

Evaluation of biomass and trophic position
for Lake Huron zooplankton

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Abstract

Lake Huron has undergone substantial changes in nutrient and community composition from reductions in phosphorus loading and the introduction of invasive mussels. Potential consequences from these alterations include changes in bottom-up and top-down controls and shifts in calanoid copepod trophic positions. Using three samples collected in Lake Huron during 2013, we studied the length-weight relationship for *Diaptomus*, *Limnocalanus*, *Senecella*, and *Epischura* compared to Great Lakes regressions published by Burgess et al. (2015), Pace and Orcutt (1981), Dumont et al. (1975), and Bottrell et al. (1976). Allometric models by Pace and Orcutt and Burgess et al. significantly underestimated dry weights ($P < 0.05$), while allometric models by Bottrell et al. and Dumont et al. were statistically similar with 84% and 83% of the variation explained by each model, respectively. Comparison of heavier calanoid copepods *Limnocalanus* and *Senecella* suggested that there was substantial variability in dry weights for animals with constant total length. Despite significantly dissimilar estimations, dry weight results remained within 90% of variation explained by the Burgess et al. equation. In this study we also aimed to examine the proposition by Jackson et al. (2013) that *Limnocalanus* and *Diaptomus* have reduced trophic positions relative to primary herbivores. Using five stations collected from 1993 to 2013, we examined $\delta^{15}\text{N}$ in *Diaptomus*, *Limnocalanus*, *Epischura*, and *Bythotrephes* to address trophic position changes relative to the putative herbivores *Daphnia mendotae* and *Holopedium gibberum*. Results did not suggest significant changes in the trophic positions for *Limnocalanus* ($P=0.14$), *Diaptomus* ($P=0.49$), *Epischura* ($P=0.50$), and *Bythotrephes* ($P=0.42$) from 1993 to 2013.

Introduction

Increases in spring silica concentration and decreases in diatom growth have indicated increased oligotrophication of Lake Huron in recent years (Evans et al., 2011). Hypothesized mechanisms for the decline in diatom production and subsequent reduction in primary productivity include the competitive effects of invasive species and policy limitations on phosphorus loading (Evans et al., 2011; Fahnenstiel et al., 2010). When combined, these changes in nutrients and community composition may alter top-down and bottom-up controls within Lake Huron, with potential consequences for biomass and trophic relations.

Historically, biomass for calanoid copepods has been estimated using an allometric model called a length-weight regression (Strickland and Parsons, 1968; Dumont et al., 1975; Bottrell et al., 1976; Downing and Rigler, 1984 and Culver et al., 1985). Using a calibration set of individual weights and body length measurements, an allometric model predicts taxon mean individual dry weights from length measurements, which can then be used to estimate population biomass (Burgess et al., 2015). The allometric length-weight regression model currently used by the USEPA (2013) for all Great Lakes Calanoida is taken from a study by Pace and Orcutt (1981). The allometric model by Pace and Orcutt (1981) was developed using limited data from a single diaptomid species, *Diaptomus siciloides*, in a small Georgia lake.

Burgess et al. (2015) recently reviewed length-weight regressions published by Pace and Orcutt (1981), Dumont et al. (1975), and Bottrell et al. (1976), and compared their predictions to original Great Lakes data from 1993 to 2013. Results revealed that the Pace and Orcutt (1981) model underestimated empirical weights, and that equations by Dumont et al. (1975) and Bottrell et al. (1976) slightly overestimated weights on average. Burgess et al. (2015) proposed an alternative regression that best fit their data drawn from all five Great Lakes, and accounted for 90% of the variance in the calibration data set. They pointed out that efforts to improve length-weight regression models further would be hampered by substantial variability within the larger glacial opportunists (Carter et al., 1986), *Limnocalanus macrurus* and *Senecella calanoides*, owing to variation in weights at constant length associated with lipid accumulation by adults (Vanderploeg et al., 1998). Burgess et al. noted that equations by Bottrell et al. and Dumont et al. were developed using maximum calanoid copepod lengths of 2.45 mm and 1.4 mm, respectively. Adult total lengths (TL) of *L. macrurus* range from 2.3 to 3 mm (Barbiero et al., 2009), and from 2.65 to 2.88 mm for female *S. calanoides* (Vyshkvartzeva, 1994).

This study was conceived in an attempt to reexamine biomass calculations in the Great Lakes, and specifically Lake Huron. Using an expanded data set, this study also aimed to explore changing trophic positions based on a proposition made by Jackson et al. (2013). From 1993 and 1995 to 2009, Jackson et al. (2013) reported a decline in the trophic position of the calanoid copepod, *L. macrurus*, after examination of $\delta^{15}\text{N}$ levels relative to reference taxa (e.g., Lajtha and Michner, 1994; Kling et al., 1992; Matthews and Mazumder 2003), *Daphnia mendotae* and *Holopedium gibberum* in Lake Huron. Additional analyses by Jackson et al. (2013) revealed up to fourfold underestimation of *L. macrurus* biomass, based on the equation used by Barbiero et al. (2009a,b) to estimate biomass. *L. macrurus* had evidentially become even more dominant than suggested by the latter authors. Two mechanisms were proposed to explain the success of *L. macrurus*: (1) a trophic position shift of *L. macrurus* with improved ecological efficiency to compensate for oligotrophic conditions, or (2) an indirect consequence from its prey shifting to a lower trophic position (Jackson et al., 2013).

