text{C}$) and nitrogen ($^{15}\text{N}:^{14}\text{N}$) were measured using continuous-flow stable isotope ratio mass spectrometry (Costech elemental analyzer attached to a Thermo Finnigan Delta^{plus} XL mass spectrometer) in the Laboratory for Stable Isotope Science at The University of Western Ontario. Measured stable isotope ratios were expressed as the per mil (‰) difference between the sample and a standard reference material using the following formula:

$$\delta X = (R_{\text{sample}}/R_{\text{standard}} - 1)$$

where X is either ^{13}C or ^{15}N , R is the $^{13}\text{C}:^{12}\text{C}$ or $^{15}\text{N}:^{14}\text{N}$ ratio, and δ is the measure of heavy to light isotopes in the sample. Isotopic measurements were calibrated to international standards using two-point curves and monitored for precision and accuracy using internal laboratory standard and replicate tissue samples. The $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of all standards and sample reproducibility were within the expected ranges of sample variability; details are given in the supplementary data¹.

The presence of lipids in tissues may result in lower $\delta^{13}\text{C}$ values compared with the isotopic composition of pure protein samples (see Kiljunen et al. 2006; Boecklen et al. 2011). Mathematical lipid correction model refined for aquatic organisms by Kiljunen et al. (2006) was used to compensate for the possible effect of lipids:

$$\delta^{13}\text{C}' = \delta^{13}\text{C} + D \times \left(I + \frac{3.90}{1 + 287/L} \right)$$

where

$$L = \frac{93}{1 + (0.246 \times \text{C:N} - 0.775)^{-1}}$$

L is the estimated lipid content of each sample based on the atomic ratio of carbon and nitrogen (C:N), $\delta^{13}\text{C}'$ is the lipid-corrected value of a sample, $\delta^{13}\text{C}$ is the measured value of each sample, D is a constant (7.02‰) representing the mean isotopic difference between lipids and pure protein, and I is a constant (0.05; Kiljunen et al. 2006).

Statistical analysis

Stomach contents were first examined within each lake for the mean contribution of the five prey types (see above) to the total fish stomach content mass. Data for the proportion of prey type mass for each fish was arcsine-square-root-transformed to address the assumptions of normality and homogeneity of variances (e.g., Jastrebski and Robinson 2004). These data were then used in separate one-factor ANOVA models, with Tukey's post hoc comparisons, to compare the contributions of each prey type to diet among the five lake-sampling periods (dependent factor: proportion of total stomach contents for a given prey type; independent factor: lake-sampling period).

The contribution of littoral prey and zebra mussels to the diets of pumpkinseed were then estimated for each lake and sampling period ($n = 5$ models) using two-source SIAR mixing models (Stable Isotope Analysis in R; R version 2.14.2; R Development Core Team 2012). Given that the isotopic composition of prey can vary temporally and spatially, we used individual models for each lake and

sampling period (e.g., Vizzini and Mazzola 2003; Syväranta et al. 2006; also see Results). Separate models for each lake and sampling period were calculated using the mean measured $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (± 1 SD) values to estimate the contribution to diet for two prey categories: (i) littoral benthic invertebrates, represented by the isotopic composition of snails (e.g., Correa et al. 2012; Jones et al. 2013); and (ii) zebra mussels. Pelagic zooplankton were not incorporated into the SIAR models because of their consistent absence from the stomach samples (see below). The prey group values were then used as the "source" estimates for each lake and sampling period within a lake. A mean trophic enrichment factor (TEF) for $\delta^{13}\text{C}$ ($0.47\text{‰} \pm 1.23\text{‰}$) was applied to the SIAR models based on multiple freshwater fish species from temperate lakes (Vander Zanden and Rasmussen 2001). Using the commonly applied $\delta^{15}\text{N}$ TEF ($3.23\text{‰} \pm 0.41\text{‰}$) reported by Vander Zanden and Rasmussen (2001) would result in implausibly high trophic positions for our pumpkinseed relative to the potential prey sources (Fig. 1); instead we applied a TEF of $5.00\text{‰} \pm 1.5\text{‰}$, reflecting the upper limits reported for fish tissues and values that have been used previously for SIAR models of pumpkinseed diet (Caut et al. 2009; Locke et al. 2014).

