

Assessing the utility of C:N ratios for predicting lipid content in fishes

Kelly-Anne Fagan, Marten A. Koops, Michael T. Arts, and Michael Power

Abstract: Numerous researchers have attempted to find suitable proxies for the lipid content of fishes. Owing to the high carbon content of lipids, C:N ratios have been used as a predictor of lipid content both for the purposes of quantifying condition and for stable isotope analyses. Here we examine the utility of C:N ratios for predicting the lipid content within and among populations, and to validate commonly used published percent lipid – C:N ratio models. No common percent lipid – C:N ratio model was found to apply; instead, population-specific influences on lipid content were observed. Published lipid prediction models significantly underestimated lipid content, and often had worse prediction error than the error obtained by using measured mean lipids as the prediction for all samples. Maximum prediction error by population ranged from a low of 50.7% to a high of 65.0%. Our results provide no support for the idea that there is a predictable relationship between bulk C:N ratios and lipid content. We recommend that sample-specific relationships be developed in situations where lipid prediction is needed, rather than relying on published models.

Résumé : De nombreux chercheurs ont essayé de trouver des variables de remplacement adéquates pour le contenu lipidique des poissons. À cause du fort contenu en carbone des lipides, les rapports C :N ont servi à prédire le contenu lipidique, tant pour la mesure de la condition que pour les analyses d'isotopes stables. Nous examinons ici l'utilité des rapports C :N pour la prédiction du contenu lipidique au sein des populations et entre les populations afin de valider les modèles % de lipides - rapport C :N les plus communément utilisés dans la littérature. Aucun des modèles communs % de lipides - rapport C :N n'est applicable; au contraire, on observe des influences spécifiques aux populations sur le contenu lipidique. Les modèles de prédiction des lipides dans la littérature sous-estiment significativement le contenu lipidique et souvent ils ont une erreur de prédiction plus importante que celle obtenue en utilisant la mesure des lipides moyens comme base de prédiction pour tous les échantillons. L'erreur de prédiction maximale par population varie d'un minimum de 50,7 % à un maximum de 65,0 %. Nos résultats n'appuient en aucune façon la proposition qu'il existe une relation prédictive entre les rapports globaux de C :N et le contenu lipidique. Nous recommandons d'établir des relations spécifiques aux échantillons pour obtenir des prédictions des lipides plutôt que de se fier aux modèles publiés.

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Introduction

Fish condition is a commonly used metric of fish health (Brown and Murphy 1991; Pangle and Sutton 2005; Hartman and Margraf 2006). Some of the factors that contribute to fish condition include gross nutritional status (Love 1970) and the level of energy reserves, particularly fat, present in the body (Gershanovich et al. 1984). Methods used to assess

fish condition include various indices (e.g., length–weight indices, hepato-somatic index) and proximate composition analyses (i.e., percent lipid and protein) (e.g., Pothoven et al. 2006; Rennie and Verdon 2008; Todd et al. 2008). The inaccuracy of length–weight based indices, and the time and expense involved in acquiring data for proximate analyses, have favored increased reliance on proxy measures of condition such as elemental bulk tissue carbon to nitrogen (C:N)

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K. Fagan and M. Power.¹ Department of Biology, University of Waterloo, 200 University Avenue West, Waterloo, ON N2L 3G1, Canada.

M.A. Koops. Great Lakes Laboratory for Fisheries and Aquatic Sciences, Fisheries and Oceans Canada, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada.

M.T. Arts. National Water Research Institute, Environment Canada, PO Box 5050, 867 Lakeshore Road, Burlington, ON L7R 4A6, Canada.

¹Corresponding author (e-mail: m3power@sciborg.uwaterloo.ca).

ratios (e.g., Sweeting et al. 2006; Al-Habsi et al. 2008; Dempson et al. 2010). The use of C:N ratios relies on the assumption that an increase in tissue total lipid concentrations correlates with increases in C:N ratios, since lipid contains mostly carbon and little-to-no nitrogen (Barnes et al. 2007). Therefore, all else being equal, individuals in better condition (higher lipid content) could be expected to exhibit higher C:N ratios.

The C:N ratio has also become a key element in mathematically based corrective procedures designed to remove the influence of lipids on bulk tissue $\delta^{13}\text{C}$ measurements. The increase in $\delta^{13}\text{C}$ at each trophic transfer is related to the fraction of assimilated diet that is respired, with the implicit recognition that variation among consumers will be affected by differences in food quality and (or) sample preparation (DeNiro and Epstein 1978; McCutchan et al. 2003). Predictable changes in $\delta^{13}\text{C}$ between predator and prey thus provide insights into aspects of ecosystem carbon cycling, provided that isotope changes arising from other causes, such as lipid storage, are appropriately factored into the inferences (McConnaughey and McRoy 1979). Lipid $\delta^{13}\text{C}$ values are typically more negative relative to other classes of biochemical compounds as a result of selectivity for the ^{12}C isotope associated with the conversion of pyruvate to acetyl coenzyme A during lipid synthesis (DeNiro and Epstein 1977; McConnaughey and McRoy 1979). In addition to adjusting for lipid content, mathematical correction has the further advantage of reducing analytical costs by eliminating the need to analyze pre- and post-extraction samples to obtain unbiased $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ measures (e.g., Kelly et al. 2006; Sweeting et al. 2006; Logan and Lutcavage 2008).

Despite the fact that lipids are a major component of energy flow in foodwebs (Arts et al. 2009), lipid removal is often suggested as a solution to avoid confusion between lipid-caused isotope variability and a habitat or dietary shift (e.g., Post et al. 2007; Logan et al. 2008). Published lipid correction models are typically based on the observed positive relationship between multi-taxa lipid content and C:N ratio data (McConnaughey and McRoy 1979; Post et al. 2007; Logan et al. 2008). For example, the corrective model developed by Post et al. (2007) was based on a wide range of aquatic species including fishes, stoneflies, unionids, and copepods. Similarly, Logan et al. (2008) and Kiljunen et al. (2006) used a wide variety of taxa in the development of their corrective models. Although statistically validated at the estimation stage (e.g., Kiljunen et al. 2006) the reported in-sample goodness-of-fit statistics of the proposed models do not guarantee correct predictions beyond the original dataset(s) (Power 1993). This principle has been recognized for some time in the statistical literature (e.g., Montgomery and Peck 1982) and justifies the use of additional measures designed specifically to evaluate the overall utility of predictive models.