In this study we aim to evaluate the length-weight regression model of Burgess et al. (2015) using data from Lake Huron that were not part of the calibration data set. We also revisit the proposition by Jackson et al. (2013) that *L. macrurus* and *D. sicilis* have reduced their trophic position relative to primary herbivores. Within these analyses we include *Epischura lacustris* and *Bythotrephes longimanus*. Analyses were performed using three samples from Lake Huron collected during September, 2013, and preserved in 5% formalin. Procedures by Burgess et al. and Jackson et al. were replicated. We hypothesized that (1) the newer model proposed by Burgess et al. would be the most accurate estimation for the individual mass of the various calanoid species and (2) results would confirm that *L. macrurus* and *D. sicilis* had shifted to a lower trophic position, compared with earlier years.

Methods

Study Area

Using a net with a 153 μm mesh aperture, and a 1-m mouth diameter, three samples were collected from each Lake Huron station during a 2013 USGS fishery survey (Table 1, Figure 1). Samples were collected by vertical tows with the vessel at anchor and were preserved in 5% formalin. As stated by Jackson et al. (2013), samples preserved in formalin cause a 0.5‰ increase in $\delta^{15}\text{N}$ values compared with fresh material (Edwards et al., 2002; Sarakinos et al., 2002). However, increases are uniform and do not affect relative differences between taxa.

Table 1. Station coordinates, depths, and sampling date(s) in Lake Huron.

| Date | Station | Latitude (N) | Longitude (W) | Depth (m) |
|-------------------------|---------|--------------|---------------|-----------|
| 27-Aug-93, 25-Jul-97 | H6 | 44° 30' | 82° 33' 18" | 65 |
| 27-Aug-93, 28-Aug-95 | H12 | 45° 45' | 83° 33' | 108 |
| 26-Aug-95 | H4 | 44° 10' | 83° 00' | 60 |
| 27-Aug-95 | H8 | 45° 11' | 82° 33' 18" | 100 |
| 25-Aug-95 | H2 | 43° 30' | 82° 13' 59" | 50 |
| 22-Sept-09 | GB3 | 45° 28.39' | 81° 34.32' | 88 |
| 20-Sept-09 | NC3 | 46° 3' | 82° 50' | 35 |
| 09-Oct-09 | MW2 | 45° 44.71' | 84° 10.79' | 38 |
| 6-Aug-13 | HU38 | 44° 44' 24" | 82° 3' 36" | 50-0 |
| 17-Sept-13 | MW4 | 45° 5' 25" | 82° 46' 52" | 170 |
| 23-Sept-13 | SB2 | 43° 44' 4" | 82° 16' 11" | 60 |
| 30-Sept-13 | ME1 | 45° 13' 36" | 82° 3' 18" | 140 |

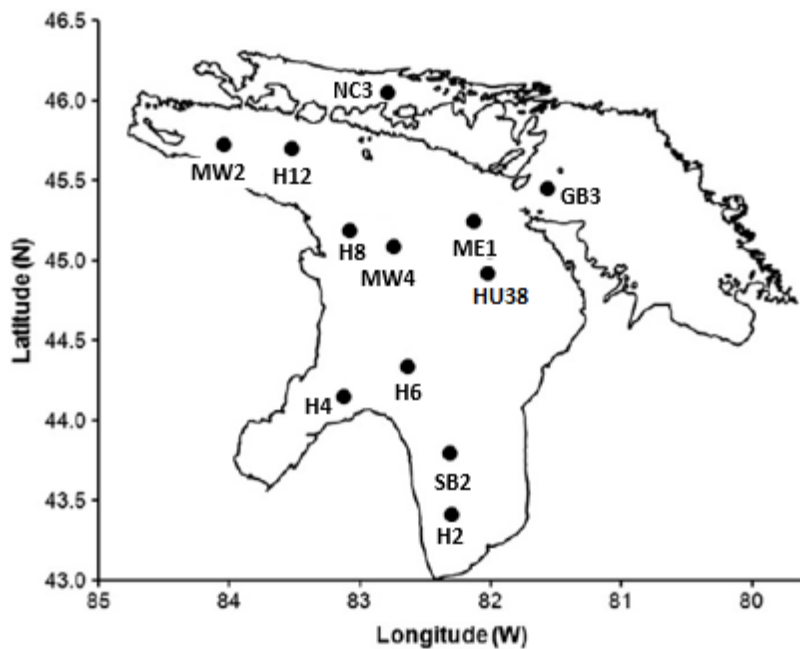


Figure 1. Location of Lake Huron stations listed in Table 1.

Length-Weight Regression

Individuals were first rinsed with reverse osmosis (RO) water to remove formalin solution. Groups of like-sized individuals were cleaned of adherent debris, measured using an ocular micrometer on a Wild M5A stereomicroscope, and placed into tared Costech™ tin capsules. Total length measurements were calculated for *D. sicilis* and *L. macrurus* from sample ME1; *D. sicilis*, *L. macrurus*, *E. lacustris* and *S. calanoides* from samples MW4 and SB2 were likewise measured. Total lengths of *D. sicilis*, *S. calanoides*, and *E. lacustris* were measured from the anterior margin of the head to the distal margin of the caudal rami. Measurements for *L. macrurus* individuals were taken from the anterior margin of the head to the posterior margin of the metasome. To calculate total length, metasome measurements were transformed using equation 1 (Burgess et al., 2015):

$$TL \text{ (mm)} = 1.192 \times ML \text{ (mm)} + 0.642 \quad (1)$$

Like-sized specimens were added to tared Costech™ tin capsules and were dried for at least 48 h at 55C, then weighed using a CAHN 29 electrobalance with silica gel desiccant inside the weighing chamber. Individual dry weights were calculated by subtracting tared mass from sample mass and dividing by the number of individuals present.

