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Use of Stable Isotopes in Benthic Organic Material as a Baseline for Estimating Fish Trophic
Positions in Lakes

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Abstract:

Estimating the trophic position of predators in food webs using stable isotopes requires establishing a 'baseline'; typically served by bivalves or snails. However, the frequent absence of such organisms in lakes leaves researchers in a difficult situation. Here we test the hypothesis that trophic position (TP) of largemouth bass (LB) in lakes can be estimated from $\delta^{15}\text{N}$ of benthic organic material (BOM) in littoral surficial sediment. $\delta^{15}\text{N}_{\text{LB}}$ as a function of $\delta^{15}\text{N}_{\text{BOM}}$ was significant across 51 lakes with an R^2 of 0.84, supporting our hypothesis. In a subset of six lakes, the mean TP_{LB} did not differ significantly when calculated using baselines of $\delta^{15}\text{N}$ in BOM, mussels (UN), gastropods (SN), or from a stable isotope mixing model. In a subset of 26 lakes, mean TP_{LB} calculated from $\delta^{15}\text{N}_{\text{BOM}}$ and $\delta^{15}\text{N}_{\text{UN}}$ baselines were not significantly different, but TP_{LB} calculated using $\delta^{15}\text{N}_{\text{BOM}}$ was not significantly correlated with TP_{LB} calculated using $\delta^{15}\text{N}_{\text{UN}}$. Although $\delta^{15}\text{N}_{\text{BOM}}$ should not be used interchangeably with $\delta^{15}\text{N}_{\text{UN}}$ as a baseline for estimating TP_{LB} , $\delta^{15}\text{N}_{\text{BOM}}$ can be useful as a separate baseline where estimates of fish TP are needed in all study lakes given that mussels are frequently absent and BOM is always available for collection.

Introduction

Researchers have used ratios of stable isotopes of nitrogen ($\delta^{15}\text{N}$) in organisms to quantify their trophic position (TP) (Cabana and Rasmussen 1994, Cabana and Rasmussen 1996; Vander Zanden et al. 1997; Vander Zanden et al. 1999; Vuorio et al. 2007; Doi et al. 2009). This provides a framework to food webs and facilitates the development of models to examine the movement of energy and contaminants and to test theoretical aspects of ecology such as environmental determinants of food chain length (Post et al. 2000; Takimoto and Post 2013). Food chain length can be assessed as the trophic position difference between a baseline and the apex predator. Comparing TP values among lakes requires use of a common organism for a baseline and knowledge of the change in isotopic value per trophic level. The baseline organism must reflect changes in lake conditions, including changes in its $\delta^{15}\text{N}$ ratio resulting from differing sources and quantities of nitrogen entering food webs.

Filter feeding bivalves, such as mussels, are often used to represent baseline values because of their long lifespans and because of their shift in $\delta^{15}\text{N}$ among lakes due to different nitrogen inputs such as wastewater from humans, or agricultural fertilizers (Vander Zanden et al. 1999; McKinney et al. 2002). Although the dietary components of mussels are not fully understood (Vuorio et al. 2007; Yasuno et al. 2014), they are often considered primary consumers and assigned a TP value of two for food web modelling because they generally consume large quantities of phytoplankton (Cabana and Rasmussen 1996; Vander Zanden et al. 1997; Post 2002). In addition to the baseline, development of TP estimates using $\delta^{15}\text{N}$ requires a knowledge of the shifts of $\delta^{15}\text{N}$ in trophic transfer between predator and prey. Comprehensive summary studies reviewing field and laboratory work in aquatic systems show mean estimates of 3.4‰ for $\delta^{15}\text{N}$

between trophic positions (Minagawa and Wada 1984; Vander Zanden and Rasmussen 2001; Post 2002).

While filter-feeding bivalves are useful as baselines in TP studies, they are often absent from lakes, present in low abundance, or occur in patches not necessarily sampled by researchers. Thus, when bivalves are unavailable, different material must be analyzed to establish a baseline. One approach is to analyze $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in particulate organic material (POM) from water samples over an extended time period (Post 2002). However, large seasonal variability of $\delta^{15}\text{N}$ in POM requires that a large number of samples is needed to represent the isotope characteristics of surface waters (Gu 2009). Such a collection regime is impractical when a large number of lakes is to be examined. However, Mitchell et al (1996) and Hodell and Schleske (1998) reported positive correlations between POM and lake sediment, while Lake et al. (2001) demonstrated a significant relationship between $\delta^{15}\text{N}$ in surface grab samples from the deepest location in lakes and muscle tissue of largemouth bass (*Micropterus salmoides*). Thus, measurement of nitrogen stable isotope ratios in benthic organic material (BOM) could provide a reliable surrogate for a baseline in the absence of mussels. However, without knowledge of the influence of temporal and spatial variability in $\delta^{15}\text{N}_{\text{BOM}}$ on the $\delta^{15}\text{N}_{\text{BOM}}$ vs $\delta^{15}\text{N}_{\text{LB}}$ relationship, its utility as a baseline is uncertain.

Here we test the hypothesis that $\delta^{15}\text{N}_{\text{BOM}}$ can serve as a useful baseline for estimating trophic position of largemouth bass (TP_{LB}) using samples from 51 lakes, in Rhode Island, USA. We examine two sub-hypotheses. H_0 S1, that there was no significant relationship between $\delta^{15}\text{N}_{\text{BOM}}$ and $\delta^{15}\text{N}_{\text{LB}}$ in the 51 study lakes. Since a significant relationship was found, we examined the utility of a $\delta^{15}\text{N}_{\text{BOM}}$ baseline by determining the impact of temporal and spatial variability in $\delta^{15}\text{N}_{\text{BOM}}$ on the $\delta^{15}\text{N}_{\text{BOM}}$ vs $\delta^{15}\text{N}_{\text{LB}}$ relationship. We assigned a trophic position value to $\delta^{15}\text{N}_{\text{BOM}}$ and used it in a model to predict Trophic Position of LB (TP_{LB}). We then tested the sub-hypothesis

H₀ S2, that there was no difference between TP_{LB} estimated by $\delta^{15}\text{N}_{\text{BOM}}$ and by those calculated with baselines of mussels $\delta^{15}\text{N}_{\text{UN}}$, snails $\delta^{15}\text{N}_{\text{SN}}$, and one calculated from a stable isotope mixing model.

Methods

Description of Study Lakes

In the present study, we sampled and measured nitrogen stable isotope ratios in BOM ($\delta^{15}\text{N}_{\text{BOM}}$) re-suspended from the surface of sediment cores taken from the littoral zones of 51 lakes, ponds and reservoirs (referred to as lakes herein) in Rhode Island, USA. These lakes were selected from those monitored in state-wide fisheries surveys conducted by the Rhode Island Department of Environmental Management from 1997 to 2013. Across the 51 lakes, surface area ranged from 3.4 to 420 ha, depths from 1.1 to 8.5m (Table A1). Fifteen of the lakes were naturally formed and 36 were constructed for drinking water and power generation during the last two centuries (Libby 2013). The lakes spanned a wide range of anthropogenic development of surrounding watersheds, from almost pristine land in the western part of the state to highly developed urban areas in the city of Providence, Rhode Island. The construction of dams at some lakes cut off access for some anadromous fish species, e.g., American shad and Atlantic salmon. Further, disruption to fish species distribution in many lakes occurred from stocking of nine predatory and forage fish species including largemouth bass (LB) during the last 130 years (Libby 2013).

