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Evaluating quantitative fatty acid signature analysis (QFASA) in fish using controlled feeding experiments

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Abstract

Accurate diet estimation has long been a challenging issue for researchers investigating predators due to constraints associated with stomach content analyses. Fatty acid signature analysis offers an alternative avenue to study long-term diet trends in consumers. Despite the wealth of experiments involving fatty acids of fish and their diets, few have evaluated quantitative fatty acid signature analysis (QFASA) with fish consumers. To this end we fed juvenile lake trout, round goby, and yellow perch various invertebrate species and back-classified each predator to its respective prey using only fatty acids. Estimates were highly accurate when metabolism of diets was natively accounted for by using fatty acid profiles of predators fed known diets as the "prey library". While highly accurate results were obtained, accounting for each predator-prey relationship limits the use of QFASA to predators that consume a limited number of species. We call for predator-prey specific knowledge of metabolism before attempting to use fatty acids for quantifying consumer's diets. Only after adequate incorporation of species metabolism will fatty acids provide an accurate view of individual's diets when stomach content data are not available or invalid.

Keywords: Lake trout, Yellow perch, Round goby, Fatty acid profile, Diet composition

Introduction

Accurate quantitative diet estimation of aquatic predators remains a pervasive challenge to resource managers. Stomach flushing or dissection have provided information on interspecific competition (Jacobs et al. 2010), intraspecific competition (Creque and Czesny 2012), individualistic prey selectivity (Roswell et al. 2013), and prey community change (Fagan et al. 2012). However, macrohistological investigations can be complicated by empty stomachs, chance events, short egestion times, and differential digestion rates between hard and soft tissues (Hyslop 1980; Parrish and Margraf 1990; Jacobs et al. 2010). As an attempt to circumvent these sources of bias, extensive work has been conducted using biochemical compounds present in all organisms as dietary tracers (Post 2002; Arts et al. 2009).

Fatty acids used as biochemical tracers conceptually follow the “you are what you eat” principle. Fatty acids, major components of energy storage molecules (triacylgylcerides), are deposited in consumer tissues in patterns reflective of fatty acids acquired through diets (Sargent et al. 2002; Budge et al. 2006; Elsdon 2010). As such, fatty acid profiles have been analyzed and compared in a wide variety of species to infer dietary relationships (Czesny et al. 2011; Kelly and Scheibling 2012; Dethier et al. 2013; Taipale et al. 2013; Strandberg et al. 2015). Qualitatively, trophic level and foraging location can be interpreted using knowledge of the fatty acid compositions of several potential prey species (Budge et al. 2006; Piché et al. 2010; Parrish et al. 2015). While such methods yield general ideas of foraging patterns, identification of actual diet composition is obscured by metabolic processes.

Consumer metabolism prevents exact matching of predator fatty acid signatures to prey even when fed a single diet (Budge et al. 2011; Taipale et al. 2011; Copeman et al. 2013). Several aquatic species (i.e., daphnids and copepods) have been shown to preferentially accumulate long chain fatty acids over others (Kainz et al. 2004; Taipale et al. 2011; Masclaux et al. 2012; Strandberg et al. 2015). Consumers' metabolism and assimilation of fatty acids has been modeled by feeding captive animals known diets and relating fatty acid compositions of consumer tissues and diets (Nordstrom et al. 2008; Käkelä et al. 2009; Galloway et al. 2015). Calibrations, derived from such feeding trials, have subsequently been employed to yield estimates of diet compositions of wild-caught predators (Nordstrom et al. 2008; Iverson 2009; Tucker et al. 2009). However, such attempts at diet composition estimation have sparsely been explored in fishes (but see: Budge et al. 2012; Magnone et al. 2015).

It has yet to be established if quantitative fatty acid signature analysis (QFASA) can yield accurate estimations of prey composition in piscine predators. The few studies that have attempted to model piscine fatty acid metabolism have relied on formulated diets. For example, Budge et al. (2012) used formulated feeds to evaluate accuracy of estimating Atlantic salmon (*Salmo salar*) diet compositions using fatty acid analysis. We question if diet estimation is possible when consumers are fed less controlled diets, i.e. natural prey organisms. Formulated diets varying in only oil composition may not provide accurate representations of complex biochemical metabolism. For example, it has been shown that varying levels of certain amino acids affect the metabolism of other amino acids (Elango et al. 2008). Similar competitive and compensatory metabolic interactions may occur with fatty acids, meaning that a predator's metabolism of each

84 individual prey species may require modeling (Rosen & Tollit 2012). Accounting for
85 consumers' assimilation of each prey item using feeding experiments has recently been
86 employed in studies of daphnia foraging (Galloway et al. 2014b; Galloway et al. 2015).
87 Such specific calibrations for each predator-prey interaction would potentially make diet
88 estimations from fatty acids too cumbersome for species in complex food-webs.

89 The purpose of this study was to develop and investigate the efficacy of a model,
90 originally constructed for marine mammals (QFASA: Iverson et al. 2004), to estimate
91 fish diet compositions. Lake trout (*Salvelinus namaycush*), round goby (*Neogobius*
92 *melanostomus*), and yellow perch (*Perca flavescens*) were selected as model species
93 due to their economic and ecological importance within the Great Lakes. Using multiple
94 consumers allowed us to ensure that our results can be interpreted and discussed in a
95 broader context that looks beyond species specific characteristics. Specifically, our
96 objectives were to 1) establish a working diet estimation model, and 2) evaluate its
97 potential in freshwater predators.

98 ## Methods

99 ### Feeding Trial Design

100 The three feeding experiments were conducted at The College at Brockport. Fish
101 were maintained under flow-through conditions in dechlorinated (carbon filter – Siemens
102 Water Technology, Warrendale, PA) city water and were exposed to natural
103 photoperiod and ambient water temperatures. Juvenile lake trout (mean ± 1 standard
104 deviation; 22.9 ± 1.0 mm; 0.09 ± 0.01 g) were kept in fifteen 38-L aquaria, yellow perch
105 (7.0 ± 0.5 mm; 3.0 ± 0.7 g) in six 400-L circular tanks, and round goby (9.6 ± 1.3 mm,

11.0 ± 4.9 g) in four 400-L rectangular tanks. To mimic the natural benthic environment of round goby, and reduce stress, small gravel and rocks were placed on the bottom of the tanks as well as half sections of PVC enclosures. Fish were fed frozen invertebrate prey: bloodworms (Diptera larva), copepods (*Cyclopoida spp.)*, daphnia (*Daphnia spp.*), mysis (*Mysis spp.*), or tubifex worms (*Tubifaceae spp.*) purchased from JEHMCO (www.jehmco.com; Table 1). Lake trout (n = 100 per aquarium) and yellow perch (n = 160 per tank) diet treatments were conducted in triplicate and round goby (n = 30 per tank) conducted in duplicate. Yellow perch and round goby were fed mysis or bloodworms at a daily feeding rate of 3 and 5% of their body weight, respectively, whereas lake trout were offered one of the five prey at 3% of tank biomass per day. Feeding rates were adjusted bi-weekly to account for growth.