Collecting new data and testing the predictive powers of proposed models against new data is clearly the most robust procedure for evaluating model predictive capabilities (Power 1993). The approach increases user confidence and helps ensure that model results are effectively and appropriately used. Most lipid correction models proposed to date, however, have not been subjected to rigorous testing and, in

the absence of such testing, their accuracy, and hence utility, remain questionable. Mintenbeck et al. (2008) tested the proposed lipid normalization and correction models of McConnaughey and McRoy (1979), Kiljunen et al. (2006), Sweeting et al. (2006), and Post et al. (2007), for application to two north-eastern Weddell Sea (Antarctica) notothenoid species, and found that all models produced biased estimates of fish tissue $\delta^{13}\text{C}$. Similarly, Kelly et al. (2006) noted that species-specific and intra-specific variation in lipid composition could make it difficult to apply generic correction factors in stable isotope studies.

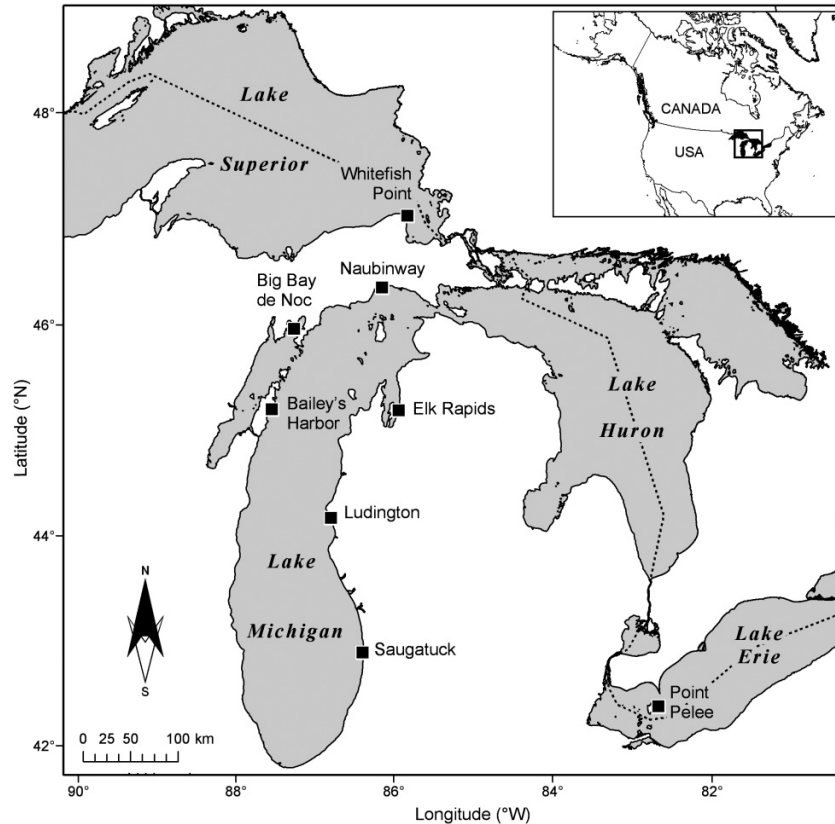
For fishes, in particular, there is substantial variation in the lipid content and composition of different tissues (Henderson and Tocher 1987), and this variation likely has significant implications for observed intra- and inter-population and species variability in $\delta^{13}\text{C}$ signatures and C:N ratios (Kelly et al. 2006). In general, patterns of lipid storage in fishes reflect life history requirements; accumulation during feeding periods and depletion during transitional (e.g., smoltification) and non-feeding periods (Sheridan 1994). Lipids can also vary within the same species by season (both intra and inter-annually, Wagner et al. 2010), habitat (Dempson et al. 2004), life-stage, and size (Shearer et al. 1994). Such species and life-stage dependent variations in lipid content and composition may require the development of species or population-specific correction factors. This is evidenced by the existing array of published lipid corrective and percent lipid – C:N ratio models (McConnaughey and McRoy 1979; Post et al. 2007; Logan et al. 2008). Differences that can occur within and among species from changes in life-stage or dietary-based protein and carbohydrate anabolism and catabolism further complicate our understanding of variation in carbon content. Finally, there is the need to consider the biochemical processes that determine tissue nitrogen composition, which can also vary with species, season, gender, and diet. For example, tissue nitrogen varies with protein and nitrogenous non-protein substances such as nucleotides, free amino acids, ammonia, and urea (Moyle and Cech 1988). Considered together, these factors suggest that predictive models driven by C:N ratios derived from other studies, especially when the ratios stem from multi-taxa analyses, may poorly characterize species or population-specific lipid content patterns.

In view of the above, this study used data from multiple populations of a single species, lake whitefish (*Coregonus clupeaformis*), sampled during the same season and at a similar life-stage in lakes Superior, Michigan, and Erie, to determine (i) if there was a statistically significant relationship between the C:N ratio determined from stable isotope analysis and measured lipid content (percent lipid – C:N ratio model); (ii) the applicability of a common percent lipid – C:N ratio model to all lake whitefish populations sampled in our study; and (iii) the predictive validity of published percent lipid – C:N ratio models for correctly inferring the lipid content of lake whitefish populations sampled in our study.