The SIAR models were also used to compare the resource use of the pumpkinseed among all five groups (lake-sampling period) with individual diet estimates available through the SIARSOLO command. The estimates of individuals' resource use were used in a one-factor ANOVA (dependent factor: proportion of diet from zebra mussels; independent factor: lake-sampling period) with Tukey's post hoc comparisons if the overall ANOVA model was statistically significant. Fish length is not reported for this analysis because we sampled fish that exceeded the ontogenetic niche shift in sunfish (see above); when included as a variable, fish length was found to be statistically nonsignificant ($P \geq 0.07$ for both white muscle and liver tissues).

Statistical analyses were completed using JMP version 10.0.0 (SAS Institute Inc., Cary, North Carolina, 2012) and the SIAR software package (Parnell et al. 2010) developed for R software (R version 3.0.1; R Development Core Team 2012). Means are presented ± 1 SD and $\alpha = 0.05$.

Results

Stomach contents

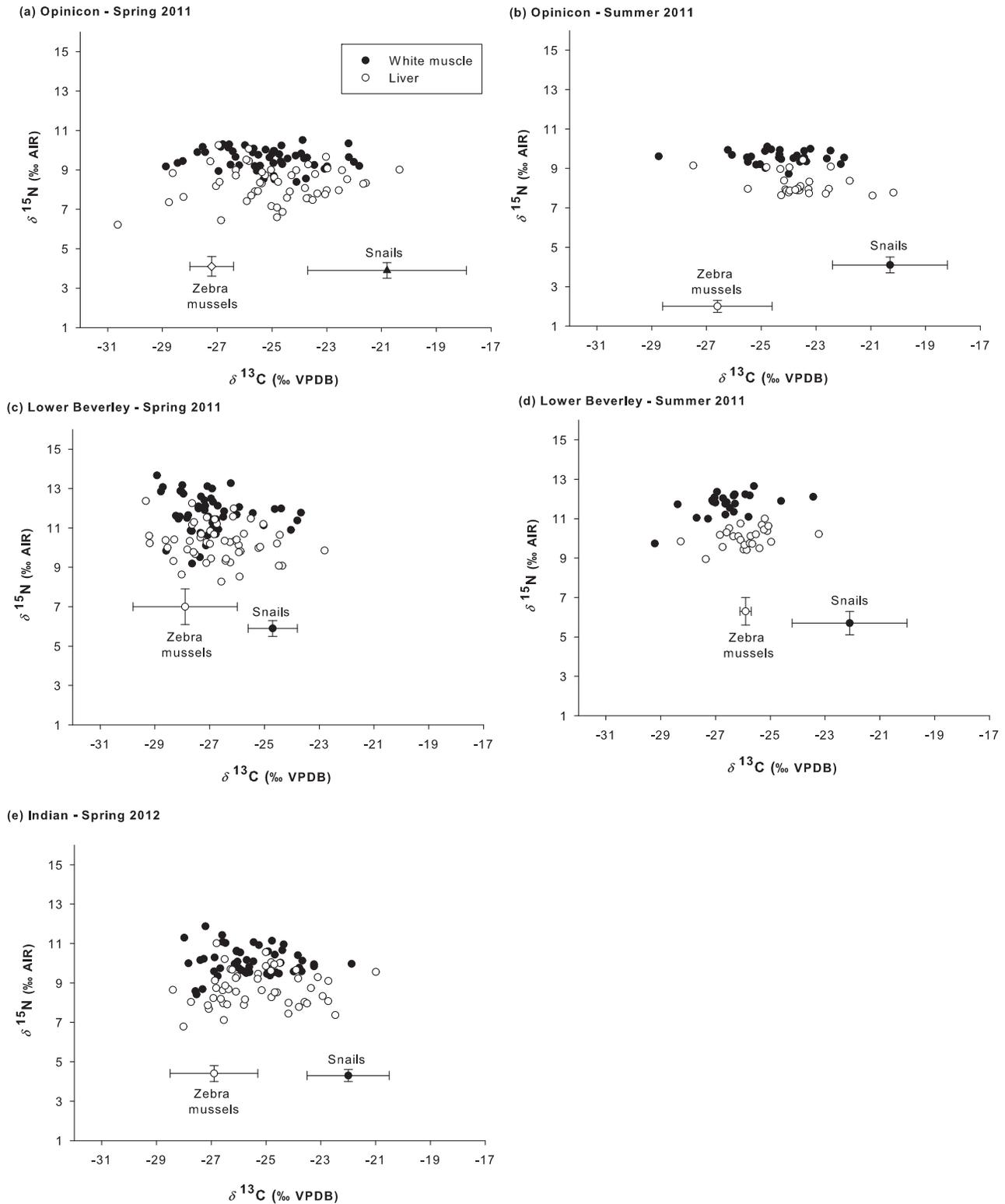
On average, across the lakes and sampling periods, zebra mussels accounted for 68% of stomach content dry mass compared with 19% for snails, 3% for benthic invertebrates (excluding snails), and 9% of the "other" prey category. Zooplankton were not identified in stomach contents of any pumpkinseed and were therefore removed as a possible prey type for analysis. There was no evidence of spatial (i.e., among lakes) or temporal (i.e., between spring and summer) differences in the contributions of zebra mussels or other benthic invertebrates to fish diet (Table 1). The pumpkinseed from Indian Lake had a larger proportion of snails in their stomach contents compared with the Lower Beverley population in both spring and summer (Tukey's, both $P \leq 0.02$). The fish collected from Opinicon Lake in summer had a greater proportion of stomach contents classified into the other category than during the spring sampling period (Tukey's, $P = 0.04$), but there were no other differences among lakes or sampling periods for this prey category.