In Microsoft Excel a one-way analysis of variance ($\alpha=0.05$) was applied to dry weight measurements from samples SB2, MW4, and ME1. Further analyses included nonparametric t-tests and the Solver application. Nonparametric t-tests were used to compare measured weights individually with the weights predicted by the allometric models of Pace and Orcutt (1981), Dumont et al. (1975), Bottrell et al. (1976), and Burgess et al. (2015). Solver was used to examine whether changes in the elevation (but not the slope) of published allometric models could improve the statistical fit of those models to empirical data, based on nonparametric comparisons of the predicted and measured $\ln DW$. Equations reproduced from Burgess et al. were as follows:

$$\text{Pace and Orcutt (1981), p. 486: } \ln(W, \mu\text{g}) = 1.05 + 2.46 \times \ln(TL, \text{mm}) \quad (2)$$

$$\text{Bottrell et al. (1976), p. 486: } \ln(W, \mu\text{g}) = 1.9526 + 2.4 \ln(TL, \text{mm}) \quad (3)$$

$$\text{Dumont et al. (1975), p. 486: } W(\text{mg}) = 0.007 \times \text{TL}(\text{mm})^{2.33} \quad (4)$$

$$\text{Burgess et al. (2015), p. 487: } \ln(W, \mu\text{g}) = 1.53 + 2.59 \ln(\text{TL}, \text{mm}) \quad (5)$$

Isotope Analysis

Dried MW4 and ME1 samples (including *L. macrurus*, *D. sicilis*, *E. lacustris*, and *B. longimanus*) were submitted to the Stable Isotope Core Laboratory at Washington State University for elemental and stable isotope analysis of $\delta^{15}\text{N}$, with *Daphnia mendotae* drawn from each sample as the reference putative herbivore. In addition to MW4 and ME1 analyses, prior estimates from 1993 through 2009 were incorporated into the data set, including the three samples published by Jackson et al. (2013). Samples H12, H8, and NC3 aggregated Diaptomidae to include *D. sicilis* and *D. oregonensis*. In Microsoft Excel, a linear regression analysis was performed on isotope data from 1993 to 2013 to determine if a temporal trend existed in the data for $\delta^{15}\text{N}$ ($\alpha=0.05$).

Results

Length-Weight Regression

Logarithmically transformed total lengths and dry weights from samples MW4, SB2, and ME1 resulted in statistically similar dry weights (ANOVA, $F=0.11$, $P=0.89$) and a linear relationship ($N=31$, $r^2=0.91$), with the highest variance occurring in the largest individuals *L. macrurus* and *S. calanoides*:

$$\ln(W, \mu\text{g}) = 1.48 (\text{SE}=0.13) + 3.28 (\text{SE}=0.19) \ln(\text{TL}, \text{mm}) \quad (6)$$

Nonparametric t-tests comparing predicted and measured dry weights revealed significant underestimates ($P<0.0001$) in $\ln\text{DW}$ for allometric models by Burgess et al. (2015) and Pace and Orcutt (1981). Underestimations for *S. calanoides* and *L. macrurus* were similarly found for models by Dumont et al. (1975) and Bottrell et al. (1976). However, the equations by Dumont et al. (1975) and Bottrell et al. (1976) also overestimated smaller total length copepods *D. sicilis* and *E. lacustris*, which when combined with underestimates in *S. calanoides* and *L. macrurus*, led to an overall lower average difference between estimated and observed dry weights. It is evident from Fig. 2 that over and underestimation of $\ln\text{DW}$ resulted in a lower sample mean, and thus a higher similarity to measured dry weights for equations by Dumont et al. ($P=0.70$) and Bottrell et al. ($P=0.27$). Comparisons between Dumont et al. (1975) $\ln\text{DW}$ estimates and those observed for *D. sicilis* and *E. lacustris* were significantly different ($P<0.005$) for samples MW4, ME1, and SB2.