After 14 wks, only 3 lake trout being fed tubifex worms remained alive and were terminally sampled. All remaining daphnia fed lake trout (n = 18), and 9 lake trout from each of the remaining dietary treatments were sampled at this point. A period of 14 wks was determined to allow for fatty acid turnover of each diet type in lake trout (Happel, A. et al. In Revision). For the round goby experiment, 5 bloodworm and 10 mysis fed individuals were sampled after 14 wks of feeding, and 5 bloodworm and 11 mysis fed individuals after 16 wks. All yellow perch were sampled after a 24 wk period of feeding either bloodworm or mysis (n = 21 for both). As feeding experiments occurred at different times, separate samples for each prey type were analyzed for fatty acids concurrently with the predators (Table 1). Data from each feeding experiment were kept separate during modelling exercises described below (Tables 1 and 2).

Individual fish were euthanized using a lethal dose of MS-222. Mass and length of each fish were recorded. Fish were individually bagged and stored at -80 °C until fatty acid analysis. Prior to analysis, individual samples were partially thawed and whole fish homogenized. Lipids were extracted according to Folch et al. (1957). Fatty acid methyl esters were prepared according to Metcalfe and Schmitz (1961), separated by gas chromatography/mass spectrometry (Agilent 7890A GC and 5975C inert XL EI/CI MSD, Agilent Technologies, Inc., Santa Clara, California, USA) and quantified as described in Czesny et al. (2011). The 24 most abundant (contributing > 1.0% in any sample group) fatty acids were expressed as relative percentages (Tables 1 and 2).

Model Creation and Testing

The quantitative fatty acid signature analysis (QFASA) model was constructed following Iverson et al. (2004) using Proc IML in SAS 9.3. Optimizations were conducted with the non-linear optimization by Quasi-Newton subroutine (Call NLPQN; default settings; estimates constrained between 0 and 1) minimizing Kullback-Liebler (KL) distances. This distance is defined as:

$$KL = \sum_j (y_j - \hat{y}_j)\log(\hat{y}_j / y_j)$$

where y_j and $\hat{y}_j$ refer to the proportion of the *j*th fatty acid in a consumer and that consumer's estimated fatty acid profile, respectively. KL distance calculation is conservative in that it gives more weight to differences among minute concentrations than large concentrations, and performs relatively well at quantifying distances between observed and estimated fatty acid signatures (Iverson et al. 2004; Bromaghin et al. 2015a). Optimizations stopped when KL distances could not be reduced further,

representing the closest attainable estimate for each predator's diet. In other words, the computer generates a mixture of prey types whose combination yields an estimated fatty acid signature that resembles the observed predator's signature the closest, and then returns the percentage values for each prey type that generated that mixture.

Our first step was to challenge the model by creating pseudo-predators using prey fatty acid signatures to investigate accuracy of the model's coding procedures. Values that did not return 100% accuracy would indicate inherent noise within the model. A single subsample of each prey type was chosen, at random with replacement, and the model was challenged to estimate which averaged prey type it originated from. Furthermore, pseudo-predators were created by randomly sampling 1 prey fatty acid signature subsample (with replacement) and subsequently multiplying by ratios, e.g., 50% of a daphnia profile and 50% of a mysis profile added together. Ratios chosen consisted of even mixtures of the two most similar prey types (daphnia and mysis), the two most different types (tubifex worms and copepods), and of all prey types (Table 1; Fig. 1). The prey library consisted of an average of all four sub-samples for each prey type from the lake trout experiment and remained unchanged throughout testing procedures. This was repeated 1000 times for each prey and each pseudo-predator to yield an average and 95% confidence interval for each pseudo-predator (Fig. 1).

Model Calibration

No significant differences in fatty acid profiles among tanks (nor time period for round goby) were noted for any species and thus individual fish are used as replicates for the following procedures (Analysis of Similarity). To account for modifications of fatty acid concentrations due to predator metabolism, calibration coefficients (CCs) were

calculated from predator and prey signatures and incorporated into models. Let x_{klj} denote the proportion of the *j*th fatty acid from the *l*th sample (from either consumer or diet type) of the *k*th diet type. Calibration coefficients were generated using two methods:

$$\mathrm{CC1}_{kj} = \overline{Predator_{klj}/Prey_{klj}} \quad \mathrm{CC2}_{kj} = \overline{Prey_{klj}/Predator_{klj}}$$

where CC1) divides fatty acid proportions in each predator by those in each sample of their respective prey type then averages (represented by a bar above the equation) across individual predators fed the same diet; and CC2) by averaging the reciprocals of those described as CC1.

The 5 lake trout, 2 round goby, and 2 yellow perch CC1s (Fig. 2) were applied to each predator independently by dividing each predator's fatty acid profile by the respective CC1. These "traditional CCs" de-metabolize each predator signature to be on par with that of the potential prey library (termed prey space optimization by Bromaghin et al. 2015b). CC1s were only applied among the relevant consumer species rather than across species (i.e., lake trout were only calibrated using lake trout derived CCs). An average of all CC1s within each predator species was tested to evaluate the ability to account for the various retention patterns that occurred across all diets. This created an additional CC1 trial for each predator species.

CC2s were concurrently applied to all prey relevant to the consumer trial (i.e., only mysis and bloodworms for yellow perch). This method involves a single trial that mathematically metabolizes the entire prey library to reflect the predator species using a CC specific to each predator-prey relationship (herein termed: prey-specific). We

194 expected this method to be more accurate than CC1 trials as it accounts for prey-
195 specific assimilation patterns in all prey items at the same time. Each prey species' fatty
196 acid signature is divided by its respective CC2, however this is nearly identical to simply
197 using the average of the predators from the feeding trial. Minute noise is added by
198 including prey fatty acid signatures in the equation. Similar to with CC1, we were also
199 interested in compiling several feeding trials to generate a mean CC that might be
200 applied to all prey items. Thus CC2s were averaged across diet treatments and
201 subsequently applied to all prey items at once.