Collection of Sediment Benthic Organic Material

Samples of BOM were collected in the littoral zone from all lakes using a hand-held, 6.5 cm diameter, piston coring sampler. The depth of the core sample was dependent on sediment composition. As the sampler was withdrawn and maintained vertically, a core cap was pushed onto the bottom of core tube. The surface layer (~ the top 1.5 to 2.0 cm) of the intact sediment core was re-suspended by stirring, and the suspended material was removed by a 30ml pipette. The suspended material was transferred to clean plastic bottles, capped, placed on ice and transported to the laboratory where they were refrigerated until processed within 72 h. Processing included stirring each sample, pouring it through a 0.5 mm Nytex screen to remove large material such as leaves, twigs, and gravel and collecting the material in 55 ml centrifuge tubes. Each tube was shaken and then centrifuged at 1000g for five minutes to separate sand from the organic material. The supernatant was poured off and a spatula was used to take samples from the top ~1.5cm of the pellet accumulated at the bottom of the centrifuge tube. Samples were dried in an oven at 40°C for at least 3 days, ground with mortar and pestle to a fine powder, weighed and analyzed by isotope ratio mass spectrometry (IRMS).

Temporal and Spatial Variability in BOM

The magnitude of temporal variability in $\delta^{15}\text{N}_{\text{BOM}}$ was evaluated using comparisons of BOM samples collected in September of 2012, and May and September of 2013 from the same locations in 35 lakes. Spatial variability was examined by sampling triplicate samples of BOM at locations around the perimeters of nine lakes collected in October and November of 2013. Two locations were sampled in three lakes, three locations were sampled in an additional five lakes, and four locations were sampled in the largest lake.

147 Biological Samples

148 Largemouth bass (LB) was selected as the representative fish for this study because it was
149 the dominant apex predator and was relatively abundant at most lakes. Samples of LB were
150 obtained from fisheries surveys conducted by the Rhode Island Department of Environmental
151 Management, Division of Fish and Wildlife. These surveys collected fish from the perimeter of
152 lakes using an electro-fishing workboat (Smith-Root H-Model). At small lakes (< 20 ha), the entire
153 perimeter was electro-fished, while the shoreline of large lakes was divided into 12 equal sections
154 and a portion of each section was electro-fished for 400 seconds. Based upon experience of the
155 collection crew, a size-representative subset of LB was taken from the live well of the boat and
156 retained for analysis. At most survey lakes, four to six fish were retained for analysis. However, at
157 some lakes only one or two LB were collected. At two additional lakes, samples of LB were
158 obtained from local fishermen. Fish were placed in a cooler on ice and transported to the
159 laboratory. The scales and skin were removed above the dorsal muscle and a sample of muscle was
160 taken. Muscle samples for stable isotope analysis were dried in an oven at 40°C and then ground
161 and weighed for analysis by IRMS.

162 Due to their use as baseline organisms in trophic position models, samples of unionid
163 mussels (*Elliptio complanata*) (UN) and snails (in the Planorbidae and Viviparidae families) (SN) also
164 were collected when present. Samples of unionid mussels were collected in the littoral zone by
165 hand or with a clam rake from only 26 of the 51 lakes. SN were hand collected and found at only 9
166 lakes. Tissue samples consisted of a section of the foot of UN and a section of the foot of SN
167 (taken behind operculum). These samples were dried, ground and weighed for IRMS analysis.

Due to the small size of the study lakes and limitations of organism availability, we considered our $\delta^{15}\text{N}$ value for LB, UN and SN as representative of each lake. These organism $\delta^{15}\text{N}$ values were single values, mean values or organism size normalized values.

Size Normalization for $\delta^{15}\text{N}_{\text{LB}}$ and $\delta^{15}\text{N}_{\text{UN}}$ in Biological Samples

We examined length to $\delta^{15}\text{N}$ relationships at each lake to determine whether size normalization was required. Mean lake $\delta^{15}\text{N}_{\text{LB}}$ and $\delta^{15}\text{N}_{\text{UN}}$ values were used where no significant length to $\delta^{15}\text{N}$ relationships were found. Significant $\delta^{15}\text{N}$ vs organism size relationships were found for LB in three of the 51 lakes and for UN at six of the 26 lakes. For these lakes, we calculated the overall mean length for LB (340mm) and for UN (74mm) from samples collected in the study. The appropriate mean length was substituted as the independent variable to calculate a lake $\delta^{15}\text{N}$ value. In this study, $\delta^{15}\text{N}$ value refers to either the mean $\delta^{15}\text{N}$ or the size corrected $\delta^{15}\text{N}$ value and was used for comparing lake values. At two lakes, only one LB was available and its single $\delta^{15}\text{N}$ ratio was used. For lakes from which multiple collections of LB and UN were available, the most recent collection was used (Table A2). The mean size, size range and $\delta^{15}\text{N}$ of LB and UN for these samples are listed (Tables S1 and S2).

At the nine lakes where SN were available, examination of SN weight to $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios showed that no correction for organism size was needed (see: Supplemental Material, Snails).

Samples of LB and UN used in the regression comparisons in this study were collected over a range of years and we were concerned that temporal changes of $\delta^{15}\text{N}$ in these organisms may have influenced the calculation of TP_{LB} using BOM and UN as baselines. Therefore, we

examined temporal variability of $\delta^{15}\text{N}$ in these organisms in lakes where two samples of LB (23 lakes) and UN (18 lakes) were available. The length of time between collections varied among lakes so we designated the collections as “early” and “recent.” Collection dates, $\delta^{15}\text{N}$ values and number of years between collections of LB and UN samples used in the organism temporal study are listed in Tables A4 and A5.

Lipids are depleted in $\delta^{13}\text{C}$ and for tissues with high lipid values or $\text{C/N} > 3.5$ a chemical extraction of lipids or normalization of $\delta^{13}\text{C}$ ratios based upon the C/N value may be needed (Post et al. 2007). The values of C/N ratios in LB samples were < 3.5 ; therefore, no adjustment of LB samples was done. In the mixing model, which was used at 6 lakes (below), we corrected the $\delta^{13}\text{C}$ ratios in UN and SN samples that had C/N ratios > 3.5 using the following equation (Post et al. 2007):

$$\text{(Eq. \# 1) } \delta^{13}\text{C normalized} = \delta^{13}\text{C untreated} - 3.32 + 0.99 (\text{C/N})$$

Mean lipid corrections for UN and SN are given in Table S3.

Stable Isotope Analyses

The nitrogen and carbon isotopic composition of samples were determined by continuous flow isotope ratio mass spectrometry at the National Health and Environmental Effects Research Laboratory, Narragansett, RI, USA. Samples were weighed ($\pm 1 \mu\text{g}$), encapsulated in combustion cups, and carbon/nitrogen isotopic compositions determined. Largemouth bass and mussel samples from 1997 through 2002 were analyzed using a Carlo-Erba NA 1500 series II elemental analyzer interfaced to a Micromass Optima mass spectrometer. All samples after 2002 were analyzed on a Vario Micro Elemental Analyzer interfaced to an Elementar Isoprime 100 Mass Spectrometer (Elementar Americas, Mt. Laurel, NJ). Replicate analyses of isotopic standard reference materials

USGS 40 ($\delta^{13}\text{C} = -26.39\text{‰}$; $\delta^{15}\text{N} = -4.52\text{‰}$) and USGS 41 ($\delta^{13}\text{C} = 37.63\text{‰}$; $\delta^{15}\text{N} = 47.57\text{‰}$) were used to normalize isotopic values of working standards (blue mussel homogenate) to the Air ($\delta^{15}\text{N}$) and Vienna Pee Dee Belemnite ($\delta^{13}\text{C}$) scales (Paul et al. 2007). Isotope values are expressed in δ notation following the formula $\delta X (\text{‰}) = [(R_{\text{sample}} / R_{\text{standard}}) - 1] \cdot 10^3$, where X is ^{13}C or ^{15}N and R is $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ isotopic ratio, respectively. Working standards were analyzed after every 24 samples to monitor instrument performance and check data normalization. The precision of the laboratory standards was $\pm 0.3\text{‰}$ for carbon and nitrogen. To demonstrate that no contamination of samples resulted from sample preparation, wheat flour was taken through all steps of the procedure used for preparing BOM samples. Results of pre- and post- flour samples showed a mean difference in $\delta^{15}\text{N}$ of $< 0.05\text{‰}$, and indicated no appreciable contamination had occurred.