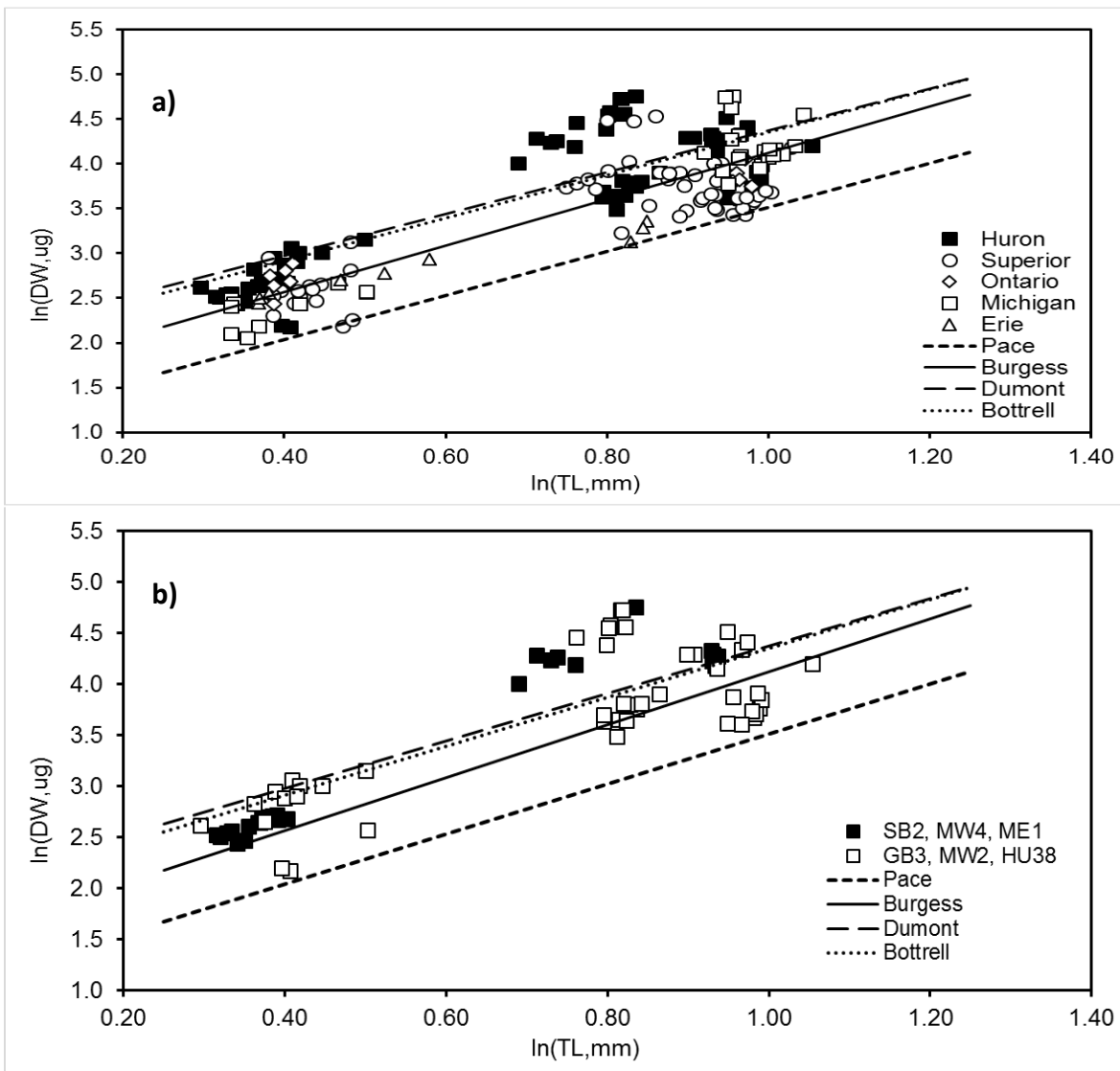


Figure 2. (a) Transformed individual total length versus dry weight for all samples from the Burgess et al. (2015) calibration data set, separated into Lakes Superior, Ontario, Michigan, and Erie. Lake Huron data includes the Burgess et al. subset and the ME1, MW4, and SB2 data. (b) Lake Huron measurements separated by Burgess et al. data (GB3, MW2, and HU38) and SB2, MW4, and ME1 data.

Using the subset of the original Burgess et al. (2015) calibration data set drawn from Lake Huron samples, we also derived a new equation that included Lake Huron data exclusively (Eq. 7). This new equation contained the Burgess et al. samples HU38 (*S. calanoides* and *L. macrurus*), MW2, and GB3 (both with *D. sicilis*, *E. lacustris*, *L. macrurus*, and *S. calanoides*). We examined the lnDW between *S. calanoides* and *L. macrurus* across all three samples, and found the observed and predicted weights from sample HU38 to be statistically different from samples GB3 and MW2 ($P < 0.001$). GB3 and MW2 were statistically indistinguishable ($P = 0.13$). Considering temporal accumulation of lipids (Vanderploeg et al., 1992, 1998) and high lnDW variability in calanoid copepods *L. macrurus* and *S. calanoides*, sample HU38 (collected early August) was excluded from the Burgess et al. calibration data set in order to observe changes in the slope of Eq. 7 for stations sampled only during the months of September and early October. Equations for the Burgess et al. Lake Huron data set Eq. 7 ($N = 49$, $R^2 = 0.72$) and Burgess et al. Lake Huron equation excluding the HU38 sample Eq. 8 ($N = 25$, $R^2 = 0.85$) were as follows:

$$\ln(W, \mu\text{g}) = 1.96 \text{ (SE=0.18)} + 2.24 \text{ (SE=0.23)} \ln(TL, \text{mm}) \quad (7)$$

$$\ln(W, \mu\text{g}) = 1.46 \text{ (SE=0.20)} + 3.36 \text{ (SE=0.30)} \ln(TL, \text{mm}) \quad (8)$$

