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Assessing trophic position quantification methods for three piscivorous freshwater fish using stable isotopes and stomach contents

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ABSTRACT

Accurate trophic position (TP) estimates are important for the development of ecosystem-based management plans. TPs can be quantified by carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotopes in tissues, but these can disagree with observed and perceived feeding ecology. A recent method that has used a scaled diet-tissue discrimination factor (DTDF), reflecting the inverse relationship between DTDF and $\delta^{15}N$, was found to better describe TPs of predatory fish species in marine ecosystems, but this has not been tested in freshwater ecosystems. Here, we compare methods of TP estimations in the Lake Huron-Erie corridor (HEC), a system where high diversity of prey items has contributed to the concern that foraging ecology of piscivorous fish species is poorly understood. Using $\delta^{15}N$ and $\delta^{13}C$, we quantified TP of longnose gar (*Lepisosteus osseus*), largemouth bass (*Micropterus salmoides*), and northern pike (*Esox lucius*) to assess the efficacy of a scaled DTDF compared to traditional DTDF isotope methods and stomach content analysis (SCA). The scaled DTDF method produced TP estimates that were at times consistent with SCA and were generally higher and with a greater range among individuals than non-scaled DTDFs. The scaled method was not sensitive to baseline choice nor influenced by incorporating carbon source in the model. Greater variability of TP estimates using a scaled DTDF suggests more complex trophic structuring in the upper trophic level guild of the HEC. These results, particularly the lack of baseline sensitivity, provide support for using the scaled DTDF in freshwater food web characterization.

Introduction

A commonly used method to assess the role of fish species in food webs is trophic position (TP), which is a continuous measure of a consumer's feeding position relative to other species in a food web that accounts for the consumption of prey across trophic levels (i.e. omnivory) (Vander Zanden and Rasmussen, 1999). TP provides a standardized ecological metric to assess food webs and species interactions between and within ecosystems (Vander Zanden and Rasmussen, 1996). Estimates of TP have traditionally been calculated using proportional diet contributions based on stomach content analysis (SCA; Elton, 1927). However, stomach contents are subject to numerous biases including: prey misidentification due to digestion, rare feeding events, empty stomachs, uneven digestion rates of prey (e.g. soft-bodied vs. hard-shelled) (Brush et al., 2012), disproportional estimates of diet based on weight or count, and a short temporal view of diet (generally < 1 day; Hyslop, 1980). Stomach content analysis also requires large sample sizes to address some of these biases, which can be difficult to acquire and may be unethical, especially for rare or endangered species (Almany and Webster, 2004). Moreover, when stomach contents are used in TP calculations it is often assumed that a single fixed TP represents each species of prey taxon, despite the evidence that prey themselves exhibit flexible foraging and ontogenetic differences. Combined, the shortfalls of stomach content analyses and the assumptions when calculating TP from these stomach contents results in inaccurate estimates of TP, resulting in an oversimplification of food web structure (Hussey et al., 2011).

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The issue of inaccurate TPs may be especially problematic in large lakes, where a wide range of fish species are often assumed to feed at the same TP at both a community and population level (e.g., perceived TP = 4.0 for piscovore fish; Krause et al., 2003; Mumby et al., 2018). Determining an accurate TP for a species or population is important for providing insights on species interactions and energy flow, knowledge of which is necessary for an ecosystem-based approach to management (Pikitch et al., 2004).

Over the past three decades, the use of nitrogen stable isotopes ($\delta^{15}N$) to estimate TP has become a well-established method (Minagawa and Wada, 1984; Peterson and Fry, 1987); converting $\delta^{15}N$ to a TP provides a method for comparing between locations and across time, especially when baseline $\delta^{15}N$ varies. Most applications which use $\delta^{15}N$ to quantify TP employ a constant diet-tissue discrimination factor (DTDF), typically 3.4‰ (derived from metanalyses), which reflects the expected change in $\delta^{15}N$ between a prey and consumer and provides a means to estimate TP (Peterson and Fry, 1987; Post, 2002; Vander Zanden and Rasmussen, 1996). However, a linear relationship where DTDF decreases with increasing dietary $\delta^{15}N$ between trophic levels or food has been demonstrated in laboratory studies (Overmyer et al., 2008) and metanalyses (Caut et al., 2008). Consequently, a scaled DTDF approach to estimating TP has been proposed to account for this inverse relationship and provide more robust estimates of TP in marine ecosystems (Hussey et al., 2014). The scaled DTDF proposed by Hussey et al. (2014) is increasingly being used in marine food studies based on significant citation increases, but few freshwater studies have used the method because of perceived shorter food chain lengths even though DTDFs have been known to vary in freshwater systems (Caut et al., 2009).

To date, a scaled DTDF has been used to determine predator TP in marine ecosystems; however despite comparable patterns of decreasing DTDF with increasing dietary $\delta^{15}N$ across different marine and freshwater environments (Caut et al., 2009), the method has not been tested in many freshwater systems. When considering species-specific isotopic fractionation between trophic levels (Caut et al., 2009; Colborne et al., 2017), scaled DTDF-derived TP estimates are found to be less sensitive to variation in baseline $\delta^{15}N$ than traditional DTDF-derived TP estimates (Hussey et al., 2014), which can be useful in determining predator TP in freshwater systems that experience seasonal changes in prey abundance (Vander Zanden and Rasmussen, 1999). The scaled DTDF model does not, however, consider adjustments to TP calculations based on dual carbon sources.

Stable isotopes of carbon ($\delta^{13}C$) are used to determine an organism's sources of primary production and habitat use (Peterson and Fry, 1987). Unlike the enrichment of $\delta^{15}N$ between trophic levels, $\delta^{13}C$ increases only slightly between trophic levels (approximately 0.47‰ in freshwater systems). Instead, differences in $\delta^{13}C$ between species are due to differences in the photosynthetic pathways of primary producers from littoral and pelagic areas (Fry, 2006). Thus, $\delta^{13}C$ is often used to determine the ultimate sources of carbon for different consumers and in this way, when the relationship of $\delta^{13}C$ between a predator and baseline deviates from expected relationship of 0.47 ± 1.23‰ $\delta^{13}C$, we can assume that the species are feeding in different habitats (France, 1995; Nilsson et al., 2012; Post, 2002). Moreover, these deviations in $\delta^{13}C$ between habitats are reflected in $\delta^{15}N$ as well, albeit more subtly than the increase of $\delta^{15}N$ (Post, 2002). Thus, to account for differences in littoral and pelagic $\delta^{13}C$ and $\delta^{15}N$ baseline values, Post (2002) proposed a dual-carbon source modification to established trophic position calculation models. Combining this dual source constant $\delta^{15}N$ DTDF model with the scaled DTDF has not yet been done but could provide valuable insight into TP of species that feed in both littoral and pelagic environments with large differences in baseline organism $\delta^{15}N$ values.