Stable isotope inferences of diet

Isotopic compositions of the pumpkinseed tissue samples varied among the lakes and sampling periods for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ (Fig. 1; Table 2; see Table S2 in supplementary information¹). Based on the SIAR models, white muscle and liver tissue estimates of the

¹Supplementary data are available with the article through the journal Web site at <http://nrcresearchpress.com/doi/suppl/10.1139/cjfas-2014-0372>.

Fig. 1. Isotopic compositions of pumpkinseed (*Lepomis gibbosus*) and potential invertebrate prey types. Fish and invertebrates were sampled from three lakes: Opinicon (a, b), Lower Beverley (c, d), or Indian (e), over multiple sampling periods. Samples of white muscle (solid circles) and liver (open circles) were collected from each fish for stable isotope analysis of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$). The isotopic compositions of prey baseline samples are also presented for snails (Gastropoda) and zebra mussels (*Dreissena polymorpha*).



proportion of pumpkinseed diet from littoral or pelagic sources differed by less than 10% units between the tissues for each of the sample groups (i.e., lake and sampling interval), with the zebra mussel contribution to diet estimated at 54%–89% of the pump-

kinseed diet (Table 3; Fig. 2). Comparing the sample groups using the diet estimates for each individual, we found differences among the groups based on both white muscle (ANOVA; $F_{[4,197]} = 11.96$, $P < 0.001$) and liver ($F_{[4,196]} = 13.28$, $P < 0.001$; Fig. 2). Tukey's

Table 1. Summary of stomach content analysis of pumpkinseed (*Lepomis gibbosus*) from three lakes.

Prey category	Opinicon		Lower Beverley		Indian	F	df	P
	Spring 2011 (n = 44 of 50)	Summer 2011 (n = 27 of 28)	Spring 2011 (n = 46 of 50)	Summer 2011 (n = 25 of 25)	Spring 2012 (n = 45 of 50)			
Snails	0.20±0.29AB	0.18±0.37AB	0.14±0.27B	0.07±0.24B	0.32±0.43A	3.38	4,182	0.01
Zebra mussels	0.69±0.34A	0.59±0.46A	0.76±0.35A	0.82±0.36A	0.57±0.46A	2.34	4,182	0.06
Benthic invertebrates	0.06±0.18A	0.01±0.05A	0.01±0.02A	0.04±0.20A	0.04±0.17A	0.84	4,182	0.50
Other	0.05±0.17A	0.22±0.38B	0.09±0.28AB	0.07±0.20AB	0.06±0.19AB	2.39	4,182	0.05