202 For comparison, we conducted another optimization trial where the prey library
203 was replaced by averaged predator fatty acid signatures for each diet treatment. This
204 trial is similar to more recent methods where daphnia fed know diets act as end points
205 for comparison of wild caught individuals (Galloway et al. 2015). As such, uncertainty in
206 CC calculation, specificity, and variation are avoided all together. To avoid confusion
207 with CC trials which involved mathematically converting fatty acids of predators to prey
208 or vice versa, we refer to this method as using average predator groups (Ave. Pred.
209 Groups; Fig. 2).

210 For a final comparison, we also conducted trials where no calibrations were
211 applied. This yielded a total of 10 trials for lake trout (5 CC1s, an averaged CC1, a CC2,
212 an averaged CC2, Ave. Pred. Groups, and finally no CCs), and 7 each for round goby
213 and yellow perch (3 less CC1s for each due to only being fed mysis and bloodworms).
214 All fatty acid signatures used were adjusted to sum to 100 post calibrations and prior to
215 optimizations.

QFASA was repeated on each individual predator for each optimization trial. Estimates attained for each individual predator were recorded and total relative mean square errors (RMSE) generated through comparison to known diets RMSE=[(estimate-true)/true]^2. For each optimization trial a total RSME was recorded through summation across all individual predator estimates. The CC trial which produced the lowest RMSE was determined to be the most appropriate (lowest error) for model employment (Figs. 2 and 3).

Model Use

Finally, we utilized data gained through model testing to investigate accuracy of the model in individual diet estimation of predators with known diets. Diet compositions were estimated for each predator sample separately, using averaged predator signatures as the prey library.

Further evaluation of the model was conducted by generating pseudo-predators using lake trout fatty acid profiles. Lake trout were fed the widest variety of prey and thus could produce more complex pseudo-predators. The “prey library” remained as the average of lake trout fatty acid profiles from each diet treatment. Pseudo-predators (n = 1000 per defined diet) were generated by sampling one individual lake trout profile from each treatment group randomly and subsequently multiplying each by a predefined diet ratio. Diet compositions (n = 6) were chosen to represent various scenarios likely to be encountered in wild age-0 lake trout: 1.) Equal = all 5 prey items in equal proportions; 2.) Most Similar = 50/50 ratio of the two most similar items; 3.) Realistic 1 = 20/5/5/70/0 mix of diet items; 4.) Realistic 2 = 70/5/0/20/5 mix of diet items; 5.) Exaggerated 1 = 90/5/0/5/0 mix of diet items; 6.) Exaggerated 2 = 5/5/0/90/0 mix of diet items. Ratios are

239 listed as bloodworm/copepod/daphnia/mysis/tubifex worms. Average diet estimates
240 were calculated for each of the six defined pseudo-predator diets. To evaluate which
241 items were over or underestimated routinely, the defined diet ratios were subtracted
242 from the respective average estimates. The difference representing the average
243 percentage point error in QFASA estimation of that item in the diet.

244 ## Results

245 ### Model Creation and Testing

246 Challenging the model to predict diet compositions of pseudo-predators yielded
247 highly accurate results. All diet ratios utilized were correctly back estimated, with little to
248 no error (Fig. 1). For single species re-estimations, erroneous estimations were all <
249 1.5% (mean: 0.41 ± 0.4%). As such, computer programming of the model was
250 considered appropriately proofed and ready for subsequent analyses using predator
251 samples.

252 ### Model Calibration

253 A more thorough description of lake trout CC1s can be found in Happel, A. et al.
254 (In Revision). In general CC1s calculated from bloodworm feeding trials were large and
255 highly variable due to low detection rates in bloodworm prey samples. This is
256 particularly true for 22:4n-6, 22:5n-6, and 22:5n-3. 22:4n-6 CC1s across all diet trials
257 were very high, except round goby that were fed mysis. Fatty acid profiles of round goby
258 fed mysis and mysis were highly similar leading to very low CC1s. These data can be
259 found as supplementary material (Table S1).

Traditional CCs (CC1) did not yield accurate dietary estimates within this study, as judged by RMSE (Figs. 2 and 3). Of the lake trout CC1 trials, mysis CC1s generated lowest RMSE for diet estimates. Similarly, round goby and yellow perch CC1s from those fed mysis had lower RMSE scores than bloodworm CC1. Accurate diet composition estimates were achieved by each CC1 when estimating the respective diet that created that coefficient (e.g., copepod consumption estimated at near 100% of the diet were only achieved when copepod CC1s were used). This is illustrated in Fig. 3 by the mysis CC1 estimating only the diet component of mysis fed lake trout correctly, compared to any other diet. Averaging CC1s or using no coefficient generally resulted in high RSME for all tested consumers.

The CC2 optimization (conducted in predator space) yielded highly accurate results (prey-specific; Figs. 2 and 3). Using averaged CC2s yielded an increased RSME that was relatively similar to that attained by using the CC1 derived from mysis feeding of each consumer. Using predator averages as the prey library yielded the most accurate results for each consumer species (Figs. 2 and 3). The difference in diet composition estimates between prey-specific and averaged predator profiles trials was minimal (Fig. 3). Using predator averages yielded the most accurate results as judged by the smallest RMSE, and thus further optimizations were conducted using that model.

Model Use

Using average predator groups in model calculations generated diet estimates generally within 10% of actual diets. For lake trout, copepod was estimated least accurately (92.3 ± 6.6%), whereas all other diets were estimated at above 94% of what the lake trout were actually fed (Fig. 3). The only 95% confidence interval to encompass

100% for lake trout was estimated for tubifex fed lake trout (94.8 ± 8.0%). Diet estimates using average predator groups for round goby and yellow perch fed bloodworm or mysis were highly accurate (97.8 ± 1.4 and 98.3 ± 1.9% respectively; Fig. 4; 97.6 ± 2.5 and 98.4 ± 1.2% respectively; Fig. 5).

Pseudo-predator diet compositions were generally estimated with a high degree of accuracy (Fig. 6). Error rates increased as diet compositions became more skewed, however remained within 15% of the true diet. Both realistic pseudo-predator diet compositions were estimated within 6.5-6.6% of the true composition. Proportions of tubifex were consistently overestimated, and contributed most (total sum across all pseudo-predators: 13.2%) to errors out of all prey species types (range: 7.2-11.5%). Items defined as having 0% in the diet were overestimated at 2.5% on average. As such, primary components of diets (bloodworm and mysis) were routinely under estimated.