The general objective of this study was to quantify and contrast methods for estimating TP of three co-occurring freshwater piscivores; longnose gar (*Lepisosteus osseus*), largemouth bass (*Micropterus salmoides*), and northern pike (*Esox lucius*) at two sites in the Lake Huron-Erie Corridor (HEC) of the Great Lakes. More specifically, we estimated and compared the TP of each species at each site using (i) stomach contents (TP_{SCA}) and stable isotopes using (ii) a constant DTDF of 3.4‰ ($TP_{Constant}$), and (iii) a scaled DTDF (TP_{Scaled}), where both a single or dual carbon source model were used for both isotope methods. Finally, we tested the influence of using baseline organisms from different trophic levels on $\delta^{15}N$-derived TP estimates. First, we hypothesize (H1) that the TP_{Scaled} method will demonstrate more variable TP estimates between individuals than $TP_{constant}$ method or currently assumed (i.e., that all individuals of these species have a TP of 4.0; Krause et al., 2003) estimates, because of a more accurate application of stable isotopes (i.e., scaled DTDF). Second, given the results from marine food webs that used a scaled DTDF (Hussey et al., 2014), we hypothesize (H2) that TP_{scaled} of these three predators will be greater than $TP_{constant}$ estimates (i.e., TP = 4.0) and more comparable to dietary TP estimates. Third, due to differences in the isotopic signature of nearshore and offshore baseline, we hypothesize (H3) that there will be differences in TP_{Scaled} estimates when using a single or dual-carbon source model. Finally, due to the findings of baseline insensitivity in scaled DTDF-derived TP estimates in Hussey et al., (2014) in comparison to constant-DTDF derived TP estimates, we hypothesize (H4) that the scaled DTDF estimates will be less sensitive to changing consumer baselines than a constant DTDF.

Methods

Sample collection

Study species were collected at two sites in the Detroit River; around Peche (42.35°N, −82.93°W) and Grass Islands (42.22°N, −83.11°W) between April 20 and June 20, 2014 (Fig. 1).

Fish were captured using trap nets, fyke, and seine nets as well as angling and a single anode boat electrofisher with a direct current (DC) of 4.0A and a pulse frequency of 30–60 Hz. All fish were euthanized with an overdose of tricaine methanesulfonate (MS-222). To avoid issues with ontogenetic changes in diet, only fish in specific size ranges were selected (longnose gar total body length range: 53–75 cm, largemouth bass: 25–42 cm, northern pike: 50 cm-70 cm), which avoid juvenile size ranges (Johnson and Davis, 1997; McGrath et al., 2013; Venturelli and Tonn, 2005). A 5 g muscle tissue sample was collected anterior to the dorsal fin and stored frozen (-80°) until stable isotope analysis. Whole stomachs were removed and preserved in 95% ethanol to prevent enzymatic degradation, and then frozen until stomach content analysis.

Stomach content analysis

For each stomach, diet items were identified to the lowest possible taxonomic level and percent frequency of occurrence (% F; the occurrence of a particular prey type across all stomachs), percentage by number (% N; the amount of a particular prey species relative to all prey species across all stomachs), and percentage by wet weight (% W; the percent weight contribution of a prey species across total mass of all prey species in all stomachs) within all stomachs were calculated. The Index of Relative Importance (IRI) (Hyslop, 1980; Cortés, 1997; Pinkas et al., 1971) was determined and expressed on a percent basis (% IRI) using all stomachs for one species at one site (Cortés, 1997) using the equations:

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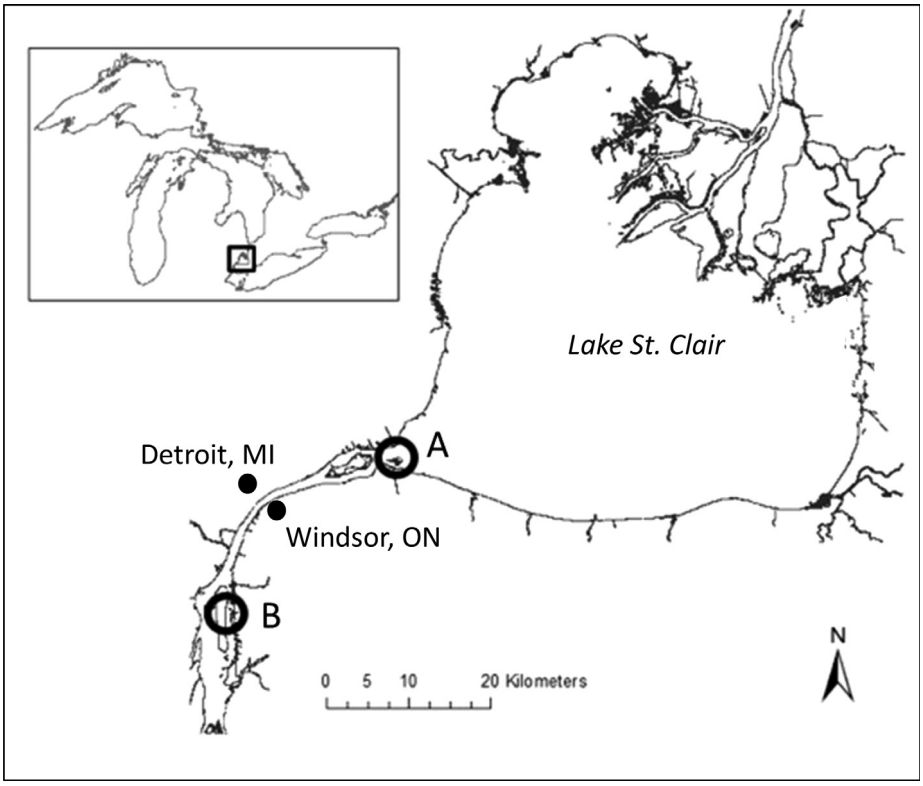

Fig. 1. Location of sampling sites (A) Peche Island and (B) Grass Island in the Detroit River of the Huron-Erie Corridor.

$$IRI = (\%N \times \%W) \times \%F \quad (1)$$

and

$$\%IRI_i = \frac{100IRI_i}{\sum_{i=1}^{n} IRI_i}, \quad (2)$$

where n is the total number of prey types and % IRI is determined for each prey type (i) using their IRI value from Eq. (1).

A rarefaction analysis was performed to determine the expected number of diet items for each predator at each site and the total number of samples needed to determine prey item diversity to a 75% confidence interval in order to adequately estimate TP_{SCA} (Table 1). This analysis was performed according to the procedure laid out in Heck et al. (1975) using the program EstimateS (Colwell, 2006), where species with enough samples to determine prey item diversity were considered to produce robust TP_{SCA} estimates.

A standardized TP estimate for each species at each location was calculated based on stomach content data and a proportional IRI value (Cortés, 1999) using an equation based on traditional (n° consumer) trophic levels (Lindeman, 1991):

$$P_i = \frac{\%IRI_i}{\sum_{i=1}^{x}(\%IRI_i)}$$

$$TP_{SCA} = 1 + (\sum_{i=1}^{x} P_i \times TP_i), \quad (3)$$

where previously estimated fixed dietary TPs of prey items (TP_i), as well as proportional IRI values (P_i) for each corresponding prey item (% IRI_i), are surmised for each predator at each site (Cortés, 1999). Prey item literature TP values were selected based off prey item body size that was also found in the HEC. Unidentifiable material present in the stomachs of predators was not included in proportional IRI, % IRI or TP_{SCA} calculations.

