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Trophic Magnification Factors: Impact of Ecology, Ecosystem and Study Design

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	with the contaminant measurements and use of $\delta^{15}N$ used for TL estimations. Recommendations are provided towards experimental design, data treatment and statistical analyses, including advice for users on reporting and interpreting TMF data. Interspecies and intrinsic ecological and organismal properties, such as thermoregulation, reproductive status, migration, and age - particularly among species at higher trophic levels with high contaminant concentrations - can influence the calculation of TMF (i.e. regression slope). Ecosystem status and lower trophic levels are important considerations in characterizing the baseline and starting point of accumulation (i.e. regression intercept). Following recommendations herein for study design, empirical TMFs are likely to be useful for understanding the food web biomagnification potential for chemicals, where the target is to definitively identify if chemicals biomagnify or not (i.e. TMF > or < 1). TMFs may be less useful in species and site-specific risk-assessments, where the goal is predicting absolute contaminant concentrations in organisms to be evaluated against threshold levels for effects of the specific chemical.

Trophic Magnification Factors: Impact of Ecology, Ecosystem and Study Design

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Abstract

Recent reviews by researchers from academia, industry and government have revealed that the criteria used by the Stockholm Convention on persistent organic pollutants (POPs) under the United Nations Environmental Programme are unable to identify the actual bioaccumulative capacity of some substances using chemical properties such as K_{OW}. Rather, trophic magnification factors (TMFs) were suggested as the most reliable tool for bioaccumulation (B) assessment of POPs for those chemicals that have been in commerce long enough to detect them in environmental samples. TMFs are increasingly used to quantify biomagnification, and represent the average prey to predator transfer of POPs through food webs, rather than the individual species biomagnification metrics that are highly variable from one predator-prey combination to another. TMF is calculated from the slope of logarithmically transformed concentrations of POPs versus trophic level (TL) of organisms in the food web; the latter is often calculated from stable nitrogen isotope ratios ($\delta^{15}N$). In this paper we give the background for the development of TMFs, identify and discuss impacts of ecosystem and ecological variables on TMF values, and discuss challenges and uncertainties associated with the contaminant measurements and use of $\delta^{15}N$ used for TL estimations. Recommendations are provided towards experimental design, data treatment and statistical analyses, including advice for users on reporting and interpreting TMF data. Interspecies and intrinsic ecological and organismal properties, such as thermoregulation, reproductive status, migration, and age - particularly among species at higher trophic levels with high contaminant concentrations - can influence the calculation of TMF (i.e. regression slope). Ecosystem status and lower trophic levels are important considerations in characterizing the baseline and starting point of accumulation (i.e. regression intercept). Following recommendations herein for study design, empirical TMFs are likely to be useful for understanding the food web biomagnification potential for chemicals, where the target is to definitively identify if chemicals biomagnify or not (i.e. TMF > or < 1). TMFs may be less useful in species and site-specific risk-assessments, where the goal is predicting absolute contaminant concentrations in organisms to be evaluated against threshold levels for effects of the specific chemical.

Keywords: TMF, POPs, trophic level, contaminants, stable isotopes, food web, field studies, regression

INTRODUCTION

Background

Recent reviews resulting from an international Pellston workshop with scientists from academia, industry and government on bioaccumulation science revealed that the bioaccumulation (B) criteria used by the Stockholm Convention on POPs (UNEP 2001) and many national risk assessment programs (e.g. European Commission 2003) were unable to identify the actual bioaccumulative capacity of some substances using chemical properties like octanol-water partitioning coefficient (K_{OW}) (Gobas et al. 2009; van Wijk et al. 2009; Weisbrod et al. 2009). Bioaccumulation is the process that causes an increased chemical concentration in an organism compared to that in its ambient environment, through all exposure routes, including dietary absorption and transport across body surfaces. Furthermore, biomagnification can be regarded as a special case of bioaccumulation in which the chemical concentration in the organism exceeds that in its prey due to dietary absorption being faster than elimination (Gobas and Morrison 2000), which may lead to concentrations that can threaten the health of top predator organisms (Fisk et al. 2005; Letcher et al. 2010).