Discussion

Provided with data on each predator-prey interaction, diet estimates calculated through quantitative fatty acid signature analysis (QFASA) appear highly accurate. Our results mirror conclusions reached by Rosen and Tollit (2012), suggesting that individual calibrations are needed for each predator-prey interaction in QFASA models. Natively accounting for consumers' diet-specific assimilation of fatty acids within quantification models has recently been advocated for and applied in aquatic invertebrate studies (Galloway et al. 2014a; Galloway et al. 2014b). Such models perform within a Bayesian context, thus allowing models to account for more variation in fatty acid profiles. While not reported, we found that multiplication of CC1 to the prey

base did not increase accuracy, despite performing on par with other QFASA methods in other studies (Bromaghin et al. 2015b). This is likely due to the high degree of dissimilarity between the fatty acid profiles of our prey and predator species. Models that involve prey and predators that have similar profiles (i.e., fish feeding on other fish rather than invertebrates) may not necessarily require predator-prey specific calibrations, but would perform better if known consumer end-points were provided.

The necessity of diet specific calibrations is likely due to fishes' capacity to elongate, desaturate, and selectively oxidize fatty acids prior to deposition in somatic tissue (Henderson and Tocher 1987; Bell et al. 2003), which adds complexity to the QFASA approach. While cumbersome, our method provided highly accurate diet estimations for three fish species that are distinct in their natural feeding habits. We are confident that QFASA can be a powerful tool for diet estimations for aquatic predators if provided with adequate data on diet specific fatty acid metabolism. Top predators (i.e., salmonines) within Lake Michigan have been reduced to consuming di- and possibly tri-chotomus diets, making them practical model species for QFASA (Jacobs et al. 2010; Roseman et al. 2014; Hares et al. 2015). As such, feeding trials with species of economic importance (i.e., lake trout) fed diets consisting of prey items captured within the same lake system would provide the groundwork needed to employ QFASA models with wild fishes.

Freshwater lakes tend to be spatially disconnected and highly disparate in their energy pathways. As such, prey species from different lake systems, despite similar diets, might have different fatty acid compositions (Dethier et al. 2013). Although variation in fatty acid profiles within species is less than that among species (Budge et

al. 2002; Czesny et al. 2011; Lau et al. 2012), zooplankton fed various algae were more correctly classified as groups according to diets rather than species of zooplankton (Persson and Vrede 2006). It is unclear if the signal to noise ratios amongst species is sufficient to allow cross system use of CCs derived from wild type diets. This is especially true for higher trophic levels, as predators' fatty acid profiles appear most distinct amongst foraging guilds rather than species (Lau et al. 2012). Therefore, it remains unknown if controlled feeding studies using prey from one location (e.g., Lake Michigan) as food, would provide accurate QFASA results when employed on predators from other lake systems (e.g., Lake Ontario). Further noise may be added to models as sub-species or ecotypes of lake trout were found to metabolize diets differently, allocating energy (lipids) in varying amounts and in different tissues (Goetz et al. 2014). With on-going studies to develop and stock various strains of commercially and ecologically valuable fishes, differences within and among prey resources, feeding calibrations, and strain type metabolism may need to be accounted for when studying biochemical tracers.

Large CCs represent high dissimilarities between diet and consumer fatty acid profiles. In general, fatty acid profiles of frozen invertebrate prey used in our experiments were relatively similar to wild samples (Desvilettes et al. 1997; Hinderer et al. 2012; Lau et al. 2012). As such, large CC calculations appear unavoidable when working across invertebrate-vertebrate interfaces. Uncertainty in CCs have led other studies to remove fatty acids from their data sets (Wang et al. 2010). Budge et al. (2012) suggested removal of fatty acids in low quantities as their low concentrations may lead to highly variable coefficients, yet not affect KL distance used in estimations.

Neubauer and Jensen (2015) reduced their fatty acid variables down to just four, to reduce computational power needed to run their Bayesian models, which is not an issue with QFASA. Furthermore, estimated pseudo-predator diets, were most accurate when an extended set of fatty acids were included in analysis by Wang et al. (2010). We believe part of the power of using fatty acids in diet studies arises from the incorporation of many variables and thus are hesitant to remove large proportions of variables from models.

Compared to consumers, our lower trophic level prey (especially bloodworms and tubifex worms) contained trace amounts of certain polyunsaturated fatty acids (PUFA), specifically 22:4n-6, 22:5n-6, 22:5n-3. Proportions of many PUFAs in fish fed these invertebrates never reached levels found in prey samples even after 14 wks, suggesting endogenous control of proportions. Accumulation of PUFA as opposed to saturated fatty acids appears common amongst carnivorous species (Persson and Vrede 2006). Specifically, 20:4n-6, 20:5n-3, and 22:6n-3 are routinely found to be selectively retained in higher quantities than offered diets in a number of aquatic taxa (Kainz et al. 2004; Taipale et al. 2011; Galloway et al. 2015; Strandberg et al. 2015). These same fatty acids are often good discriminators amongst prey species, and predators fed various diets (e.g., Happel, A. et al. In Revision), and thus removal of these fatty acids seems unjustified. Furthermore, reducing fatty acids produces little to no increase in accuracy when predators have been fed known diets for extended periods (Budge et al. 2006; Iverson et al. 2007; Wang et al. 2010). Currently fatty acid profile calibrations are a simplified algebraic attempt to account for a highly complex

374 metabolic system; elaboration of this method may alter how fatty acid estimations are
375 calculated in future studies.

376 Optimizations carried out with pseudo-predators suggest that highly uneven prey
377 compositions may be difficult to estimate in wild populations. In our study, these diets
378 were estimated within 10% of the defined diet ratios, primarily due to consistent
379 overestimation of non-present items. This overestimation was highest for tubifex and
380 daphnia prey, especially when not a part of the defined diet. Potentially feeding
381 consumers for longer durations could yield more distinct fatty acid profiles between
382 predator groups used as reference points. Certainly longer feeding durations would be
383 necessary for larger, slower growing consumers compared to the juvenile fishes we
384 used. QFASA estimates obtained using Atlantic salmon fed for 22 wks still included a
385 small estimated percentage of the initial diet (Budge et al. 2006). As such, estimates
386 from QFASA should yield good indicators of more important prey species, but will likely
387 misrepresent species consumed in minute proportions.

