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PRIMARY RESEARCH PAPER

Spatial and temporal variabilities of $\delta^{13}C$ and $\delta^{15}N$ within lower trophic levels of a large lake: implications for estimating trophic relationships of consumers

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Abstract Stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) often have unique values among lake habitats (e.g. benthic, littoral, pelagic), providing a widely used tool for measuring the structure and energy flow in aquatic food webs. However, there has been little recognition of the spatial and temporal variabilities of these isotopes within habitats of aquatic ecosystems. To address this, $\delta^{13}C$ and $\delta^{15}N$ were measured in seston, zebra mussels (*Dreissena polymorpha*) and young-of-year (YOY) yellow (*Perca flavescens*), and white perch (*Morone americana*) collected from four sites across the offshore habitat of the western basin of Lake Erie during June–September 2009. Values of $\delta^{13}C$ and $\delta^{15}N$ showed significant spatial and temporal variations, with month accounting for >50% of the variation, for both stable isotopes and all the species except seston. Such variation in isotope values has the potential to significantly influence or confound interpretation of stable isotopes in measures, such as trophic position (TP) which use lower trophic level organisms as their baseline. For example, TP was found to vary up to 0.7 for yellow and white perch ($TP = \delta^{15}N_{fish} - \delta^{15}N_{zebra\ mussel}$/diet-tissue fractionation factor) depending on the zebra mussel data used (e.g., from a different location or a different collection month). As the use of stable isotopes continues to move from qualitative to more quantitative measures of trophic structure, food web research must recognize the importance of stable isotopes' variability in lower trophic level organisms, especially in large lake systems.

Keywords Stable isotopes · Lake Erie · Fish · Yellow Perch · White Perch · Food webs

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Introduction

Food webs represent energy and nutrient flows within an ecosystem and have for a long time been a central theme in ecology (Lindeman, 1942; Martinez, 1995). The study of food webs provides insight into species' interactions and enhances the understanding of the processes that structure ecosystems (Vander Zanden & Rasmussen, 1996; Hobson et al., 2002; Post, 2002). One of the most common tools used for studying the structure and the energy flow within food webs are the stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$). Stable isotopes of carbon can provide insight into the sources of primary producers in aquatic food webs, and in lakes are often employed to differentiate between littoral (nearshore)/benthic and pelagic

(open water) primary production (Peterson & Fry, 1987; France, 1995; France & Peters, 1997). Stable isotopes of nitrogen provide a means to quantify the trophic position (TP) of organisms, where consumers become enriched in ^{15}N relative to their prey by an average of 3.4‰ for $\delta^{15}N$ providing a space- and time- integrated measure of TP (Minagawa & Wada, 1984; Peterson & Fry, 1987; Cabana & Rasmussen, 1994). In order to overcome across-system variations in $\delta^{13}C$ and $\delta^{15}N$ values, carbon sources and TPs are often normalized to the stable isotope values of a common primary consumer, such as unionid mussels to represent baseline values of pelagic and littoral food webs (Cabana & Rasmussen, 1996; Post, 2002).

It is well established that stable isotopes vary among habitats within (pelagic/benthic/littoral) and among lakes (France, 1995; Vander Zanden & Rasmussen, 1999), and that this variation is key to the understanding of the food web relationships. However, it is often assumed that spatial and temporal variation of isotope values within a single habitat type are relatively minor compared with food web fractionation processes. If within-habitat variation exists, then it can confound the interpretation of stable isotopes and ultimately result in an erroneous assessment of food web structure and dynamics. Often, this variation in stable isotopes within lakes is associated with anthropogenic sources (Steffy & Kilham, 2004), such as sewage out flows (Savage & Elmgren, 2004) or near areas of increased urban populations, as seen on Lake Superior (Harvey & Kitchell, 2000). Stable isotopes have also been found to vary seasonally in particulate organic matter (Gu, 2009) and exhibit temporal variability in zooplankton due to changes in lipid content, growth rate (Matthews & Mazumder, 2005), and food source (Grey et al., 2001).

Recent research, however, has demonstrated that stable isotopes can also vary within a single lake habitat. For instance, Syvaranta et al. (2006) found temporal variation of $\delta^{15}N$ in pelagic particulate organic matter and zooplankton and spatial variation of $\delta^{13}C$ and $\delta^{15}N$ values within both littoral and profundal communities of Lake Jyvasjari in Finland. Spatial variations of $\delta^{13}C$ and $\delta^{15}N$ were also found in single species among canals in Xochilmilco, Mexico, a small, shallow, heterogeneous canal system with constant depth and sediment characteristics (Zambrano et al., 2010) and among sites of similar environmental characteristics in a variety of invertebrates and fish in Lake Kyoga, Africa (Mbabazi et al., 2010). There has been little effort, however, to quantify spatial and temporal variabilities of stable isotopes in important large lake systems, such as the Laurentian Great Lakes.

The western basin of Lake Erie represents one of the most productive and resilient food webs in the Great Lakes system and contributes approximately 30% of total Canadian freshwater commercial fish catches (Regier & Hartman, 1973; DFO, 2006). We examine the spatial and temporal variabilities of stable isotope across multiple lower trophic levels within the same habitat types, in the wellmixed western basin of Lake Erie. We hypothesized that $\delta^{13}C$ and $\delta^{15}N$ would vary spatially (within a single habitat zone), as a result of contrasting carbon/energy inputs in the lake. Because Lake Erie is temperate, we also hypothesized that $\delta^{13}C$ and $\delta^{15}N$ would vary temporally throughout the growing season (JuneSeptember), because of changes in nutrient inputs and changes in algal and zooplankton biodiversities. Finally, we examine the potential influence of spatial and temporal variabilities on food web structure assessment by examining TP estimates. Specifically, we address the following questions: 1. Do spatial scale and temporal (four month period) variability in $\delta^{13}C$ and $\delta^{15}N$ exist within lower trophic level species of the offshore habitat within the western basin of Lake Erie? 2. What are the implications of spatial and temporal variations of $\delta^{13}C$ and $\delta^{15}N$ on estimating TP and carbon sources of YOY piscivorous fish [e.g. white perch (*Morone americana*) and yellow perch (*Perca flavescens*)]?