Traditionally, environmental risk assessments of persistent organic pollutants (POPs) have been based on results extrapolated from controlled laboratory tests. Many of these studies have provided measures of bioaccumulation and calculations of bioconcentration factor (BCF), bioaccumulation factor (BAF), biota-sediment accumulation factor (BSAF), or biomagnification factor (BMF) for different organisms under varying exposure conditions including different abiotic conditions (e.g. pH, salinity), contaminant properties (e.g. K_{OW} and octanol-air partitioning coefficient (K_{OA})), and/or biotic (e.g. habitat, feeding mode, food quantity /quality, trophic transport) factors (Gobas et al. 2009; Weisbrod et al. 2009; Burkhard et al. 2010; Selck et al. 2010). Although we have a relatively good understanding of the factors controlling bioaccumulation of non-ionic organic contaminants under laboratory conditions for traditional terrestrial and aquatic test species, empirical BCFs from laboratory studies and BAFs from field samples can differ by several orders of magnitude (e.g. Arnot and Gobas 2006; Borgå et al. 2005). Laboratory results do not translate easily, or perhaps not at all, to similar metrics in the

field when considering bioaccumulation and biomagnification of contaminants (e.g. Weisbrod et al. 2009; Burkhard et al. in prep; Selck et al. in prep).

The Pellston workshop concluded that BCF was not a good descriptor of the biomagnification capacity of chemical substances. In aquatic food webs, poorly metabolized hydrophobic chemicals with log K_{OW} >5 generally biomagnify, while chemicals with log K_{OW} <5 do not (Gobas et al. 1999). In terrestrial food chains, however, some chemicals with log K_{OW}<5 and BCFs <5000 have been shown to biomagnify (e. g. chlorobenzenes, lindane, PFAs) (Kelly et al. 2007; 2009). The water solubility and vapor pressure of the chemical affects the rate of elimination in water-, and air-breathing organisms respectively, and Kelly et al. (2007) suggested that K_{OW} alone cannot be used to identify all bioaccumulative substances in food webs. They concluded that, for air-breathing organisms, K_{OW} and BCF in fish are not good predictors of biomagnification for chemicals with log $K_{OA} \geq 6$ and $K_{OW} > 2$. In addition, BCF is determined using tests that are (a) difficult to perform for very poorly water soluble organic chemicals with high bioaccumulation potential, (b) time consuming, and (c) costly. Rather, trophic magnification factors (TMF) were suggested as the most reliable tool for contaminant bioaccumulation (B) assessments of chemicals that have been in commerce long enough to be detectable in environmental samples (Gobas et al. 2009; Weisbrod et al. 2009). TMFs were earlier called food web magnification factors (FWMF) and food web concentration factors (FWCF). As described below, TMFs are currently determined empirically using field measures of both contaminant concentrations and relative trophic position or level [TL; estimated from stable nitrogen isotope ratios ($\delta^{15}N$) calculated from tissue ratios of $^{15}N/^{14}N$] in food webs, and represent average food web biomagnification (Fisk et al. 2001a; Jardine et al. 2006). Unlike bioconcentration, the TMF approach assumes that the diet is the major exposure route for contaminants, and biomagnification, due to dietary absorption being faster than elimination (Gobas and Morrison 2000), will result in TMFs above 1. TMFs above 1 imply increasing disequilibrium between trophic level and media (water or air), thus chemical properties enhancing or reducing disequilibrium are particularly important to consider in the TMF approach.

In the 1980s a long-standing debate about the significance of trophic position on the extent of non-ionic organic chemical bioaccumulation by aquatic biota was resolved when Connolly and Pedersen (1988) showed that fugacity ratios of PCBs between fish and water were generally greater than 1 and increased with trophic level and hydrophobicity of the chemical. Thomann (1989), using a food chain model, showed optimum log K_{OW} and molecular size for biomagnification, i.e. log K_{OW} 5.0-8.0, while Clark et al. (1990) developed a fugacity based food chain model which included the dependence of fish concentration on rates of metabolism and growth, and the effect of reduced bioavailability. However, the models used hypothetical food chains and did not consider the actual trophic position of the organisms. In the early 1990s, biomagnification of contaminants (selected polychlorinated dioxins (PCDDs) and dibenzofurans (PCDFs)) was assessed by an integrated approach using two whole food webs in the Northern Baltic (Broman et al. 1992; Rolff et al. 1993), rather than using single predator-prey relationships as in biomagnification factors (BMFs). The new approach quantified biomagnification by first assessing the organisms' relative positions in the food web based on the biological fractionation of $\delta^{15}N$, and then regressing the measured contaminant content against $\delta^{15}N$, to quantify the rate of trophic transfer of POPs. This method was soon applied to different compounds and food webs, e.g. mercury (Hg) and POPs in lakes (Kidd et al. 1995a; 1995b), and was used to study how different factors such as lipid content and trophic position influenced the transfer of contaminants within the food web (e.g. Kidd et al. 1998). Over the past two decades, many studies have used $\delta^{15}N$ to assess the trophic transfer of contaminants through marine and freshwater food webs.