Note: The total number of fish collected with stomach contents are indicated for each lake and sampling period; fish with empty stomach contents were not included in statistical analyses. Zooplankton were included as a prey type during sorting of stomach contents, but are not shown in this table because zooplankton were absent from all fish sampled. Means are expressed as plus or minus one standard deviation. Stomach contents were collected from fish sampled in three lakes (Opinicon, Lower Beverley, and Indian) over sampling periods during 2011 and 2012. Stomach contents were sorted into four prey types: snails (Gastropoda); zebra mussels (*Dreissena polymorpha*); benthic invertebrates (excluding snails and zebra mussels); and “other” (including plant material and unidentifiable contents) and are shown here as the proportion of the total dry mass of all stomach contents. The *F*, *df*, and *P* values represent the results of analysis of variance (ANOVA) models comparing the proportion for each prey type among the lakes and sampling periods (see Materials and methods for details). Letters beside the mean proportions indicate the results of Tukey’s post hoc comparisons for a given prey type; different letters indicate statistically significant ($\alpha = 0.05$) differences among lakes and sampling periods for that prey type.

Table 2. Summary of the stable isotopic compositions of pumpkinseed (*Lepomis gibbosus*).

Lake	Sampling period	White muscle			Liver		
		n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$	n	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Opinicon	Spring 2011	50	-25.2±1.7	+9.6±0.5	50	-24.9±2.0	+8.3±0.9
	Summer 2011	28	-24.3±1.4	+9.6±0.3	27	-23.6±1.4	+8.2±0.5
Lower Beverley	Spring 2011	50	-26.9±1.2	+11.7±1.0	50	-26.7±1.4	+10.2±0.9
	Summer 2011	25	-26.6±1.1	+11.7±0.6	25	-25.9±0.9	+10.0±0.5
Indian	Spring 2012	50	-25.6±1.4	+10.1±0.7	50	-25.3±1.6	+8.7±0.9

Note: Mean values are presented as plus or minus one standard deviation. The liver isotopic composition for one summer 2011 fish was unavailable owing to a technical error during freeze-drying of the sample. The isotopic compositions are based on white muscle and liver samples collected from fish sampled from three lakes (Opinicon, Lower Beverley, and Indian) over multiple samplings periods from 2011 to 2012.

Table 3. Summary of pumpkinseed (*Lepomis gibbosus*) diet estimates based on SIAR (Stable Isotope Analysis in R) two-source mixing models.

Lake	Collection period	White muscle		Liver	
		Proportion littoral	Proportion zebra mussels	Proportion littoral	Proportion zebra mussels
Opinicon	Spring 2011	0.27 (0.20–0.34)	0.73 (0.66–0.80)	0.31 (0.22–0.39)	0.69 (0.61–0.78)
	Summer 2011	0.35 (0.26–0.44)	0.65 (0.56–0.74)	0.46 (0.36–0.55)	0.54 (0.45–0.64)
Lower Beverley	Spring 2011	0.32 (0.21–0.42)	0.68 (0.58–0.79)	0.41 (0.27–0.55)	0.59 (0.45–0.73)
	Summer 2011	0.11 (0–0.21)	0.89 (0.79–1.0)	0.20 (0.06–0.35)	0.80 (0.65–0.94)
Indian	Spring 2012	0.19 (0.12–0.26)	0.81 (0.74–0.88)	0.25 (0.17–0.32)	0.75 (0.68–0.83)

Note: Means are presented with the 95% Bayesian credibility intervals in parentheses. The isotopic composition ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) of fish white muscle and liver samples were used to estimate the proportion of littoral benthic invertebrates and zebra mussels to the diets of fish sampled from three lakes (Opinicon, Lower Beverley, and Indian) over multiple sampling periods during 2011 and 2012.

post hoc comparisons using both muscle and liver tissues indicated that Opinicon Lake and Indian Lake pumpkinseed consumed similar quantities of zebra mussels during the spring sampling period, and both had higher levels than Lower Beverley Lake pumpkinseed (Fig. 2). Examination of the summer sampling period indicated that Lower Beverley Lake pumpkinseed increased their zebra mussel consumption and Opinicon Lake pumpkinseed decreased their consumption as compared with the spring sampling period, and a significant difference in zebra mussel consumption was maintained between the two populations (Fig. 2).

