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Estimating Trophic Levels and Trophic Magnification Factors Using Bayesian Inference.
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24 **ABSTRACT**

25 Food web biomagnification is increasingly assessed by estimating trophic magnification
26 factors (TMF) where solvent (often lipid) normalised contaminant concentration is regressed
27 onto trophic level, and TMFs are represented by the slope of the relationship. In TMF
28 regressions, the uncertainty in the contaminant concentrations is appreciated, whereas the
29 trophic levels are assumed independent and not associated with variability or uncertainty
30 pertaining to e.g. quantification. In reality, the trophic levels may vary due to measurement
31 error in stable isotopes of nitrogen ($\delta^{15}\text{N}$) of each sample, in $\delta^{15}\text{N}$ in selected reference
32 baseline trophic level, and in the enrichment factor of $\delta^{15}\text{N}$ between two trophic levels (ΔN),
33 which are all needed to calculate trophic levels. The present study used a Markov Chain
34 Monte Carlo method, with knowledge about the food web structure, which resulted in a
35 dramatic increase in the precision in the TMF estimates. This also lead to a better
36 understanding of the uncertainties in bioaccumulation measures; instead of using point
37 estimates of TMF, the uncertainty can be quantified (i.e. $\text{TMF} > 1$, namely positive
38 biomagnification, with an estimated X % probability).

39

40 Keywords: biomagnification, trophic level, food web, contaminants

41

42 **INTRODUCTION**

43 Recent reviews and studies have suggested the implementation of trophic relations in the
44 assessment guidelines of contaminant accumulation¹⁻⁴. This includes evaluating the
45 bioaccumulation potential of contaminants by quantifying their magnification through diet,
46 either by specific predator-prey relations (biomagnification factor - BMF) or as an average
47 factorial change from one trophic level to the next in a specified food web (trophic
48 magnification factor –TMF; previously also referred to as Food Web Magnification Factor).
49 Whereas the BMF is the ratio of contaminant concentration between predator and prey
50 ($BMF=C_{PREDATOR}/C_{PREY}$), the TMF is estimated by regressing the contaminant concentrations
51 in representatives of a food web onto their relative trophic positions, and the TMF is the slope
52 of the regression line^{3,5,6}. Although the TMF is currently recognized as the most realistic
53 quantitative measure of food web accumulation of contaminants^{1,4}, several issues remain
54 regarding scientific understanding, feasibility of test protocols, and thus regulatory
55 acceptance^{7,8}. One of the greater challenges is to obtain a better understanding of the
56 variability in TMF estimates and whether this variability comes about through natural
57 variation in relevant processes or uncertainties surrounding our knowledge of them, or if it is
58 the result of measurement errors, poorly defined concepts and statistical analyses. Despite
59 this, the European Community Regulation on chemicals and their safe use (REACH)_recently
60 amended to Annex XIII that accumulation of chemicals from the diet (BMF) and in the food
61 web (TMF) could be used in the weight of evidence assessment of the chemical as a
62 contaminant of concern due to bioaccumulation (REACH, Annex XIII⁹).

63

64 The trophic level of a species reflects its approximate feeding position in a food web,
65 where primary producers (plants/algae) constitute the first trophic level, followed by primary
66 consumers (herbivore) on the second trophic level, secondary and tertiary consumers

67 (carnivore) on the third and fourth trophic level, and so on. However, the simple concept of
68 unidirectional linear food chains rarely apply to natural ecosystems, where more complex
69 network models more appropriate describe the food webs¹⁰. Thus, the feeding position of a
70 species is not an integer trophic level (e.g. 2, 3 or 4), but rather a continuous descriptor of a
71 trophic position (e.g. 2.1, 2.7, 3.9), which can easily be calculated using a dietary matrix of
72 the food web. Traditionally, trophic position of a species has been evaluated by stomach
73 content analysis, but in the past decades stable nitrogen isotopes ratios ($\delta^{15}\text{N}$ measured as
74 the $^{15}\text{N}/^{14}\text{N}$ ratio compared to a standard) has been more commonly used to assess a relative
75 trophic position of organisms. The heavier isotope ^{15}N is retained in the organism to a larger
76 extent than ^{14}N , with a relative increase of ^{15}N over ^{14}N ($\delta^{15}\text{N}$) of 3-5‰ per trophic level,
77 depending of species comparison and ecosystem^{11,12}. The $\delta^{15}\text{N}$ ratios thus provide a non-
78 discrete measure of the relative trophic positions along a continuum, and has been utilized in
79 ecotoxicology (either as $\delta^{15}\text{N}$ or converted to trophic position) since the early 1990s^{3,5,6,13,14}.

81 In studies of biomagnification, measurements of $\delta^{15}\text{N}$ and contaminants are reflecting
82 accumulation over time. As such they are assumed to be good estimators of the average
83 ecological (diet) and contaminant status of the respective species. Although there is increasing
84 knowledge of ecological and analytical factors that affect the variance in the contaminants
85 quantified, fewer ecotoxicological studies appreciate the unknowns and evaluate the
86 uncertainty associated to measured $\delta^{15}\text{N}$ values, and the estimated trophic positions³. In
87 addition to a switch in diet that affect the $\delta^{15}\text{N}$, the isotopic ratio may vary within a species
88 depending on the productivity of the ecosystem, e.g. in phytoplankton and zooplankton the
89 $\delta^{15}\text{N}$ vary up to 5‰ depending on bloom stage¹⁵. This difference corresponds to a difference
90 of more than one trophic level, using the scaling factor relating relative $\delta^{15}\text{N}$ measurements
91 with trophic levels (ΔN)¹² in the range 3-5‰. Unless other information is available, a value