The initial studies described above showed that concentrations of POPs or Hg were significantly related to the increase in $\delta^{15}N$ from primary consumers to top predators in aquatic food webs (Broman et al. 1992; Kidd et al. 1995a). Later, the method was refined by calculating integer-based TL (or trophic position, TP) from $\delta^{15}N$ using enrichment factors (fractionation of ^{15}N into predator, called Δ15N; Fisk et al. 2001a; Eqs. 1-2) and assumptions that the primary producers and primary consumers included in the calculations occupied discrete trophic levels of 1 and 2, respectively.

$$TL_{consumer} = ((\delta^{15}N_{consumer} - \delta^{15}N_{primary\ producer}) / \Delta 15N) + 1 \quad (1)$$

or

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

This refinement allowed assessment of the average change in contaminant concentration per relative trophic level (rather than per $\delta^{15}N$) in the food web and is analogous to the average biomagnification of a contaminant through the system (Jardine et al. 2006). In addition, this method corrects for the baseline variation in $\delta^{15}N$ that occurs among systems as a result of human inputs of nitrogen from wastewaters or agriculture (e.g. Anderson and Cabana 2005).

The use of relative TL rather than $\delta^{15}N$ also allows unique enrichment factors for ecosystems, species or groups of animals to be incorporated into Eqs. 1 and 2 as needed to refine TMF calculations. This approach was soon applied in more recent studies (e.g. Hop et al. 2002; Hoekstra et al. 2003; Muir et al. 2003, Mackintosh et al. 2004; Kelly et al. 2007; 2009; Tomy et al. 2009) to quantify TMFs in diverse aquatic ecosystems. Indeed, this technique has been used in Arctic, temperate, and tropical lake and ocean food webs to understand the rate of trophic transfer of contaminants.

Due to differences in biomass and contaminant transfer efficiency, contaminant concentrations often increase exponentially through the food web (**Fig. 1**). Therefore, the regression is usually, but not always, log-normal (Eq. 3), and TMF calculated as the antilog of the regression slope with base 10 or e depending on the logarithmic transformation (Eq. 4). Thus, in its most simple form, TMFs are calculated as follows:

$$Log[Contaminant] = a + bTL + \varepsilon \quad (3)$$

$$TMF = 10^{b} \text{ or } e^{b} \quad (4)$$

In the absence of significant metabolism, contaminants with log K_{OW} values less that 5 tend to achieve concentrations that represent a thermodynamic equilibrium between the fish (predator or prey) and surrounding water. A contaminant is said to 'biomagnify' when lipid-normalized concentrations (or fugacity) of accumulated chemical residues in biological organisms increase with increasing trophic position (Fisk et al. 2001a). Therefore, TMFs can be used to understand whether a chemical does or does not biomagnify through aquatic food webs.

For TMF = 1 (b=0), the chemical does not biomagnify, on average, through the food web. For TMF > 1 (b>0), the chemical biomagnifies through the food web, on average a factor of TMF per trophic level. For TMF < 1 (b<0), the chemical decreases, on average, in concentration with each trophic level in the food web, also called trophic dilution. TMFs can then be compared across systems and chemicals to understand how biomagnification varies with properties of the ecosystems or chemicals of interest.

Baseline variability among ecosystems due to different $\delta^{15}N$ or inputs of contaminants at the base of the food webs, such as between different lakes, or between ice, pelagic or benthic food webs, is accounted for by the intercept (a) in the regression (**Fig. 1**), so that the "rate of increase" per unit in the food web can be studied independently of exposure level (Broman et al. 1992). However, most of the focus of TMF studies has been on the relationship between the contaminant versus TL (regression slope) rather than on the ecosystem properties (regression intercepts), and the significance of the latter, including interaction between the two, is not yet well understood.