388 Bootstrapping methods may provide estimates for individual fishes
389 encompassing variability within prey fatty acid profiles. We used averaged prey libraries
390 to encompass variation and obtained estimates for each consumer's diet, then
391 averaged across individuals within a diet type to estimate accuracy for each diet
392 composition. Averaging individual consumer diet compositions by a factor (i.e. location,
393 season, age, etc.) is akin to the employment of stomach content analysis of diets in
394 many contemporary studies. However, with QFASA it is possible to get confidence
395 intervals for each individual predator/consumer, using randomized sampling of prey
396 signatures to generate new prey libraries repeatedly (Iverson et al. 2004; Bromaghin et

al. 2013). Galloway et al. (2014a) used a similar method in a Bayesian approach to QFASA, yielding confidence intervals for each item estimated within an individual consumer's diet. The use of this technique with large prey databases may better incorporate known seasonal, spatial, and temporal variation within prey communities and yield less ambiguous diet descriptions for predators.

In summary, diet estimates gained through the use of fatty acid signatures may require further refinement but provide crude estimates of predator's diet composition when other methods are not possible. Calibrating QFASA models to each possible predator-prey pairing has yielded highly accurate results and likely provides an emerging tool ecologists can add to their toolbox. Quantitative abilities may aid investigations into on-going recruitment failures plaguing top predator populations in large lakes or help understand competitive interactions among fishes by identifying dietary shifts and overlaps within and among species. If solely based on stomach contents, such studies are subject to biases and shortcomings associated with this "snapshot" method. Alternatively, the QFASA approach may offer valuable insights into long term feeding habits alleviating some shortfalls of traditional diet assessments. Further investigations into non-lethal methods of sampling tracers may increase applicability to species of conservation concern. We remain confident that biochemical tracer analyses provide alternative tools for monitoring foraging habits and food web interactions in freshwater ecosystems.

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568

569

570 **Table 1.**

571 Average (± standard error; %ww) fatty acid profiles of invertebrate diets, analyzed separately for each feeding experiment.

	Lake Trout					Round Goby		Yellow Perch	
	Bloodworm	Copepod	Daphnia	Mysis	Tubifex	Bloodworm	Mysis	Bloodworm	Mysis
n	4	4	4	4	4	3	3	6	6
14:0	1.8 ± 1.4	3.9 ± 2.2	3.8 ± 2.2	5.2 ± 3.0	2.7 ± 2.0	3.6 ± 2.6	4.9 ± 3.5	2.1 ± 1.1	5.4 ± 2.4
15:0	1.1 ± 0.6	0.7 ± 0.4	1.1 ± 0.6	0.6 ± 0.3	1.1 ± 0.6	1.2 ± 0.9	0.6 ± 0.4	0.8 ± 0.4	0.6 ± 0.3
16:0	17.8 ± 10.3	18.9 ± 10.9	20.6 ± 11.9	16.1 ± 9.3	8.1 ± 4.7	18.0 ± 12.7	16.2 ± 11.5	18.0 ± 8.0	16.0 ± 7.2
16:1n-9	1.2 ± 0.7	0.2 ± 0.1	1.9 ± 1.1	0.5 ± 0.3	0.8 ± 0.6	1.0 ± 0.7	0.4 ± 0.3	0.8 ± 0.4	0.4 ± 0.2
16:1n-7	8.3 ± 4.9	17.0 ± 9.8	13.2 ± 7.6	8.4 ± 4.9	4.4 ± 2.6	7.7 ± 5.5	6.7 ± 4.8	8.9 ± 4.0	8.4 ± 3.8
17:0	1.4 ± 0.8	0.6 ± 0.4	0.8 ± 0.5	0.3 ± 0.2	1.6 ± 1.0	1.6 ± 1.1	0.3 ± 0.2	1.3 ± 0.6	0.4 ± 0.2
17:1	0.9 ± 0.5	3.9 ± 2.3	0.3 ± 0.2	0.3 ± 0.2	0.1 ± 0.1	1.4 ± 1.0	0.4 ± 0.3	0.8 ± 0.4	0.3 ± 0.1
18:0	10.1 ± 5.9	3.2 ± 1.8	3.4 ± 2.0	0.9 ± 0.5	6.3 ± 3.6	9.9 ± 7.1	1.1 ± 0.8	9.9 ± 4.5	0.9 ± 0.4
18:1n-9	12.7 ± 7.3	1.4 ± 0.9	14.3 ± 8.3	15.3 ± 8.9	1.9 ± 1.1	12.8 ± 9.1	17.2 ± 12.2	12.3 ± 5.5	14.9 ± 6.7
18:1n-7	5.6 ± 3.2	3.3 ± 1.9	3.8 ± 2.2	4.0 ± 2.3	9.5 ± 5.5	6.5 ± 4.6	4.2 ± 3.0	5.4 ± 2.4	3.9 ± 1.8
18:2n-6	17.3 ± 10.2	1.8 ± 1.0	4.3 ± 2.5	5.3 ± 3.1	13.0 ± 7.5	18.0 ± 12.8	4.9 ± 3.4	16.2 ± 7.4	5.4 ± 2.4
18:3n-3	12.0 ± 7.3	6.5 ± 3.8	8.7 ± 5.0	6.4 ± 3.8	2.4 ± 1.4	6.8 ± 4.8	5.5 ± 3.9	13.5 ± 6.3	6.8 ± 3.1
18:4n-3	1.3 ± 0.7	3.6 ± 2.1	1.4 ± 0.8	3.9 ± 2.3	0.3 ± 0.2	1.4 ± 1.0	3.1 ± 2.2	1.4 ± 0.6	4.3 ± 2.0
20:1n*	0.4 ± 0.3	0.4 ± 0.2	0.2 ± 0.1	2.1 ± 1.2	15.6 ± 9.0	0.8 ± 0.6	2.6 ± 1.9	0.4 ± 0.2	1.5 ± 0.8
20:2n-6	0.1 ± 0.1	0.5 ± 0.3	0.1 ± 0.1	1.5 ± 0.9	6.8 ± 4.0	0.2 ± 0.2	1.4 ± 1.0	0.1 ± 0.0	1.6 ± 0.7
20:3n-6	0.1 ± 0.0	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.1	2.2 ± 1.3	0.1 ± 0.1	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
20:4n-6	2.6 ± 1.6	0.8 ± 0.5	2.8 ± 1.6	3.0 ± 1.7	13.3 ± 7.7	3.1 ± 2.2	3.2 ± 2.2	2.4 ± 1.1	2.9 ± 1.3
20:3n-3	0.1 ± 0.0	0.7 ± 0.4	0.3 ± 0.2	0.9 ± 0.5	0.5 ± 0.3	0.1 ± 0.1	0.8 ± 0.6	0.0 ± 0.0	1.0 ± 0.4
20:4n-3	0.2 ± 0.1	1.0 ± 0.6	0.3 ± 0.3	1.3 ± 0.8	0.6 ± 0.3	0.2 ± 0.2	1.3 ± 0.9	0.1 ± 0.1	1.4 ± 0.6
20:5n-3	4.7 ± 2.8	18.5 ± 10.7	18.1 ± 10.4	12.1 ± 7.0	7.5 ± 4.4	4.2 ± 3.0	12.3 ± 8.7	5.2 ± 2.4	12.1 ± 5.4
22:4n-6	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.0	0.6 ± 0.5	0.0 ± 0.0	0.1 ± 0.1	0.0 ± 0.0	0.1 ± 0.1
22:5n-6	0.0 ± 0.0	0.8 ± 0.5	0.0 ± 0.0	1.4 ± 0.8	0.0 ± 0.03	0.1 ± 0.1	1.6 ± 1.1	0.0 ± 0.0	1.5 ± 0.7
22:5n-3	0.0 ± 0.0	1.0 ± 0.6	0.0 ± 0.0	0.7 ± 0.4	0.3 ± 0.2	0.1 ± 0.1	0.9 ± 0.6	0.0 ± 0.0	0.8 ± 0.3
22:6n-3	0.2 ± 0.2	11.0 ± 6.4	0.2 ± 0.1	9.5 ± 5.5	0.2 ± 0.1	0.8 ± 0.7	10.0 ± 7.1	0.2 ± 0.1	9.3 ± 4.2