92 of 3.4‰ is commonly applied in ecotoxicological studies for the estimation of trophic
93 position and TMF^{3,16}. Lastly there are analytical considerations that affect the quantified
94 $\delta^{15}\text{N}$, such as extraction method, and removal of lipid and carbonate or not¹⁷. Using
95 measurements of the isotope ratios to estimate the trophic position of individuals (as opposed
96 to estimating trophic position of a species) will make sure that some of the natural variability
97 in diets is taken into account, and this will directly affect the TMF, especially the precision.
98 On the other hand, it is still a model relating the individual isotope levels to trophic positions.
99 To our knowledge, no examination of the effect of variability in either enrichment factor
100 (ΔN), baseline $\delta^{15}\text{N}$ or individual sample $\delta^{15}\text{N}$ on estimated trophic level has been performed.
101 Fortunately, ecotoxicology and risk assessment is developing in the direction of appreciating
102 and quantifying uncertainties, including an increased focus on probabilistic risk assessment
103 e.g.^{18,19}. Thus, focus on assessing uncertainty and variability in bioaccumulation models e.g.
104 ^{20,21}, methods are needed for reducing uncertainty in TMF estimates while incorporating
105 variability in these factors. However, most TMF studies lack of appreciation of this
106 variability, i.e. most TMFs are calculated only using traditional regression methods that only
107 take into account (or try to minimize) error in the measured values of contaminant
108 concentrations. Some simple methods have been performed, e.g. removing one of the
109 measured compartments from TMF calculation, as in^{5,22,23}. Ways forward should include
110 direct quantification and treatment of the trophic level variability associated with TMF
111 estimates.

112

113 In the present study, we utilized both measurements of $\delta^{15}\text{N}$ as well as knowledge about
114 the structure of a food web (in the form of a binary (0/1) dietary matrix) to predict $\delta^{15}\text{N}$ values
115 (and hence trophic levels). The model also estimated parameters used in relating $\delta^{15}\text{N}$ values
116 to trophic levels (baseline/reference $\delta^{15}\text{N}$ and enrichment factor ΔN), and the error variance of

117 $\delta^{15}\text{N}$. Together the estimated parameters use the links between dietary information and
118 isotope enrichment to generate probability distributions of trophic levels, and these in turn are
119 used to generate probability distribution of TMFs.

120

121 **THEORY AND METHODS**

122 Trophic magnification factors are assumed to reflect the magnitude of contaminant
123 accumulation in a food web, and are defined as the estimated slope of the solvent (often lipid)
124 normalized contaminant concentrations (C_{lipid}) on trophic level (TL) (eq. 1);

$$\log_{10}(C_{lipid}) = a + b \cdot TL + \varepsilon, \quad (\text{Eq. 1})$$

$$TMF = 10^b$$

125 Regressions like these are often performed by traditional least-squares regression or other
126 maximum likelihood measures attempting to minimize the squared error (ε), i.e. the best
127 estimate of TMF are achieved through minimizing the (squared) difference between predicted
128 and observed (log) contaminant concentrations. Implicitly this means that all variability in
129 trophic levels (including measurement errors, and estimates of isotope enrichment factors etc.)
130 are ignored; or more correctly trophic levels are seen as independent. Though methods for
131 inclusion of errors or variability in the independent variable (so-called errors-in-variables
132 models, e.g. Deming regression) exist, to our knowledge no such examples exist for TMF
133 estimation. Thus TMFs as measures of contaminant biomagnification does not include any
134 treatment of the potential variability of trophic levels among individuals or samples of the
135 same species or population.

136

137 **Trophic level estimation from food webs and isotope ratio measurements.** Estimation
138 of trophic levels using $\delta^{15}\text{N}$ is performed using equation 2:

$$TL_{consumer} = \frac{\delta^{15}N_{consumer} - \delta^{15}N_{primary\ consumer}}{\Delta N} + TL_{primary\ consumer} \quad (\text{Eq 2})$$

139 where $TL_{consumer}$ is the trophic level of an individual with a measured $\delta^{15}N_{consumer}$.
 140 $\delta^{15}N_{primary\ consumer}$ is the isotope ratio measured for a primary consumer assumed to occupy a
 141 trophic level of $TL_{primary\ consumer}$. Isotope enrichment factors (ΔN) of 3.4 ‰ are commonly
 142 used^{3,16}.

143

144 Describing the community using a food web dietary matrix yields another way to estimate
 145 trophic levels. Effective trophic levels can be defined as the weighted average length of all
 146 energetic pathways originating from outside a system to a specific compartment. For a
 147 secondary consumer feeding on only one primary consumer this corresponds to an effective
 148 trophic level of 3 (abiotic environment (TL 0) → primary producer (TL 1) → primary
 149 consumer (TL 2) → secondary consumer (TL 3)). With mixed diets one calculates a weighted
 150 average for each compartment in the food web matrix e.g. ^{24,25}. For each species or population
 151 i with a diet consisting of G other species according to the fraction F_{ij} , effective trophic level
 152 is then calculated as:

$$TL_i = 1 + \sum_{j \in G} F_{ij} TL_j. \quad (\text{Eq 3})$$

153 Or equivalently in matrix notation for the vector of trophic levels:

$$\mathbf{TL} = \sum (\mathbf{I} - \mathbf{F})^{-1} \quad (\text{Eq 4})$$

154 where \mathbf{I} is the identity matrix and \mathbf{F} is the dietary matrix describing the food web.