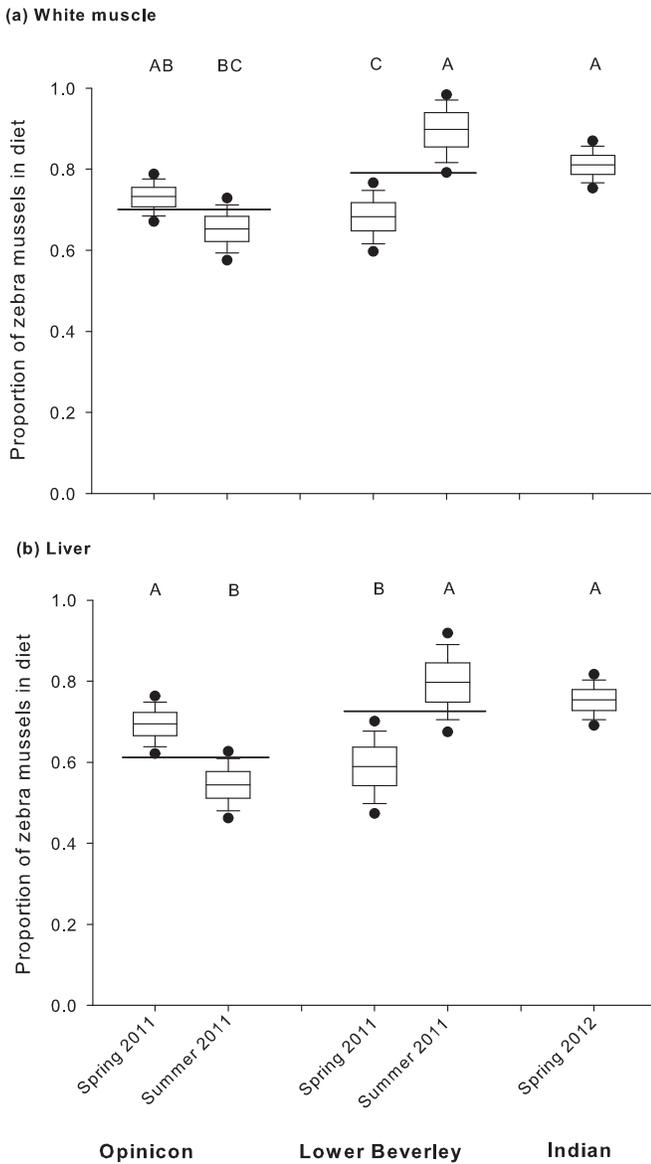
Discussion

Assessing the foraging ecology of native fishes can help to evaluate the impact of zebra mussel invasions on freshwater communities around the world. Here, we examined the diet of pumpkinseed, which are native to the Great Lakes area — the region of initial zebra mussel introduction to North America. Using stomach content and stable isotope analyses, which allow for inferences of diet over a period of hours to months, we tested for the incorporation of zebra mussels into pumpkinseed diet from

smaller temperate lakes that characterize the Canadian Shield region. Zebra mussels were present in the stomachs of 81% of the pumpkinseed we collected and represented 57%–82% of diet by mass across the three study populations. In larger North American lakes, stomach content analyses have found comparable levels of zebra mussels in the diet of pumpkinseed from Lake Erie (63% by volume; Andraso 2005), but lower levels were reported in Lake Champlain (mean of 32% dry mass over 3 sampling years; Watzin et al. 2008). The stable isotope estimates of diet indicated that, on average, zebra mussels represented a minimum of 54% of the diet across the lakes and sampling periods. A similar stable isotope mixing model of Opinicon pumpkinseed sampled during July 2010 estimated that zebra mussels comprised 71.5% of diet during the midsummer period (Locke et al. 2014), consistent with the results we found during the spring and late summer sampling periods.