Statistical Tests Used in Study

Least squares regressions of organism length vs. $\delta^{15}\text{N}$ ratios of UN and LB at each lake were used to determine whether size normalization of $\delta^{15}\text{N}$ ratios was required. Size and stable isotope ratios for UN, LB and SN are in supplemental material (Table S1 and S2).

For our first sub hypothesis, $H_0 S1$, we used least squares regression to test the significance of the relationship of $\delta^{15}\text{N}_{\text{BOM}}$ vs $\delta^{15}\text{N}_{\text{LB}}$ ($n = 51$ lakes).

We tested temporal variability in $\delta^{15}\text{N}_{\text{BOM}}$ across 35 lakes. We used a randomized block design to test for differences in $\delta^{15}\text{N}_{\text{BOM}}$ among the three sampling dates with the collection site as the blocking factor. We tested spatial variability (intra-lake) of $\delta^{15}\text{N}_{\text{BOM}}$ in 9 of these lakes using analysis of variance (ANOVA). We then determined the influence of a combination of temporal and spatial variability on the $\delta^{15}\text{N}_{\text{BOM}}$ vs $\delta^{15}\text{N}_{\text{LB}}$ relationships using the above subsets of 35 and 9 lakes. This test used a bootstrap resampling procedure. For each lake a single $\delta^{15}\text{N}_{\text{BOM}}$ from the

combined spatial and temporal sub-studies was randomly selected and paired with the $\delta^{15}\text{N}_{\text{LB}}$ largemouth bass $\delta^{15}\text{N}$ result from that lake. The linear regression model parameters and R-squared value were then calculated using the generated data set of 35 BOM and largemouth bass $\delta^{15}\text{N}$ pairs. This process was repeated 1,000 times with each generated data set yielding a distribution of slopes, intercepts and R-squared values. The means of the 1,000 slopes, intercepts and R-squared values were used as estimates of the overall association. Additionally, the 2.5th and 97.5th percentiles of the 1,000 generated slopes, intercepts and R-squared values were determined; these correspond to 95% confidence bounds for these three measures of association. The widths of these confidence intervals were used to determine how the localized spatial and temporal variability affected the estimated associations between BOM and largemouth bass measurements.

For our second sub hypothesis, H_0 S2, we used t-tests and ANOVA to test whether mean TP_{LB} values were significantly different when calculated with different baselines. T-tests used in the present study were preceded by an F-test for equality of variance. The appropriate t-test was then used for analysis of data. At 26 lakes where UN were present, we compared lake means of TP_{LB} calculated using $\delta^{15}\text{N}_{\text{UN}}$ in eq. # 2 and $\delta^{15}\text{N}_{\text{BOM}}$ in eq. #4 with a t-test. We also tested the relationship of the TP_{LB} calculated with $\delta^{15}\text{N}_{\text{UN}}$ and $\delta^{15}\text{N}_{\text{BOM}}$ baselines among lakes with least squares regression to determine whether TP_{LB} from the two baselines moved together.

At six lakes, we used ANOVA to compare TP_{LB} calculated using $\delta^{15}\text{N}_{\text{UN}}$ in eq. # 2, $\delta^{15}\text{N}_{\text{SN}}$ in eq. # 3, $\delta^{15}\text{N}_{\text{BOM}}$ eq. # 4 and a baseline derived from a mixing model. The mixing model (eq. # 5 and # 6) used $\delta^{13}\text{C}$ from gastropods (SN) and mussels (UN) to estimate the fractional inputs of carbon from the littoral and pelagic zone (respectively) and adjusted the $\delta^{15}\text{N}$ values of SN and UN prior to their use in estimating TP_{LB} .

259 (Eq. # 2) $TP_{LB} = 2 + ((\delta^{15}N_{LB} - \delta^{15}N_{UN}) / 3.4)$

260 (Eq. # 3) $TP_{LB} = 2 + ((\delta^{15}N_{LB} - \delta^{15}N_{SN}) / 3.4)$

261 (Eq. # 4) $TP_{LB} = 1 + ((\delta^{15}N_{LB} - \delta^{15}N_{BOM}) / 3.4)$

262 (Eq. # 5) $TP_{LB} = 2 + ((\delta^{15}N_{LB} - (\delta^{15}N_{UN} \times \alpha + \delta^{15}N_{SN} \times (1-\alpha))) / 3.4)$

263 (Eq. # 6) $\alpha = (\delta^{13}C_{LB} - \delta^{13}C_{SN}) / (\delta^{13}C_{UN} - \delta^{13}C_{SN})$

264

265 The assigned constant of 2 in Eq. # 2, 3 and 5 is the assumed TP represented by a primary
 266 consumer. The constant 1 (Eq. # 4) is the TP assigned to BOM (see Results). The constant 3.4 is
 267 the part per thousand (‰) difference in $\delta^{15}N$ for one trophic step.

268 We tested the significance of temporal variability in relationships of $\delta^{15}N$ recent, as
 269 dependent variable, and $\delta^{15}N$ early, as independent variable, for UN and LB with least squares
 270 regressions. This was done to determine whether the 95% confidence interval surrounding the
 271 slopes of regressions overlapped with 1, where lack of this overlap would indicate significant
 272 temporal variability. We used a paired t test to examine the temporal variability in $\delta^{15}N_{LB}$.

273 Correlations, least squares regressions, ANOVA, ANCOVA, randomized block design
 274 test, paired t test and Bootstrap estimation procedures were done using SAS software version 9.4
 275 (SAS Institute Cary, NC, USA). Student's t-tests, F-tests for equality of variance, and regression
 276 analyses were done using Microsoft Excel. The level of significance for all tests was $\alpha \leq 0.05$.

277

278 Results

279 A Summary of the Subsets Comparing $\delta^{15}N$ in Organisms with BOM

280 Across the 51 lakes, the mean $\delta^{15}N_{LB} = 13.7$, range = 12.2 ‰, the mean of $\delta^{15}N_{BOM} = 4.2$
 281 ‰, range = 11.4 ‰. In the subset of 26 lakes that contained UN, the mean $\delta^{15}N_{LB} = 14.5$, range =

10.5 ‰, the mean of $\delta^{15}\text{N}_{\text{BOM}} = 5.0$ ‰, range = 9.1 ‰ and the mean of $\delta^{15}\text{N}_{\text{UN}} = 8.0$ ‰, range = 12.1 ‰. The ratios of $\delta^{15}\text{N}$ in 6 lakes, where the mixing model could be applied, were: mean $\delta^{15}\text{N}_{\text{LB}} = 15.3$ ‰, range = 9.6 ‰, mean $\delta^{15}\text{N}_{\text{UN}} = 9.0$ ‰, range = 11.2 ‰, mean $\delta^{15}\text{N}_{\text{SN}} = 9.3$ ‰, range = 10.7 ‰. The ratios of $\delta^{13}\text{C}$ in 6 lakes, where the mixing model could be applied, were: mean $\delta^{13}\text{C}_{\text{LB}} = -26.6$ ‰, range = 6.9 ‰, mean $\delta^{13}\text{C}_{\text{UN}} = -29.8$ ‰, range = 3.7 ‰, mean $\delta^{13}\text{C}_{\text{SN}} = -24.7$ ‰, range = 9.0 ‰ (Table 1).