155

156 By rearranging equation 2 we can use trophic levels from a dietary matrix to predict
 157 isotope ratios:

$$\delta^{15}N_i = (TL_i - TL_j)\Delta N + \delta^{15}N_j \quad (\text{Eq 5})$$

158

159 **A Bayesian model of $\delta^{15}\text{N}$ ratios inferred from food webs.** In Bayesian statistics, the
 160 goal is to arrive at distributions of parameters that reflect our degree of belief in their values.
 161 The main ingredient of Bayesian analysis is Bayes rule;

$$p(\theta|y) = \frac{p(y|\theta)p(\theta)}{p(y)}, \quad (\text{Eq 6})$$

162 where θ represents a set of estimated parameters and y represents data or observations. Our
 163 main goal is to get an estimate of the distribution on the left-hand-side (called a *posterior*
 164 distribution); a probability distribution of (a set of) parameters, given our data. In a simple
 165 case it could be the estimate of a regression coefficient, given a sample and the distribution
 166 ($p(\theta|y)$) could be described in terms of percentiles and a visual representation of the posterior
 167 distribution. Bayes rule gives us a way to calculate such posterior distributions since they are
 168 (by definition) the product of the *likelihood* (the probability of the observations, *given* the
 169 parameters, $p(y|\theta)$) and a prior distribution ($p(\theta)$). A likelihood is a formal measure of the
 170 similarity between predictions and observations, most often directly related to sums of squares
 171 and a *prior* distribution is reflecting our current knowledge about the probability of the
 172 parameters. In the case of estimating a regression coefficient (like the TMF), we might for
 173 instance have prior knowledge (from other studies or common sense) about its expected
 174 distribution. In the case of estimating the regression coefficient b in eq 1, we could form a
 175 *prior distribution* which would encapsulate our current knowledge about the system, say with
 176 a mean of 2 and a given standard deviation, if such priors were warranted based on earlier
 177 analyses. In other cases we have little information about the expected value and choose
 178 *uninformative priors*, distributions that are uniform or in other ways express vague

179 information about a parameter. In most cases the likelihood, $p(y|\theta)$, is a combination of a
 180 mathematical model that yields predictions and a model for the distribution of the errors, i.e.
 181 the expected deviances between observed and predicted values. The denominator in eq 6
 182 gives the probability of the observations. This is independent of the parameters of the model
 183 (θ) and is therefore often reduced to an unknown constant yielding

$$p(\theta|y) \propto p(y|\theta)p(\theta). \quad (\text{Eq 7})$$

184 In other words, since $p(y)$ is constant we can estimate the posterior distribution, $p(\theta|y)$, as
 185 proportional to the prior distribution, $p(\theta)$, multiplied by the likelihood $p(y|\theta)$.

186 A model (Figure 1) was set up using the equations 4 and 5 to predict the population means
 187 of $\delta^{15}\text{N}$ ratios in the food web compartments, by estimating a set of parameters through
 188 Bayesian inference. The parameters to be estimated were the non-zero entries in the dietary
 189 matrix (F in eq 3), the isotope enrichment factor (ΔN in eq 5) and the population mean $\delta^{15}\text{N}$
 190 for one of the diet matrix compartments (*Daphnia*, as primary consumer in eq 2). All of these
 191 parameters can be combined with an error variance (σ^2) estimated (common for all
 192 populations) to predict $\delta^{15}\text{N}$ in an individual (technically this error variance is a combination
 193 of variance in the population and observational error). The data points of $\delta^{15}\text{N}$ measurements
 194 ($y_{ij}, i = 1, \dots, n_j, j = 1, \dots, J$) are modelled as independently normally distributed within each
 195 population (j) with means μ_j and variance σ^2 . The group or population means are assumed to
 196 be related through the food web, according to equation 5.

197

198 Letting θ denote the parameters of the dietary matrix, μ_D the estimated population mean
 199 level of $\delta^{15}\text{N}$ for *Daphnia*, σ^2 the variance of the $\delta^{15}\text{N}$ distributions (common for all
 200 populations) and ΔN the isotope enrichment factor we will explore the posterior distribution

$$\begin{aligned}
p(\theta, \mu_D, \Delta N, \sigma^2 | y) \\
\propto p(\theta, \delta^{15}N_D, \Delta N, \sigma^2) p(y|\theta, \delta^{15}N_D, \Delta N, \sigma^2) \quad (\text{Eq 8})
\end{aligned}$$

201 where the likelihood is defined by:

$$\begin{aligned}
p(y|\theta, \mu_D, \Delta N, \sigma^2) &= \prod_{j=1}^J \prod_{i=1}^{n_j} p(y_{ij}|\theta, \mu_D, \Delta N, \sigma^2) \\
&= \prod_{j=1}^J \left(\frac{1}{2\pi\sigma^2} \right)^{n_j/2} e^{-\left(\frac{\sum_{i=1}^{n_j} (y_{ij} - \mu_j)^2}{2\sigma^2} \right)}. \quad (\text{Eq 9})
\end{aligned}$$

202 In equation 9, $y_{i,j}$ are observed isotope ratios in sample i belonging to population j , and μ_j
203 are the mean isotope ratios for the population j , given by:

$$\mu_j = f(\theta, \Delta N, \mu_D) = \left(TL_j(\theta) - TL_D(\theta) \right) \Delta N + \mu_D \quad (\text{Eq 10})$$

204 where TL_j is the trophic level calculated using the food web matrix as in eq 4.