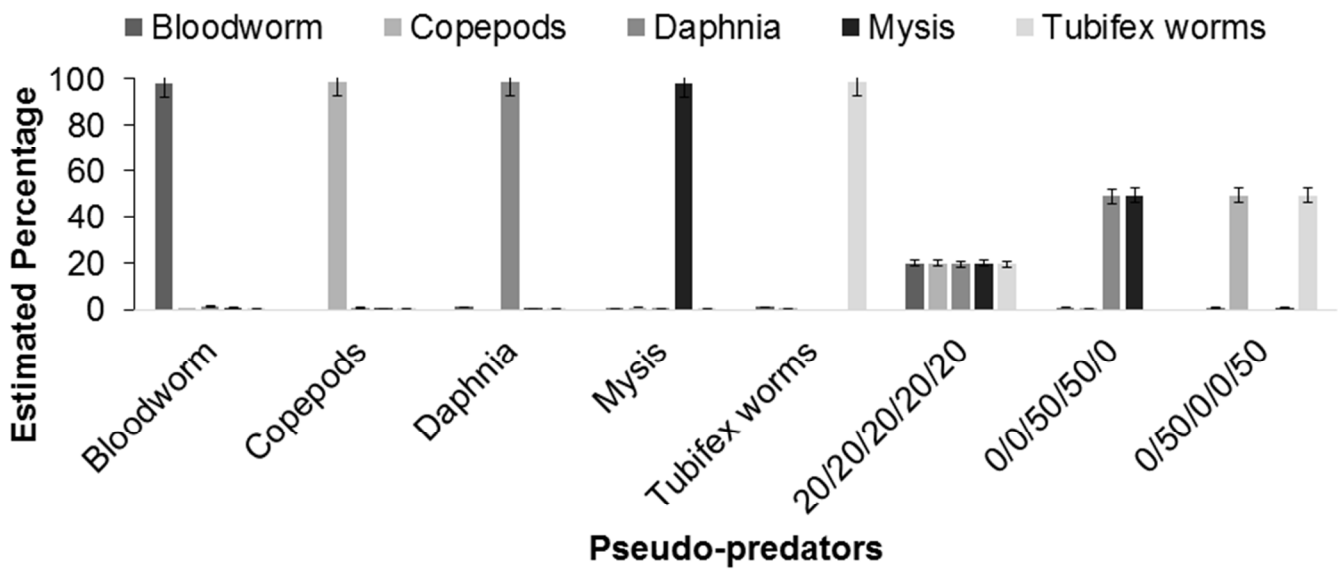
572 *20:1n-9 and 20:1n-11 coeluted and thus are summed

573 **Table 2.**

574 Average (± standard error; %ww) fatty acid profiles of lake trout, round goby, and yellow perch fed invertebrate diets.