288

Sub hypothesis H₀ S1. Significance of $\delta^{15}\text{N}_{\text{BOM}}$ to $\delta^{15}\text{N}_{\text{LB}}$ Regression

We found a regression of stable isotopes in $\delta^{15}\text{N}_{\text{BOM}}$ vs size normalized $\delta^{15}\text{N}_{\text{LB}}$ was significant $F_{1,50} = 260$, $p < 0.001$, $n = 51$, $R^2 = 0.84$ (Figure 1).

292

Assessment of Temporal and Spatial Variability in BOM

Regression comparisons of $\delta^{15}\text{N}_{\text{BOM}}$ in temporal samples taken at the same locations in 35 lakes in 9/2012, 5/2013 and 9/2013 showed a mean R^2 of 0.93 for the three comparisons 9/2012 to 5/2013, 9/2012 to 9/2013, and 5/2013 to 9/2013. A randomized block design was used to test for differences in $\delta^{15}\text{N}_{\text{BOM}}$ among the three sampling dates. The overall time factor was ($F_{2,34} = 2.4$, $p = 0.099$, $n = 105$), which indicated no significant differences in $\delta^{15}\text{N}_{\text{BOM}}$ among these sampling dates. We calculated TP_{LB} using $\delta^{15}\text{N}_{\text{BOM}}$ for the three sample dates and the $\delta^{15}\text{N}_{\text{LB}}$ with Eq. #4. Across the 35 lakes, we found a mean range of $\text{TP}_{\text{LB}} = 0.19$ (range = 0.66).

Results of ANOVA showed significant spatial variability for $\delta^{15}\text{N}_{\text{BOM}}$ among locations within 6 of the 9 lakes (Table A3). Across the 9 lakes the maximum within lake range of difference of individual samples of $\delta^{15}\text{N}_{\text{BOM}}$ was 2.0‰. We calculated the maximum error introduced to TP_{LB} estimates within each lake using the lowest and highest $\delta^{15}\text{N}_{\text{BOM}}$ of individual BOM samples and

eq. #4. Across the 9 lakes, these results showed a mean range of $TP_{LB} = 0.34$ (range of ranges = 0.45).

Bootstrap resampling showed little effect of temporal and spatial variability on the BOM to LB relationships for $\delta^{15}N$, as evidenced by small changes in the slopes, intercepts and R^2 and their upper and lower confidence limits (Table 2). We used the mean slopes and intercepts and their confidence intervals from the bootstrap (Table 2) to calculate estimates of $\delta^{15}N_{LB}$. For this calculation we used 4.17 ‰, the mean $\delta^{15}N_{BOM}$ of a combination of the temporal and spatial data sets ($n = 180$), as the independent variable. We then calculated the mean TP_{LB} and its confidence intervals using Eq. # 4. The results from the temporal data showed a mean $TP_{LB} = 4.06$ and a confidence interval of ± 0.12 . For the combination of temporal and spatial data mean $TP_{LB} = 4.05$ and a confidence interval of ± 0.14 . Therefore, sampling location and date did not have much influence on these relationships. Overall, these results supported the use of $\delta^{15}N_{BOM}$ for calculating TP_{LB} in lakes.

Variability in Organisms

Temporal variability was assessed by comparing means and/or size normalized values of $\delta^{15}N_{LB}$ and $\delta^{15}N_{UN}$ with least square regressions. The mean, minimum and maximum range of years between early and recent samples for $\delta^{15}N_{LB}$ were: 11, 5 and 16, for $\delta^{15}N_{UN}$ the corresponding results were: 13, 9 and 17 (Tables A4 and A5). Least squares regressions comparing $\delta^{15}N$ ratios recent samples as a function of early samples for LB and UN from lakes showed highly significant relationships for $\delta^{15}N_{LB}$ ($F_{1, 22} = 439$, $p < 0.0001$, $n = 23$, $R^2 = 0.95$) and for $\delta^{15}N_{UN}$ ($F_{1, 17} = 128$, $p < 0.001$, $n = 18$, $R^2 = 0.89$) (Figures 2 and 3). For $\delta^{15}N_{UN}$, the 95% confidence interval surrounding the slope = 1.04 overlapped with 1, which indicated temporal independence for

328 $\delta^{15}\text{N}_{\text{UN}}$. However, for $\delta^{15}\text{N}_{\text{LB}}$ the 95% confidence interval surrounding the slope = 0.84 did not
329 overlap with 1, which indicated temporal changes in $\delta^{15}\text{N}_{\text{LB}}$. However, the magnitude of temporal
330 variability was small mean $\delta^{15}\text{N}_{\text{LB}}$ recent - $\delta^{15}\text{N}_{\text{LB}}$ early = -0.27‰ and was not significant, paired t
331 test (t stat = -1.78, p = 0.089, n = 23).

332 Snails were found at nine lakes. The lake mean for $\delta^{15}\text{N}_{\text{SN}}$ was 8.8‰, range = 11.4 ‰. The
333 lipid corrected $\delta^{13}\text{C}_{\text{SN}}$ mean was -26.5‰, range = 13.9 ‰. Intra-lake variability in $\delta^{15}\text{N}_{\text{SN}}$ and
334 $\delta^{13}\text{C}_{\text{SN}}$ were large at some lakes. In one lake, individual snails collected within 2 m² showed ranges
335 of 5.3‰ in $\delta^{15}\text{N}$ and 5.2‰ in $\delta^{13}\text{C}$. In addition, $\delta^{13}\text{C}_{\text{SN}}$ was below $\delta^{13}\text{C}_{\text{LB}}$ at three lakes which
336 prohibited calculation of trophic position values using the mixing model (Eq. #5 and 6).

337 A comparison of relationships for $\delta^{15}\text{N}_{\text{LB}}$ vs $\delta^{15}\text{N}_{\text{UN}}$, $\delta^{15}\text{N}_{\text{LB}}$ vs $\delta^{15}\text{N}_{\text{BOM}}$ and $\delta^{15}\text{N}_{\text{UN}}$ vs
338 $\delta^{15}\text{N}_{\text{BOM}}$ showed R² of 0.91, 0.78 and 0.82, respectively (Figure 4).

339

340 Assignment of a Trophic Position Value to BOM

341 To evaluate $\delta^{15}\text{N}_{\text{BOM}}$ as a baseline, we needed to assign it a TP value. Therefore, for
342 comparisons of TP results with those of models based upon other baselines, we assigned a TP of
343 1.0 (equivalent to 3.4‰) to BOM. We compared our assigned TP value of 1 with another
344 calculated from the difference between $\delta^{15}\text{N}_{\text{BOM}}$ and $\delta^{15}\text{N}_{\text{UN}}$ across 26 lakes. The mean difference
345 between these values across these 26 lakes was 3.0‰. Subtraction of this value from 6.8‰, the
346 $\delta^{15}\text{N}$ ratio for the UN (at trophic position 2), resulted in 3.8‰ or a surrogate trophic position of
347 1.12 for BOM. Thus, assignment of a TP of 1 for BOM appeared to be reasonable.

348

349 Sub hypothesis, Ho S2, Comparisons of TP_{LB}

In the UN data set ($n = 26$) mean TP_{LB} calculated with the $\delta^{15}N_{UN}$ baseline (mean $TP_{LB} = 3.91$ (eq. # 2)) or the $\delta^{15}N_{BOM}$ baseline (mean $TP_{LB} = 3.77$ (eq. # 4)) were not significantly different (t stat = -1.46, $p = 0.15$, $n = 26$). Calculations of deviations of TP_{LB} made with the $\delta^{15}N_{BOM}$ baseline relative to those made with the $\delta^{15}N_{UN}$ baseline showed mean deviations of 8% and a range of 0 to 18% (Table 3). A least squares regression of TP_{LB} calculated using $\delta^{15}N_{UN}$ and $\delta^{15}N_{BOM}$ as baselines showed no significant relationship across the 26 lakes ($F_{1,25} = 1.78$, $p = 0.19$, $n = 26$, $R^2 = 0.07$).