Carbon and nitrogen stable isotope analysis

Only fish with identifiable stomach contents were used in the stable isotope analyses to be consistent with the proportional IRI, % IRI, and TP_{SCA} calculations. All fish white muscle tissue samples were lyophilized at −48 °C and 133 × 10^3 mbar for 48 h, ground by hand, and lipid-extracted using a 2:1 chloroform:methanol mixture (Bligh and Dyer, 1959). Following lipid extraction, ~400–600 µg of sample per individual was weighed into tin cups. The carbon and nitrogen isotopic composition of each sample were determined using a Delta V Advantage Thermoscientific continuous flow mass spectrometer (Thermo Electron Corporation, Bremen, Germany) coupled to a 4010 Elemental Combustion System (Costech Instruments, Valencia, CA, USA). Stable isotope values are reported as per mil (δ) and were calculated using the equation:

$$\delta X = \left(\left[\frac{Rsample}{Rstandard}\right] - 1\right) \times 1000, \quad (4)$$

where X represents ^{13}C or ^{15}N and R is represented by ^{13}C:^{12}C and ^{15}N:^{14}N. Vienna Pee Dee Belemnite and atmospheric nitrogen were used as standard reference materials for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$), respectively. Analytical precision was assessed by examining variation in replicate tissue samples (every 10th sample was run in triplicate), all were within the acceptable ± 0.2‰ standard deviation range (0.1‰ for $\delta^{13}C$ and 0.1‰ for $\delta^{15}N$, n = 30). Measures of NIST and internal laboratory standards were also < 0.2‰ from known values for $\delta^{13}C$ and $\delta^{15}N$.

Trophic position estimates using stable isotopes

Trophic position for a species population was first calculated using the traditional method ($TP_{Constant}$) that uses the same DTDF value of 3.4‰ for all three species (Vander Zanden et al., 1997) and a single carbon source:

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Table 1
Stomach content sample size needed to provide 75% measures of diversity based on cumulative frequency rarefaction curves of stomach contents for each species; largemouth bass (LMB; *Micropterus salmoides*), longnose gar (LNG; *Lepisosteus osseus*), and northern pike (NP; *Esox lucius*), and site (Peche Island and Grass Island) from the Lake Huron-Erie Corridor (Colwell, 2006). S_{obs} represents the number of diet items observed in predator stomachs; S_{exp} represents the estimated number of diet items in the assemblage, and $S_{75\%}$ represents the required number of stomachs to determine 75% dietary diversity. N/A was assigned to species that had a linear relationship between number of stomachs and diversity, and as such we were unable to approximate the asymptote. Species that were denoted with (*) represent adequate stomach contents to estimate 75% dietary diversity and is determined by the presence and frequency of prey items.

Species	n (# of stomachs)	n (# of stomachs containing prey)	S_{obs}	S_{exp}	$S_{75\%}$
Peche Island					
LMB	35	25	9.8	5.7	21*
LNG	6	4	5.2	3.9	11
NP	12	11	6.9	2.7	11*
Grass Island					
LMB	30	22	6.2	4.6	11*
LNG	31	18	8.1	6.1	12*
NP	16	10	N/A	N/A	N/A

$$TP_{constant,single} = \frac{\delta^{15}N_{predator} - \delta^{15}N_{baseline}}{3.4} + TP_{baseline}, \quad (5)$$

where $\delta^{15}N_{predator}$ represents predator δ^{15}N, $\delta^{15}N_{baseline}$ represents baseline δ^{15}N, and $TP_{baseline}$ is a literature TP estimate of a baseline organism that is also reflective of an expected increase of 0.47 ± 1.23‰ between trophic levels. In comparison, a dual carbon-source baseline model was used to calculated TP:

$$TP_{constant,dual} = \frac{\delta^{15}N_{predator} - (\delta^{15}N_{baseline1} \times \alpha + \delta^{15}N_{baseline2} \times (1-\alpha))}{3.4} + (TP_{baseline1} \times \alpha) + (TP_{baseline2} \times (1-\alpha)), \quad (6)$$

where variables were the same as Eq. (5) and $\alpha = \frac{(\delta^{13}C_{consumer} - \delta^{13}C_{baseline2})}{(\delta^{13}C_{baseline1} - \delta^{13}C_{baseline2})}$ represents the proportional contribution of carbon (δ^{13}C) from baseline species used to represent littoral and pelagic environments (Post, 2002).

Trophic position was then calculated using a scaled DTDF method that accounts for decreasing DTDFs with increasing predator δ^{15}N (TP_{scaled}, Hussey et al., 2014) using the following equation:

$$TP_{scaled,single} = \frac{\ln(\delta^{15}N_{lim} - \delta^{15}N_{baseline}) - \ln(\delta^{15}N_{lim} - \delta^{15}N_{predator})}{k} + TP_{baseline}, \quad (7)$$

where $\delta^{15}N_{lim}$ represents the rate at which ^{15}N and ^{14}N uptake equals the rate of ^{15}N and ^{14}N excretion respectively (resulting in no net change in δ^{15}N between consumer and prey, Δ^{15}N = 0) and was determined to be 21.93, and k represents the rate at which $\delta^{15}N_{predator}$ approaches $\delta^{15}N_{lim}$ per TP increment and determined to be 0.14 through meta-analyses for fish (Hussey et al., 2014).

The TP_{scaled} method was also modified to account for proportional baseline contribution to provide a dual-carbon source model:

$$TP_{scaled,dual} = \frac{\ln(\delta^{15}N_{lim} - \delta^{15}N_{baseline1} * \alpha) - \ln(\delta^{15}N_{lim} - \delta^{15}N_{predator})}{k} + (TP_{baseline1} * \alpha) + \frac{\ln(\delta^{15}N_{lim} - \delta^{15}N_{baseline2} * (1-\alpha)) - \ln(\delta^{15}N_{lim} - \delta^{15}N_{predator})}{k} + (TP_{baseline2} * (1-\alpha)), \quad (8)$$

where variables were the same as Eq. (7). Individual predator-baseline scaled DTDFs (Δ^{15}N) were also calculated through rearranging Eqs. (7) and (8) (See Supplementary Materials S4 in Hussey et al., 2014).

TP baseline substitutions

Values of δ^{15}N vary with habitat in lakes (e.g., nearshore vs pelagic) (Fry, 2006), which needs to be considered when calculating TPs (Post, 2002). For all TP calculation methods multiple trophic level baseline species were used (trophic levels ≈ 2–3) to assess the sensitivity of these methods to baseline species choice. Baseline species selected for the dual carbon-source model were representative of littoral and pelagic feeding, respectively, while baseline selection for a single-carbon source model was decided by selecting a species with predictable ^{13}C enrichment between trophic levels (±0.47; Vander Zanden and Rasmussen, 2001).

Data analysis

All data and residuals were found to be normal using Shapiro-Wilks and Levene's test, respectively. Trophic position estimates calculated using both a constant DTDF and a scaled DTDF (for single and dual-carbon source models) were compared using two-way ANOVAs (for carbon source and TP calculation method, including an interaction effect) for each species separately at each site. A one-way ANOVA was used to test the effect of different baseline species on TP estimations for the different calculation methods for a given site and given species followed by a Tukey's Significance Difference, post-hoc test for those sites and species which were significant. Interspecific comparisons of predator TP were also performed using a one-way ANOVA testing for differences in TP among species at a given site for a given method followed by a Tukey's Significance Difference, post-hoc test. All statistical analyses were performed using R (Version 0.98.1083, R Core Team, 2014) and statistical significance was set at $\alpha = 0.05$.