Methods

Study site

This study was implemented in the western basin of Lake Erie, a shallow (mean depth, 7.5 m; maximum depth, 10 m), flat basin that comprises the western third of Lake Erie. The basin is classified as mesotrophic (Kane et al., 2009), and is well mixed vertically with little or no significant summer stratification. Spatial complexity in the western basin of Lake Erie is a function of tributary and connecting channel hydraulic inputs. The basin has two major sources of nutrients, the Detroit and Maumee Rivers.

Although the Detroit River's mean annual discharge is > 35 times that of the Maumee (5,100 Vs 135 m^3 s^{-1}), the Maumee River contributes ~35% of the total phosphorus load to the basin (Di Toro et al., 1987; Baker & Richards, 2002; Dolan & McGunagle, 2005) and provides warm nutrient-rich waters which circulate in the southwest portion of the lake. The Detroit River provides a much larger flow of cooler nutrient limited waters, and its plume extends well out into the basin (Reichert et al., 2010) (Fig. 1). Both the Maumee and Detroit Rivers not only provide spatial and seasonal subsidies of nutrients, but also contribute to environmental heterogeneity with respect to water temperatures, plankton communities, plankton and zooplankton production dynamics, and fish assemblages (Barbiero et al., 2001; Reichert et al., 2010).

Sample collection/analysis

Samples were collected from four locations across the western basin of Lake Erie (Fig. 1). Detroit River Plume and Middle Sister Island receive much of their water from Lake Huron, while Maumee River Plume and Bass Islands are highly affected by spring melt water from the Maumee River basin, an area dominant in agriculture. At each location, seston samples were collected monthly from June to September 2009 using a 63-μm zooplankton net. In an effort to incorporate seston from the entire water column, vertical tows were conducted from one-half meter off bottom to the water surface. Bulk seston samples were frozen at −20°C in hexane-rinsed polyethylene jars. Zebra mussels (*Dreissena polymorpha*) (June-September 2009) and YOY yellow perch (4.4–9.5 cm), and white perch (3.2–8.4 cm) were collected by bottom trawls conducted as part of the Ontario Ministry of Natural Resources and the Ohio Department of Natural Resources summer inter-agency trawls during July–September 2009. Both zebra mussels and YOY fish were frozen whole and brought back to lab in polyethylene sample bags. Zebra mussels were shucked to remove shells and were rinsed with distilled water. Samples were then pooled using 5–10 individuals of similar size to achieve sufficient sample quantity for analysis and placed into cryo vials, and frozen at −20°C. Individual YOY fish dorsal muscle plugs were removed and placed into cryo vials and frozen at −20°C. For all the species sampled, a minimum of three samples were

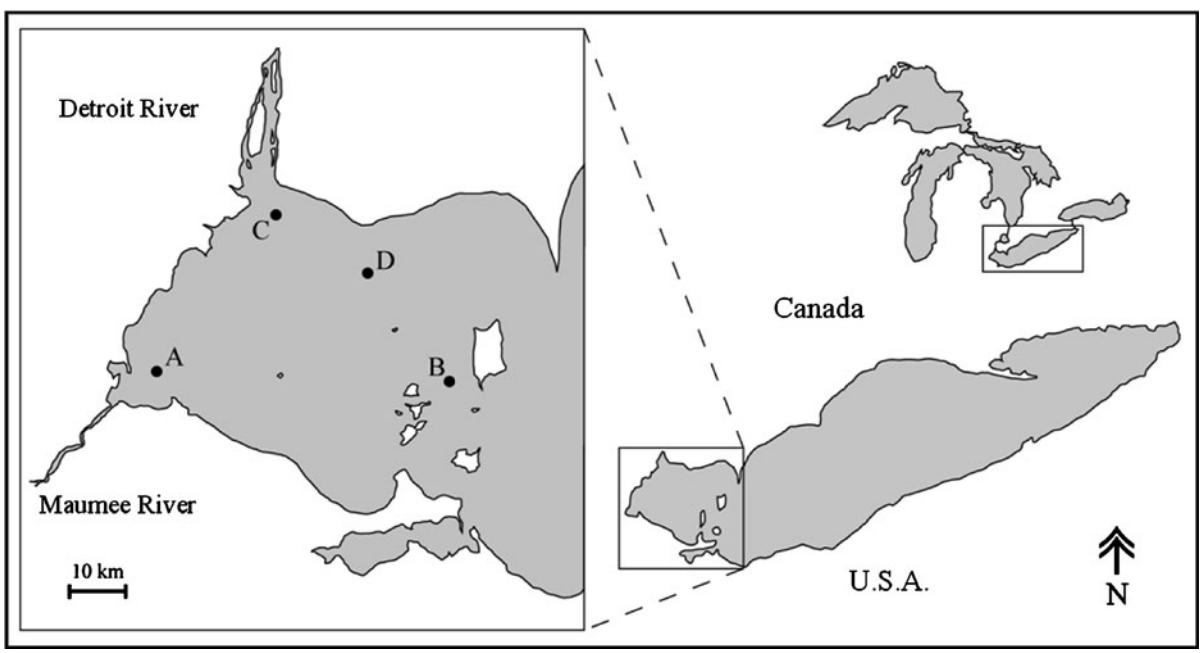

Fig. 1 Location of sampling sites in the western basin of Lake Erie, sampled during June–September 2009. Letters *A–D* represents fixed sampling site. *A* Maumee River Plume, *B* Bass Islands, *C* Detroit River Plume, *D* Middle Sister Island

collected per site, per sampling period (Table 1). Sample sizes for stable isotopes ranged from 3 to 9 replicates per species/site/month.