Assumptions when Calculating and Using TMFs

The TMF approach and Equation 3 assume that the diet is the major route of exposure to contaminants, and that trophic level is the main driver of accumulation for contaminants in organisms and food webs. If other factors are important for the observed contaminant residue in an organism (e.g. age, size, reproductive status, biotransformation efficiency, omnivorous feeding), the regression of contaminant level onto trophic level will become increasingly obscured the more these factors differ among species included in the calculation. When estimating the average increase of contaminant concentrations with trophic position in the food web, the main interest is to identify and quantify biomagnification both in terms of assessing actual "B" in the environment and in terms of risk assessment. Thus, other factors that may influence bioaccumulation in organisms should be acknowledged and accounted for in the regression model, or assumed to be negligible compared to the influence of trophic position, for the relationship to be significant. Without considering other drivers of contaminant accumulation, the TMF approach assumes that, e.g., the energy transfer efficiency and

biotransformation ability is comparable among trophic levels (Broman et al. 1992) and that the $\delta^{15}N$ fractionation is similar (or at least known) among trophic levels. The assumptions described below introduce different challenges and assumptions for the estimation and use of TMFs, which will be addressed in the subsequent sections.

When assessing the change in contaminant load per trophic level, it is important to ensure that the trophic transfer of contaminants is the process quantified rather than changes in the cellular medium to which the contaminant is associated (Kidd et al. 1995b). Because lipid content and contaminant concentrations are often correlated across organisms, results are typically normalized to lipid content before the regression analysis such that the TMF values are calculated and reported on the basis of lipid-equivalent concentrations. Contaminants associated with other cellular media (e.g. proteins) should also be normalized in the same manner (e.g. Kelly et al. 2009), although there has been much less study of this. However, alternative approaches for TMF estimations are also presented in the subsequent section on data treatment and statistical analyses.

The main assumption for estimating TMFs, and all other bioaccumulation metrics, is that the organism or consumer is at steady state with its environment, or here, its diet. Steady state is critical not only for the contaminant concentrations but also for the reflection of dietary habits of the organism. For example, $\delta^{15}N$ may vary temporally as much as 5‰ in phytoplankton depending on the stage of the bloom (Tamelander et al. 2009) and this variation, if not considered in the sampling design, could result in an estimated trophic level difference of about 1.5 in the consumer. Along the same lines, one must ensure that the species included in a food web relationship are actually trophically related and representatives of the same food web, e.g. benthic or pelagic (see section on Organismal Properties). To better define a food web, individual species can be assigned to food chains or to a more narrowly defined food web using the stable isotope signatures of elements that are not biologically fractionated during trophic transfer and used mainly to assess energy flow to consumers. This can be done by using stable carbon ($\delta^{13}C$) or sulfur ($\delta^{34}S$) isotope ratios. Carbon and sulfur isotope ratios are conserved or only slightly enriched from prey to predator and can be used to assess whether consumers are supported by the same primary producers (Peterson and Fry 1987), and reflect the flow of energy

within a food web (Post 2002). Similarly, one must also ensure that the contaminant being evaluated originates from a common source at the base of the food web and not from multiple sources that may occur at different trophic levels throughout the food web (see section on Spatial Variation). Slowly accumulating contaminants, seasonal changes in diet and migratory species are examples of other challenges to the steady state assumption, as is differences in the time needed to obtain steady state for contaminants versus stable isotopes, which will all be addressed in subsequent sections. The challenge of steady state has wide implications as the estimated TMF is assumed to represent the average biomagnification in a local food web, where the actual food web is represented.

Challenges and Uncertainties with TMFs

There are several challenges and uncertainties related to the use of TMFs that are described in this manuscript. They include biological factors such as the differences between poikilotherms (cold-blooded) and homeotherms (warm-blooded) in their energy requirements and abilities to metabolize chemicals, and the uncertainties regarding the assumed steady state in contaminants and stable isotopes between a consumer and its diet. Chemical challenges include the current restriction of TMFs to entirely field-based measurements, and the major analytical limitations (detection and otherwise) in using this technique for contaminants other than the legacy POPs. Methodological challenges include the analysis of tissues or organs, rather than whole body, for chemicals and the assumption that the sub-sample is representative of biomagnification in the whole body. This is particularly a problem for mammals and birds, where whole samples are generally not available, and for non-lipophilic substances, where solvent normalization practices are less standardized. Statistical treatment is also a major challenge as the TMF is affected by choices made during collection and analysis of samples (i.e. experimental design), data processing and calculations. The present paper addresses most of these issues and provides recommendations to improve future study designs and, ultimately, a better understanding of biomagnification. Refinement and application of the TMF technique should improve our ability to assess and predict biomagnification of chemicals and their risk to the environment and humans.