Results

Stomach contents

Of the 130 longnose gar, largemouth bass, and northern pike stomachs examined, 71% (n = 90; largemouth bass, n = 47; longnose gar, n = 22; northern pike, n = 21) contained identifiable prey items. Stomach content data met the 75% dietary diversity criteria, except for northern pike at Grass Island and longnose gar at Peche Island (Table 1). Stomach contents differed between largemouth bass, longnose gar, and northern pike, and showed variability between sites. By %IRI, largemouth bass diet was consistent at both sites; rusty crayfish (*Orconectes rusticus*) were major contributors to diet at both Grass (%IRI = 59.8) and Peche Island (% IRI = 62.9; Table 2). Longnose gar stomach contents consisted of bluegill (*Lepomis machrochirus*; % IRI = 36.5) and invertebrates (% IRI = 40.5) at Grass Island, while spotfin (*Cyprinella spiloptera*)

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Table 2
Stomach content analysis of largemouth bass (LMB; *Micropterus salmoides*), longnose gar (LNG; *Lepisosteous osseus*), and northern pike (NPK; *Esox lucius*) across two sampling sites (Grass Island and Peche Island, columns coloured light and dark grey, respectively) in the Lake Huron-Erie corridor. Values reported are the Index of Relative Importance (%IRI). Predator TP_{SCA} estimates were calculated using aggregated proportional IRI and previously estimated prey TP values (see methods for details) and these are reported at the bottom of the table. Expanded diet metrics can be found in ESM Table S1.

Species	Trophic Guild	Lit TP	Grass Island			Peche Island		
			LMB (n = 22)	LNG (n = 18)	NPK (n = 10)	LMB (n = 25)	LNG (n = 4)	NPK (n = 11)
Invertebrates	Omnivorous zoobenthos[1]	2.5[1]	0.8	40.5	4.9	18.4	0	0
Spottail Shiner[A]	Insectivores[2,3]	2.7[4]	6.1	9.8	4.9	2.9	0	14.2
Striped Shiner[B]		2.5[1]	0	0	0	3.2	34.7	0
Emerald Shiner[C]		2.9[4]	1.2	0	0	0	0	0
Brook Silverside[D]		2.7[5,6]	0	0	0	1.0	0	0
Black Bullead[E]		3.8[7]	32.1	0	0	0	0	0
Spotfin Shiner[F]	Zoobenthivores[2]	2.5[1]	0	4.0	0	3.8	65.3	4.0
Rusty Crayfish[G]		3.0[1]	59.8	0	0	62.9	0	0
Bluegill[K]	Omnivores[2]	3.2	0	36.5	0	0	0	52.4
Common Carp[M]		3.1[8]	0	0	26.9	0	0	0
Pumpkinseed[N]		3.3[9,10]	0	0	42.5	0	0	0
Round Goby		3.2	0	0	0	0	0	14.9
Silver Bass[O]	Piscivores[2]	3.5[1]	0	0	15.8	0	0	0
Yellow Perch[I]		3.7[1]	0	9.2	5.0	0.7	0	14.6
Northern Pike[J] (juvenile)		4.2[1,4]	0	0	0	1.6	0	0
TP_{SCA}			**4.2**	**4.0**	**4.3**	**3.9**	**3.5**	**4.2**

[A]*Notropis hudsonius*, [B]*Luxilus chrysocephalus*, [C]*Notropis atherinoides*, [D]*Labidesthes sicculus*, [E]*Ameiurus melas*, [F]*Cyprinella spiloptera*, [G]*Orconectes rusticus*, [H]*Neogobius melanostomus*, [I]*Perca flavescens*, [J]*Esox Lucius*, [K]*Lepomis machrochirus*, [L]*Micropterus salmoides*, [M]*Cyprinus carpio*, [N]*Lepomis gibbosus*, [O]*Morone chrysops*.
[1] Vander Zanden et al., 1997, [2]Uzarski et al., 2005, [3]Bhagat et al., 2007, [4]McLeod et al., 2015, [5]Keast and Welsh, 1968, [6]Keast, 1985, [7]Turner, 1966, [8]Brush et al., 2012, [9]Maitland and Campbell, 1992, [10]Froese and Pauly, 2000.

and striped shiner (*Luxilus chrysocephalus*) species were the dominant diet items at Peche Island (% IRI = 65.3 and 34.7, respectively; Table 2). Northern pike stomach content items were not consistent between the sites; the major contributors to diet by % IRI at Peche Island included common carp (*Cyprinus carpio*) (% IRI = 26.9), pumpkinseed (*Lepomis gibbosus*) (% IRI = 42.5), and silver bass (*Morone chrysops*) (% IRI = 15.8; Table 2), while bluegill were mostly consumed at Grass Island (% IRI = 52.4; Table 2). Expanded diet metrics can be found in Electronic Supplementary Material (ESM) Table S1.

Trophic position estimated using stomach contents and a constant and scaled DTDF

TP_{SCA} estimates ranged from 3.5 for longnose gar to 4.2 for northern pike at Peche Island, and 4.0 for longnose gar to 4.3 for northern pike at Grass Island (Table 2).

Species used as baseline values (TL ≈ 2–3) for TP calculations using stable isotopes had a wide range in mean $\delta^{15}N$ (Table 3), however except for dreissenids, the majority of collected littoral baseline species had comparable $\delta^{13}C$ values (Fig. 2). TP_{Scaled} estimates did not differ when using multiple baseline species (TL ≈ 2–3; ANOVA, $p > 0.05$), with the exception of northern pike at Peche Island, while $TP_{Constant}$ estimates differed (ANOVA, $p < 0.05$; Table 3), with the exception of longnose gar at Peche Island. TP_{Scaled} estimates did not differ when using a single (ANOVA, $p > 0.05$) or dual-carbon source (ANOVA, $p > 0.05$) while $TP_{Constant}$ did differ when using a single (ANOVA, $p < 0.05$) or dual-carbon source (ANOVA, $p < 0.05$) at either site, with the exception of northern pike (Table 3; see ESM Tables S2 and S3 for individual df, f-values, and p-values). ANOVAs revealed that the interaction effect between source (i.e. single or dual-carbon source) and method (scaled or constant DTDF) was not significant for any species at either site ($p > 0.1$). Trophic position estimates using the scaled DTDF were higher than using a constant DTDF across all three species at Grass Island (ANOVAs, $p < 0.001$), and had a greater range than those using a constant DTDF (Fig. 3), a range that generally increases when TP > 4.4 (Fig. 4). There were no differences between TP_{Scaled} and $TP_{Constant}$ at Peche Island for all three species (ANOVAs, $p > 0.05$), however TP ranges were greater for TP_{Scaled} than $TP_{Constant}$ across all three species (Figs. 3 and 4). Finally, TP estimates did not differ when using a single or dual-carbon source for either TP_{Scaled} or $TP_{Constant}$ at Peche or Grass Island (ANOVAs, $p > 0.05$). Irrespective of calculation method, all three species exhibited significantly different TP from each other at both sites (expanded statistics for these findings can be found in ESM Tables S4 and S5 followed by post-hoc comparisons in ESM Tables S6 and S7, however, the reasons for and importance of these species differences are not the focus of this paper).