	Lake Trout					Round Goby		Yellow Perch	
	Bloodworm	Copepod	Daphnia	Mysis	Tubifex	Bloodworm	Mysis	Bloodworm	Mysis
n	9	9	18	9	3	10	21	21	21
14:0	1.1 ± 0.4	1.5 ± 0.5	1.3 ± 0.3	2.5 ± 0.9	1.5 ± 1.1	2.8 ± 0.9	2.5 ± 0.6	3.3 ± 0.7	0.6 ± 0.2
15:0	0.6 ± 0.2	0.4 ± 0.1	0.6 ± 0.1	0.4 ± 0.1	0.4 ± 0.3	0.6 ± 0.2	0.4 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
16:0	18.4 ± 6.5	16.5 ± 5.9	21.4 ± 5.2	15.4 ± 5.4	17.8 ± 12.6	15.5 ± 5.2	12.7 ± 2.8	15.0 ± 3.4	16.5 ± 3.7
16:1n-9	1.4 ± 0.5	0.5 ± 0.2	1.4 ± 0.3	0.7 ± 0.2	1.4 ± 1.0	1.2 ± 0.4	0.5 ± 0.1	0.9 ± 0.2	1.5 ± 0.3
16:1n-7	2.6 ± 0.9	11.5 ± 4.2	4.8 ± 1.2	4.9 ± 1.7	2.4 ± 1.7	10.5 ± 3.6	6.9 ± 1.6	9.3 ± 2.1	3.4 ± 0.8
17:0	1.1 ± 0.4	0.4 ± 0.2	0.7 ± 0.2	0.4 ± 0.1	0.8 ± 0.6	0.5 ± 0.2	0.2 ± 0.0	0.3 ± 0.1	0.8 ± 0.2
17:1	0.8 ± 0.3	0.2 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.2 ± 0.1	1.6 ± 0.5	0.4 ± 0.1	0.4 ± 0.1	0.8 ± 0.2
18:0	8.7 ± 3.1	5.3 ± 1.9	7.2 ± 1.8	2.9 ± 1.0	7.9 ± 5.6	5.3 ± 1.8	2.2 ± 0.5	1.8 ± 0.4	9.6 ± 2.1
18:1n-9	13 ± 4.6	6.2 ± 2.3	15.6 ± 3.8	17.3 ± 6.1	10.9 ± 7.8	17.9 ± 6.0	15.7 ± 3.5	16.1 ± 3.6	12.2 ± 2.7
18:1n-7	4.2 ± 1.5	4.7 ± 1.7	3.9 ± 0.9	4.4 ± 1.6	5.5 ± 3.9	7.5 ± 2.5	4.7 ± 1.0	3.7 ± 0.8	3.7 ± 0.8
18:2n-6	7.6 ± 2.7	1.3 ± 0.5	1.6 ± 0.4	3.8 ± 1.4	3.5 ± 2.5	15.7 ± 5.3	4.3 ± 1.0	4.6 ± 1.0	7.9 ± 1.8
18:3n-3	1.8 ± 0.7	3.3 ± 1.2	2.1 ± 0.5	3.4 ± 1.2	0.5 ± 0.4	5.1 ± 1.7	4.5 ± 1.0	4.3 ± 1.0	1.9 ± 0.4
18:4n-3	0.8 ± 0.3	1.5 ± 0.6	0.6 ± 0.2	1.4 ± 0.5	0.1 ± 0.1	1.2 ± 0.4	2.8 ± 0.6	2.3 ± 0.5	0.5 ± 0.1
20:1n*	0.5 ± 0.2	0.4 ± 0.2	0.2 ± 0.1	1.9 ± 0.7	1.3 ± 0.9	0.6 ± 0.2	1.2 ± 0.3	1.2 ± 0.3	0.7 ± 0.2
20:2n-6	0.8 ± 0.3	0.4 ± 0.1	0.1 ± 0.0	1.3 ± 0.4	1.3 ± 0.9	0.2 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	0.3 ± 0.1
20:3n-6	2.0 ± 0.7	0.2 ± 0.1	0.2 ± 0.1	0.3 ± 0.1	0.8 ± 0.6	0.2 ± 0.1	0.2 ± 0.0	0.2 ± 0.0	0.7 ± 0.2
20:4n-6	8.8 ± 3.1	2.2 ± 0.8	5.1 ± 1.2	4.0 ± 1.4	10.8 ± 7.6	5.4 ± 1.9	4.5 ± 1.0	3.7 ± 0.8	10.2 ± 2.3
20:3n-3	0.3 ± 0.1	0.8 ± 0.3	0.2 ± 0.0	0.8 ± 0.3	0.2 ± 0.1	0.1 ± 0.0	0.7 ± 0.2	0.8 ± 0.2	0.4 ± 0.2
20:4n-3	1.6 ± 0.6	1.3 ± 0.5	0.8 ± 0.3	1.2 ± 0.4	0.6 ± 0.4	0.2 ± 0.1	1.1 ± 0.3	1.0 ± 0.2	1.5 ± 0.5
20:5n-3	3.7 ± 1.3	9.4 ± 3.3	9.1 ± 2.2	7.6 ± 2.7	3.8 ± 2.7	4.2 ± 1.4	11.5 ± 2.6	8.9 ± 2.0	4.6 ± 1.0
22:4n-6	0.4 ± 0.2	0.2 ± 0.1	0.4 ± 0.1	0.5 ± 0.2	1.3 ± 1.0	0.2 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.1 ± 0.0
22:5n-6	2.1 ± 0.7	0.7 ± 0.3	0.1 ± 0.0	1.3 ± 0.5	2.4 ± 1.7	0.2 ± 0.1	1.9 ± 0.4	2.0 ± 0.5	2.5 ± 0.6
22:5n-3	1.9 ± 0.7	3.0 ± 1.1	3.6 ± 0.9	2.1 ± 0.8	2.8 ± 2.0	1.6 ± 0.6	4.2 ± 1.0	1.5 ± 0.3	1.6 ± 0.4
22:6n-3	15.6 ± 5.5	28.2 ± 10	18.9 ± 4.6	21.2 ± 7.6	22 ± 15.6	1.7 ± 0.6	15.5 ± 3.5	16.8 ± 3.8	17.5 ± 3.9

575 *20:1n-9 and 20:1n-11 coeluted and thus are summed



576

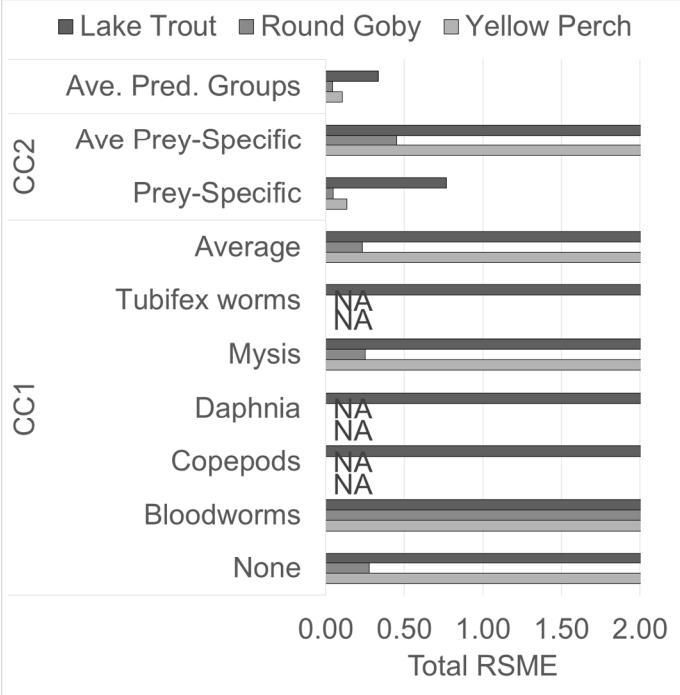
577 **Fig 1.**

578 Diet estimates from QFASA of pseudo-predators created through randomized prey

579 samplings and average prey signatures as possible diet categories. Ratios are listed as

580 bloodworm/copepod/daphnia/mysis/tubifex worms.