205

206 **MCMC implementation and prior probabilities.** To explore the posterior values (i.e.
207 arriving at a distribution for the parameters in eq 8) we used standard Markov Chain Monte
208 Carlo (MCMC) simulations where the proposal values were generated by a normal
209 distribution around the current value²⁶. The proposed values were accepted using the
210 Metropolis Hastings algorithm. The step size was in an initial run found so as to achieve well
211 mixed chains with an acceptance rate around 0.23 and was fixed for the main analysis²⁶. We
212 simulated 10 independent chains for 100 000 iterations each and used the last 25 000
213 iterations as parameter estimates and for posterior predictive sampling. To evaluate the effect
214 of including knowledge about the structure of the food web we also performed a Bayesian
215 analysis of the regression in eq 1 through Gibbs sampling, also with 10 chains for 100 000
216 iterations. This essentially copies the standard methods for TMF estimation³, which was also

217 applied for this specific food web²³, by using a Bayesian estimation of the TMF values, while
218 assuming the isotope enrichment factors and all other measurements to be fixed. The analysis
219 was implemented in Matlab ²⁷.

220

221 Dirichlet distributions with concentration parameter $\alpha = 1$ were used as priors for the
222 diets; essentially this entails a uniform distribution over all possible combinations. Gaussian
223 priors were used for the isotope enrichment factor (ΔN) and mean $\delta^{15}\text{N}$ for *Daphnia* (μ_D) with
224 means and standard deviations of (0.0035, 3×10^{-4}) and (8, 1) respectively. For the error
225 variance (σ^2) a uniform prior with range [0...10] was applied.

226

227 **Posterior predictive sampling and TMFs estimation.** The probability distributions of
228 the estimated parameters can be used for posterior predictive sampling, essentially generating
229 distributions of $\delta^{15}\text{N}$ values for individual samples of the different compartments in the food
230 web. For each of the $\delta^{15}\text{N}$ data we also have contaminant data, and by resampling $\delta^{15}\text{N}$ values
231 from the estimated distributions of $\delta^{15}\text{N}$ we can thereby quantify the uncertainty in trophic
232 magnification factors arising from the variability in the trophic levels assigned to the analysed
233 individuals. We did this by randomly drawing n number of the last 25 000 iterations, using the
234 parameter values at that point in the chain to draw simulated $\delta^{15}\text{N}$ values for the individual
235 samples (see Data sources below). Using these simulated $\delta^{15}\text{N}$ values together with ΔN , we
236 then performed a regression to get n number of estimates of TMFs for selected compounds.
237 These estimates were pooled to generate a probability distribution of TMFs given the
238 structure of the food web, the prior distributions of the parameters and observed levels of
239 contaminant and $\delta^{15}\text{N}$.

240 **Data sources and food web structure.** Empirical data used in the present study are
241 previously presented ^{23,28} and details on contaminant levels, sampling and analysis can be
242 found therein. In brief, representatives of the pelagic food web of Lake Mjøsa, Norway, were
243 collected mid-lake near Helgøya in September-October 2010. The food web representatives
244 included the top predator piscivorous brown trout (*Salmo trutta*), the zooplanktivorous fish
245 smelt (*Osmerus eperlanus*) and vendace (*Coregonus albula*). The invertebrate representatives
246 included *Mysis relicta* and zooplankton (*Daphnia galeata* and *Limnocalanus macrurus*). The
247 samples were analysed for lipids and legacy persistent organic pollutants including
248 polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs) and
249 dichlorodipenyldichloroethylene (*p,p'*-DDE)²⁸. $\delta^{15}\text{N}$ and cyclic volatile methyl siloxanes
250 (decamethylcyclopentasiloxane - D5) were analysed as described in Borgå et al. ²³.

251
252 Based on previous ecological studies of Lake Mjøsa, or similar lakes, a binary dietary
253 matrix representing who eats whom (but not the proportions) for each food web representative
254 was developed. All entries in the dietary matrix were estimated; however, which entries were
255 non-zero was based on earlier studies and constitutes all the knowledge about the food web
256 included in the model. In addition to the food web compartments described above that were
257 analysed for contaminants, lipids and $\delta^{15}\text{N}$, particulate organic matter (POM),
258 microzooplankton, small size group of vendace (< 15 cm) and smelt (< 15 cm) were included
259 in the binary dietary matrix.

260

261

262 **RESULTS AND DISCUSSION**

263 Our analysis consists of two major parts; the first part uses the assumed structure of the
264 food web (i.e. who eats who), the relations in eq 4 and 5 and observations of isotope levels to

265 estimate the relevant parameters (diets, enrichment factor, isotope ratios for *Daphnia* and an
266 error term) of our model. The second part uses these estimates to generate ranges of likely
267 isotope ratios. These generated isotope ratios ($\delta^{15}\text{N}$), baseline isotope ratios for *Daphnia* (μ_D)
268 and enrichment factor (ΔN) are then used to calculate trophic levels and the probability
269 distributions of TMFs. In essence we are estimating a mean isotope ratio for each
270 compartment and then simulating likely $\delta^{15}\text{N}$ measurements given our model, and combining
271 these simulated isotope ratios with observed contaminant concentrations to estimate TMFs.

272

273 The MCMC algorithm applied was successful in estimating the posterior distribution of
274 diets, enrichment factor, mean isotope ratio for *Daphnia* and the error variance of the model.
275 The chains converged quickly and arrived at an acceptance rate of 0.189 during the last 25
276 000 iterations of all the 10 chains. The posterior dietary matrix (Figure 2) shows that there is
277 quite a large range of uncertainty with regard to the feeding relations in some compartments
278 (especially the small smelt and vendace, and brown trout), whereas for other populations a
279 narrower posterior was found. As $\delta^{15}\text{N}$ values were not available for small smelt and vendace
280 (only large fish), this may explain the larger uncertainty for these compartments in the
281 posterior dietary matrix, as well as for trout that assumed to have small smelt and small
282 vendace as their main prey.