Advantages of TMFs

The main advantage with TMF is that it validates or augments the "B" criteria for chemicals, i.e. by quantifying biomagnification potential in the field. Field-derived TMF values are considered to represent a more accurate and holistic measure of biomagnification compared to laboratory-derived metrics and chemical properties, or BMFs between single predator and prey, all of which can mis-characterize chemicals (Gobas et al. 2009; Weisbrod et al. 2009). For example, certain high molecular weight phthalate esters are very hydrophobic chemicals with a high Kow (log Kow > 5) and BCFs > 1,000, but they are not biomagnifying in aquatic food chains as demonstrated by their TMF < 1 (Mackintosh et al. 2004). Polyaromatic hydrocarbons (PAHs) and cyclic methylsiloxanes (CMS) are other examples of substances with this bioaccumulation behavior (Wan et al. 2007; Powell et al 2009; 2010a). In contrast, substances like perfluorooctane sulfonate (PFOS) exhibit a relatively low BCF < 5,000 but are known to biomagnify in some aquatic food webs as demonstrated by TMFs > 1 (Houde et al. 2006; Tomy et al. 2009).

For site- or region-specific risk assessment of chemical effects on specific species, the TMF and underlying regression model can be used to predict chemical concentrations for unmeasured trophic levels. However, prediction of absolute concentrations on a trophic level basis is more problematic than the "B" assessment (TMF > or < 1), because a better species-specific understanding of the relationships between trophic position, diet, and contaminant accumulation is required. This is particularly the case for species where other factors are important for bioaccumulation, such as age, size, reproduction and biotransformation, and where these factors differ among species. Additionally, in risk assessment, the estimation of concentrations in biota is not the only desired data. Concentrations at the food web base (i.e. the intercept with the Y-axis), and their relationship to concentrations in abiotic media (e.g. sediment, soil, water) are often of interest in chemical management. An understanding of these relationships is not provided by the TMF values alone but may be obtained from the regression model used to derive the TMF values.

Objectives

The aims of the present paper are to identify and discuss:

- the effect of ecosystem and ecological variables such as organism properties, food web structure and spatial and temporal distribution on TMFs.
- chemical and environmental properties that affect TMF directly or its baseline conditions, such as exposure concentrations, primary production, dissolved organic carbon (DOC).
- methodological aspects regarding stable isotope and contaminant measurements, as well as statistical considerations and data treatment for TMFs.
- recommendations for conducting food web studies and reporting and interpreting TMF data.

In the following we focus primarily on legacy-type organic contaminants (i.e. PCBs and other POPs), but also include knowledge on Hg and emerging chemicals of concern such as CMS when appropriate.

TMFs – WHAT AFFECTS THEM?

As TMFs are calculated from the regression of the sample (organism) contaminant concentrations onto their trophic position, the degree to which the assumptions are fulfilled will influence the slope of the regression, and thus the TMF. When calculating the TMF for a set of species or samples, it is assumed that the relationship between the contaminant concentrations and trophic level is the same across all species, and that contaminant concentrations are mainly driven by trophic level (and thus diet) (Broman et al. 1992). These may not be valid assumptions both between and within species. The main factors that may affect the biomagnification of chemicals, in addition to diet, are discussed below.

Organismal Properties

Species at the same relative trophic level may have very different levels of contaminants depending on their energy demands (e.g. Braune and Norstrom 1989) and biotransformation rates (e.g. Borgå et al. 2005). Several studies have documented lower TMFs for recalcitrant