581



582

583 **Fig 2.**

584 Total relative mean square error (RSME; smaller is better) for QFASA trials (Y-axis)

585 using different calibration coefficients (CC1, CC2; see methods) or using predator

586 averages as the prey library (Ave. Pred. Groups). QFASA was conducted on individual

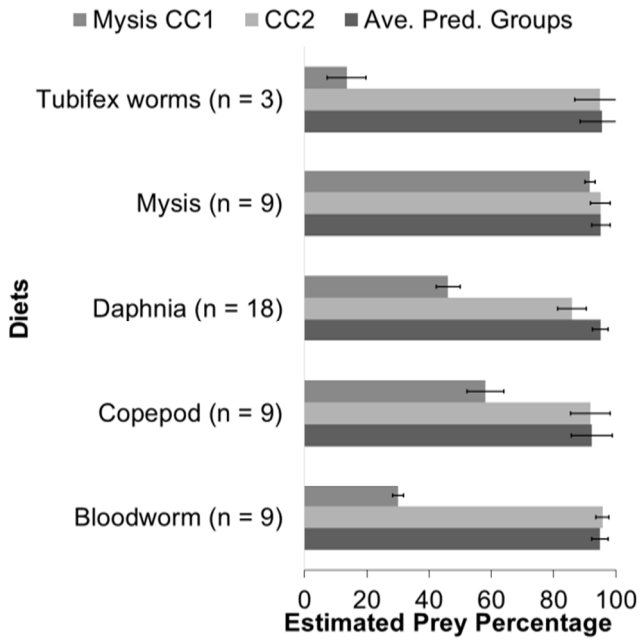
587 lake trout, yellow perch, and round goby, and the RSME summed across individual

588 estimates. “NA” is used to denote that CC1 Tubifex worms, Daphnia, and Copepod

589 trials were not conducted with round goby and yellow perch as they were not fed those

590 items. Figure cut off at a RSME of 2.0 for ease of comparison of smaller, more

591 important numbers.



592

593 **Fig 3.**

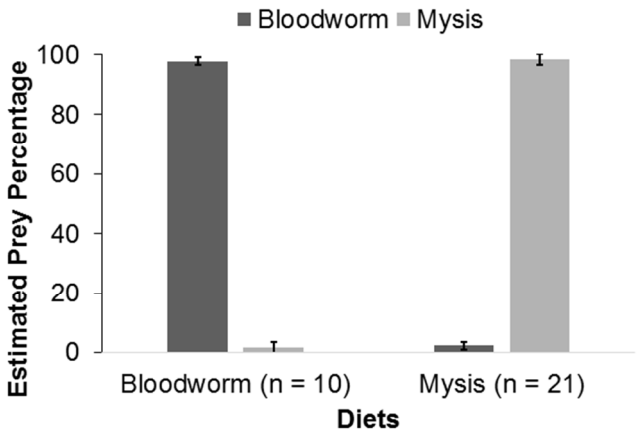
594 Average (95% CL) estimates the diets fed to lake trout for 14 wks from three different

595 QFASA methods (top legend). Depicted is how correct the 100% diet of each individual

596 predator was estimated. Not depicted is the erroneous estimates of non-consumed

597 items. As such, the best method yields 100% bars across all diet categories, which is

598 the Ave. Pred Group method.



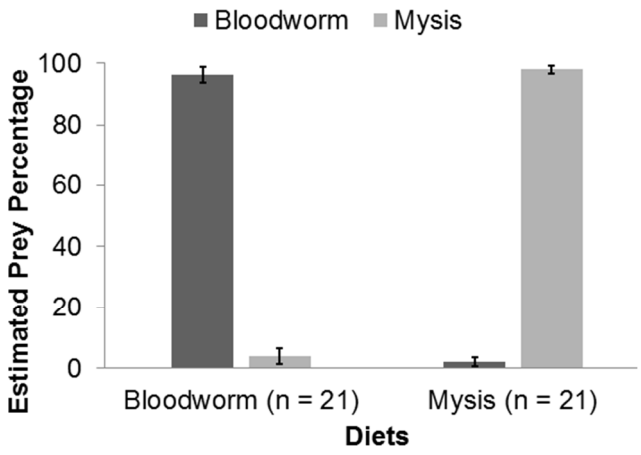
599

600 **Fig 4.**

601 Average diet composition estimates (95% CL) attained through QFASA on individual

602 round goby fed two separate diet treatments for 14-16 wks. The prey library consisted of

603 the average of round goby fed each item.



604

605 **Fig 5.**

606 Average diet composition estimates (95% CL) attained through QFASA on yellow perch

607 fed two separate diet treatments for 24 wks. The prey library consisted of the average of

608 yellow perch fed each item.

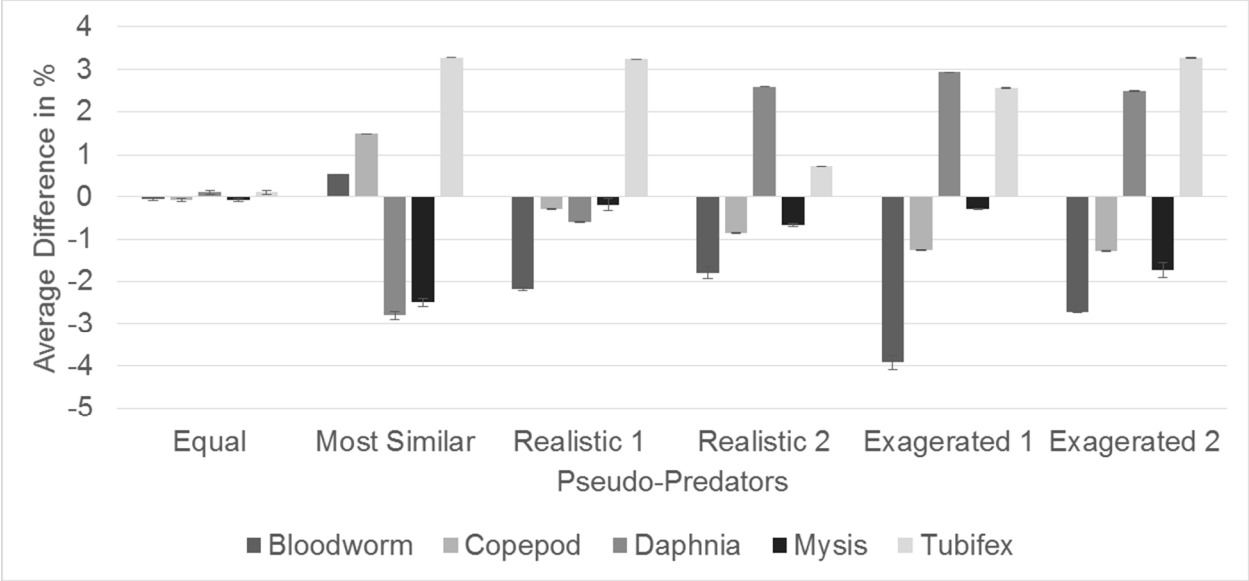


Fig 6.

Differences between defined diet compositions of pseudo-predators and the average diet estimates (n = 1000 per diet). Error bars represent 95% confidence intervals. Positive values indicate overestimation of true proportional contribution of that prey item, conversely negative values represent underestimation. See text for defined compositions.