We also calculated the TP_{LB} using $\delta^{15}N_{UN}$, $\delta^{15}N_{SN}$, $\delta^{15}N_{BOM}$ in equations # 2, 3, and 4, and a mixing model, which uses the $\delta^{13}C$ and $\delta^{15}N$ values in equations # 5 and 6 (Post 2002). At three of the 9 SN lakes, $\delta^{13}C_{SN}$ ratios were below $\delta^{13}C_{LB}$ and the mixing model could not be used to generate a baseline for calculation of TP_{LB} values. At six remaining lakes, TP_{LB} estimates were made with the mixing model and these estimates were compared with those made with different baselines (Eq. #2,3,4). These comparisons showed mean TP_{LB} estimates from the models were not statistically different (ANOVA, $F_{3,23} = 0.27$, $p = 0.85$, $n = 24$). Comparisons of the model estimates of TP_{LB} relative to those made with the mixing model showed mean deviations and ranges of 5% (2% to 9%) for $\delta^{15}N_{UN}$ based model (eq. #2), 3% (1 to 8%) for the $\delta^{15}N_{SN}$ based model (eq. #3) and 5% (0 to 13%) for the $\delta^{15}N_{BOM}$ based model (eq. #4) (Table 4). However, the largest difference across the four mean TP_{LB} estimates was 0.11 TP (Table 4).

Discussion

The relationship between $\delta^{15}N_{BOM}$ and $\delta^{15}N_{LB}$ was strong and supported the use of a $\delta^{15}N_{BOM}$ baseline. To allow prediction of TP of aquatic biota, we assigned a value of one as a TP position for BOM. This assignment was done with the knowledge that BOM was not an organism, for which a trophic position could be easily defined, and that BOM was formed from a wide

variety of processes in littoral and pelagic zones. The littoral zone of lakes receives inputs of carbon and nitrogen from the water column above it, terrestrial sources upstream, surrounding vegetation, human wastewater and runoff from watersheds. Once deposited in sediments, $\delta^{15}\text{N}$ ratios are changed by biotic production and a variety of diagenetic processes (Lehmann et al. 2002; Harvey et al. 1995). Others have suggested that determination of TP for organisms, that incorporate inputs from the complex of microbial processing and consequent isotope fractionations, will be problematic (Middelburg 2014). Though our use of BOM as a baseline will incorporate microbial inputs, stable isotope ratios in periphyton (biofilms) have been used to represent littoral production in lakes (Post 2002) and aquatic production in streams (Rasmussen 2010; Jardine et al. 2012; Smucker et al. 2018). We view our use of BOM as a baseline in lakes as an extension of those approaches. Furthermore, the use of $\delta^{15}\text{N}_{\text{BOM}}$ baseline was well supported because its TP_{LB} estimates agreed with those calculated with biota.

The use of a $\delta^{15}\text{N}_{\text{BOM}}$ baseline offers considerable advantages over other baselines for among lake comparisons of TP because its relatively low temporal and spatial variability result in little impact on TP_{LB} estimates. In contrast, large seasonal and interspecific variability in $\delta^{15}\text{N}$ ratios found for zooplankton (Matthews and Mazumder 2003) and chironomid larvae (Grey et al. 2004) may limit their utility as baselines. In addition, our data showed estimates for TP_{LB} calculated with either $\delta^{15}\text{N}_{\text{BOM}}$ or $\delta^{15}\text{N}_{\text{UN}}$ as baselines were statistically similar (Table 3). Also, statistically similar results for TP_{LB} were found in the six lakes where $\delta^{15}\text{N}_{\text{BOM}}$, $\delta^{15}\text{N}_{\text{UN}}$, $\delta^{15}\text{N}_{\text{SN}}$ and the mixing model were used as baselines (Table 4).

While our TP_{LB} estimates were not significantly different using the selected models, we found the application of models limited by organism abundance and distribution and variability of stable isotopes in baseline organisms. The mixing model approach to TP estimation (Post 2002)

had limited utility in the present study due to the paucity of UN and SN in our lakes. SN were only found at 9 lakes and the variability in $\delta^{15}\text{N}_{\text{SN}}$ and $\delta^{13}\text{C}_{\text{SN}}$, which has been reported elsewhere (Doi et al. 2010), limited application of the mixing model at our lakes. UN were found in only 26 of 51 lakes, and were often scarce and/or had patchy distributions when they were present.

While our data support a conclusion of using $\delta^{15}\text{N}_{\text{BOM}}$ as a baseline, it is well known that lake trophic status influences $\delta^{15}\text{N}$ ratios in sediments and biota. However, our data set does not include lakes at the hypereutrophic end of the spectrum; so, we are unable to extend the generality of using $\delta^{15}\text{N}_{\text{BOM}}$ across the entire lake trophic spectrum.

Other studies have shown that the increase in $\delta^{15}\text{N}$ in particulate and sediment organic matter in lakes resulted from the primary production of non-N-fixing algae in lakes with adequate supplies of N, but in highly eutrophic, nitrogen-limited lakes, fixation of relatively light atmospheric nitrogen by cyanobacteria becomes dominant (Howarth et al. 1988; Gu et al. 1996; Scott et al. 2008). This mix of nitrogen sources to particulate organic matter (POM) and to sediments has been identified as a potentially important factor in the use of $\delta^{15}\text{N}_{\text{POM}}$ to predict aqueous nutrient concentrations in lakes. Also, these different sources have been identified as potential problems for the use of $\delta^{15}\text{N}_{\text{POM}}$ and $\delta^{15}\text{N}_{\text{BOM}}$ as baselines for TP estimations (Gu 2009). However, another study reported that $\delta^{15}\text{N}_{\text{POM}}$ reflects the ecosystem mixture of N from different sources, and N entering the food web via N fixation is used throughout the food web, which provides support to the use of a $\delta^{15}\text{N}_{\text{BOM}}$ baseline for TP estimation (Jankowski et al. 2012).

Lake size is another possible limitation to the use of $\delta^{15}\text{N}_{\text{BOM}}$ baselines. At the small lakes (mean = 55 ha) in the present study, the range of spatial variability in $\delta^{15}\text{N}_{\text{BOM}}$ from littoral zones in combination with temporal variability of $\delta^{15}\text{N}_{\text{BOM}}$ did not have a large impact on $\delta^{15}\text{N}_{\text{BOM}}$ to $\delta^{15}\text{N}_{\text{LB}}$ regressions. In large lakes ($> 2.5 \times 10^6$ ha), however, variations in stable isotope values in

419 sediment resulting from differences in terrestrial inputs and land use on the shore have been found
420 (Guzzo et al. 2011; Machiwa 2010). Also, differences in energetic and isotope fractionation
421 processes in littoral and profundal based food webs have been found in large lakes (Turschak
422 2013, Vander Zanden and Rasmussen 1999). These findings suggest that for some applications, a
423 single sample of $\delta^{15}\text{N}_{\text{BOM}}$ from the littoral zone likely would be inadequate to represent a baseline
424 value. Further work should assess sampling procedures needed for developing $\delta^{15}\text{N}_{\text{BOM}}$ baselines
425 in a larger size range of lakes.