Materials and methods

Adult lake whitefish were sampled at six sites around Lake Michigan and at one site in each of lakes Superior

Fig. 1. Map of sampling locations for the eight populations of lake whitefish (*Coregonus clupeaformis*) populations used in this study.



and Erie (Fig. 1). Lake whitefish collected at sample sites were considered to belong to separate spawning populations for the purpose of this and other co-occurring research. Fish were collected between October and December in 2004 and 2005, using either commercial trap nets with 40 cm stretched mesh and a pot composed of 11.4 cm stretched mesh, or gill net sets with stretched mesh sizes ranging from 4 cm to 15 cm. At each site, up to 30 pre-ovulatory females and 30 ripe males were collected. Fish ranged in total length from 37.5 cm to 75.0 cm and in age from 3 to 15 years as determined using scales defined and identified by Muir et al. (2008). Paired skinless, boneless muscle plugs (1 cm × 2 cm) were collected from landmarked positions from either side of the dorsal fin of each fish and frozen (−85 °C) for later C:N ratio and lipid analyses. As lipid and stable isotope measurements are known to vary spatially (e.g., ventral, dorsal, and caudal areas) within a fish, but to be statistically equivalent within tissue types (e.g., left and right dorsal fillet) (Persson et al. 2007), landmarking was used to control for possible confounding factors resulting from known spatial variation in both lipid and stable isotope measurements.

C:N ratio analysis

The first muscle plug was dried at 50 °C for 48 h and pulverized to an homogenate with a Retsch MM 301 ball mill grinder. Approximately 0.3 mg of prepared material was used for stable isotope analysis (SIA) completed with a Delta Plus Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled

to a Carlo Erba elemental analyzer (CHNS-O EA1108, Carlo Erba, Milan, Italy) with an analytical precision of $\pm 0.2\text{‰}$ ($\delta^{13}\text{C}$) and $\pm 0.3\text{‰}$ ($\delta^{15}\text{N}$) at the Environmental Isotope Laboratory, University of Waterloo (Waterloo, Ontario). Nitrogen and carbon compositions used for computation of C:N ratio data were calculated based on Carlo Erba Elemental Standards B2005, B2035, and B2036, with an error of < 1%. Sample weights necessary for percentage composition computations were obtained from a high precision ultra microbalance (Model XP2U, Mettler-Toledo GmbH, Greifensee, Switzerland). All results were corrected to internal laboratory standards calibrated against the international standards defined for carbon (Craig 1957) and nitrogen (Mariotti 1983). Internal laboratory standards included the ammonium sulphate IAEA-N1 and IAEA-N2 standards for nitrogen and IAEA-CH6 (sugar), EIL-72 (cellulose), and EIL-32 (graphite) for carbon. NIST organic materials, e.g., bovine liver, were additionally used to crosscheck all percentage composition results. A 1-in-10 repeat protocol was used, with repeat analysis indicating a precision ($n = 80$) of 0.49% and 0.33%, respectively, for carbon and nitrogen percent composition calculations.

Lipid analysis

The second muscle plug was freeze-dried and used for total lipids analysis following Folch et al. (1957) at the National Water Research Institute, Environment Canada (Burlington, Ont.). The selected method is known to yield reliable estimates of percent lipids across a broad range of lipid content values (Iverson et al. 2001) and, unlike the

rapid extraction method of Bligh and Dyer (1959), does not significantly underestimate lipid content from samples containing >2% lipid (Iverson et al. 2001). Approximately 31 mg of dry muscle tissue were extracted in triplicate by grinding freeze-dried materials in 2 mL of 2:1 (v/v) chloroform–methanol (Folch et al. 1957) and the resulting three extracts (i.e., 6 mL) thus obtained were combined in a 15 mL, acid-washed, centrifuge tube. After centrifugation (4000 rpm), to remove non-lipid containing material, the supernatant was evaporated to ~2 mL and then transferred to a 2 mL Kuderna-Danish receiving vial (Sigma No. 6-4689U) so that evaporation to a final volume of 2 mL could be achieved with accuracy. From this final volume, duplicate 200 µL aliquots of sample extracts were pipetted into pre-weighed, seamless, tin cups (Elemental Microanalyses Ltd., catalogue No. D4057). The solvent was then evaporated at room temperature and the remaining lipid weighed on a Sartorius ME-5 microbalance to provide a gravimetric measure of percent of dry weight of total lipid content. In all cases, maintenance of a high solvent-to-sample ratio and multiple extractions were used to ensure measurement accuracy and that extraction efficiency would not be limited by saturation of the lipids with respect to solvent volume (Iverson et al. 2001).

Model testing

Variation in obtained carbon and nitrogen percentage values was characterized using the coefficient of variation (CV) and among-population differences in mean C:N ratios were assessed using analysis of variance (ANOVA) followed with the Tukey–Kramer post-hoc honestly significant difference (HSD) test (Zar 1999). Measured lipid (%) and C:N ratios data were used to fit literature described log-linear (McConnaughey and McRoy 1979) and nonlinear (Kiljunen et al. 2006) models for all individual populations. Residual testing was then used to assess the statistical integrity of all estimated models as recommended for linear regression (e.g., Draper and Smith 1981) and nonlinear regression (e.g., Bates and Watts 1988). The applicability of a common slope model as a description of the percent lipid – C:N ratio relationship for all populations was assessed using analysis of covariance (ANCOVA; Zar 1999). Nonlinear models were similarly assessed for the applicability of a common model using analysis of residual sums of squares (Chen et al. 1992; Haddon 2001).

The validity of proposed lipid prediction models was assessed for lake whitefish using four published percent lipid – C:N ratio models. Three models (McConnaughey and McRoy 1979; Post et al. 2007; Logan et al. 2008) were developed from multi-species data sets that included fishes. The fourth (Bodin et al. 2007) was developed for a single marine decapod, the spider crab *Maja brachydactyla*. Three of the models (Bodin et al. 2007; Post et al. 2007; Logan et al. 2008) proposed linear relationships between percent lipid and the C:N ratio. The fourth model (McConnaughey and McRoy 1979), also used in Kiljunen et al. (2006), proposed a nonlinear relationship between the variables. All models were evaluated by comparing predicted lipid content to measured values obtained via chemical extraction (i.e., percent lipid on a dry weight basis) as described above.