compounds in aquatic food webs including poikilothermic species only, whereas the TMFs were significantly greater when homeothermic species were included (Fisk et al. 2001a; Hop et al. 2002). Homeotherms have higher energy requirements compared to poikilotherms, and higher weight-specific metabolic rates resulting from higher food intake. Thus, they are more exposed to contaminants through food intake resulting in potentially higher biomagnification of recalcitrant contaminants in a bird than in a fish of comparable size and trophic position (Braune and Norstrom 1989). In contrast, if the apex predator can biotransform the chemical then this may result in lower TMFs. A food web that contains an apex predator with the ability to biotransform a compound that is poorly biotransformed by lower trophic level organisms would result in varying BMFs between different predator-prey pairs. This effect has been well documented in the polar bear food web by Letcher et al. (1995) for compounds such as 4,4'-DDE. The BMF for 4,4'-DDE between polar bear and its major food, ringed seal, is very low (0.6) due to the extensive biotransformation of 4,4'-DDE into methyl-sulfone metabolites in polar bears. Conversely, the BMF for 4,4'-DDE between ringed seal and polar cod, a major food item for ringed seals, is 39. Similar biotransformation effects have also been observed by these researchers for a variety of PCB congeners in this food web. Other recent studies that included birds in the upper trophic levels had lower TMFs compared to results for the same food web without birds (Hallanger et al. in press a). This was particularly the case for less recalcitrant compounds and chemicals susceptible to biotransformation, and demonstrates that physiological characteristics of individual species can affect determinations of TMFs for food webs. Marine mammals are often included in TMF studies usually as apex predators. Biotransformation ability among marine mammal groups can vary widely depending on the type of compound. For example, seals tend to have a relatively good ability to biotransform PCBs with vicinal hydrogens in the *meta*- and *para*-positions while the opposite is true for cetaceans (Tuerk et al. 2005). This can also be a concern when comparing food webs with different species of fish. Stapleton and colleagues, for example, have found that sculpin are able to produce methyl-sulfone PCB metabolites, thereby reducing their PCB burden relative to other fish (Stapleton et al. 2001). Differing degrees of polybrominated diphenyl ethers (PBDE) biotransformation have also been observed between carp and rainbow trout (Stapleton et al. 2006). Differing elimination abilities can also occur between sexes of the same species, as has been previously observed for perfluorooctanoic acid (PFOA) in rats (Kemper and Jepson 2003) and more recently in fish (Lee

and Schultz 2009). The effect of bioenergetics and biotransformation needs to be recognized in TMF studies as a potentially confounding factor. Failure to identify these differences will result in over- or under-prediction of the TMF.

Another property that will influence the TMF is the route of contaminant uptake into the organisms. When regressing the contaminant concentrations onto trophic level, one assumes that the main route of exposure for an organism is from its diet. However, all invertebrates and also fish are, to a large degree, influenced by direct uptake across respiratory surfaces, and the relative importance of food versus water exposure for a particular chemical will likely influence the magnitude of its TMF in the food web. This is more of a consideration for the lower-trophic-level organisms with high surface area to body ratios and for the chemicals that are more water soluble (Borgå et al. 2004).

For some organisms such as fish at higher trophic levels, size or age affects bioaccumulation of the contaminant as the larger, older fish are slower growing with lower elimination rates (Jardine et al. 2006; Swanson and Kidd 2010). It is well known that larger, slower-growing individuals are typically higher in POPs and Hg concentrations than younger, faster-growing conspecifics. Comparisons of TMFs across systems may be confounded if one system is dominated by slow growing top predators compared to another. One way to address this is to standardize the sampling of fish or data included in the calculations of TMFs to a certain range of sizes. Another option would be to remove the variability associated with size or age of fish by regressing residuals (after the effects of size or age are removed) in contaminant concentrations against TL. This approach has not been commonly used in calculations of contaminant-$\delta^{15}N$ (or TL) relationships but may improve estimates of TMFs to be more reflective of trophic transfer by decreasing variability. For recent studies of Hg in Arctic lake food webs, removing the effects of size and age of the fish decreased the slope in some systems (Swanson and Kidd 2010).

Gender is particularly important for POPs because female mammals can reduce their concentration by transfer due to lactation and via the placenta. Loseto et al. (2008) showed that beluga whales will segregate geographically by length, sex, and reproductive status leading to

distinct feeding habits that ultimately result in different Hg concentrations in the muscle and livers of the segregated populations. Although not well explored, it is possible that a food web that is dominated by reproductively active females of an apex predator may have a lower TMF values for lipophilic POPs (due to higher maternal elimination rates of contaminants) than a food web that is dominated by males of the same species.

If the relationship between contaminant load and trophic level is strong, and the regression is significant, the range on the axis, e.g. the number of trophic levels, should not influence the regression. If, however, the food web includes high trophic level species with large leverage on the regression, i.e. has an unbalanced design, then the number of trophic levels included in the regression (or more precisely, species included), will affect the slope and thus TMF. This was seen for methylmercury (MeHg) in Arctic char food webs in Canadian Arctic lakes (Gantner et al. 2010b), and for organochlorines in the Barents Sea marine pelagic food web (Hop et al. 2002; Borgå et al. in prep).