Before the stable isotope analysis, samples were freeze dried for 48 h and then ground with mortar and pestle in liquid nitrogen. Samples were weighed (800–1,000 μg for seston, 400–600 μg for zebra mussel and fish) into 0.5 mg tin capsules and analyzed using a Delta V Advantage isotope ratio mass spectrometer (Thermo Electron Corporation, Bremen, Germany) and 4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA). At least three different lab and one NIST (8414) reference standards were used for quantification of stable isotope values, and every tenth sample was run in triplicate to assess within-run precision. Stable isotope values are conveyed in δ notation using the following equation:

$$\delta X = \left[R_{\text{sample}}/R_{\text{standard}} - 1\right] \times 1,000$$

where X is ^{13}C or ^{15}N, and R is the ratio of $^{13}C/^{12}C$ or $^{15}N/^{14}N$. The standard reference material was Pee Dee Belemnite carbonate for C and atmospheric nitrogen N_2 for N. The analytical precision was based on the standard deviation of two standards (NIST 8218 bovine liver and internal fish standard; $n = 33$ for each standard) and was 0.17–0.21‰ for $\delta^{15}N$ and 0.04–0.07‰ for $\delta^{13}C$. The analysis of NIST standards (sucrose and ammonia sulfate; $n = 3$ for each) during the analysis of samples generated values that were within 0.01 and 0.07‰ of certified values for $\delta^{15}N$ and $\delta^{13}C$, respectively.

Data analysis

We used several statistical approaches to evaluate the effect of sampling site and sampling month on the isotopic values of western Lake Erie food web components and the estimated TP of white and yellow perch. To compare the isotopic composition of food web components between sampling sites, we used repeated, linear mixed-effects models. Mixed-effects models are appropriate for these data structure encountered in this study, where samples collected across multiple months represent repeated measures of the same site (Raudenbush & Byrk, 2002). Therefore, to account for monthly variation both within and between sites, our analytic design incorporated the random effect of monthly samples (treated as random intercepts) nested within study site (treated as fixed effect). Further, to identify the proportional effect of monthly sampling within sites, we calculated the intraclass correlation coefficients (ICC), reflecting the proportion of variance attributable to each level of the model (i.e., sites and months within sites: see Raudenbush & Byrk, 2002).

To estimate the effects of sampling site and month on the TP of white and yellow perch, we first estimated the TPs of the fish sampled using the following equation:

$$\text{TP} = \left[\left(\delta^{15}N_{\text{fish}} - \text{mean } \delta^{15}N_{\text{mussel}}\right)/3.4\right] + 2$$

where the value 3.4 was adopted to denote an increase of one trophic level, assuming that zebra mussels occupy a TP of 2 (Post, 2002). We then developed a series of orthogonal contrasts to compare means of the estimated TPs of white and yellow perch between months within sites. We also used linear-mixed models, controlling for the random effects of month, to see if the TP of white and yellow perch differed between sites. We then calculated TP of yellow and white perch from Detroit and Maumee using zebra mussels from different sites and months to demonstrate the relative importance of spatial and temporal variations in estimating TP in food webs of large lake ecosystems.

All the statistical analyses were performed using the statistical package R (Version 2.11.1; R Development Core Team, 2008). Before the analysis, all stable isotopes' data were tested for normality using probability plots and transformed where appropriate. For post-hoc multiple comparisons among fixed effects in models, we used Tukey tests (Hothorn et al., 2008).

Results

Samples from the Maumee River Plume and Bass Island had lower (more negative) $\delta^{13}C$ and higher $\delta^{15}N$ values than species from the Detroit River Plume and Middle Sister Island (Table 1). Our analysis revealed significant differences in both the $\delta^{13}C$ and $\delta^{15}N$ of seston ($\delta^{13}C$: $F_{3,61} = 9.06$, $P < 0.001$; and $\delta^{15}N$: $F_{3,61} = 22.42$, $P < 0.001$), zebra mussels ($\delta^{13}C$: $F_{3,47} = 16.93$, $P < 0.001$; and $\delta^{15}N$: $F_{3,47} = 45.53$,

Table 1 Spatial and temporal variabilities of $\delta^{13}C$ and $\delta^{15}N$ for seston, zebra mussels, yellow perch, and white perch from the western basin of Lake Erie