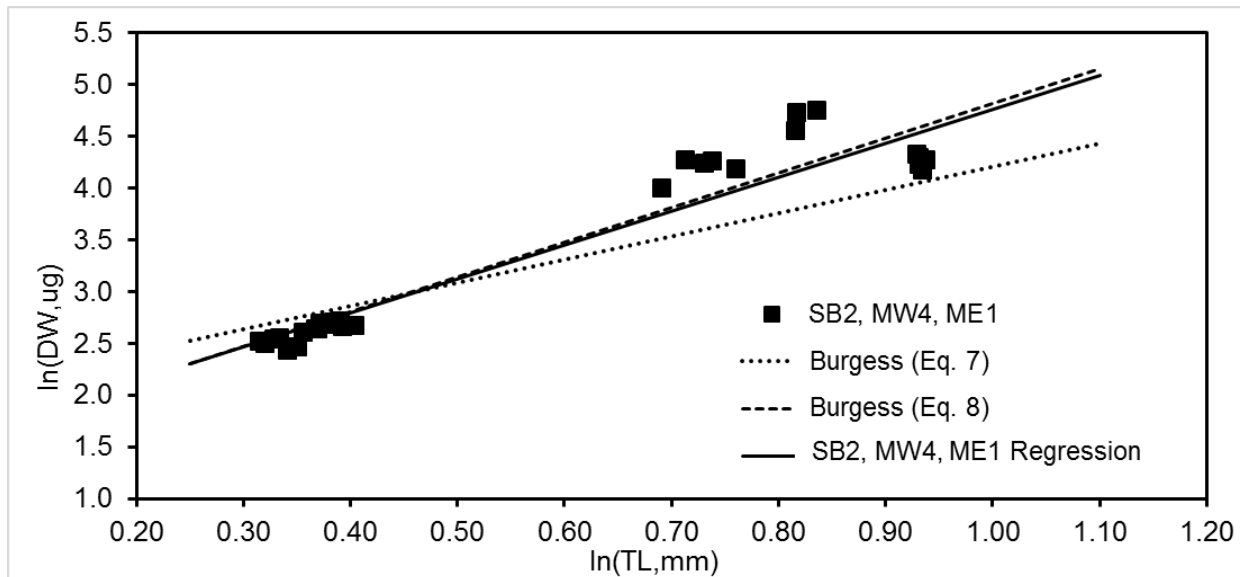


Figure 3. Burgess et al. (2015) Lake Huron regression (Eq. 7) and the regression excluding sample HU38 (Eq. 8) are plotted against SB2, MW4, and ME1. The SB2, MW4, and ME1 regression (Eq. 6) is displayed as a solid line.

Minimized square errors and variances were additionally calculated to evaluate Eqs. 2 through 5 by adjusting the elevation of each model regression line to minimize residual error. Equations published by Dumont et al. (1975) and Bottrell et al. (1976) did not require substantial adjustment in their position relative to MW4, SB2, and ME1 data (<0.10 lnDW units), compared to equations by Burgess et al. (2015) and Pace and Orcutt (1981) that required 0.38 and 0.94 lnDW unit adjustments, respectively (Table 2). After adjustment, Dumont et al. (1975) remained at 83% of the variance explained by the line, and Bottrell et al. (1976) increased by 1% to 85% of the variance explained by the line. Significant underestimates by Burgess et al. were improved from 68% to 87% from the original equation, and from 79% to 82% for Eq. 7. The Burgess et al. Lake Huron equation excluding station HU38 (Eq. 8) required little elevation adjustment (<0.01 lnDW units), and remained at 91% of the variance explained by the line. The original Pace and Orcutt equation differed so significantly from the empirical data that the sum-of-squares for predicted versus observed was greater than the sum of squares of error (SS_{reg}) from a horizontal line (SS_{tot}) at mean lnDW. Once squared errors were minimized by increasing the equation intercept, the adjusted Pace and Orcutt model accounted for 85% of the total variance in lnDW.

Table 2. Minimized squared error adjustments for equations by Burgess et al. (2015), Bottrell et al. (1976), Dumont et al. (1975), and Pace and Orcutt (1981) are indicated by the adjusted y-value (lnDW). Percent variation is the percent of variation that is explained by the line after adjustment.

| Equation | Adjusted y (lnDW) | % Variation |
|-----------------|-------------------|-------------|
| Burgess et al. | 0.38 | 87 |
| Bottrell et al. | 0.07 | 85 |
| Dumont et al. | 0.02 | 83 |
| Pace and Orcutt | 0.94 | 85 |
| Eq. 7 | 1.08 | 82 |
| Eq. 8 | <0.01 | 91 |

Nonparametric t-tests comparing measured dry weights from SB2, MW4, and ME1 with the estimated lnDW from Eq. 7 ($P=0.02$) and Eq. 8 ($P=0.63$) yielded statistically similar results for only Eq. 8. As noted by Burgess et al. (2015), there was significant heterogeneity across lakes and time in the original calibration set ($P<0.0002$). Temporal homogeneity in our results, compared to the results from Burgess et al. confirm this association, with 91% of the variation of SB2, MW4, and ME1 explained by Eq. 8. Of the 90% variation determined from upper and lower bounds by the original Burgess et al. equation, our regression (Eq. 6) fit within these specified bounds. The following equation is the upper bound from the original calibration set (their Eq. 9a, p. 487):

$$\ln(W, \mu\text{g}) = 2.0 + 3.2 \ln(TL, \text{mm}) \quad (9)$$

In absence of the original data used to construct the Bottrell et al. (1976), Dumont et al. (1975), and Pace and Orcutt (1981) equations, we were unable to estimate upper and lower bounds associated with their model parameters.

Isotope Analysis – Nitrogen

Putative trophic level distinctions were based nominally on 3.4‰ elevation of $\delta^{15}\text{N}$ ($\Delta\text{N} = \delta^{15}\text{N}_{\text{taxa}} - \delta^{15}\text{N}_{\text{reference}}$) relative to reference taxa *Daphnia* or *Holopedium* (Post, 2002). In 2013 *L. macrurus* individuals were about 1.24 trophic levels greater than *D. sicilis* individuals in sample ME1 and 1.46 trophic levels greater in sample MW4 (Appendix Table 1). Across all years, trophic levels between *Diaptomus* and *L. macrurus* ranged from 0.67 in 1995 to 1.46 in 2013 ($N=12$, $\bar{x}=1.06$, $SD=0.24$). Linear regressions indicate that the differences in $\delta^{15}\text{N}$ between *Diaptomus* and *L. macrurus* did not change significantly from 1993 to 2013 ($P=0.12$). Overall differences in $\delta^{15}\text{N}$ relative to the reference taxa also did not change significantly for *L. macrurus* ($P=0.14$), *D. sicilis* ($P=0.49$), *E. lacustris* ($P=0.50$), and *B. longimanus* ($P=0.42$) from 1993 to 2013 (Fig. 4).