The normalization model of McConnaughey and McRoy

(1979) was derived by McConnaughey (1978). The model includes two equations, one equation to predict lipid content (%) in muscle tissue as a function of the C:N ratio and a second equation to predict a lipid corrected $\delta^{13}\text{C}$ signature based on the calculated lipid content. The first of these equations is validated in this study, where L and C:N, respectively, are the predicted sample ash-free dry weight lipid content and the measured C:N ratio:

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

The derivation of this model assumes that (i) lipid, protein, and carbohydrate are the only major constituents of biomass, (ii) protein is the only nitrogenous component of biomass, and (iii) protein plus lipid content is constant at 93% (McConnaughey 1978).

The second model, proposed by Post et al. (2007), was based on a variety of aquatic animals, including both freshwater and marine fishes and invertebrates:

$$(2) \quad L = -20.54 + 7.24C : N$$

The lipid values used by Post et al. (2007) were obtained from dried muscle tissue extracted using a 50:50 chloroform–methanol mixture following Folch et al. (1957) as revised by Post and Parkinson (2001) and Arrington et al. (2006).

The third model tested was proposed by Logan et al. (2008), based on an assemblage of freshwater Australian fishes including silver tanderan *Porochilus argenteus*, bony bream *Nematalosa erebi*, spangled perch *Leiopotherapon unicolor*, and golden perch *Macquaria ambigua*:

$$(3) \quad L = -6.56 + 2.47C : N$$

Tissue percent lipid measures on a dry weight basis were determined via a modified Bligh and Dyer (1959) method and bulk C:N ratios were obtained from SIA.

The fourth model (Bodin et al. 2007) relied on percent lipid data from muscle, gonad, and hepatopancreas tissue obtained with a derived Soxhlet method (Manirakiza et al. 2001; Schlechtriem et al. 2003) that used an 80:20 hexane–acetone mixture. The percent lipid vs. C:N model was derived from a set of two equations describing the relationship between $\delta^{13}\text{C}$ values and the C:N ratio and lipid corrected and uncorrected $\delta^{13}\text{C}$ values (see Bodin et al. 2007) as follows:

$$(4) \quad L = \frac{0.322C : N - 1.175}{0.0588}$$

The Bodin et al. (2007) model was chosen specifically for comparative purposes because it was not created for use with fish and, therefore, intuitively cannot be expected to predict well in comparison to models estimated for use with fish fauna.

The predictive ability of each model was assessed using mean absolute error (MAE), mean absolute percent error (MA%E), and paired *t* tests to determine the statistical significance of differences in model predicted and actual lipid content (Power 1993). MAE and MA%E are measures of predictive accuracy. Models with smaller MAE and MA%E are preferred over larger values. Linear regression was used

Table 1. Lake whitefish skinless dorsal muscle percent of dry weight total percent lipid and stable isotope analysis measured C:N ratio (mean \pm CV), associated elemental carbon and nitrogen CV and mean stable isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$).

Site	<i>n</i>	Percent lipid	C:N	Carbon CV	Nitrogen CV	$\delta^{13}\text{C}$	$\delta^{15}\text{N}$
Lake Michigan							
Bailey's Harbor	111	10.53 \pm 0.58b,c	3.80 \pm 0.12b,c,d	8.68	11.81	-24.67	10.64
Big Bay de Noc	118	14.02 \pm 0.64b	3.76 \pm 0.09b,c,d	5.40	6.24	-24.93	10.51
Naubinway	119	8.81 \pm 0.49c	3.45 \pm 0.08e	6.00	4.73	-20.89	10.15
Elk Rapids	117	8.42 \pm 0.51c	3.64 \pm 0.11c,d,e	6.78	9.03	-22.82	11.73
Saugatuck	89	10.01 \pm 0.60c	3.69 \pm 0.12d,e	10.82	13.33	-24.80	11.66
Ludington	59	11.38 \pm 0.39b,c	3.92 \pm 0.14b,c	11.97	16.37	-25.95	11.45
Lake Superior							
Whitefish Point	106	13.79 \pm 0.70b	3.97 \pm 0.14b	8.51	11.12	-20.28	5.95
Lake Erie							
Point Pelee	107	36.69 \pm 0.51a	5.53 \pm 0.31a	7.71	19.86	-24.94	15.69

Note: Values followed by the same letter are not significantly different ($P > 0.05$) as established using Tukey's post-hoc honestly significant different (HSD) test.

to test for significant relationships between actual and predicted lipid values, and to determine whether individual regression intercepts and slopes statistically approximated zero and unity, respectively, as would be expected under the perfect fit null hypothesis (Zar 1999). All linear regressions were further assessed for statistical adequacy using residual testing to ensure conformance with the underlying assumptions of regression analysis (Sokal and Rohlf 1995). Finally, modeling efficiency (EF), a dimensionless statistic that relates model predictions to observed data (Loague and Green 1991),

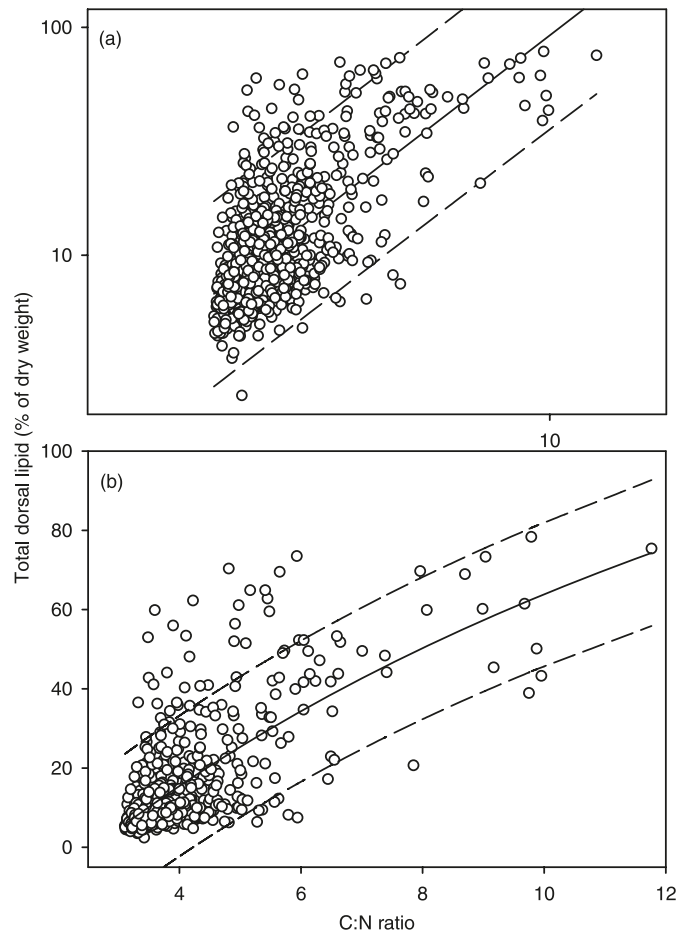
$$(5) \quad EF = 1 - \frac{\sum_{i=1}^n (y_{ai} - y_{pi})^2}{\sum_{i=1}^n (y_{ai} - y_{am})^2}$$