All TP_{Scaled} ranges were greater than $TP_{Contstant}$ ranges, using both single and dual-carbon source models (Fig. 3). Longnose gar had the greatest intraspecific TP_{Scaled} ranges, and standard errors (SE), which accounted for differences in sample sizes, for single and dual carbon-source models compared to largemouth bass and northern pike at both Peche Island (TP range = 2.3 TLs, SE single = 0.21 and SE dual = 0.16) and Grass Island (TP range = 1.5 TLs, SE single = 0.05 and SE dual = 0.05; Fig. 3 b). Northern pike, on the other hand, had the smallest range in TP_{Scaled} estimates at both sites (Peche Island TP range = 1.2 TLs; Grass Island TP range = 0.6 TLs), and the smallest standard error at Grass Island (SE single = 0.04 and SE dual = 0.03; Fig. 3 c). It was, however, comparable to largemouth bass at Peche (SE single = 0.03 and SE dual = 0.04 for northern pike and largemouth bass, respectively; Fig. 3 a & c).

Discussion

Accurate TP estimates are important for quantifying structure and energy-flow within food webs. Mean TP estimated of the three predator fish species from two lower Detroit River sites demonstrated more variability than currently assumed, irrespective of TP calculation method used (TPs ranged from 3.9 to 5.1, while current assumption is a TP of 4; Krause et al., 2003), supporting our first hypothesis (H1). Depending on site, most stable isotope-calculated TPs were significantly higher when using a scaled rather than constant DTDF and showed larger interspecific ranges, consistent with H2. However, comparisons to TP_{SCA} produced mixed results, and estimates of TP using a scaled or constant DTDF were similar for individuals at higher TPs (TP > 4.4) but overestimated

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Table 3
Trophic position (mean ± SD) estimates of largemouth bass (*Micropterus salmoides*), longnose gar (*Lepisosteus osseus*), and northern pike (*Esox lucius*) using different baseline species at Peche Island and Grass Island. ANOVAs were used to determine whether the use of different baseline species showed significant intraspecific differences in trophic position using either a scaled DTDF (TP_{Scaled} ± SD) or a constant DTDF ($TP_{Constant}$ ± SD) and either single- or dual-source models. Scaled DTDFs for each baseline species were calculated from the negative linear relationship between $\Delta^{15}N$ and consumer $\delta^{15}N$ from Hussey et al., (2014). Significant differences in TP using different baselines are denoted with (*), and results from the post-hoc Tukey's Significance Difference test for baseline items are given with superscript lowercase letters.

Peche Island

							Single-Source		Dual-Source	
Species	Baseline	n	$\delta^{13}C$ ± SD	$\delta^{15}N$ ± SD	Lit TP_{SCA}	Calculated Scaled DTDF	TP_{Scaled}	$TP_{Constant}$	TP_{Scaled}	$TP_{Constant}$
Largemouth Bass	Oligochaete[B]	10	−17.2 ± 0.9	7.2 ± 0.2	2.3[1]	3.9	4.4 ± 0.4	4.4 ± 0.2^{*b}	4.4 ± 0.4	4.4 ± 0.2^{*b}
	Bluegill[C]	16	−16.0 ± 1.1	11.1 ± 0.7	3.2[2]	2.9	4.4 ± 0.3	4.2 ± 0.2^{*a}	4.4 ± 0.3	4.2 ± 0.2^{*a}
	Pumpkinseed[D]	10	−15.4 ± 1.0	10.7 ± 0.4	3.3[3,4]	3.0	4.6 ± 0.3	4.4 ± 0.3^{*b}	4.6 ± 0.3	4.4 ± 0.2^{*b}
Longnose Gar	Dreissenids[F]	6	−16.6 ± 1.1	5.5 ± 0.3	2.0[1]	4.2	4.9 ± 0.6	4.9 ± 0.4	5.0 ± 0.7	4.8 ± 0.4
	Bluegill	16	−16.0 ± 1.1	11.1 ± 0.7	3.2	2.9	4.9 ± 0.7	4.8 ± 0.4	5.1 ± 0.7	4.8 ± 0.4
	Pumpkinseed	6	−15.4 ± 1.0	10.7 ± 0.4	3.3	3.0	5.1 ± 0.7	4.9 ± 0.4	5.2 ± 0.7	5.0 ± 0.4
Northern Pike	Oligochaete	10	−17.2 ± 0.9	7.2 ± 0.2	2.3	3.9	4.3 ± 0.3^{*ab}	4.1 ± 0.2^{*a}	4.3 ± 0.3^{*a}	4.3 ± 0.2^{*a}
	Bluegill	16	−16.0 ± 1.1	11.1 ± 0.7	3.2	2.9	4.2 ± 0.3^{*a}	4.3 ± 0.2^{*b}	4.0 ± 0.3^{*b}	3.9 ± 0.2^{*b}
	Pumpkinseed	10	−15.4 ± 1.0	10.7 ± 0.4	3.3	3.0	4.4 ± 0.3^{*b}	4.3 ± 0.2^{*ab}	4.1 ± 0.3^{*ab}	4.0 ± 0.2^{*b}

Grass Island

							Single-Source		Dual-Source	
Species	Baseline	n	$\delta^{13}C$ ± SD	$\delta^{15}N$ ± SD	Lit TP_{SCA}	Calculated Scaled DTDF	TP_{Scaled}	$TP_{Constant}$	TP_{Scaled}	$TP_{Constant}$
Largemouth Bass	Spottail Shiner[H]	10	−15.4 ± 1.3	10.7 ± 0.5	2.7[5]	2.8	4.1 ± 0.3	3.9 ± 0.2^{*a}	4.2 ± 0.3	4.0 ± 0.2^{*a}
	Bluegill	8	−14.5 ± 1.6	12.1 ± 0.5	3.2	2.7	4.2 ± 0.3	3.9 ± 0.2^{*ab}	4.3 ± 0.3	4.1 ± 0.2^{*ab}
	Yellow Perch[I]	5	−15.9 ± 0.5	13.2 ± 0.7	3.7[1]	2.4	4.3 ± 0.3	4.1 ± 0.2^{*b}	4.3 ± 0.3	4.2 ± 0.2^{*b}
Longnose Gar	Spottail Shiner	10	−15.4 ± 1.3	10.7 ± 0.5	2.7	2.8	4.6 ± 0.4	4.2 ± 0.3	4.4 ± 0.4	4.2 ± 0.3^{*a}
	Bluegill	8	−14.5 ± 1.6	12.1 ± 0.5	3.2	2.7	4.4 ± 0.4	4.1 ± 0.2	4.5 ± 0.4	4.3 ± 0.3^{*ab}
	Yellow Perch	5	−15.9 ± 0.5	13.2 ± 0.7	3.7	2.4	4.6 ± 0.4	4.3 ± 0.3	4.6 ± 0.4	4.3 ± 0.3^{*b}
Northern Pike	Spottail Shiner	10	−15.4 ± 1.3	10.7 ± 0.5	2.7	2.8	4.1 ± 0.2	3.9 ± 0.1^{*b}	4.2 ± 0.2	4.0 ± 0.1^{*a}
	Bluegill	8	−14.5 ± 1.6	12.1 ± 0.5	3.2	2.7	4.2 ± 0.2	4.0 ± 0.1^{*b}	4.3 ± 0.2	4.1 ± 0.1^{*b}
	Yellow Perch	5	−15.9 ± 0.5	13.2 ± 0.7	3.7	2.4	4.3 ± 0.2	4.2 ± 0.1^{*a}	4.4 ± 0.2	4.2 ± 0.1^{*b}