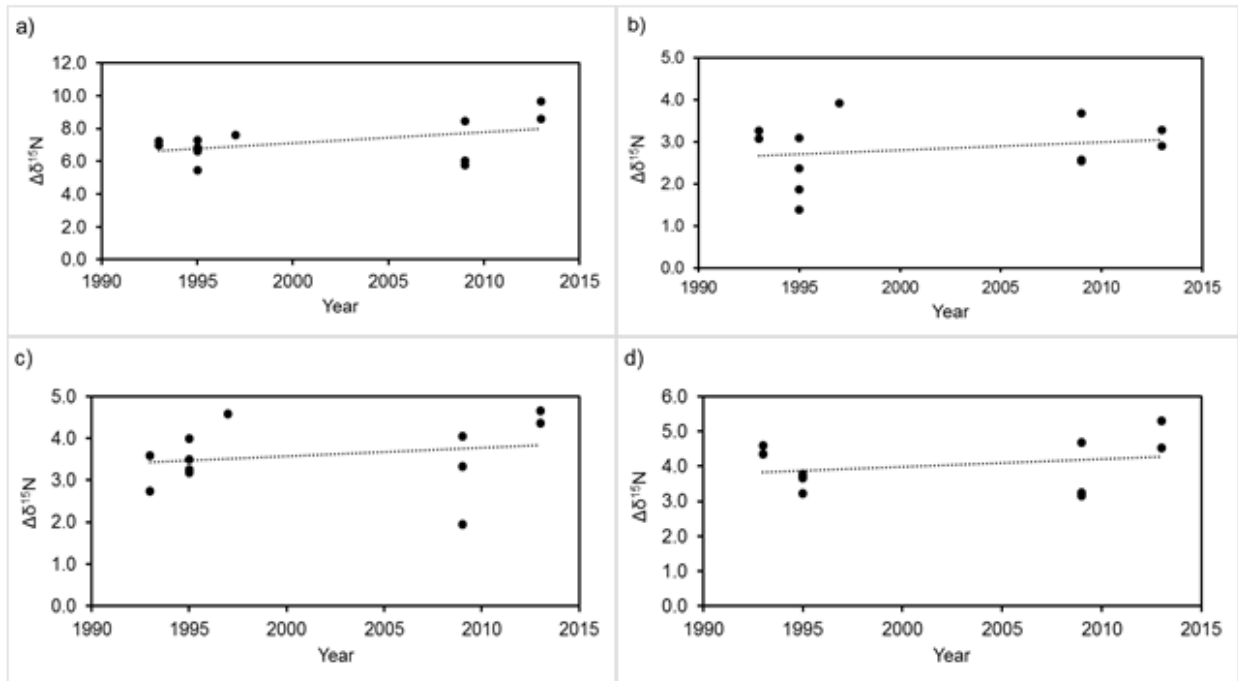


Figure 4. $\Delta\delta^{15}\text{N}$ plots relative to the primary herbivore from stations MW4, ME1, H12, H8, and NC3: (a) *Limnocalanus* (b) *Episichura* (c) *Diaptomus* (d) *Bythotrephes*.

Table 3. ANOVA and regression summary for $\Delta\delta^{15}\text{N}$ values from Figure 4.

| Taxon | Slope | R² | P |
|---------------------|--------------|----------------------|----------|
| <i>Limnocalanus</i> | 0.07 | 0.20 | 0.14 |
| <i>Epischura</i> | 0.02 | 0.05 | 0.50 |
| <i>Diaptomus</i> | 0.02 | 0.05 | 0.49 |
| <i>Bythotrephes</i> | 0.02 | 0.07 | 0.42 |

Discussion

Length – Weight Regression

In this study, we aimed to investigate the calanoid copepod length-weight regressions published by Pace and Orcutt (1981), Bottrell et al. (1976), Dumont et al. (1975), and Burgess et al. (2015) using three samples collected from Lake Huron in 2013. Our results coincide with prior results published by Burgess et al. (2015), including similarities between $\ln\text{DW}$ estimates by the Dumont et al. ($P=0.70$) and Bottrell et al. ($P=0.27$) models, and significant $\ln\text{DW}$ underestimates by the equation of Pace and Orcutt ($P<0.05$). However, our original hypothesis that the Burgess model would provide the most similar average $\ln\text{DW}$ estimates for Lake Huron samples MW4, SB2, and ME1 was not supported ($P<0.05$).

Using the original calibration data set, and a regression summarizing our three samples, we hypothesize the variability between the two data sets involves (1) temporal differences associated with lipid accumulation at constant total lengths during adulthood (Vanderploeg et al., 1992, 1998) and (2) substantial variability among taxa within the five Great Lakes. As indicated in Figure 2, there was substantial variation in dry weights of identical taxa between Lake Huron and Lake Superior. Lake Superior exhibited overall lower estimates compared to our MW4, SB2, and ME1 data from Lake Huron. Given the increasing oligotrophication of Lake Huron (Evans et al., 2011) and the resultant lower primary productivity before and after summer stratification, we may observe less accumulation of lipids and results closer to those observed in Lake Superior and sample HU38 from Lake Huron. However, some of the observed differences may be a consequence of the different temporal sampling of the lakes with respect to taxa that are univoltine.