Species	Month	n[a]	Maumee River Plume		n	Bass Island		n	Detroit River Plume		n	Middle Sister Island	
			$\delta^{13}C$ ‰	$\delta^{15}N$ ‰		$\delta^{13}C$ ‰	$\delta^{15}N$ ‰		$\delta^{13}C$ ‰	$\delta^{15}N$ ‰		$\delta^{13}C$ ‰	$\delta^{15}N$ ‰
Seston	June	3	−28.0 ± 0.38	8.59 ± 0.06	5	−27.1 ± 0.06	6.72 ± 0.06	3	−24.6 ± 0.04	7.00 ± 0.02	3	−25.4 ± 0.14	5.77 ± 0.30
	July	7	−25.4 ± 0.03	6.22 ± 0.09	5	−26.7 ± 0.03	8.78 ± 0.09	3	−24.1 ± 0.09	6.10 ± 0.34	3	−25.0 ± 0.04	6.66 ± 0.08
	August	7	−23.7 ± 0.06	7.96 ± 0.06	5	−25.8 ± 0.05	10.2 ± 0.15	3	−25.0 ± 0.10	6.75 ± 0.11	3	−22.7 ± 0.03	3.63 ± 0.45
	September	3	−24.6 ± 0.04	10.0 ± 0.11	3	−24.0 ± 0.19	9.72 ± 0.01	5	−25.9 ± 0.04	5.68 ± 0.07	5	−24.6 ± 0.09	6.08 ± 0.13
Zebra mussel	June	3	−26.6 ± 0.04	8.07 ± 0.06	5	−25.3 ± 0.18	10.1 ± 0.08	3	−23.3 ± 0.16	6.82 ± 0.19	3	−25.1 ± 0.17	6.87 ± 0.18
	July	3	−25.6 ± 0.07	7.31 ± 0.03	3	−26.2 ± 0.26	7.32 ± 0.08	3	−23.3 ± 0.14	6.46 ± 0.05	5	−24.9 ± 0.20	6.09 ± 0.09
	August	3	−26.3 ± 0.74	7.38 ± 0.05	5	−25.4 ± 0.50	7.23 ± 0.15	3	−22.4 ± 0.09	6.43 ± 0.11	3	−22.8 ± 0.07	5.99 ± 0.06
	September	3	−22.8 ± 0.10	9.12 ± 0.15	3	−22.5 ± 0.15	9.30 ± 0.27	3	−22.8 ± 0.22	6.90 ± 0.16	3	−21.7 ± 0.83	6.71 ± 0.21
Yellow perch	July	3	−24.8 ± 0.06	11.3 ± 0.05	5	−24.6 ± 0.07	11.4 ± 0.10	3	−22.0 ± 0.04	11.1 ± 0.05	3	−22.9 ± 0.04	11.0 ± 0.07
	August	5	−23.3 ± 0.18	11.5 ± 0.13	4	−23.0 ± 0.17	13.1 ± 0.15	7	−21.8 ± 0.17	11.6 ± 0.16	3	−22.1 ± 0.06	11.4 ± 0.09
	September	7	−21.4 ± 0.16	12.2 ± 0.20	6	−22.4 ± 0.05	13.2 ± 0.07	9	−20.8 ± 0.17	12.1 ± 0.21	7	−21.0 ± 0.25	11.7 ± 0.18
White perch	July	3	−25.1 ± 0.33	11.8 ± 0.22	7	−24.7 ± 0.04	12.2 ± 0.13	3	−20.7 ± 0.08	10.8 ± 0.06	3	−21.2 ± 0.15	10.9 ± 0.02
	August	5	−22.3 ± 0.18	12.0 ± 0.11	5	−23.9 ± 0.11	13.8 ± 0.27	4	−20.4 ± 0.07	11.3 ± 0.13	3	−20.7 ± 0.10	11.3 ± 0.03
	September	5	−21.6 ± 0.09	12.8 ± 0.17	5	−23.1 ± 0.10	14.4 ± 0.10	4	−20.2 ± 0.12	12.9 ± 0.16	4	−20.8 ± 0.18	12.3 ± 0.08

Values are means ± 1 SE

[a] n refers to the number of samples, samples were pools of multiple individuals for seston and zebra mussel only

$P < 0.001$), and yellow ($\delta^{13}C$: $F_{3,54} = 37.97$, $P < 0.001$; and $\delta^{15}N$:$F_{3,54} = 24.17$, $P < 0.001$) and white perch ($\delta^{13}C$: $F_{3,45} = 102.18$, $P < 0.001$; and $\delta^{15}N$: $F_{3,45} = 60.81$, $P < 0.001$) between sites (Table 2). Temporal changes within site were found to contribute a significant proportion of the variation in the $\delta^{13}C$ and $\delta^{15}N$ signatures for all the species tested (Fig. 2) accounting for >50% of variability in all the species except seston (Table 2).

The TP of yellow and white perch differed significantly between sample sites and months within western Lake Erie (Site: $F_{11,46} = 23.68$, $P < 0.001$; and Month: $F_{11,39} = 28.92$, $P < 0.001$; Table 3). The TP of white and yellow perch from Maumee were significantly lower than the other sampling sites, which produced similar estimates. The TP of white and yellow perch were also found to differ between months within a site, with the highest TP for each species across all sites found in August (Table 3). TP estimates for yellow and white perch also varied up to 0.7 when zebra mussels of non-corresponding sites and months were utilized to estimate TP (Fig. 3). In general, using zebra mussels from Maumee to estimate TP for Detroit fish resulted in an underestimation of TP, while using zebra mussels from Detroit to calculate TP of Maumee fish resulted in an

Table 2 Tukey post-hoc comparisons among sites for $\delta^{13}C$ and $\delta^{15}N$ of all species from the western basin of Lake Erie