283 **Enrichment factor – $\Delta\delta^{15}\text{N}$.** The posterior for the isotope enrichment factor (ΔN) was not
284 very different from the prior (Figure 3), meaning that our model and observations could not
285 adequately narrow down the distribution, thus underlining the importance of the variability in
286 this scaling factor. For future analyses we would recommend an even wider prior range for
287 the enrichment factor since the analysis did not narrow down the distribution substantially.
288 The 95% credibility interval²⁵ for the enrichment factor spanned from 2.77 to 3.97 ‰ with a
289 median of 3.29 ‰, lower than the commonly used value of 3.4 ‰. This suggests a lower

290 enrichment for the Mjøsa food web than previously have been assumed²⁹. In general, the
291 relationship between isotope enrichment factor and TMF is such that an increase in the
292 enrichment factor will make the estimated TMF tend away from 1. This means that assuming
293 a low enrichment factor will increase the risk of Type II error, i.e. increase the likelihood of
294 classifying a magnifying compound as non-magnifying by 'pushing' the estimate towards 1.
295 Such issues will be even more problematic in a frequentist approach, where the main
296 questions asked is 'how probable are these contaminant observations in the food web given no
297 magnification' where non-magnifying compounds are defined as chemicals which does not
298 exhibit a TMF significantly above 1.

299 The estimated ΔN in our model are generally lower than the assumed value of 3.4 ‰ used
300 in²³, the probability of the enrichment factor being lower than 3.4 ‰ is 0.64 and the
301 probability of the factor being lower than 3.0 ‰ is also substantial (0.13). This is one of the
302 major factors that lead to our estimates of TMF being slightly lower (i.e. closer to 1) for all
303 analysed compounds (Table 1) compared to the earlier analysis²³ and in the simple Bayesian
304 regression.

305 The enrichment factor ΔN is obviously associated with variability across time, space and
306 trophic level, and may be more appropriate on some specific trophic steps than others. This is
307 in contrast to previous studies that report one similar enrichment factor throughout the food
308 web³⁰, except for birds. Experimental studies on cormorants indicate that the ΔN from bird
309 diet to muscle tissue is 2.4‰³¹, which is less than the recommended 3.4‰. A Bayesian
310 approach (or more generally a distributional approach) to performing analyses with ΔN has
311 the possibility of including this uncertainty and quantifying it. Our model explicitly takes this
312 uncertainty in ΔN into account by using a distribution of the enrichment factor derived from
313 our observations and the structure of the food web. Extending this approach to include
314 distributions of ΔN for separate groups could be valuable.

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Predicted trophic level and TMF. One of the benefits of a Bayesian approach to parameter estimation is that instead of point estimates of parameters or regression coefficients, whole probability distributions are generated. These parameter distributions can then be used to generate more realistic predictions, since the natural variability in parameters, such as the enrichment factor, will be included in the estimate and the generation of the prediction distributions. Figure 4 show the predicted trophic levels of the populations in the food web when taking the uncertainty in diets, enrichment factor and error variance into account. By using these simulated trophic levels a narrower estimate of TMFs for all compounds are achieved, when compared to a standard Bayesian regression analysis of the observations alone (Table 1, Figure 5), despite the considerable uncertainty in some of the parameters (e.g. the diets). Using such Bayesian approaches can also lead to a better understanding of the uncertainties in bioaccumulation measures. Instead of using point estimates of TMF, as previously done in most TMF studies e.g. ^{5,14,22}, we can quantify the uncertainty. For our model here, for instance, we can quantify the total uncertainty; given our model and parameter estimates, there is a 89 % probability that the TMF for PCB-153, is greater than 2. For the cyclic siloxane D5, there is a 56% probability that the TMF is greater than 2.

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In summary, we have utilized Bayesian inference on the model relating relative isotope levels and trophic levels together with the structure of the food web to reduce the uncertainty in TMF estimates. With relatively few data points the method manages to estimate the diets of the species in the system, and use these diets to restrict the plausible values of trophic position of the species, and thereby also reducing the uncertainty surrounding TMF estimates. Such

339 reduction of uncertainty in the TMF estimate is especially of interest in cases where TMF is
340 close to 1, i.e. where there is a question of biomagnification, or not.

341

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436

437 **Table 1.** Distributions of trophic magnification factors (TMF; 2500 draws from each chain =
438 25000 simulated TMFs) for simple Bayesian regression (simple) and posterior predictive
439 simulation of the full model (full). The TMFs were determined for lipid normalized
440 concentrations. The simple model is identical to the regression model presented in the
441 empirical study²², adapted to a Bayesian framework.

TMF	Model	2.5%	Median	97.5%
PCB-153	Full	3.54	4.67	6.20
	Simple	3.06	4.91	7.77
PCB-180	Full	4.05	5.56	7.62
	Simple	3.65	6.01	9.85
P,P',DDE	Full	2.99	3.8	4.87
	Simple	2.55	3.89	5.92
BDE-47	Full	4.11	5.58	7.68
	Simple	3.48	5.83	9.93
BDE-99	Full	1.82	2.32	3.04
	Simple	1.09	2.44	5.4
D5	Full	1.66	2.03	2.45
	Simple	1.09	2.29	4.75

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443

444 **FIGURE LEGENDS**

445

446 **Figure 1.** Conceptual diagram of the Bayesian Food Web isotope Level Estimator. Boxes
447 are estimated parameters; rounded corners imply calculated values and circle represent
448 observations. The diets of all populations (except for mikrozooplankton) are estimated. These
449 diets are used to calculate trophic levels for all compartments, using equation 4. Together with
450 independently estimated μ_D and the isotope enrichment factor (ΔN) these values are used to
451 calculate isotope population means for all compartments, using equation 5. With an estimated
452 error variance these can be used to predict the observed $\delta^{15}\text{N}$ values. For the estimation of
453 these parameters the only information used are the observed $\delta^{15}\text{N}$ values as well as the
454 structure of the food web.