was used to assess model predictions where y_{ai} and y_{pi} are the actual and predicted lipid values of the i th sample and y_{am} is the measured mean lipid value. An EF of 1 indicates perfect prediction, while an EF < 0 indicates that prediction error using the model is worse than the error that would be obtained by simply using the observed mean as the prediction for all samples (Loague and Green 1991). Maximal type I error in all statistical tests was set at $\alpha = 0.05$.

Results

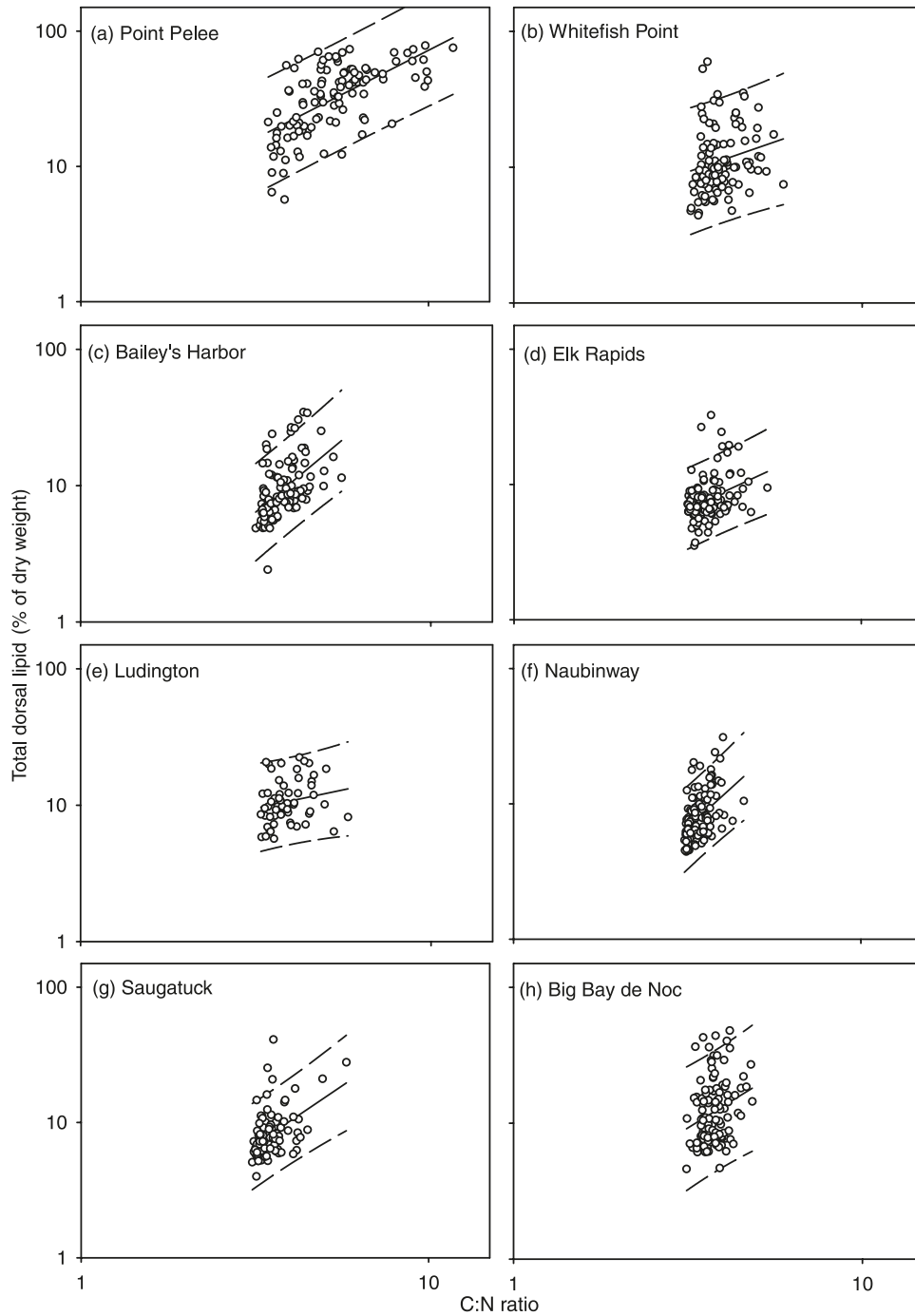
Significant differences existed among lake whitefish populations with respect to mean C:N ratios (ANOVA $P < 0.001$) as identified by the Tukey-Kramer post-hoc HSD test ($P < 0.05$, Table 1). With the exception of Naubinway lake whitefish, variation in elemental nitrogen was greater than variation in elemental carbon in skinless, boneless, dorsal muscle tissue. On average, lake whitefish muscle tissues from the Point Pelee specimens were more lipid rich (mean = 36.7%) than any other population. There was no relationship between body size and the C:N ratio when all population data were combined ($r^2 = 0.09$), and only weak evidence for a relationship in the population subsets, with r^2 values for the body size - C:N ratio relationships ranging from a high of 0.38 to a low of 0.01 (mean among the eight populations = 0.12). Similarly, no relationship was found for

Fig. 2. Skinless, boneless, dorsal muscle tissue samples from lake whitefish (all populations combined; $n = 826$) analyzed with (a) a log transformed linear regression and 95% prediction intervals, and (b) the nonlinear model form used by McConnaughey and McRoy (1979) and 95% prediction intervals.



regression of percent lipids on body size for the data set as a whole ($r^2 = 0.03$) or for seven of eight population subsets ($r^2 \leq 0.06$). The exception was the Point Pelee population where evidence of a correlation between percent lipids and body size was found ($r^2 = 0.21$).