426 Due to the ease of sampling and low cost of analysis, $\delta^{15}\text{N}_{\text{BOM}}$ is useful for determining a
427 TP baseline. Samples of BOM are available at all lakes while other baselines require the
428 availability of organisms or combinations of organisms, which may introduce seasonal or
429 intraspecific variability in baselines and TP estimates. Overall, our results support the use of
430 $\delta^{15}\text{N}_{\text{BOM}}$ as a baseline for estimating trophic positions of fish in small, shallow lakes, which are
431 typical of many lakes in southern New England. In our lakes, a constancy of exposure is indicated
432 by the lack of temporal variability in $\delta^{15}\text{N}_{\text{UN}}$. However significant temporal changes in $\delta^{15}\text{N}_{\text{LB}}$
433 were indicated by regression comparisons, but were small and not significant when analyzed by a
434 paired t test.

435 We rejected our first sub hypothesis, $H_0 \text{ S1}$, because we found a significant relationship of
436 $\delta^{15}\text{N}_{\text{BOM}}$ vs. $\delta^{15}\text{N}_{\text{LB}}$ across 51 lakes, which showed 84% of $\delta^{15}\text{N}$ variability in largemouth bass was
437 explained by the respective $\delta^{15}\text{N}_{\text{BOM}}$ ratios (Figure 1). A combination of temporal and spatial
438 variability in $\delta^{15}\text{N}_{\text{BOM}}$ had only a minimal impact on the $\delta^{15}\text{N}_{\text{BOM}}$ to $\delta^{15}\text{N}_{\text{LB}}$ relationships, further
439 supporting the use of $\delta^{15}\text{N}_{\text{BOM}}$ as a stable isotope baseline. We accepted our second sub hypothesis,
440 $H_0 \text{ S2}$ due to finding mean TP_{LB} calculated with $\delta^{15}\text{N}_{\text{BOM}}$ were not significantly different than
441 those calculated with baselines of $\delta^{15}\text{N}_{\text{UN}}$, $\delta^{15}\text{N}_{\text{SN}}$, or one generated from a mixing model across

six lakes. Further, mean TP_{LB} calculated with $\delta^{15}N_{UN}$ and $\delta^{15}N_{BOM}$ baselines were not significantly different across a subset of 26 lakes. While the above attributes supported the use of $\delta^{15}N_{BOM}$ as a baseline, some of our findings indicated that it should not be interchanged with $\delta^{15}N_{UN}$ in the same study.

We found the $\delta^{15}N_{UN}$ vs. $\delta^{15}N_{LB}$ relationship was stronger ($R^2 = 0.91$) than that between $\delta^{15}N_{BOM}$ vs. $\delta^{15}N_{LB}$ ($R^2 = 0.78$), and there was not a significant relationship between TP_{LB} estimated using $\delta^{15}N_{UN}$ and $\delta^{15}N_{BOM}$ as baselines. The absolute value of differences in TP_{LB} calculated with $\delta^{15}N_{BOM}$ and $\delta^{15}N_{UN}$ baselines was 0.3 and ranged up to 0.76 across lakes. This lack of correspondence in findings likely results from different sources: UN is thought to represent pelagic inputs to lake food webs, but BOM is formed from, and represents organic material from pelagic, littoral and allochthonous sources. While these results indicate potential problems with interchanging $\delta^{15}N_{UN}$ and $\delta^{15}N_{BOM}$ as baselines, $\delta^{15}N_{BOM}$ can be used as a separate, independent baseline in studies requiring TP values of aquatic biota at all study lakes. The use of $\delta^{15}N_{BOM}$ as a baseline for TP estimation will be useful for quantitatively linking contaminant bioaccumulation to TP and for examining aspects of ecological theory where food chain length is calculated from the $\delta^{15}N$ difference between an apex predator and a baseline (Takimoto and Post 2013).

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Table 1. Summary data of subsets used for comparisons of $\delta^{15}\text{N}_{\text{BOM}}$ vs $\delta^{15}\text{N}_{\text{LB}}$ and evaluation of TP_{LB} .

	BOM to LB ^a		26 UN lakes ^b			For mixing model ^c					
	$\delta^{15}\text{N}_{\text{LB}}$	$\delta^{15}\text{N}_{\text{BOM}}$	$\delta^{15}\text{N}_{\text{LB}}$	$\delta^{15}\text{N}_{\text{BOM}}$	$\delta^{15}\text{N}_{\text{UN}}$	$\delta^{15}\text{N}_{\text{LB}}$	$\delta^{15}\text{N}_{\text{UN}}$	$\delta^{15}\text{N}_{\text{SN}}$	$\delta^{13}\text{C}_{\text{LB}}$	$\delta^{13}\text{C}_{\text{UN}}$	$\delta^{13}\text{C}_{\text{SN}}$
Mean	13.7	4.2	14.5	5	8	15.3	9	9.3	-26.6	-29.8	-24.7
SD	2.4	2.3	2.2	2.2	2.7	3.7	4.2	3.8	2.8	1.5	3.5
Min	9	-0.4	10.7	1.8	3.4	11.6	4.3	5.3	-29.6	-31.9	-28.8
Max	21.2	11	21.2	11	15.5	21.2	15.5	16.1	-22.6	-28.2	-19.8
Range	12.2	11.4	10.5	9.1	12.1	9.6	11.2	10.7	6.9	3.7	9
Number of lakes	51	51	26	26	26	6	6	6	6	6	6

Used for comparisons of $\delta^{15}\text{N}_{\text{BOM}}$ vs $\delta^{15}\text{N}_{\text{LB}}$.

^b Subset of 26 lakes where UN were found. Used for comparisons of TP_{LB} calculated with $\delta^{15}\text{N}_{\text{BOM}}$ and $\delta^{15}\text{N}_{\text{UN}}$ baselines.

^c Subset of 6 lakes where snails were found. Used for comparisons of TP_{LB} calculated with baselines of $\delta^{15}\text{N}_{\text{BOM}}$, $\delta^{15}\text{N}_{\text{UN}}$, $\delta^{15}\text{N}_{\text{SN}}$ and one from a mixing model.

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Table 2. Evaluation of temporal and a combination of temporal and spatial variability of $\delta^{15}\text{N}_{\text{BOM}}$ on the linear relationship of $\delta^{15}\text{N}_{\text{LB}} = \delta^{15}\text{N}_{\text{BOM}} \times (\text{slope}) + \text{Intercept}$ is shown.

	(slope)	R ²	(Intercept)
Temporal	0.92 [± 0.045] ^a	0.81 [± 0.035]	9.95 [± 0.225]
Temporal and spatial	0.91 [± 0.055]	0.80 [± 0.04]	9.98 [± 0.245]

Estimates of slopes, R², intercepts and confidence intervals resulting from largemouth bass (LB) to benthic organic material (BOM) linear relationships ($\delta^{15}\text{N}_{\text{LB}} = \delta^{15}\text{N}_{\text{BOM}} \times (\text{slope}) + b$) for $\delta^{15}\text{N}$ calculated with $\delta^{15}\text{N}_{\text{BOM}}$ data from the temporal data set (n = 35 lakes), and a combination of the temporal data set and the spatial data set (n = 9 lakes) in a resampling procedure. LB samples are $\delta^{15}\text{N}$ values from lakes taken during fisheries surveys.

^a Lower and upper Confidence Intervals are within brackets.
Confidence Intervals were calculated from 1000 bootstrap runs.

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Table 3. Comparisons of model estimates of trophic position of largemouth bass (TP_{LB}) from 26 lakes using $\delta^{15}\text{N}$ in mussels (UN) and benthic organic material (BOM) as baselines are shown.