Species	Isotope	Site differences						Variability attributed by month (%)
		DET-BS	ME-BS	MSI-BS	ME-DET	MSI-DET	MSI-ME	
Seston	$\delta^{13}C$							
	Estimate[a]	1.01	0.89	1.6	−0.12	0.62	0.74	47
	P	0.0089*	0.012*	<0.001*	0.98	0.28	0.10	
	$\delta^{15}N$							
	Estimate[a]	−2.4	−0.86	−3.08	1.6	−0.67	−2.2	27
	P	<0.001*	0.11	<0.001*	0.0012*	0.45	<0.001*	
Zebra mussel	$\delta^{13}C$							
	Estimate[a]	1.9	−0.42	1.2	−2.4	−0.76	1.6	57
	P	<0.001*	0.63	0.0033*	<0.001*	0.17	<0.001*	
	$\delta^{15}N$							
	Estimate[a]	−1.9	−0.57	−2.1	1.3	−0.205	−1.5	75
	P	<0.001*	0.42	<0.001*	<0.001*	0.79	<0.001*	
Yellow perch	$\delta^{13}C$							
	Estimate[a]	1.7	0.36	1.3	−1.3	−0.37	0.95	68
	P	<0.001*	0.21	<0.001*	<0.001*	0.21	<0.001*	
	$\delta^{15}N$							
	Estimate[a]	−1.03	−0.94	−1.3	0.089	−0.26	−0.35	58
	P	<0.001*	<0.001*	<0.001*	0.94	0.37	0.16	
White perch	$\delta^{13}C$							
	Estimate[a]	3.4	1.1	2.9	−2.3	−0.50	1.8	58
	P	<0.001*	<0.001*	<0.001*	<0.001*	0.19	<0.001*	
	$\delta^{15}N$							
	Estimate[a]	−1.7	−1.3	−1.9	0.42	−0.18	−0.61	68
	P	<0.001*	<0.001*	<0.001*	0.058	0.74	0.0027*	

ME Maumee River Plume, *BS* Bass Islands, *DET* Detroit River Plume, *MSI* Middle Sister Island

[a] Estimate denotes the mean difference between sites, while *P* reflects statistical significance with $\alpha < 0.05$ considered statistically significant. Percent variability by month reflects the percent of variation attributed by month in the model for the $\delta^{13}C$ and $\delta^{15}N$ of each species

* Indicates comparison is significant at $\alpha = 0.05$

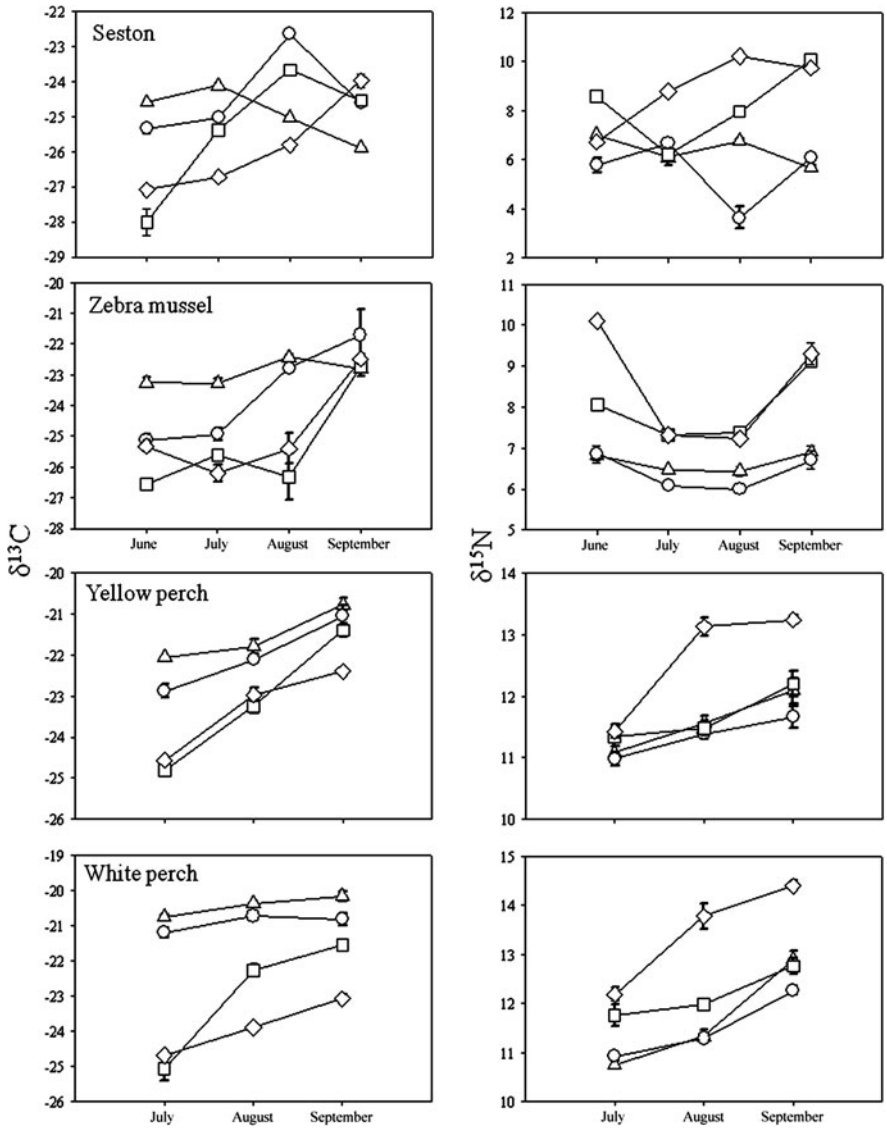

Fig. 2 Temporal patterns of mean stable isotope (δ^{13}C and δ^{15}N) values (±1 SE) in the western basin of Lake Erie during 2009. In each graph, *squares* Maumee River Plume, *diamonds* Bass Islands, *circles* Middle Sister Island, *triangles* Detroit River Plume

overestimation of TP. Using zebra mussels from the same site but incorrect months also resulted in variable TP estimates in YOY white and yellow perch: however, these differences were more prominent in fish from Maumee River Plume (Fig. 3).