Fig. 3. Log transformed linear regressions and 95% prediction intervals measured from skinless, boneless, dorsal muscle tissue samples from individual lake whitefish, by population.



Linear, log linear, and nonlinear regression of lake whitefish laboratory measured percent lipid on SIA-determined C:N ratio data resulted in models with poor explanatory power, whether the relationship was estimated using data from all populations ($r^2 = 0.49$) or if data were separated by population (all $r^2 \leq 0.40$; Figs. 2 and 3; Table 2). With the exception of the combined data model and Point Pelee population model, all linear percent lipid – C:N ratio regression model residuals were non-normal and heteroscedastic. The log percent lipid vs. log C:N ratio regression model residuals were normal for the combined data model only. When

using nonlinear regression, only the Point Pelee model residuals met basic statistical test requirements. Thus, in addition to having poor explanatory power, linear, log linear, and nonlinear regression models failed, in most instances, to meet basic tests of statistical adequacy (e.g., Draper and Smith 1981; Bates and Watts 1988).

For the linear models, ANCOVA indicated both C:N ratio ($F_{[1,817]} = 216.06$, $P < 0.001$) and population ($F_{[7,817]} = 32.46$, $P < 0.001$) significantly influenced percent lipids and that no common slope model applied to the populations ($F_{[7,810]} = 2.56$, $P = 0.013$). An ANCOVA for the log linear

Table 2. Range of measured percent lipid and percent explained variation (r^2) by study site for tested linear, log-linear and nonlinear models relating percent lipids to elemental C:N ratios.

Site	Lipid range (%)	Linear r^2	Log-linear r^2	Nonlinear r^2
All sites combined	2.42–78.37	0.49**	0.45**	0.49**
Lake Michigan				
Bailey's Harbor	2.42–34.67	0.19**	0.27**	0.21 *
Big Bay de Noc	4.60–48.11	0.04 *	0.06 *	0.04ns
Naubinway	4.43–31.07	0.17**	0.20**	0.18 *
Elk Rapids	3.53–32.62	0.07 *	0.12**	0.09ns
Saugatuck	3.40–41.10	0.18**	0.21**	0.18ns
Ludington	5.69–22.44	0.03ns	0.04ns	0.05ns
Lake Superior				
Whitefish Point	4.42–59.85	0.01ns	0.04 *	0.03ns
Lake Erie				
Point Pelee	5.70–78.37	0.35**	0.39**	0.40**

Note: ns, not significant; *, represents significant models where $P < 0.05$; and **, represents significance where $P < 0.001$.

Table 3. Statistical measures of validation using all population data combined, including: mean absolute error (MAE), mean absolute percent error (MA%E), linear regression coefficients (slope, intercept), and associated diagnostic statistics (r^2 , F) and modelling efficiency (EF).

Model	Deviance measures		Linear regression				
	MAE	MA%E	Slope	Intercept	r^2	F	EF
McConnaughey and McRoy (1979)	6.64	55.87	0.58	4.98	0.47	716.33	0.43
Post et al. (2007)	6.93	42.87	0.38	2.60	0.49	781.23	0.23
Bodin et al. (2007)	12.56	97.67	0.29	-2.48	0.49	781.23	-0.57
Logan et al. (2008)	10.95	72.48	0.13	1.35	0.49	781.23	-0.52

Note: Results computed by comparing percent lipid predictions using each model with laboratory measured values. All linear regressions were significant ($P < 0.001$) and significantly different from the 1:1 line (see Fig. 4). Loague and Green's (1991) modelling efficiency statistic was calculated for each model to assess predictive capacity.

model also indicated both log C:N ratio ($F_{[1,817]} = 164.06$, $P < 0.001$) and population ($F_{[7,817]} = 26.81$, $P < 0.001$) significantly influenced log percent lipids and that no common slope model applied to the populations ($F_{[7,810]} = 2.18$, $P = 0.034$). For nonlinear models, analysis of residual sums of squares indicated that the population-specific models varied significantly from one another ($F_{[21,802]} = 7.02$, $P < 0.001$).

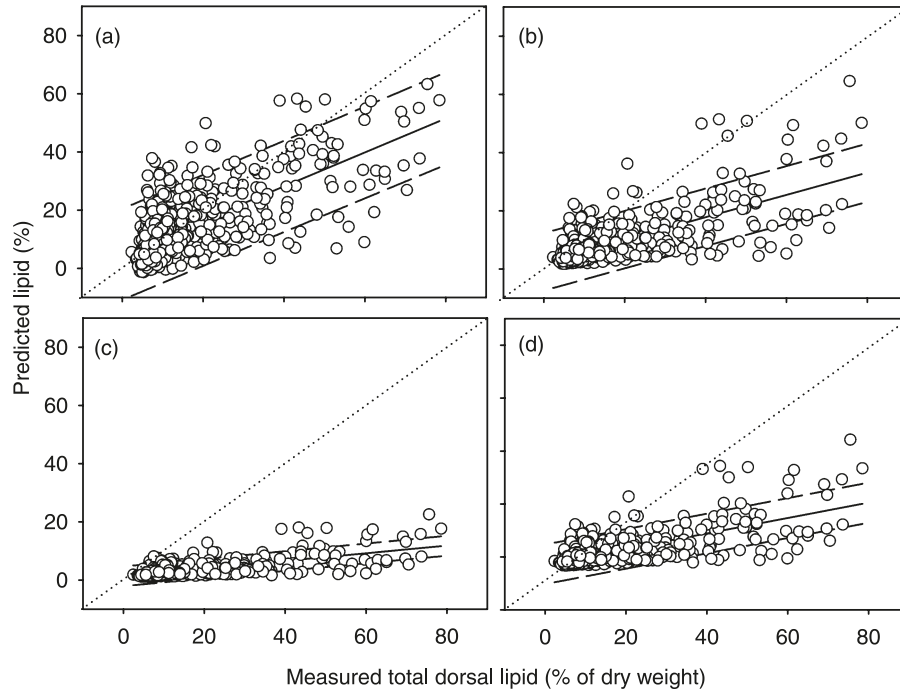
The analyses of measured versus predicted lipids yielded significant regression models (all: $r^2 \geq 0.47$, $P < 0.001$) for each of the tested lipid prediction models (Table 3). However, all the estimated regression slopes differed significantly (all t test $P < 0.001$) from the hypothetical 1:1 line with the size of the prediction error positively related to percent lipid content (Fig. 4). In addition, all estimated regression intercepts differed significantly from zero (all t test $P < 0.001$). When compared with the 1:1 perfect fit line, most models under-predicted lipid content at high lipid values and over-predicted observed lipid content at low lipid values. Thresholds for shifts between over-prediction and under-prediction, respectively, for the McConnaughey and McRoy (1979), Post et al. (2007), Logan et al. (2008), and Bodin et al. (2007) models were: 11.9%, 4.2%, 1.6%, and 0.0%. Maximal prediction error was large, ranging from 50.7% for the McConnaughey and McRoy (1979) model to 65.0% for the Logan et al. (2008) model.