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Baseline estimate	TP _{LB} mean ^a	Sites (n)	Mean deviation ^b
TP $\delta^{15}\text{N}_{\text{UN}}$ Base (Eq.# 2)	3.91	26	
TP $\delta^{15}\text{N}_{\text{BOM}}$ Base (Eq.# 4)	3.79	26	8% (0-18%)

^a Mean TPs were not significantly different (t stat = -1.46, p = 0.15, n =26).

^b Mean deviation of TP for LB calculated with UN baseline % (% range).

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36 Table 4. Comparisons of model estimates of trophic position of largemouth bass (TP_{LB}) from
37 lakes using different baselines are shown.

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Baseline estimate	TP _{LB} ^a mean	Sites (n)	Mean deviation ^b
Nominal Estimate (mixing model) (Eq. # 5, 6)	3.78	6	
TPδ ¹⁵ N _{UN} Base (pelagic only) (Eq. # 2)	3.85	6	5% (2-9%)
TPδ ¹⁵ N _{SN} Base (littoral only) (Eq. # 3)	3.78	6	3% (1-8%)
TPδ ¹⁵ N _{BOM} Base (Eq. # 4)	3.74	6	5% (0-13%)

^a Mean TPs were not significantly different ($F_{3,23} = 0.27, p = 0.85, n = 24$).
^b Mean deviation of TP for LB from nominal estimate made with mixing model % (% range)

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Figure 1. Relationship of $\delta^{15}\text{N}_{\text{LB}}$ as a function of $\delta^{15}\text{N}_{\text{BOM}}$ in 51 lakes is shown. Equation for line is $\delta^{15}\text{N}_{\text{LB}} = 0.96 \delta^{15}\text{N}_{\text{BOM}} + 9.7$, $R^2 = 0.84$.

Figure 2. Relationship of $\delta^{15}\text{N}_{\text{LB}}$ recent as a function of $\delta^{15}\text{N}_{\text{LB}}$ early in 23 lakes is shown.

Equation for line is $\delta^{15}\text{N}_{\text{LB}}$ recent = $0.84 \delta^{15}\text{N}_{\text{LB}}$ early + 2.1, $R^2 = 0.95$.

Figure 3. Relationship of $\delta^{15}\text{N}_{\text{UN}}$ recent as a function of $\delta^{15}\text{N}_{\text{UN}}$ early in 18 lakes is shown.

Equation for line is $\delta^{15}\text{N}_{\text{UN}}$ recent = $1.04 \delta^{15}\text{N}_{\text{UN}}$ early – 0.82, $R^2 = 0.89$.

Figure 4. Relationships of $\delta^{15}\text{N}_{\text{BOM}}$, $\delta^{15}\text{N}_{\text{UN}}$, and $\delta^{15}\text{N}_{\text{LB}}$ in 26 lakes are shown. Comparisons were of the relationships $\delta^{15}\text{N}_{\text{LB}} = 0.91 \delta^{15}\text{N}_{\text{BOM}} + 9.9$, $R^2 = 0.78$ (●), $\delta^{15}\text{N}_{\text{LB}} = 0.78 \delta^{15}\text{N}_{\text{UN}} + 8.2$, $R^2 = 0.91$, (▲), and $\delta^{15}\text{N}_{\text{UN}} = 1.15 \delta^{15}\text{N}_{\text{BOM}} + 2.3$, $R^2 = 0.82$ (■). Values for dependent variable, $\delta^{15}\text{N}_{\text{LB}}$ or $\delta^{15}\text{N}_{\text{UN}}$, are on the y axis for all comparisons.

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1 Appendix

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3 Table A1. Lake names, areas, depths, and subsets of BOM samples

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5 Table A2. Lake names, LB and UN collection dates, LB and UN $\delta^{15}\text{N}$ values, $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$
6 values for LB, UN, SN

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8 Table A3. ANOVA statistics of spatial variability in nine lakes

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10 Table A4. Lake names, sample dates, $\delta^{15}\text{N}_{\text{LB}}$ values and number of years between samples

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13 Table A5. Lake names, sample dates, $\delta^{15}\text{N}_{\text{UN}}$ values and number of years between samples

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Table A1

Lake Name	Area	Mean depth	BOM Samples ^a
	(ha)	(m)	
Alton	16	1.2	A,T
Asa	11	1.1	A
Ashville	10	1.2	A,T
Belleville	64	1.5	A
Boone	19	3.7	A,T
Bowdish	92	1.7	A,T
Breakheart	18	1.2	A,T,S
Browning Mill	19	1.2	A,T,S
Burlingame	28	0.9	A
Carbuncle	14	4.6	A
Chapman	66	0.9	A,T
Deep	7.7	5.5	A,T
Eisenhower	23	1.5	A,T,S
Gilbert Stuart	27	2.4	A
Gorton	25	4.9	A,T,S
Hundred Acre	34	5.5	A,T
Indian	221	2.1	A
Jamestown	11	2.1	A
JL Curran	12	3	A
Keech	52	1.4	A,T
Larkin	18	4.3	A,T,S
Little	10	3.7	A,T
Locustville	34	2.4	A,T
Lower Beach	18	2.3	A,T
Meadowbrook	16	2.4	A,T
Mishnock	19	2.4	A,T,S
Nonquint	82	2.4	A
Oak Swamp	47	1.5	A,T
Pasquisett	28	2.4	A
Quidnick	69	3.7	A,T
Schoolhouse	34	3	A,T
Secret	16	1.1	A
Simmons Mill	8	1.5	A
Spring Green	3.4	2.1	A,T
Stafford	193	3	A
Tarbox	12	2.1	A,T
Tiogue	87	1.8	A,T
Tucker	41	3.4	A,T,S

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Turner	94	1.5	A
U.Dam	8	2.1	A,T,S
U. Slatersville	56	2.4	A,T
Wakefield	29	1.8	A,T
Wallum	130	8.5	A
Warwick	34	4.3	A
Watchaug	232	2.4	A,T
Waterman	124	2.1	A,T
Wilson	45	2.1	A,T
Wincheck	54	2.1	A,T
Worden's	420	1.2	A,T,S
Wyoming	12	2.7	A,T
Yawgoo	55	2.7	A,T

^aA = BOM samples were taken at all 51 lakes in September 2013.

^aT = Temporal study BOM samples were taken at each lake in September 2012, May and September 2013.

^aS = Spatial study BOM samples were taken at different locations at each lake August through September 2013.