Characterizing Food Webs with Stable Isotopes

An organism's position in the food web can be quantified using relative abundances of naturally occurring stable isotopes of nitrogen ($^{15}N/^{14}N$, referred to as $\delta^{15}N$) (e.g. Peterson and Fry 1987). The enrichment in $\delta^{15}N$ is generally 3.0 to 5.0 ‰ between trophic levels in aquatic food webs (average of about 3.4-3.8‰; Hobson and Welch 1992; Jardine et al. 2006), whereas for birds the $\delta^{15}N$ between its diet and muscle tissue is only 2.4 ‰ (Mizutani et al. 1991). Increases in $\delta^{15}N$ occur because of the preferential retention of the heavier isotope compared to the lighter isotope in the predator relative to its prey. This technique provides a continuous measure of longer-term feeding habits of an organism than those available from gut contents alone.

Several assumptions are made when using $\delta^{15}N$ to estimate the trophic position of organisms within a food web. The first, and perhaps most important, is that ^{15}N fractionates in a predictable manner from prey to predator. For aquatic organisms, fractionation and enrichment factors (Δ15N) used to calculate TL are often assumed to be about 3.4 ‰ and this is based on a

number of feeding experiments or syntheses of the literature (e.g. Vander Zanden and Rasmussen 2001; Post 2002; McCutchen et al. 2003). However, there are several organism-level factors (e.g. nutritional status and age) that can affect this enrichment factor and contribute some uncertainties when calculating relative TL within food webs. These factors contribute to the variability in $\Delta^{15}N$ that is observed among individuals either in laboratory or field studies and that may lead to violations in the assumption that ~ 3.4‰ is appropriate for calculations of TL. Nonetheless, an enrichment factor of 3.4‰ per trophic level step is recommended for constructing food webs without *a priori* knowledge of $\Delta^{15}N$ or the ecology of the system (Jardine et al. 2006).

Fractionation of ^{15}N is affected by the physiology of the organism. Animals undergoing periods of rapid growth where protein demands for new tissue are high have lower enrichment factors than those with slower growth rates (e.g. Hesslein et al. 1993). Starvation or higher metabolic rates will also result in some catabolism of body proteins and an enrichment of the ^{15}N of the predator relative to those with adequate food or lower metabolic rates (e.g. Gaye-Siessegger et al. 2004). Along these lines, composition of an individual's diet can also contribute to the magnitude of ^{15}N fractionation. Several studies that have shown that fractionation of ^{15}N is higher into animals consuming protein-poor than protein-rich foods. In addition, even at a constant diet, $\Delta^{15}N$ from diet to predator may be affected by age. Though the literature remains equivocal, older walleye on a constant diet had higher enrichment factors than younger walleye (Overman and Parrish 2001). For this reason, it may be important to normalize $\delta^{15}N$ to species size or remove the variation caused by intraspecific differences in size prior to calculating an individual's TL (e.g. Swanson and Kidd 2010).

Second, use of enrichment factors in TL calculations assumes steady state between an animal and its diet. However, wild animals are often opportunistic feeders with diets that vary over seasons or with life stage. The tissues used for isotope analyses could therefore reflect either shorter or longer term dietary habits of that individual because some tissues are more metabolically active and have higher turnover times than others. For example, liver tissues have higher tissue turnover rates and reflect changes in an organism's diet much more quickly than muscle tissues; isotope analyses of liver and muscle tissues in both poikilotherms and

homeotherms will reflect feeding habits over shorter (weeks to months) and longer (months to years) periods, respectively (Hobson and Clark 1992; MacNeil et al. 2006).

As described above, stable isotope signatures in different tissues reflect different time spans (Hobson and Clark 1992), since isotopic turnover and incorporation rates can vary markedly among different tissues of a single animal (Tieszen et al. 1983; Hobson and Clark 1992). If the turnover rates for contaminants and stable isotopes differ in a given tissue, a thorough understanding of the co-variation is important to be able to link the two and calculate TMFs. Also, if the diet of an organism changes over time, exposure to contaminants with long half-lives, such as PCBs and DDE, may be misrepresented by measuring tissues with a fast turnover of $\delta^{15}N$ but not contaminants.

The other critical information when calculating TL is adequate characterization of the baseline of the food web. As shown in Eqns. 1 and 2 above, TL is calculated using both an assumed $\Delta^{15}N$ and baseline $\delta^{15}N$ value. Human activities such as agriculture and municipal wastewater inputs can affect the $\delta^{15}N$ signature of primary producers supporting the food web (e.g. Anderson and Cabana 2005) and short-lived (typically lower-trophic-level) organisms are known to be more variable over time in $\delta^{15}N$. As a result, $\delta^{15}N$ for a longer-lived primary consumer is used to standardize baselines before any comparisons across systems can be made (Vander Zanden and Rasmussen 1999; Post 2002).