Assessment of model predictive abilities with MAE, MA%E, and modelling efficiency statistics underscored the relatively poor predictive abilities of all models (Table 3). MAE values did not approach zero, and when expressed in percentage terms (MA%E), indicated mean predictive errors in the 43%–98% range. In only two of four cases, McConnaughey and McRoy (1979) and Post et al. (2007), was EF positive. Negative values for Bodin et al. (2007) and Logan et al. (2008) indicated that better predictions of lipid content could have been achieved using the mean of the measured values. Paired t tests of measured versus predicted values further indicated significant predictive bias for all populations when subdivided by sex, with the exception of the gender-specific predictions made by the McConnaughey and McRoy (1979) model. For that model, bias was not significant for predictions made for four of eight groups of males and three of eight groups of females (Table 4).

Discussion

Variation in elemental nitrogen was greater than variation in elemental carbon for all but one assessed population of lake whitefish. Bulk C:N ratio data for Great Lakes lake whitefish were also significantly related to percent lipid content. The predictive ability of tested literature lipid models

Fig. 4. Values for skinless, boneless, dorsal muscle tissue samples of individual lake whitefish using data from all populations. Predicted values were generated from each of four published percent lipid vs. C:N ratio models (a) McConnaughey and McRoy (1979), (b) Post et al. (2007), (c) Logan et al. (2008), and (d) Bodin et al. (2007). Unbroken lines and broken lines represent the fitted relationships and 95% predictive confidence limits, respectively, and the dotted lines indicate 1:1 agreement.



for our large lake whitefish data set was poor. There was some evidence of significant population effects on observed percent lipids, but no common model was found to describe the pattern of percent lipid within or between populations. When the data were split by sampling site, no statistically adequate population-specific percent lipid – C:N ratio models were found, whether linear, log linear, or suggested non-linear model forms were used. All models had low explanatory power, with relationships varying by population, and there was no evidence for the applicability of a common model. When assessed for their ability to accurately predict measured lipid content, all published models produced large predictive errors, had substantial predictive bias and, in many cases, under-performed as predictive tools when compared to an alternative forecast model defined by the mean.

Our results contrast sharply with those reported elsewhere in the literature. Published coefficients of determination for multi-species percent lipid – C:N ratio models are high, ranging from 0.81 to 0.97 (Bodin et al. 2007; Post et al. 2007; Logan et al. 2008). However, models published to date vary in mathematical form and reported coefficient estimates. Differences in model coefficients and functional forms suggest that underlying differences in data sets, including species composition, tissue type, and life-stage affect the coefficient estimates obtained and drive the significance of relationships. In most cases, no underlying biological principle is provided to justify the choice of model form, with model selection appearing to rest solely on the fit of the estimated model. From a practical point of view, none of the suggested models accurately predict percent lipid content from bulk C:N ratio data for lake whitefish from the Great Lakes. Furthermore, the assumption that

relative variation in carbon due to changing lipid content is the only factor contributing to variation in C:N ratio is inaccurate. As reflected in this large lake whitefish dataset the relative variation of elemental nitrogen is higher than the relative variation of elemental carbon in most cases.

Although variation in percent N in lake whitefish exceeded variation in percent C, the variation in percent N observed here (mean for all populations = 11.6%) approximated that reported in other studies. For example, comparative studies have indicated that fish N content varies little (8%–11%), with a mean of ~10% (Sterner and George 2000). Inter-specific variation typically exceeds intra-specific variation (Hendrixson et al. 2007), with variations in elemental composition having been related to life-history characteristics (e.g., sex, maturity stage and size), habitat and feeding history (Hendrixson et al. 2007). Experimental studies with brown trout (*Salmo trutta*) have shown that excretory losses high in N content (ammonia and urea) vary with body size, ration, and temperature (Elliott 1976). Experiments with minnows (*Phoxinus phoxinus*) have yielded similar results, with nitrogenous excretion having been found to depend on ration and temperature (Cui and Wootton 1988). Although variation in body size and maturity status were largely controlled for by the sampling of larger and mature individuals, variation in nutritional status was nonetheless evident as exemplified by the high variability in muscle lipid content. The spatial variability inherent in sampling locations is also likely to have included significant differences in thermal regimes that will have had implications for the temperature-dependent N-relationships noted above.

Methodologically and interpretatively, it is wrong to use erroneously predicted lipid values when attempting to com-

Table 4. Two-tailed *P* values indicating significant overestimates (+) or underestimates (−) for listed lipid prediction models (matched pairs *t* test for predicted and measured percent lipids) by site and grouped data by gender.