[A]*Micropterus salmoides*, [B]*Oligochaeta spp.*, [C]*Lepomis machrochirus*, [D]*Lepomis gibbosus*, [E]*Lepisosteus osseus*, [F]*Dreissena polymorpha*, [G]*Esox lucius*, [H]*Notropis hudsonius*, [I]*Perca flavescens*.
[1] Vander Zanden et al., 1997, [2]Keast, 1985, [3]Froese and Pauly, 2000, [4]Keast and Walsh, 1968, [5]McLeod et al., 2015.

for lower TP individuals (TP generally < 4.4), which was not consistent with H2. The use of a dual or single source model did not influence TP estimates calculated using a scaled DTDF, which does not support H3. The scaled DTDF approach was more robust to different baseline species used to calculate TP then the conventional constant DTDF method, supporting H4. While the relationship between TP calculation methods varied, these results suggest greater variability and range in TP of individuals and species in the Great Lakes, and highlight the need for refining trophic ecology quantification methods. This is particularly relevant given increasing stress and anthropogenic pressure on freshwater ecosystems, which require greater resolution and understanding of Great Lakes food webs and are necessary for supporting or challenging similar ecosystem-based management decisions as seen in marine systems (e.g. mean annual global decrease of 0.1 marine fish TP; Pauly et al., 1998).

Intraspecific TP range estimates (H1)

While evidence for differences between TP_{Scaled} and $TP_{Constant}$ varied across site and species, TP_{Scaled} consistently estimated a greater mean and population range compared to traditional estimates of TP (e.g., assumed TP of 4.0 in Great Lakes piscivores; Krause et al., 2003). The greater range in TP_{Scaled} estimates, as well as the wide range in $\delta^{13}C$ variation for predator species across species and site, may be important for understanding individual specialization and discrete habitat utilization by consumers, where differing prey assemblages across habitats and individual diet variation may result in greater TP ranges (Newsome et al., 2007). This lack of resolution in individual TP estimates using a constant DTDF may confound understanding individual-level feeding strategies and may lead to a false sense of dietary generalism in niche characterization of piscivores at higher trophic levels (Vander Zanden et al., 2000). Although there is evidence that the trophic structure of Great Lakes food webs and top predator foraging ecology is oversimplified (Ives et al., 2018), this issue warrants further study.

Ecological implications of underestimating TP (H2)

The generally higher TP estimate using a scaled DTDF approach for the three species at Grass Island was consistent with results for multiple marine ecosystems that utilized the scaled DTDF approach (Espinoza et al., 2017; Hussey et al., 2014; Linnebjerg et al., 2016). Using the same rationale as Hussey et al. (2014), we found TP estimated from stomach contents at Grass Island were sometimes consistent with TP estimates from the scaled rather than constant DTDF when dietary diversity criteria was met. For example, TP_{scaled} estimates for largemouth bass at Grass Island were comparable to TP_{SCA} and are likely supported by a greater consumption of crayfish (TL = 3.0, Vander Zanden et al., 1997) and smaller piscivores, including yellow perch (*Perca flavescens*), suggesting that largemouth bass are feeding at TL = 4.0 or higher. In contrast, longnose gar TP_{SCA} at Grass Island was more comparable to $TP_{Constant}$ and may be attributed to a greater consumption of *Cyprinidae* spp. that feed at TL ≈ 2.7 (McLeod et al., 2015; McGrath, 2010; 2013) suggesting a specialized feeding strategy possibly due to gape width limitations, i.e., long and narrow snout (Fletcher et al., 2015). The inability to meet minimum stomach content criteria in northern pike for diet comparisons may be due to documented varied prey item consumption in this species throughout freshwater studies (Diana, 1979; Venturelli and Tonn, 2005, 2006), suggesting generalist feeding behaviour (Almany and Webster, 2004). Seasonality and heterogeneous prey distribution have likely influenced spring time diets in the HEC (Lapointe et al., 2006), and thus have driven variable TP_{SCA} estimates. These results highlight that ecological differences between piscivorous species, including habitat usage, are important considerations when choosing the appropriate trophic position calculation

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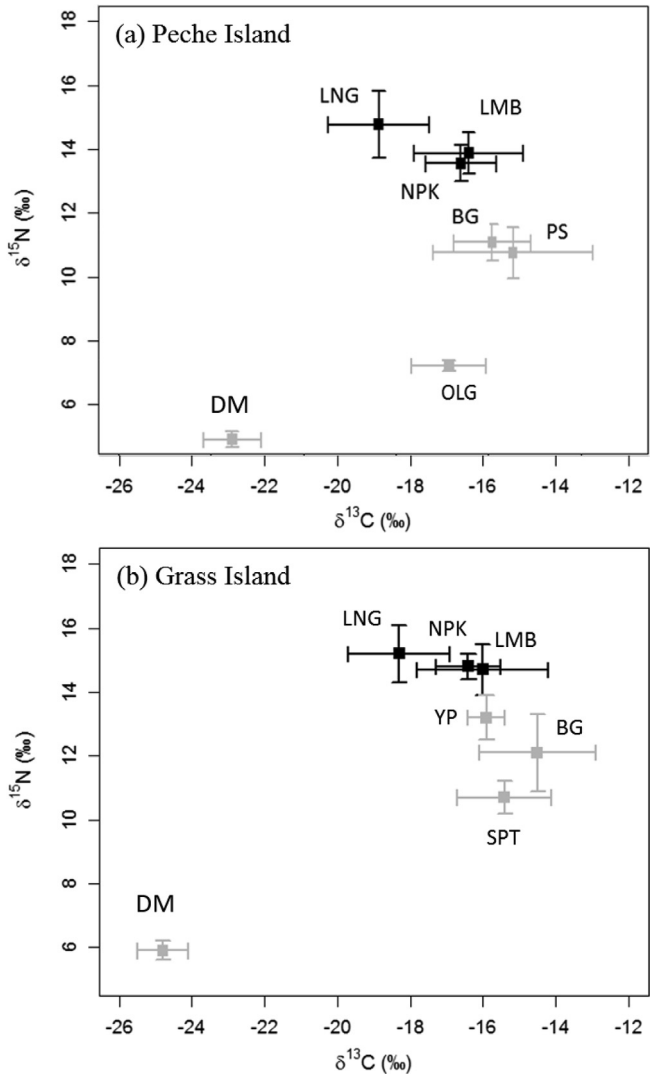

Fig. 2. Mean (±1 SD) $\delta^{13}C$ and $\delta^{15}N$ values for largemouth bass (LMB; *Micropterus salmoides*), longnose gar (LNG; *Lepisosteus osseus*), and northern pike (NPK; *Esox lucius*) at (a) Peche Island and (b) Grass Island in the Lake Huron-Erie Corridor. Bluegill (BG; *Lepomis machrochirus*), pumpkinseed (PS; *Lepomis gibbosus*), Dreissenids (DM; *Dreissena* spp.), oligochaete (OLG) *spp.*, spottail shiner (SPT; *Notropis hudsonius*), and yellow perch (YP; *Perca flavescens*) are representative of baseline species used in trophic position calculations for both single and dual-carbon source models.