Studies of several other native fishes in North America have found the presence of zebra mussels in stomach contents (e.g., freshwater drum (*Aplodinotus grunniens*), yellow perch (*Perca flavescens*), and redear sunfish (*Lepomis microlophus*); Morrison et al. 1997; Magoulick and Lewis 2002). These studies, however, did not

Fig. 2. SIAR (Stable Isotope Analysis in R) mixing model estimates of the proportion of zebra mussels (*Dreissena polymorpha*) in the diet of pumpkinseed (*Lepomis gibbosus*). Estimates are presented for (a) white muscle and (b) liver fish tissue samples collected for fish from three lakes (Opinicon, Lower Beverley, Indian). Boxes represent the inner 50% of observations, the line inside each box represents the overall mean value, the whiskers are the 90th and 10th percentiles, and the dots represent the 95th and 5th percentiles. For lakes with multiple sample periods (Opinicon and Lower Beverley), the line across the SIAR estimates represents the mean zebra mussel consumption across the sample periods. The letters above the boxplots represent the pairwise comparisons of each group using the Student's *t* test with a Bonferroni correction ($\alpha = 0.005$); different letters represent statistically significant differences between groups.



estimate the total proportion of diet composed of zebra mussels. The proportion of zebra mussels in the stomach contents of native species has been reported at 66% in redhorse suckers (*Moxostoma* spp.) and 73% in common carp (*Cyprinus carpio*) of the Upper Mississippi River (Bartsch et al. 2005). Our analysis of diet not only indicates that zebra mussels have become the primary resource group for native pumpkinseed in northern temperate lakes, but that there

are similar degrees of consumption across multiple populations and high levels of consumption throughout the primary foraging period for pumpkinseed (i.e., June through August) in these lakes. We found that pumpkinseed diet contained a large proportion of zebra mussels across all three population lakes and over multiple sampling time periods.

We also assessed the usefulness of isotopic compositions for differentiating zebra mussels from other common invertebrate prey types. We found that snails, zebra mussels, and zooplankton from our lakes separated into two isotopic groups (see supplementary data for details of the comparisons¹). The similarity in isotopic compositions between zooplankton and zebra mussels is consistent with their similar diets of seston (i.e., phytoplankton, small zooplankton, and other particulate matter floating in the water column) that are suspended in the water column (Strayer 2009). Indeed, other native filter-feeding bivalves that consume seston have been found to have similar isotopic compositions to zooplankton (e.g., Vander Zanden and Rasmussen 1999). These results support the use of zebra mussels to generate the pelagic resource baseline in temperate freshwater lakes. This significant isotopic overlap between some prey types (e.g., zooplankton and zebra mussels), however, could limit the ability to distinguish them based on isotopic composition alone. In our case, we were able to refine our isotopic analysis by including dietary information from stomach content analysis of the same fish. Specifically, zooplankton were not present in any of the pumpkinseed stomachs we collected. We ruled out any technical issues with detecting zooplankton in stomach contents because we have successfully identified zooplankton in the stomachs of pumpkinseed in other lakes using the same methods (S. Colborne and B. Neff, unpublished data; also see Keast 1978; Robinson et al. 1993; Jastrebski and Robinson 2004). Thus, based on stomach contents we were able to eliminate zooplankton as a pelagic resource and use isotopic composition to directly assess zebra mussel consumption by pumpkinseed compared with other littoral benthic invertebrates.