	<i>n</i>	McConnaughey and McRoy (1979)	Post et al. (2007)	Bodin et al. (2007)	Logan et al. (2008)
Bailey's Harbor					
Male	60	0.8032	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Female	51	0.0025 ⁺	0.0003 [−]	<0.0001 [−]	<0.0001 [−]
Big Bay de Noc					
Male	59	0.5840	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Female	59	0.0023 [−]	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Naubinway					
Male	59	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Female	60	0.0008 [−]	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Elk Rapids					
Male	59	0.1914	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Female	58	0.4823	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Saugatuck					
Male	38	0.0019 [−]	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Female	51	0.9673	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Ludington					
Male	29	0.0485 ⁺	0.0033 [−]	<0.0001 [−]	<0.0001 [−]
Female	30	0.2518	0.0005 [−]	<0.0001 [−]	<0.0001 [−]
Whitefish Point					
Male	49	0.4213	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Female	57	0.0101 ⁺	0.0088 [−]	<0.0001 [−]	<0.0001 [−]
Point Pelee					
Male	50	0.0040 [−]	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Female	57	0.0053 [−]	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
All sites pooled					
Male	403	0.0051 [−]	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]
Female	423	0.1854	<0.0001 [−]	<0.0001 [−]	<0.0001 [−]

plete trophic analyses. Continued use of the results of existing models portends poorly for our ability as ecologists to eventually decipher the trophic complexity of the systems we study simply because, in many instances, we will be working with erroneous data. Model error has, in connection with the consistent under-estimation of $\Delta\delta^{13}\text{C}$, been addressed in analyses of McConnaughey and McRoy (1979), Sweeting et al. (2006), and Post et al. (2007). Model error has also been suggested by Kiljunen et al. (2006), who argued for the need to re-estimate the lipid normalization model first proposed by McConnaughey and McRoy (1979).

The utility of the C:N ratio as a proxy for lipid content, and the condition of individual fish, is based on the assumption that the C:N ratio and lipid content will vary predictably across a wide range of conditions within and among individuals and species. As our data show, the variation of percent lipid, carbon, and nitrogen of lake whitefish dorsal muscle tissue at a similar life-stage and season (i.e., mature and spawning) can be great. There is also substantial variation in lipid content among species, with species such as vendace (*Coregonus albula*), northern pike (*Esox lucius*), and yellow perch (*Perca flavescens*) having muscle lipid contents < 2% wet tissue weight, and species such as com-

mon carp (*Cyprinus carpio*), sockeye salmon (*Onchorynchus nerka*), and lake whitefish having muscle lipid contents that can exceed 10% wet tissue weight (Henderson and Tocher 1987; Kelly et al. 2006).

Seasonal fluctuations in lipid content are also known to occur in many freshwater fishes (e.g., Jonsson and Jonsson 2003; Wagner et al. 2010). For example, some lipid classes, such as triglycerides, decline during periods of low food consumption while others, such as phospholipids, remain relatively stable. Lipids are also known to vary within the same species by habitat (Dempson et al. 2004) and life-stage (Shearer et al. 1994), and lipid composition may vary by species. For example, the lipid (0.9% wet weight) of snakehead (*Channa argus*) fillets comprises 72% neutral lipid, 25% phospholipid, and 3% glycolipid, while the lipid content from common carp muscle comprises 77% neutral lipid, 21% phospholipid, and 2% glycolipid (Henderson and Tocher 1987).

Furthermore, variations in the fatty acid composition, as influenced by temperature, seasonal feeding habits, and environment (Henderson and Tocher 1987), are associated with varying isotopic signatures. For example, there are significant differences in the fatty acid composition of fresh-

water and marine fishes, with lipids in freshwater fishes typically, but not always, containing higher proportions of saturated fatty acids and C18 polyunsaturated fatty acids (PUFA) and lower levels of C20 and C22 unsaturated fatty acids than marine fishes (Henderson and Tocher 1987 and references therein). Additionally, as fatty acid composition studies of deep-sea shrimp *Rimicaris exoculata* have shown, the $\delta^{13}\text{C}$ isotopic signatures of individual fatty acids can be highly variable (Pond et al. 2000), ranging from having a coefficient of variation of 15.3% for 18:1n-9 in polar fatty acids to a coefficient of variation of 48.7% for 16:1n-7 in polar fatty acids. Thus, comparisons among individuals and tissues of differing fatty acid composition will have limited value, since differences in lipid content will influence the associated stable isotope metrics (C:N ratio and $\delta^{13}\text{C}$ signature) derived from an analysis of those tissues. Given such variability, it is important to validate whether the C:N ratio is an accurate predictor of lipids and whether the variability observed in fatty acid composition is accurately reflected using C:N ratio data.

Our results suggest that available percent lipid – C:N ratio models are not appropriate for predicting lipid content in Great Lakes' lake whitefish. The failure of models to accurately predict lipid content in such a comprehensive test does not portend well for their use with other taxa. Furthermore, even relationships developed for a single species may not be applicable across populations, as shown here and elsewhere (e.g., Syväranta and Rautio 2010). Differences among populations in habitat and diet can lead to differences in body lipid composition and the C:N ratio, requiring the estimation of population-specific relationships when attempting to infer lipids from measured C:N ratios, and then only when the appropriate statistical requirements have been met (e.g., normal, homoscedastic residuals). The low explanatory power of the percent lipid – C:N relationships for lake whitefish suggest that in many instances attempts to develop accurate predictive models will fail. Therefore, we conclude that it is advisable to avoid the use of generalized lipid prediction models. Perhaps the best solution, as has been suggested by Logan et al. (2008), is to extract a subset of samples for the estimation of a population-specific equation. In doing so, one would capture differences in species physiology and environment that inevitably contribute to the observed variation in lipid content and composition. Where statistically sound relationships exist, they could be used to reduce the effort and costs associated with completing lipid extraction on all samples. Where sound relationships cannot be estimated, duplicate analysis of extracted and unextracted samples are required to avoid the stable isotope signature biases caused by the extraction process (e.g., Kelly et al. 2006; Sweeting et al. 2006; Logan and Lutcavage 2008).

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