455

456 **Figure 2.** Estimated parameters of the food web from the Bayesian analysis. All priors
457 used were uninformative Dirichlet distributions (i.e. uniform in n -dimensional space), the
458 only previous knowledge included in the estimation was which entries in the matrix that were
459 non-zero. Note that the distributions are highly correlated, also across compartments. X-axes
460 are from 0 to 1, and Y is scaled to highest probability for the 51 bins used to generate the
461 histograms.

462

463 **Figure 3.** Isotope enrichment factor (ΔN). Prior (line) and posterior (histogram)
464 probability distribution of the ΔN . The posterior distribution has 2.5 50 and 97.5 percentiles
465 of 2.77 ‰, 3.29 ‰ and 3.97 ‰.

466

467 **Figure 4.** Posterior predictive simulation of trophic levels for the biological
468 compartments. Lines span from 2.5 to 97.5 percentiles, bars at 25 and 75 % with diamond

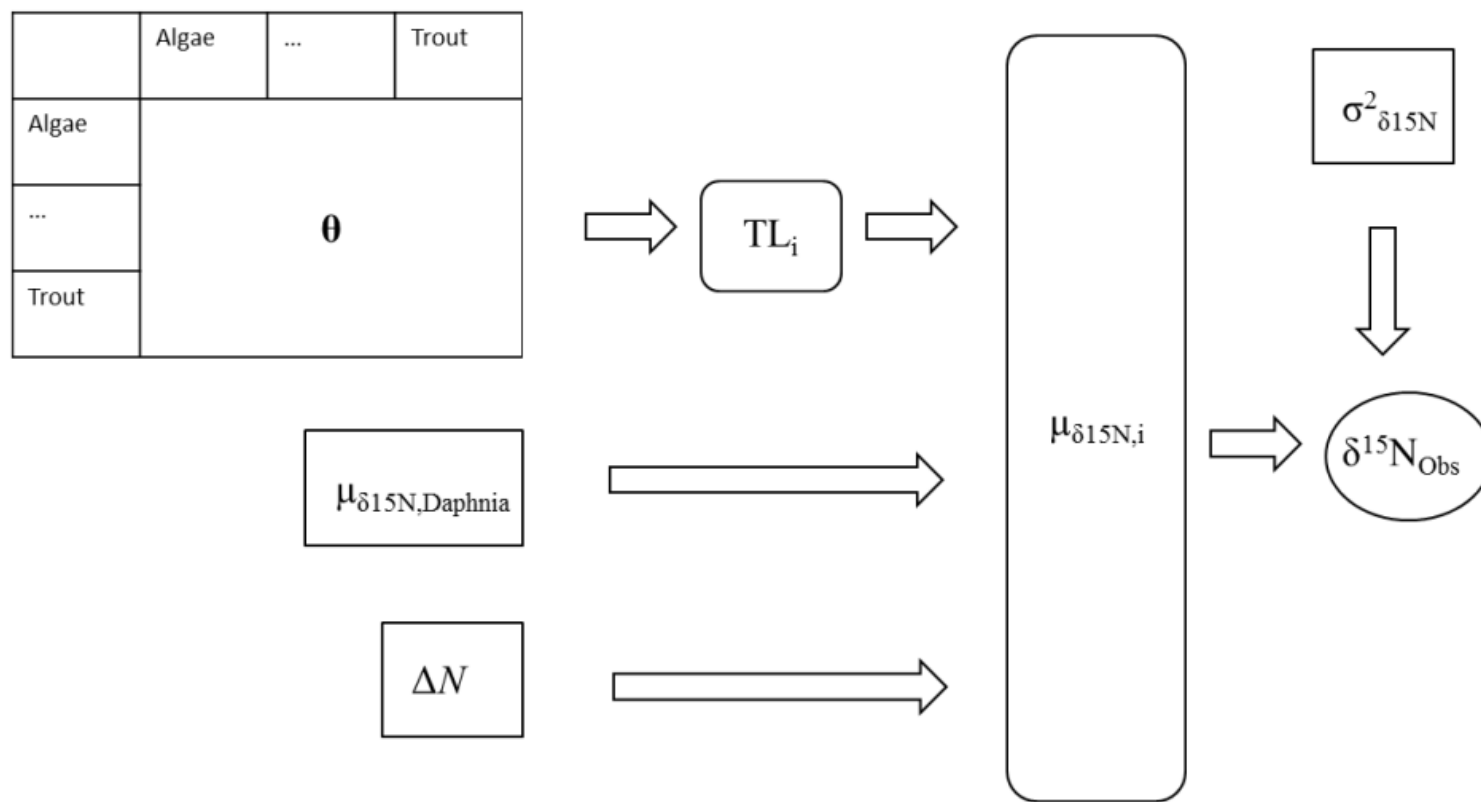
469 indicating the median value. The trophic levels were simulated by selecting sets of parameters
470 from the converged chains (i.e. diets, μ_D for daphnia, ΔN and variance estimate of $\delta^{15}\text{N}$
471 estimates). The $\delta^{15}\text{N}$ means were then calculated for all compartments and a deviation was
472 added using the variance estimation. These ‘simulated’ $\delta^{15}\text{N}$ values were then back calculated
473 to trophic levels using eq 2. Note that these estimates will be correlated, i.e. a higher trophic
474 level for trout is accompanied by higher trophic levels for the species in its diet. The
475 independently estimated isotope level for Daphnia used to fix the relationship in eq 5 had a
476 median value of 8.107 with a 95% confidence interval from 7.229 to 9.035.