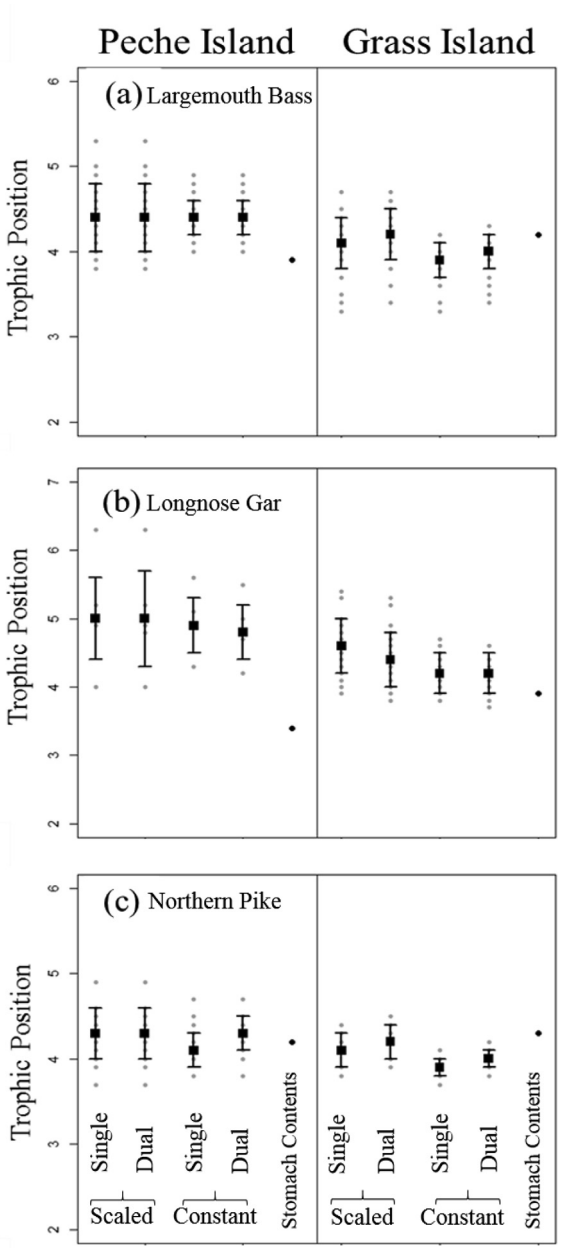

Fig. 3. Trophic position estimates of (a) largemouth bass (*Micropterus salmoides*), (b) longnose gar (*Lepisosteus osseus*), and (c) northern pike (*Esox lucius*) at Peche Island and Grass Island in the Huron-Erie corridor. Grey circles represent individual trophic position estimates, while black squares represent (1) TP_{Scaled} (±1 SD) estimates using a single carbon-source, (2) TP_{Scaled} (±1 SD) estimates using a dual carbon-source, (3) $TP_{Constant}$ (±1 SD) estimates using a single carbon-source, (4) $TP_{Constant}$ (±1 SD) estimates using a dual carbon-source, and (5) black circles represent dietary TP values.

method to use. Further, the results demonstrate that while we are using TP_{SCA} as the best approximation of a true trophic position, for reasons discussed in the introduction, stomach content analyses are not perfect. With recent advances in genetic techniques using DNA analyses to determine stomach contents, TP_{SCA} based on this would provide more accurate assessment of diet with better insight into the relationship between habitat usage and TP.

Contrary to our predictions, there were instances where there were no differences between TP_{scaled} and $TP_{constant}$. The scaled DTDF method reflects differences in dietary discrimination factors as trophic level increases, something that is very evident in marine systems (Caut et al., 2009; Hussey et al., 2014). At sites where there were no significant differences between TP_{scaled} and $TP_{constant}$, the consumers were feeding at lower trophic levels. This is most evident at Peche Island where the individual DTDFs calculated for each baseline at Peche Island were comparable (2.9–4.2‰) to traditional DTDFs of 3.2 or 3.4‰ (Minagawa and Wada, 1984; Post et al., 2007), resulting in mean TP estimates that were not statistically different at Peche Island across methods. This lack of difference in TP_{Scaled} and $TP_{Constant}$ may also be attributed to a comparatively small range in $\delta^{15}N$ in this study system, ultimately masking differences in mean TP estimates otherwise seen in larger freshwater systems that have more differing community assemblages and longer food chain lengths, such as Lake Ontario (Vander Zanden et al., 2000; Mumby et al., 2018).

Influence of dual-source carbon TP estimates (H3)

Previous freshwater TP studies demonstrated significant differences in TP estimates when accounting for single or dual-source

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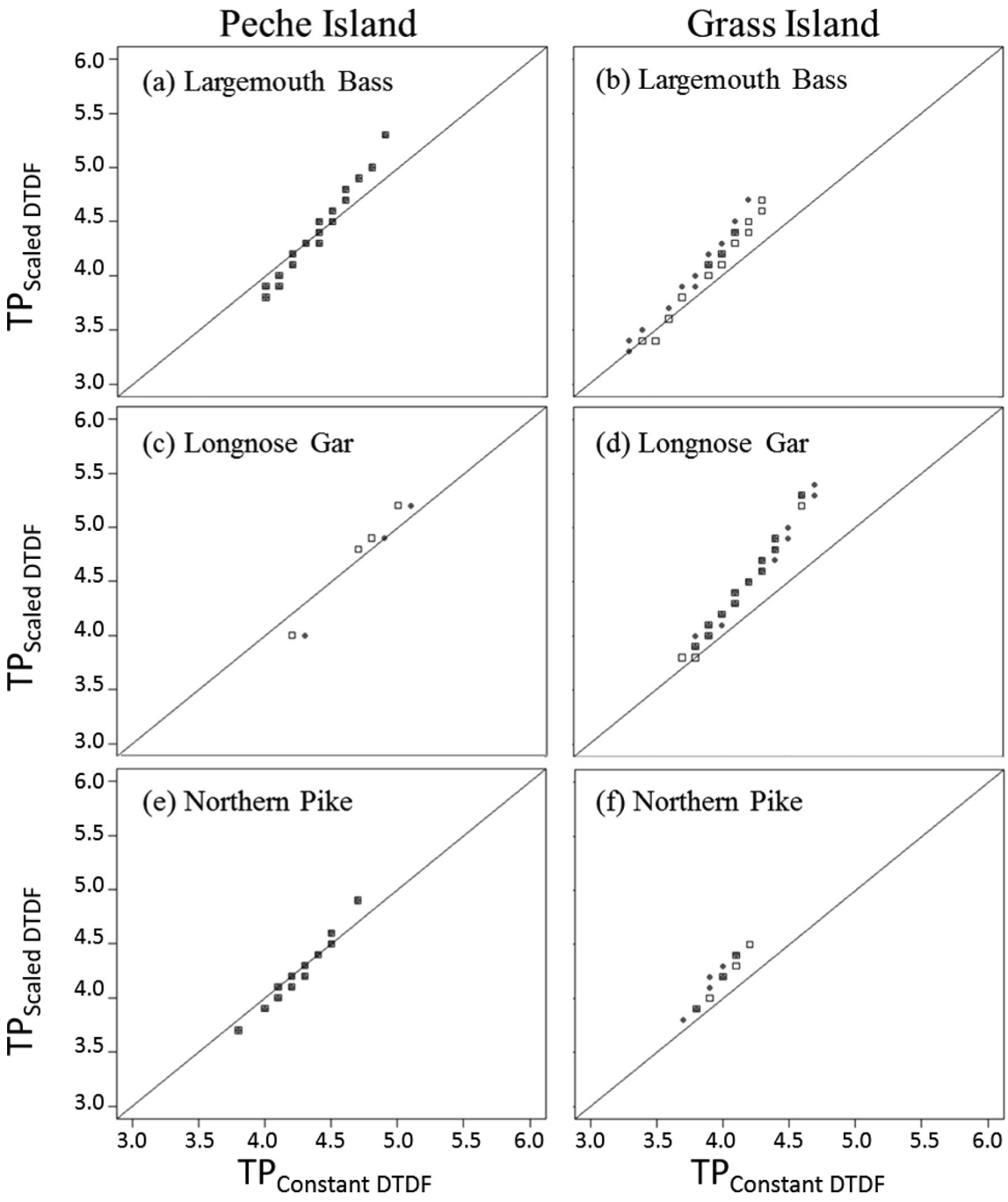