Overall, unadjusted Dumont et al. (1975) and Bottrell et al. (1976) equations most accurately fit the data with around 83% and 84% of the variation explained by the lines, respectively. However, we cannot assume these findings inevitably apply to all five lakes, in absence of upper and lower boundaries within the calibration data set. Considering the significant variability in Lake Huron data, and comparison between Lake Huron with the other Great Lakes, we must consider upper and lower boundaries for a general equation to be applied. We presume that estimates from the Pace and Orcutt equation exceed the bounds of the calibration data set. On average, there were 1.4 unit underestimates in $\ln\text{DW}$, which improved greatly with adjustments in the y-intercept. This is likely the consequence of basing the generic equation on a single species. As noted with respect to the high variability in the *Senecella* and *Limnocalanus* individual dry weights, it would be unlikely to account for this substantial variability through measuring solely *Diaptomus* samples.

Isotope Analysis – Nitrogen

Despite alterations in primary productivity and hypothesized adjustments in the community structure of Lake Huron, our data did not suggest significant changes in the trophic position of *L. macrurus*, *D. sicilis*, *E. lacustris*, and *B. longimanus* from 1993 to 2013. Our hypothesis that *L. macrurus* had improved ecological efficiency from a direct trophic position shift, or an indirect trophic position shift in *D. sicilis*, was not supported by our results, which displayed consistent $\delta^{15}\text{N}$ levels for *L. macrurus* ($P=0.14$) and *D. sicilis* ($P=0.49$). Hypotheses regarding the success of *Limnocalanus* were based on limited data (three samples differing in both time and place) which as noted by Jackson et al. (2013), may not have accurately represented the entirety of Lake Huron. Indeed, the limited scope of that original study is what gave impetus to this one. Our results suggest instead that there are disparities among stations, but that there was limited change in $\delta^{15}\text{N}$ from 1993 to 2013. The 2009 estimate for *Limnocalanus* $\delta^{15}\text{N}$ at station NC3 was 2.68‰ lower than that measured at station MW2 during the same year.

We find no evidence of progressive shifts in trophic positions despite evidence of top-down and bottom-up influences mediated by invasive benthic filter-feeders and nutrients influencing primary productivity. The interactions of invasive quagga and zebra mussels in combination with altered nutrient inputs do not seem to have changed the fundamental trophic structure of the zooplankton taxa that we examined in Lake Huron. Importantly, this study establishes a baseline against which future changes can be measured, especially in an era of increasing globalization and anthropogenic introduction of invasive species. Presently, the Great Lakes are at risk of infiltration by other invasive species, including the golden mussel (Oliveira et al., 2010) and Asian carp (Cooke et al., 2010). Ecologically, the introduction of another benthic filter-feeder or a piscivore may trigger shifts from present trophic position stability, and alter planktivorous fish densities in the Great Lakes.

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Appendix

Table 1. Original (MW4, ME1) and previously published analyses of mean $\delta^{15}\text{N}$, calculated per station and taxon. New samples are in addition to stations H12 (27-Aug-93), H8 (23-Aug-95), NC3 (20-Sep-09) published by Jackson et al. (2013).