477

478 **Figure 5.** Distributions of trophic magnification factors (TMF; 2500 draws from each chain =
479 25000 simulated TMFs) for simple Bayesian regression (grey lines) and posterior predictive
480 simulation of the full model (black). See Table 1 for median and 95% confidence intervals.

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

















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Figure 1.

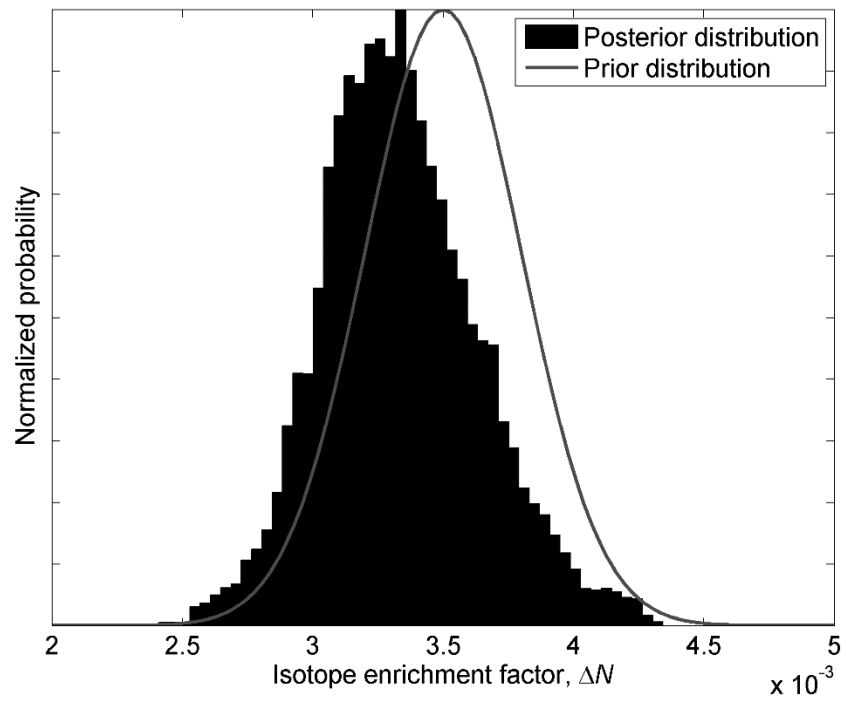
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	POM	Micro-zooplankton	Daphnia	Copepod	Mysis	Vendace small	Vendace large	Smelt small	Smelt large	Trout
POM										
Micro-zooplankton	1									
Daphnia										
Copepod										
Mysis										
Vendace small										
Vendace large										
Smelt small										
Smelt large										
Trout										

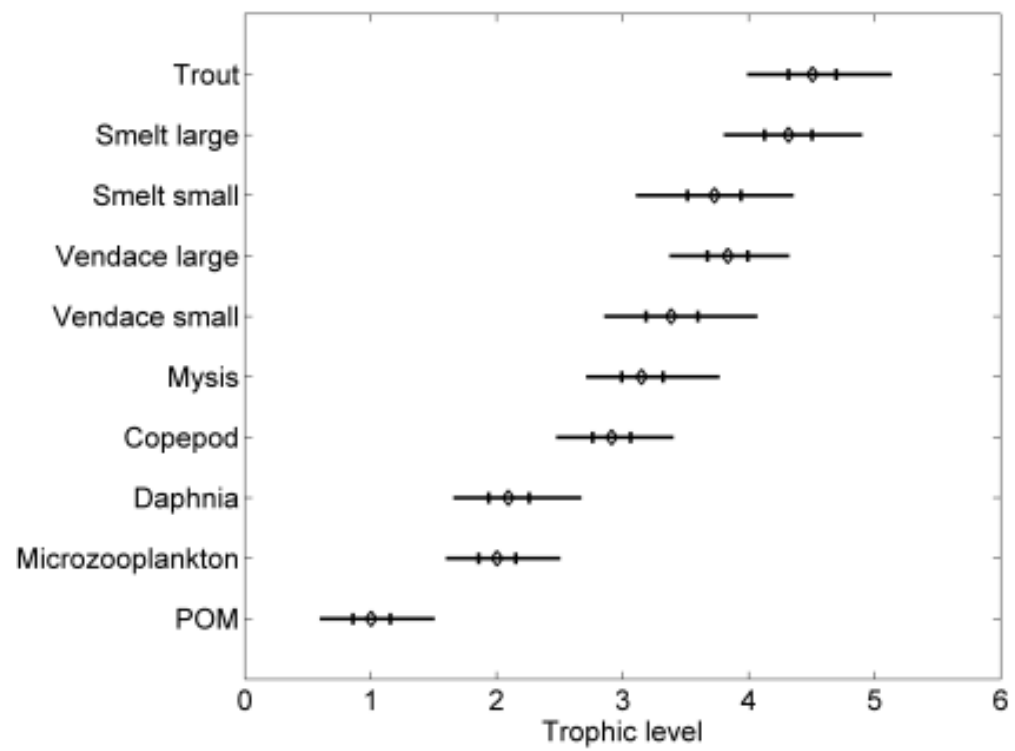
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1 **Figure 2.**



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Figure 3.