Fig. 4. Trophic position estimates of largemouth bass (*Micropterus* salmoides) (a,b), longnose gar (*Lepisosteus osseus*) (c,d), and northern pike (*Esox lucius*) (e,f) from the Huron-Erie corridor at Peche Island and Grass Island respectively, using a scaled DTDF and a constant DTDF with single and dual carbon source models. The comparison between TP_{Scaled} and $TP_{Constant}$ is represented by grey circles for a single-carbon source model and black-bordered boxes for a dual-carbon source model.

carbon (Nilsson et al., 2012; Post, 2002). Our findings contradict this, however, as no significant differences were found when we compared single and dual-carbon source calculations using a scaled DTDF. Previous studies have found littoral and pelagic $\delta^{15}N$ to influence freshwater TP calculated with stable isotopes (Nilsson et al., 2012; Post, 2002), however this was not present in this study. The lack of dual carbon source influence was likely due to either comparable $\delta^{15}N$ across trophic level for the pelagic baseline (dreissenids) and littoral baselines (e.g. oligochaetes, bluegill), or that greater $\delta^{13}C$ of predators were more representative of littoral feeding, thus pelagic $\delta^{15}N$ had negligible contributions to dual-carbon source TP estimates. This may also be attributed to the morphology of the Detroit River, which is an area of rich littoral primary production (Lapointe et al., 2006) and experiences a large influx of pelagic resources (Baustian et al., 2013). Furthermore, wide ranges in baseline $\delta^{13}C$ could be representative of varying degrees of predator site fidelity and indiscrete habitat utilization throughout the HEC. Indeed, largemouth bass home ranges are comparatively small in other freshwater systems (Ridgeway, 2002), while longnose gar have exhibited low site fidelity in riverine-lake systems (McGrath, 2010), and northern pike have shown high intraspecific variation in site fidelity and home

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range movement (Diana et al., 1977; Ovidio and Philippart, 2003). Such movements would influence $\delta^{13}C$ isotopic compositions and demonstrate the need to include movement and behaviour studies to understand predator foraging ecology. While the lack of proportional carbon source contribution to TP calculations was not relevant in this study system, it may be in large, deep freshwater ecosystems (Post, 2002), such as Lake Ontario and Lake Superior, which have more distinct pelagic and littoral baseline $\delta^{15}N$ (Keough et al., 1996; Matisoff and Ciborowski, 2005; Mumby et al., 2018).

TP sensitivity to chosen baseline species (H4)

TP_{Scaled} estimates did not differ when baseline species of different trophic positions were used but did for $TP_{Constant}$, demonstrating that the scaled DTDF method is more robust when baseline species are not available, and may be a better choice in complex ecosystems with mobile predators, multiple carbon sources, and seasonal fluctuations in prey communities. Indeed, the scaled DTDF proposed by Hussey et al. (2014) has been found to provide better estimates of trophic structure in marine systems, explain comparable predator–prey body mass ratios across different food webs, and yield TP estimates that were more comparable to dietary TP (Jennings and Collingridge, 2015; Kiszka et al., 2015; Reum et al., 2015). This consistency in TP_{Scaled} estimates among baselines chosen is important to consider in field studies using stable isotopes, where it is often difficult to standardize baseline species across ecosystems and geographic distribution of species (Vander Zanden and Rasmussen, 1999). Seasonal variation in $\delta^{13}C$ and $\delta^{15}N$ of both consumers and baselines can also confound absolute TP estimates (Post, 2002; Woodland et al., 2012). In particular, baseline insensitivity to TP_{Scaled} estimates are important to consider in freshwater systems such as the Great Lakes where the introduction of aquatic invasive species such as round goby (*Neogobious melanostomus*) and dreissenid mussels have altered the strength of existing predator–prey trophic linkages (Campbell et al., 2009; Nalepa et al., 1996). The scaled method may also have utility for addressing questions that pertain to historical TP trends using archived samples, where baseline species are often not available.

Limitations to stable isotope derived TP estimates

One of the shortfalls of comparing TP_{SCA} and estimates using muscle stable isotopes is that muscle data provide a longer-term integrated assessment of diet (Newsome et al., 2007), while TP_{SCA} is reflective of an instantaneous "snapshot" of prey consumed (Cortés, 1997). Despite these limitations, our study demonstrates some similarities between stomach content and stable isotope analysis for determining TP, at least for species studied. This supports the use of stable isotopes, as stomach content analyses are time consuming, are subject to viewer bias, and require high sample numbers. Recent improvements in DNA techniques, however, suggest that smaller sample numbers could be used to get increasingly accurate diet information, thus allowing us to integrate across temporal scales improving our TP_{SCA} estimates (Valentini et al., 2009). A drawback of stable isotope analysis is that TP estimates using this method may not be able to detect seasonal changes in prey compositions due to long tissue turnover times, thus resulting in uncertain or generalized stable isotope TP calculations (Newsome et al., 2007). These seasonal differences in stable isotope values are further amplified by seasonality in baseline species where fluctuations in system productivity and high tissue turnover of baseline organisms, in part because they are small and short lived, results in seasonal fluctuation in $\delta^{15}N$ and $\delta^{13}C$ (Leggett et al., 1999). This can be overcome by frequent sampling of baseline over the course of a season, integrating the stable isotope estimates across these sampling time points (Fry, 2006). Changes in TP estimates may also be subject to differential isotopic routing, although this phenomenon is poorly understood (Fry, 2006).

Conclusion

This study provides evidence that the method used for calculating TP from stable isotopes in freshwater piscovore fish can produce variable estimates. The use of a scaled DTDF method (TP_{scaled}) generated TP estimates that were generally higher and with a greater range for each species than the commonly used constant DTDF ($TP_{constant}$), with the scaled DTDF method at times being closer to TP estimates from stomach contents. The scaled DTDF method was not influenced by the choice of baseline species nor when carbon source was included, while baseline selection influenced $TP_{constant}$ estimates. The use of a scaled DTDF method should be considered in studies using stable isotopes to quantify TP or estimate diets, particularly if baseline data is lacking or there are temporal or spatial considerations. As we progress into the Anthropocene, environmental change is going to increase and there is a need for accurate TPs for effective management and conservation decisions. TP estimates need to reflect these changes, both through their sensitivity and their incorporation of uncertainty, to make plausible inferences about ecosystem stressors, including changing species assemblages and overfishing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jglr.2020.03.017.

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