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Table A2

Lake Name	LB			UN					
	Collection			Collection					
	Date	$\delta^{15}\text{N}_{\text{LB}}$	$\delta^{15}\text{N}_{\text{BOM}}^a$	Date	$\delta^{15}\text{N}_{\text{UN}}$	$\delta^{15}\text{N}_{\text{SN}}$	$\delta^{13}\text{C}_{\text{LB}}$	$\delta^{13}\text{C}_{\text{UN}}^b$	$\delta^{13}\text{C}_{\text{SN}}^b$
Alton	7/26/11	14.1	5.4	7/27/11	8.3 ^c				
Asa	5/23/00	14.1	3.9						
Ashville	5/24/97	9.5	0.2						
Belleville	8/24/11	15.3	5.0	6/5/13	9.0 ^c	7.8	-28.0	-30.3	-29.8
Boone	5/12/99	14.2	5.4						
Bowdish	10/27/08	11.1	0.8						
Breakheart	10/11/12	13.0	2.3						
Browning Mill	11/16/12	12.8	3.0	10/21/13	6.1				
Burlingame	5/21/02	9.0	-0.4						
Carbuncle	10/14/09	13.2	3.8						
Chapman	9/12/06	14.7	4.3	9/12/11	9.4				
Deep	6/20/05	10.6	2.1						
Eisenhower	5/24/12	11.6	2.1	6/18/13	4.3 ^c	5.3	-29.4	-31.0	-28.8
Gilbert Stuart	5/30/00	16.3	5.4						
Gorton	8/18/11	17.7	8.7	6/13/13	12.1	10.1	-24.8	-28.2	-21.5
Hundred Acre	5/19/98	16.0	4.3	7/15/12	7.6				
Indian	9/26/11	12.2 ^c	4.7						
Jamestown	10/1/12	15.2	5.7						
JL Curran	4/30/97	15.8	6.0						
Keech	10/23/07	13.9	2.9						
Larkin	6/24/13	14.5	5.1	6/20/12	7.7				
Little	5/29/02	15.0	6.7	6/12/13	8.3				
Locustville	6/8/99	14.5	2.9	6/3/13	7.0				
Lower Beach	11/1/11	10.9	2.8						
Meadowbrook	8/3/11	13.6 ^c	4.9	8/3/11	7.5 ^c				
Mishnock	5/14/97	14.6	5.6	5/8/13	9.1	8.6	-29.6	-31.9	-27.8
Nonquint	9/4/13	14.6	6.1	7/8/13	8.4				
Oak Swamp	5/23/96	15.1	6.4	5/6/13	7.8	9.6	-22.6	-28.2	-19.8
Pasquisett	5/21/07	12.6	4.4	9/23/13	5.8				
Quidnick	5/31/12	11.8	2.2	6/18/13	5.3	5.9	-27.8	-30.1	-25.6
Schoolhouse	5/31/05	10.7 ^c	2.6	9/12/11	3.4				
Secret	5/27/03	16.1	5.6						
Simmons Mill	6/12/01	10.8	1.7						
Spring Green	6/17/03	16.4	5.4						
Stafford	10/10/07	12.8	4.7	7/23/12	7.1 ^c				
Tarbox	8/25/04	10.0	1.6						
Tiogues	9/9/13	17.3	8.3	6/17/13	12.2				
Tucker	10/15/13	11.3	1.8	6/6/12	4.0 ^c				
Turner	7/29/13	21.2	11.0	7/15/13	15.5	16.1	-25.3	-29.5	-24.4

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U. Dam	10/17/11	16.9	7.3	5/6/13	11.6	11.0	-26.4	-29.4	-27.2
U. Slatersville	10/18/04	15.7	4.2	6/6/12	9.6	4.7	-27.9	-31.6	-33.7
Wakefield	9/30/13	10.7	1.0						
Wallum	10/22/12	11.6	1.2						
Warwick	8/16/12	17.3	8.1						
Watchaug	9/26/02	12.4	3.0						
Waterman	9/19/11	15.7	6.3						
Wilson	10/8/13	13.3	3.2						
Wincheck	9/25/00	10.4	0.3						
Worden's	8/22/13	13.9	4.4	6/27/12	6.8				
Wyoming	9/1/11	14.3	4.1	6/19/13	8.0				
Yawgoo	5/27/98	13.9	3.7	9/11/13	5.5				

Subscripts refer to sample type, LB = largemouth bass, BOM = benthic organic material, UN = mussels, SN = snails

^a BOM samples collected 9/2013

^b $\delta^{13}\text{C}$ for samples were lipid corrected

^c Size normalized values

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Table A3 Spatial Variability in Benthic Organic Material at nine lakes^a

Lake name	<i>F</i> Value	<i>p</i> > <i>F</i>	Number of locations	Number of samples	Max within-lake range of δ ¹⁵ N _{BOM} (‰) ^b
Breakheart	91.76	<.0001	3	9	1.33
Browning Mill	16.21	0.0038	3	9	1.26
Eisenhower	19.65	0.0114	2	6	0.98
Gorton	2.24	0.1881	3	9	1.49
Larkin	0.21	0.8138	3	9	0.66
Mishnock	11.43	0.009	3	9	2.01
Tucker	44.5	0.0026	2	6	0.89
Upper Dam	0.65	0.464	2	6	0.51
Worden	60.25	<.0001	4	12	1.33

^a Results of ANOVA analyses

^b Maximum within lake range of δ¹⁵N_{BOM} (‰) using individual BOM samples

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74 Table A4. Lake names, sample dates, $\delta^{15}\text{N}_{\text{LB}}$ values and number of years between samples.

Lake name	Early date	Early $\delta^{15}\text{N}_{\text{LB}}$	Recent date	Recent $\delta^{15}\text{N}_{\text{LB}}$	Years between samples
Alton	5/15/2000	14.4	7/26/2011	14.1	11
Belleville	6/7/2000	15.2	8/24/2011	15.3	11
Bowdish	7/1/2002	11.4	10/27/2008	11.1	6
Breakheart	5/14/2000	11.4	10/11/2012	13.0	12
Browning Mill	5/31/1997	12.4	11/16/2012	12.8	15
Chapman	5/3/2000	15.0	9/12/2006	14.7	6
Eisenhower	4/29/1999	12.5	5/24/2012	11.6	13
Gorton	6/14/2000	19.1	8/18/2011	17.7	11
Indian	5/16/2000	12.6	9/26/2011	12.2	11
Keech	7/1/2002	13.4	10/23/2007	13.9	5
Larkin	5/13/1998	15.5	6/24/2013	14.5	15
Meadowbrook	5/8/2000	14.0	8/3/2011	13.6	11
Nonquint	5/17/2001	15.5	9/4/2013	14.6	12
Quidnick	7/28/1997	12.0	5/31/2012	11.8	15
Tarbox	5/20/1997	10.4	8/25/2004	10.0	7
Tiogou	5/8/1997	18.8	9/9/2013	17.3	16
Tucker	10/14/1997	11.1	10/15/2013	11.3	16
Turner	4/23/2001	22.8	7/29/2013	21.2	12
Wakefield	10/13/2004	10.8	9/30/2013	10.7	9
Warwick	6/20/2000	17.7	8/16/2012	17.3	12
Wilson	9/18/2006	12.8	10/8/2013	13.3	7
Wordens	9/6/2006	14.2	8/22/2013	13.9	7
Wyoming	9/6/1998	13.5	9/1/2011	14.3	13

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85 Table A5. Lake names, sample dates, $\delta^{15}\text{N}_{\text{UN}}$ values and number of years between samples.

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Lake name	Early date	Early $\delta^{15}\text{N}_{\text{UN}}$	Recent date	Recent $\delta^{15}\text{N}_{\text{UN}}$	Years between samples
Alton	5/15/2000	8.9	7/27/2011	8.3	11
Belleville	6/7/2000	10.2	6/5/2013	9.0	13
Browning Mill	7/13/2004	6.4	10/21/2013	6.1	9
Chapman	5/3/2000	9.9	9/12/2011	9.4	11
Eisenhower	4/29/1999	6.1	6/18/2013	4.3	14
Gorton	6/14/2000	11.7	6/13/2013	12.1	13
Hundred Acre	5/19/1998	8.8	7/15/2012	7.6	14
Larkin	5/13/1998	8.5	6/20/2012	7.7	14
Little	8/17/2004	8.1	6/12/2013	8.3	9
Locustville	7/28/2004	8.3	6/3/2013	7.0	9
Meadowbrook	5/8/2000	7.6	8/3/2011	7.5	11
Mishnock	5/14/1997	9.1	5/8/2013	9.1	16
Nonquint	5/17/2001	9.1	7/8/2013	8.4	12
Oak Swamp	5/23/1996	7.8	5/6/2013	7.8	17
Quidnick	7/28/1997	4.9	6/18/2013	5.3	16
Tiogue	5/8/1997	12.0	6/17/2013	12.2	16
Wyoming	9/26/1998	9.6	6/19/2013	8.0	14
Yawgoo	5/27/1998	6.1	9/11/2013	5.5	15

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