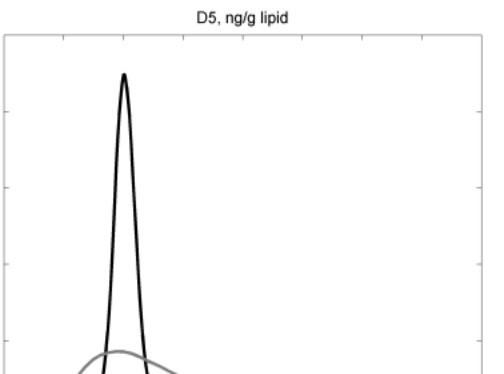
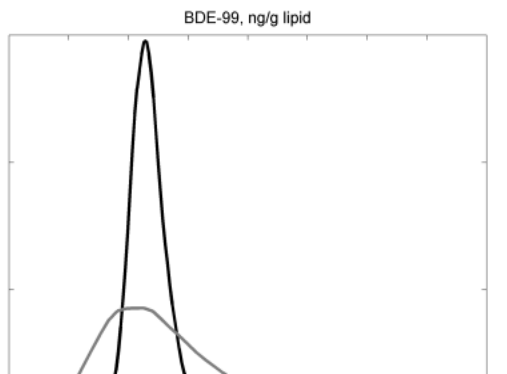
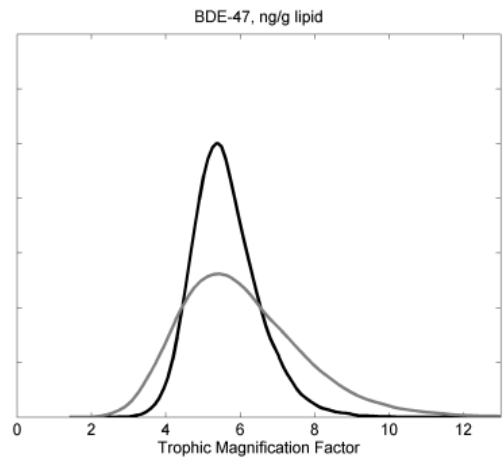
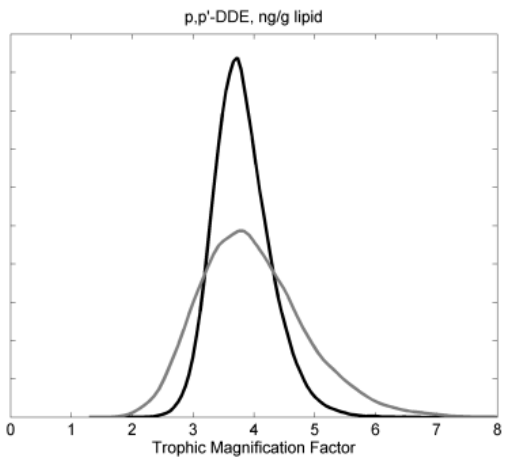
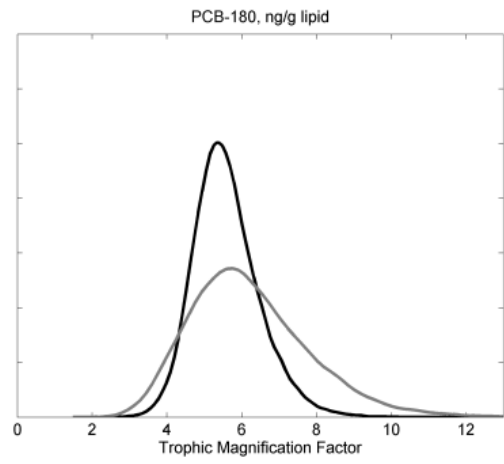
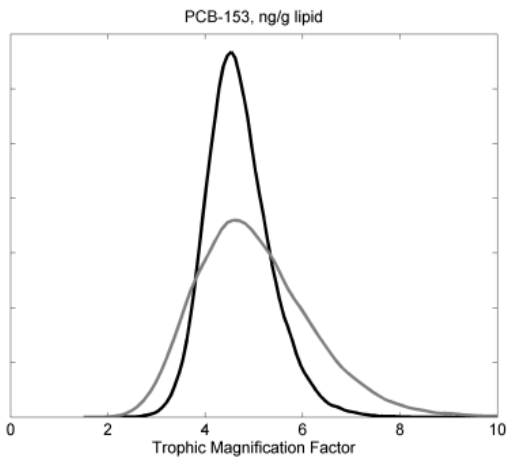


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2 **Figure 4.**

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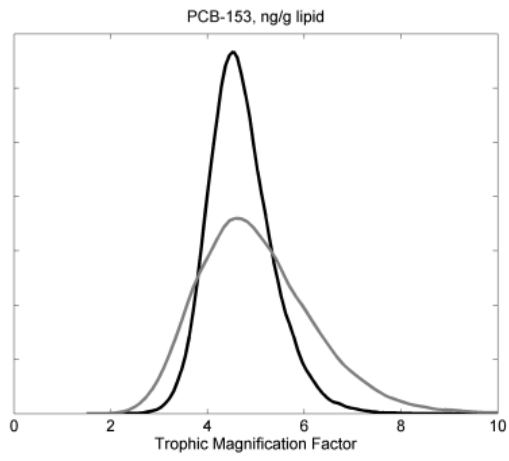
6 **Figure 5.**

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1 TOC / Abstract art figure:

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