In addition to demonstrating the current diets of pumpkinseed, our study also afforded the opportunity to compare the current diets with that prior to the invasion of zebra mussels. For two of our study lakes, Opinicon and Lower Beverley, pumpkinseed diet has been studied within the 20 years prior to the introduction of zebra mussels. Stomach content estimates of Opinicon pumpkinseed, sampled between 1969 and 1971, estimated 33% snails, 65% other benthic invertebrates, and 2% zooplankton (Keast 1978). Similar estimates of Lower Beverley pumpkinseed, sampled in 1981, estimated 11% snails and 89% other littoral benthic invertebrates (Deacon and Keast 1987). Compared with the historical stomach content data, our stomach content analyses show that pumpkinseed have decreased benthic invertebrate consumption by more than 60% and snail consumption by 14%. The pumpkinseed thus have shifted from a diet of primarily snails and soft-bodied invertebrates to a diet composed primarily of zebra mussels in both study populations.

It is conceivable that pumpkinseed, along with other species found to consume zebra mussels (e.g., Magoulick and Lewis 2002; Bartsch et al. 2005; Carlsson et al. 2011), may decrease both the overall densities and rates at which zebra mussels spread. For example, wintering water birds in Lake Constance, Germany, have been shown to decrease the density of adult zebra mussels by more than 90% during their winter stopover period (Werner et al. 2005). If the total predation on invasive mussel populations is great enough, natural biological controls may develop to reduce the zebra mussel populations. Additionally, dietary shifts in native species may further change freshwater lake food webs, adding to the indirect effects of invasive mussels. On one hand, the observed drop in consumption of native littoral benthic invertebrates by the pumpkinseed in our study populations as compared with the pre-invasion diets (Keast 1978; Deacon and Keast 1987)

could indicate that there has been predator release from one of the most abundant littoral fishes in these lakes, which can cause further changes in foraging patterns (Fraser et al. 2004; Burkepile and Hay 2007), overall species composition (Duffy 2002; Sieben et al. 2011b), and trophic cascades (Eriksson et al. 2009; Sieben et al. 2011a). Indeed, in Opinicon Lake, map turtles (*Graptemys geographica*) now also consume zebra mussels in their diets (5%–28% of diet depending on sex and size; Bulté and Blouin-Demers 2008), supporting the possibility of simultaneous shifts occurring among multiple species within the same environment. On the other hand, as pumpkinseed change their diet it is possible that other fish (e.g., bluegill) will also alter their resource patterns, having the net effect of maintaining similar overall predation pressure even though the predator composition may change. Given the variety of implications, both ecological and evolutionary, that come with the shifting resource dynamics resulting from the introduction of zebra mussels, this is a promising area for future research to understand how populations respond to large-scale environmental disturbances.

In summary, we have found that across three populations, pumpkinseed are consuming large quantities of zebra mussels during both the spring and summer foraging periods. As a result, pumpkinseed have shifted away from diets that consisted predominantly of snails and soft-bodied benthic invertebrates to diets of zebra mussels and snails. This spatially replicated and temporally stable shift in diet has important ecological consequences for not only the pumpkinseed and zebra mussels, but also other species within lakes. As we move beyond establishing whether native species are consuming invasive species, more detailed assessment of foraging interactions and changing food web dynamics will provide insights into both the direct and indirect effects of these introduced species on the communities of which they are now members.

Acknowledgements

We thank C. Rodgers, M. Roubakha, T. Hain, M. Lau, and A. Berchtold for assistance in the field. We also thank K. Law for assistance with the stable isotope analysis in the laboratory. This manuscript has been improved by comments from M. Lau and four anonymous reviewers. This research was carried out with approval of The University of Western Ontario Council on Animal Care (animal use protocol No. 2010-214) and the Ontario Ministry of Natural Resources (license Nos. 1057026 and 1062735). This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants to B.D. Neff and F.J. Longstaffe; Canada Foundation for Innovation (CFI) and Ontario Research Fund (ORF) infrastructure awards to F.J. Longstaffe; and The University of Western Ontario Graduate Thesis Research Fund, Queen Elizabeth II Graduate Scholarship in Science and Technology (QEIGSST), and Ontario Graduate Scholarship (OGS) awards to S.F. Colborne. This work was also partially supported through release time provided to F.J. Longstaffe through the Canada Research Chairs program. This paper is Laboratory for Stable Isotope Science contribution No. 306.

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