Discussion

The results of this study reveal significant spatial and temporal variations in the δ^{13}C and δ^{15}N values of primary consumers and YOY fish in the offshore habitat of the western basin of Lake Erie. These results coincide with similar studies on other lake systems which have found spatial variation in stable isotope values of organisms within the same habitat type (Syvaranta et al., 2006; Mbabazi et al., 2010; Zambrano et al., 2010). For our model, month of collection explained the majority of the variation for all species except seston, indicating that temporal variability is a driving force of isotope's variation within lower trophic levels of lake ecosystems. These findings are consistent with the previous studies that have documented temporal variation in stable isotope values in particulate organic matter and zooplankton (Grey et al., 2001; Matthews & Mazumder, 2005;

Table 3 Mean TP estimates from bootstrapping with orthogonal contrasts of months within site and Tukey post-hoc comparisons between sites from the western basin of Lake Erie

Species	Mean TP estimates				Monthly differences within site				
					Statistical significance of orthogonal contrasts (*P*)		Site differences		
	Site	July	Aug	Sept	July–Aug	Aug–Sept	Comparison	Estimate[a]	*P*
Yellow perch							BS-ME	0.083	<0.001*
	ME	3.2	3.2	2.9	0.10	<0.001*	DET-ME	0.12	<0.001*
	BS	3.2	3.7	3.2	<0.001*	<0.001*	MSI-ME	0.13	<0.001*
	DET	3.4	3.5	3.6	0.03*	0.11	DET-BS	0.041	0.079
	MSI	3.4	3.6	3.5	0.29	0.40	MSI-BS	0.048	0.059
							MSI-DET	0.006	0.98
White perch							BS-ME	0.11	<0.001*
	ME	3.3	3.4	3.1	0.08	<0.001*	DET-ME	0.083	<0.001*
	BS	3.4	3.9	3.5	<0.001*	<0.001*	MSI-ME	0.098	<0.001*
	DET	3.3	3.4	3.8	<0.001*	<0.001*	DET-BS	−0.030	0.39
	MSI	3.4	3.6	3.6	0.75	<0.001*	MSI-BS	−0.015	0.86
							MSI-DET	0.014	0.90

ME Maumee River Plume, *BS* Bass Islands, *DET* Detroit River Plume, *MSI* Middle Sister Island

[a] Estimate denotes the mean differences among sites; *P* reflects statistical significance

* Indicates comparison is significant at $\alpha = 0.05$

Syvaranta et al., 2006; Gu, 2009). Since trends in $\delta^{13}C$ and $\delta^{15}N$ values follow similar seasonal patterns across sites, it suggests that spatial variation of stable isotopes in lower trophic level organisms is more a function of baseline effects rather than food web differences in Lake Erie. In essence, the variation in isotope values among lower trophic level species represents the underlying biogeochemical and abiotic differences among sites within a lake as suggested by Zambrano et al. (2010).

Spatial variation of $\delta^{13}C$ and $\delta^{15}N$ values were observed despite the fact that our samples were collected from sites of similar depth and habitat characteristics within the offshore habitat of the western basin of Lake Erie. The different characteristics of the two major rivers and their watersheds are very likely the sources for the observed variation. Stable isotope values at Detroit/Middle Sister and Maumee/Bass were closely related to one another as would be predicted by water current patterns within the lake (Kovacik, 1972; Bolsenga & Herdendorf, 1993). These results indicating lower (more negative) $\delta^{13}C$ values at Maumee Plume and Bass Islands relative to Detroit Plume and Middle Sister Island were consistent with the studies that found $\delta^{13}C$ values to decrease (more negative) with decreased river discharge (Junger & Planas, 1994; Doucett et al., 1996; Finlay et al., 1999). These results, however, do not coincide with previous studies that have reported $\delta^{13}C$ to increase (less negative) with higher temperatures and lower light penetration (Junger & Planas, 1994; Doucett et al., 1996; Hemminga & Mateo, 1996; MacLeod & Barton, 1998; Finlay et al., 1999), as Maumee Plume is both warmer and more turbid than Detroit Plume (Reichert et al., 2010). Higher (less negative) $\delta^{13}C$ values at Detroit Plume and Middle Sister Island are likely due to the Detroit River receiving its water from the Lake Huron which has $\delta^{13}C$ values representative of autochthonous lake sources (see Hebert et al., 1999; Fox et al., 2002; Paterson et al., 2006). The Maumee River catchment area is dominated by agriculture (~76% of watershed area) and urban run-off (Bolsenga & Herdendorf, 1993; Forster et al., 2000), both of which have been shown to result in decreased (more negative) $\delta^{13}C$ values. Agricultural runoff may yield decreased (more negative) $\delta^{13}C$ values because of differential fractionation of ^{13}C by C4 plants (e.g.,