| Date | Station | Taxon | N | $\delta^{15}\text{N}$ (SE) | $\Delta \delta^{15}\text{N}$ Herbivore (SE) | 95% CI |
|-----------|---------|--------------------------|---|----------------------------|---|-----------|
| 27 Aug 93 | H6 | <i>Daphnia</i> | 3 | 2.01 (0.13) | | |
| | | <i>Epischura</i> | 3 | 5.09 (0.13) | 3.08 (0.18) | 2.83-3.33 |
| | | <i>Diaptomus</i> | 4 | 5.60 (0.24) | 3.59 (0.27) | 3.12-4.06 |
| | | <i>Bythotrephes</i> | 3 | 6.59 (0.04) | 4.59 (0.14) | 4.50-4.66 |
| | | <i>Limnocalanus</i> | 3 | 8.97 (0.19) | 6.96 (0.23) | 6.59-7.33 |
| 27 Aug 93 | H12 | <i>Daphnia</i> | 3 | 3.03 (0.01) | | |
| | | <i>Epischura</i> | 1 | 6.30 (NA) | 3.27 (NA) | NA |
| | | <i>Diaptomus</i> | 3 | 5.77 (0.51) | 2.75 (0.51) | 4.78-6.77 |
| | | <i>Bythotrephes</i> | 3 | 7.38 (0.17) | 4.35 (0.17) | 4.02-4.69 |
| | | <i>Limnocalanus</i> | 3 | 10.29 (0.10) | 7.26 (0.10) | 7.03-7.49 |
| 25 Aug 95 | H2 | <i>Daphnia</i> | 3 | 2.80 (0.08) | | |
| | | <i>Epischura</i> | 3 | 4.19 (0.04) | 1.39 (0.09) | 1.31-1.47 |
| | | <i>Diaptomus</i> | 3 | 5.98 (0.20) | 3.18 (0.21) | 2.79-3.57 |
| | | <i>Bythotrephes</i> | 3 | 6.04 (0.24) | 3.23 (0.25) | 2.76-3.70 |
| | | <i>Limnocalanus</i> | 3 | 8.25 (0.13) | 5.45 (0.15) | 5.20-5.70 |
| 27-Aug 95 | H8 | <i>Daphnia</i> | 3 | 2.41 (0.05) | | |
| | | <i>Epischura</i> | 3 | 4.27 (0.16) | 1.86 (0.17) | 3.96-4.58 |
| | | <i>Diaptomus</i> | 3 | 5.66 (0.46) | 3.25 (0.47) | 2.34-4.15 |
| | | <i>Bythotrephes</i> | 3 | 6.07 (0.07) | 3.66 (0.09) | 3.51-3.80 |
| | | <i>Limnocalanus</i> | 3 | 9.24 (0.07) | 6.83 (0.08) | 9.11-9.38 |
| 26 Aug 95 | H4 | <i>Daphnia</i> | 3 | 2.60 (0.13) | | |
| | | <i>Epischura</i> | 3 | 5.69 (0.01) | 3.09 (0.13) | 3.07-3.11 |
| | | <i>Diaptomus</i> | 3 | 6.09 (0.04) | 3.49 (0.14) | 3.41-3.57 |
| | | <i>Bythotrephes</i> | 3 | 6.31 (0.18) | 3.71 (0.22) | 3.41-3.57 |
| | | <i>Limnocalanus</i> | 3 | 9.20 (0.08) | 6.60 (0.15) | 6.44-6.76 |
| 28 Aug 95 | H12 | <i>Daphnia</i> | 3 | 2.10 (0.11) | | |
| | | <i>Epischura</i> | 3 | 4.47 (0.13) | 2.37 (0.17) | 2.12-2.62 |
| | | <i>Diaptomus</i> | 3 | 6.10 (0.10) | 4.00 (0.15) | 3.80-4.20 |
| | | <i>Bythotrephes</i> | 3 | 5.87 (0.16) | 3.77 (0.19) | 3.46-4.08 |
| | | <i>Limnocalanus</i> | 2 | 9.37 (0.06) | 7.27 (0.13) | 7.15-7.39 |
| 25 Jul 97 | H6 | <i>Daphnia</i> | 3 | 1.22 (0.07) | | |
| | | <i>Epischura</i> | 3 | 5.14 (0.18) | 3.92 (0.19) | 3.57-4.27 |
| | | <i>Diaptomus</i> | 3 | 5.80 (0.21) | 4.58 (0.22) | 4.17-4.99 |
| | | <i>Limnocalanus</i> | 3 | 8.80 (0.04) | 7.58 (0.08) | 7.50-7.66 |
| 20 Sep 09 | NC3 | <i>Holopedium</i> | 3 | 5.32 (0.06) | | |
| | | <i>Epischura</i> | 3 | 7.89 (0.03) | 2.57 (0.07) | 2.51-2.62 |
| | | <i>Diaptomus</i> | 3 | 7.26 (0.16) | 1.94 (0.17) | 1.63-2.25 |
| | | <i>Bythotrephes</i> | 3 | 8.57 (0.21) | 3.25 (0.22) | 2.83-3.66 |
| | | <i>Limnocalanus</i> | 3 | 11.08 (0.09) | 5.76 (0.11) | 5.58-5.94 |
| 22 Sep 09 | GB3 | <i>Holopedium</i> | 3 | 3.08 (0.01) | | |
| | | <i>Epischura</i> | 3 | 5.62 (0.14) | 2.54 (0.14) | 2.27-2.81 |
| | | <i>Diaptomus</i> | 3 | 6.41 (0.01) | 3.33 (0.01) | 3.31-3.35 |
| | | <i>Bythotrephes</i> | 3 | 6.24 (0.05) | 3.16 (0.05) | 3.06-3.26 |

| | | | | | | |
|-----------|-----|-----------------------|---|--------------|-------------|------------|
| 9 Oct 09 | MW2 | <i>Limnocalanus</i> | 3 | 9.12 (0.33) | 6.04 (0.33) | 5.39-6.69 |
| | | <i>Daphnia</i> | 3 | 2.17 (0.16) | | |
| | | <i>Holopedium</i> | 3 | 3.74 (0.19) | 1.57 (0.25) | 1.20-1.94 |
| | | <i>Epischura</i> | 3 | 5.86 (0.17) | 3.69 (0.23) | 3.36-4.02 |
| | | <i>Diaptomus</i> | 3 | 6.22 (0.10) | 4.05 (0.19) | 3.85-4.25 |
| | | <i>Bythotrephes</i> | 3 | 6.85 (0.02) | 4.68 (0.16) | 4.64-4.72 |
| 17 Sep 13 | MW4 | <i>Limnocalanus</i> | 3 | 10.61 (0.29) | 8.44 (0.33) | 7.87-9.01 |
| | | <i>Daphnia</i> | 3 | 2.03 (0.03) | | |
| | | <i>Epischura</i> | 3 | 5.32 (0.02) | 3.28 (0.04) | 3.25-3.33 |
| | | <i>Diaptomus</i> | 3 | 6.70 (0.05) | 4.67 (0.06) | 4.57-4.77 |
| | | <i>Bythotrephes</i> | 3 | 7.34 (0.15) | 5.31 (0.15) | 5.02-5.60 |
| | | <i>Limnocalanus</i> | 3 | 11.67 (0.26) | 9.64 (0.26) | 9.13-10.15 |
| 30 Sep 13 | ME1 | <i>Daphnia</i> | 3 | 1.79 (0.03) | | |
| | | <i>Epischura</i> | 3 | 4.69 (0.02) | 2.90 (0.04) | 2.86-2.94 |
| | | <i>Diaptomus</i> | 3 | 6.16 (0.04) | 4.37 (0.05) | 4.29-4.45 |
| | | <i>Bythotrephes</i> | 4 | 6.31 (0.04) | 4.52 (0.05) | 4.44-4.60 |
| | | <i>Limnocalanus</i> | 3 | 10.37 (0.10) | 8.58 (0.10) | 8.38-8.87 |