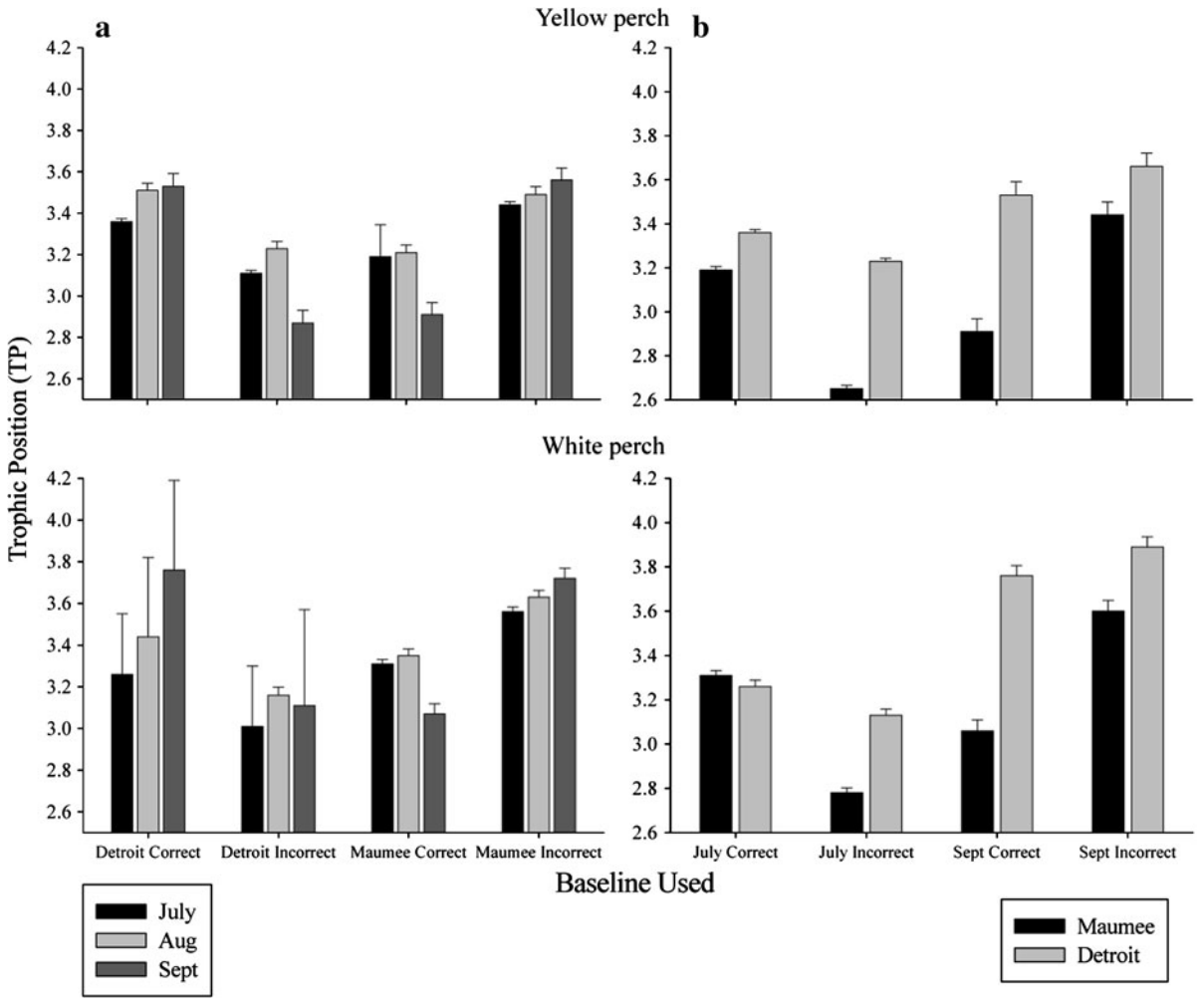

Fig. 3 Trophic position estimates for yellow and white perch using zebra mussel baselines collected from the same site/month as fish (Correct) and using zebra mussels from a different month/site (Incorrect). For graph "*a*'" YOY yellow and white perch collected from Maumee River Plume and Detroit River Plume are calculated using zebra mussels from the corresponding location and using zebra mussels from non-corresponding locations (i.e., Detroit fish using Maumee mussels), respectively. For graph "*b*" YOY yellow and white perch collected from July to September are calculated using zebra mussels from the corresponding month and using zebra mussels from non-corresponding months (i.e. July fish using September mussels)

corn crops) and C3 plants (e.g. deciduous and coniferous trees) (Forsberg et al., 1993): however, it is also possible that residues of C4 plants may sometimes enter aquatic systems as particulate organic matter, which could result in higher (less negative) δ^{13}C values in filtering mussels (DeBruyn & Rasmussen, 2002). Raw sewage runoff from natural agricultural fertilizers (e.g., manure), have also been shown to decrease (more negative) δ^{13}C values (Rau et al., 1981; VanDover et al., 1992; DeBruyn & Rasmussen, 2002): however, raw urban sewage does not explain elevated δ^{15}N values unless the sewage had time to undergo the nitrification process. Recently, Diebel & Vander Zanden (2009) have reported that inorganic fertilizers are more likely than organic fertilizers (manure), to drive variability in δ^{15}N and to cause increased nitrogen concentrations in fluvial systems. Therefore, the decreased (more negative) δ^{13}C and elevated δ^{15}N values evident at Maumee Plume and Bass Islands are likely the result of agricultural (crop) runoff and inorganic fertilizers.

Month was found to be a more important variable than location in contributing to the variations of $\delta^{13}C$ and $\delta^{15}N$ in zebra mussels and YOY yellow and white perch across the western basin of Lake Erie. These temporal contributions reflect the fact that Lake Erie is located in a temperate climate area, and as a result, seasonal runoff, primary production, algal content, and food web dynamics vary through the open-water season, all of which have been shown to influence stable isotope values. Stable isotope values in all the samples in June and July followed expected patterns based on the river of influence, but in August and September, stable isotope values were relatively similar across sampling sites. In general, species increased in both $\delta^{13}C$ and $\delta^{15}N$ from early summer to fall. Increases in $\delta^{13}C$ could be a result of greater demands of CO_2 by increased phytoplankton biomass which typically occurs as the summer progresses. If CO_2 becomes limited, then primary producers will discriminate less against ^{13}C, in turn enriching their $\delta^{13}C$ values (Yoshioka et al., 1994; Finlay, 2001). These primary producers serve as a primary food for YOY yellow and white perch, and therefore temporal changes in $\delta^{13}C$ would be reflected in the isotope values of YOY fish as well. Increases in $\delta^{15}N$ values could be the result of a loss of dilution of high spring $\delta^{15}N$ from inorganic fertilizers when water levels drop later in summer, particularly in the Maumee River. In addition to this loss of dilution, increased denitrification through summer could also yield higher $\delta^{15}N$ values (DeBruyn & Rasmussen, 2002). While isotopic values for all species varied among sites, these values exhibited similar trends in both $\delta^{13}C$ and $\delta^{15}N$ values from spring through fall, confirming that these isotopic differences are linked to baseline effects rather than to food web differences (Zambrano et al., 2010).

Differences among species were more pronounced in $\delta^{15}N$ rather than in $\delta^{13}C$ values. Seston had a slightly higher $\delta^{15}N$ signature than zebra mussels, which could be a result of sediment disturbance events, potentially increasing the organic/inorganic content of seston. Increases in $\delta^{15}N$ in the seston could reflect increases in zooplankton population later in the season (Fahnenstiel et al., 1998); seston samples were not sorted. Zebra mussels $\delta^{15}N$ values spiked in June and September, while decreasing during July and August. Increases in YOY fish $\delta^{15}N$ are most likely a function of fish growth and resulting diet changes similar to YOY smallmouth bass (*Micropterus dolomieu*) whose $\delta^{15}N$ of which were correlated with growth (Vander Zanden et al., 1998), but could also partially be due to an increase in the value of $\delta^{15}N$ in the system due to a loss of nitrogen dilution or increased denitrification. Yellow perch spawn in late April or early May in Lake Erie, while white perch typically spawn in late May or early June (Thoits, 1958; Thorpe, 1977; Schaeffer & Margraf, 1986, 1987; Norton, unpublished). This difference in spawning allows YOY yellow perch to be larger than YOY white perch throughout their first growing season and in turn have access to larger prey items (Norton, unpublished): however, this does not explain the higher $\delta^{15}N$ values of white perch relative to yellow perch. During this first growing season yellow perch typically undergo a diet shift from pelagic to demersal when they reach about 20–25 mm in length (Wu & Culver, 1992), while white perch remain in shallow waters. White perch have a more terminal mouth, advantageous for feeding up in the water column on plankton, while yellow perch, on the other hand have a sub-terminal mouth, allowing for more efficient benthic feeding (Parrish & Margraf, 1990). While YOY of both species have been found to feed relatively similarly in laboratory studies (Parrish & Margraf, 1990), the $\delta^{13}C$ observed in this study suggests that feeding strategies of the two species may be different, thus providing a potential explanation for differences in $\delta^{15}N$ among the two species.

Estimates of TP based on the $\delta^{15}N$ of fish relative to a sessile baseline species have become increasingly popular in food web studies (Vander Zanden et al., 1997). Evidence of spatial zones and monthly variability of $\delta^{15}N$ in this study suggest a potential impact on the accuracy of TP estimations. Monthly differences in TP could be a result of changes in feeding behavior with growth of YOY fish as found in YOY black bass (Vander Zanden et al., 1998) or differences in baseline $\delta^{15}N$ values in zebra mussels due to variation in nitrogen flow across the basin. We quantified large variations in the TP estimates for both yellow and white perch when using a baseline collected from an incorrect site or month in the calculation. These differences were most pronounced when mussels from incorrect sites were used for TP calculation of the fish. Using zebra mussels from incorrect months led to relatively smaller variations, suggesting that baseline differences among sites are

an important factor to be considered when estimating TP using δ^{15}N values of species relative to δ^{15}N of sessile baseline organism. This also highlights the importance of consistency in sampling design, ensuring that sessile baseline organisms used in TP calculations are sampled from the same location/timeframe as the organisms being estimated for.

In general, many studies that have utilized stable isotopes to examine food webs have been coarse in their descriptions of food web structure and links. As the use of stable isotopes becomes more quantitative (e.g., Layman et al., 2007; Hoffman et al., 2010), it becomes necessary that we understand and incorporate this variation in stable isotopes values into studies on food web structure and function. Within-habitat variation of isotopes in large lakes could be problematic when trying to distinguish carbon and nitrogen sources in systems with multiple nutrient inputs. The calculation of TP for organisms acquiring nitrogen from multiple sources requires the use of base δ^{13}C and δ^{15}N values from each nutrient input (Post, 2002). However, if significant temporal variation exists within single sites, even in primary consumers, which are thought to absorb temporal variance of isotopic values (Vander Zanden & Rasmussen, 1999), then uncertainties could arise in the determination of true isotopic values of each contributing nutrient source. This problem is complicated further by the fact that greater number of TP fish integrate isotope values over longer timescales and are more mobile than baseline organism, allowing them to integrate isotope values from multiple sites (Perga & Gerdeaux, 2005).

The significance of incorrect TP assignment was demonstrated recently; where Branch et al. (2010) discovered that a 0.5 change in TP of anchoveta resulted in an erroneous report of steep declines in global fisheries landings since the 1970s, as described in the benchmark study of Pauly et al. (1998). While this degree of error in TP estimates would typically be considered minor or inconsequential in many food web studies, it altered the global catch mean trophic level trend reported by Pauly et al. (1998). These findings highlight the sensitivity of fisheries management techniques to variation in TP estimates. While primary consumers do provide appropriate baselines for qualitative use of stable isotopes, we have demonstrated that they are susceptible to substantial spatial and temporal variations, which could hinder the evolution of stable isotopes as a quantitative tool in food web studies.

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