Most of the focus in the TMF literature has been on the use of N isotopes rather than other isotopes that are common in the field of aquatic ecology. Of the other stable isotopes used to understand food web structure and habitat use in aquatic systems (i.e., isotopes of the elements C, S, H, O), C and S likely have the most promise in improving how we calculate TMFs. Ratios of both C and S isotopes are conserved as energy moves from prey to predator (e.g. Peterson and Fry 1987). For this reason, C is used to determine reliance of primary through tertiary consumers on terrestrial vs. aquatic or benthic vs. pelagic production because isotopic ratios of these elements are often distinct in primary producers at the base of the food web (e.g. Hecky and Hesslein 1995). Sulfur isotopes vary with geology and are most commonly used to distinguish organisms relying on freshwater versus marine subsidies (i.e. freshwater versus

marine contributions for anadromous fishes; e.g. Hesslein et al. 1991, Swanson et al. 2010). There is also evidence that S may be useful for distinguishing sediment from pelagic sources of energy within lake systems (Croisetière et al. 2009). Prior to regressing contaminants versus $\delta^{15}N$ to calculate TMFs, it is important to demonstrate energy flow between food web organisms and this can be achieved by examining bi-plots of $\delta^{15}N$ versus $\delta^{13}C$ or $\delta^{34}S$. Any organisms that do not rely on others within the isotope mixing space should be removed prior to running regressions (e.g. Wyn et al. 2009).

In some systems, albeit only a few thus far, it is possible to assess the influence of carbon source on TMFs. To date, this has only been done in systems where C flow to upper-level consumers is distinct. In temperate and Arctic lakes, estuaries or oceans, understanding the importance of benthic or pelagic carbon sources in the TMFs of Hg and POPs is challenging. Even though distinct $\delta^{13}C$ signatures (differences of up to 20 ‰) exist in algae or macrophytes supporting the base of these food webs, increasing omnivory in primary through tertiary consumers is common and top predators often (Hecky and Hesslein 1995), but not always (Kidd et al. 2001; Stewart et al. 2004; Wyn et al. 2009), reflect reliance on several sources of energy.

It should be noted that variation of lipid content among organisms or among tissue types has the potential to introduce considerable bias into $\delta^{13}C$ measurements because lipids are depleted in ^{13}C and typically have $\delta^{13}C$ values that are more negative than those for proteins and carbohydrates. Both lipid extraction of samples and mathematical adjustment using C:N ratios have been used to adjust $\delta^{13}C$ (McConnaughey and McRoy 1979; Hobson and Clark 1992). Post et al. (2007) concluded that normalization using C:N ratios was a better approach than lipid extraction in order to preserve the integrity of samples for $\delta^{15}N$ analysis and that lipid normalization was necessary to reduce bias in differences in $\delta^{13}C$ in food webs. Stable isotope methodology is a growing research discipline therefore it is beyond the scope of this paper to address this in more detail herein.

To date, C isotopes have mainly been used to look within species at how contaminant concentrations are affected by feeding habits (Eagles-Smith et al. 2008) or to identify lower-trophic-level organisms that are appropriate to include in TMF relationships (Wyn et al. 2009).

Few have been able to separate out distinct food webs relying mainly on benthic or pelagic carbon. For those that have, TMFs are higher for DDT in organisms relying on pelagic than benthic carbon (e.g. Kidd et al. 2001; Houde et al. 2008), this was not found for Hg (Kidd et al. 2003). In Lakes Simcoe and Champlain, the TMFs for *p,p'*-DDE were 0.9 and 1.3 for food webs with benthic feeders only (mysids, sculpin, smelt), i.e. no significant biomagnification from invertebrates to bottom feeding fish (Houde et al. 2008). However, there was limited sampling of benthic animals because the main focus of this study was on pelagic organisms, thus the uncertainty of the TMF estimates for benthic feeders is high.

Effects of Ecosystem Characteristics on TMFs

Although $\delta^{15}N$ and $\delta^{13}C$ have been used in some biomagnification studies, few systematic comparisons have been done of how TMFs are affected by other ecosystem characteristics such as productivity, species composition, size, latitude and longitude. Data now exist to start comparing TMFs between freshwater systems (i.e. stream vs lake food webs), marine and freshwater food webs, and systems with high and low productivities for a range of biomagnifying compounds. For example, TMFs for three CMSs in the freshwater benthic food web of Lake Pepin (range 0.3 to 0.4; Powell et al. 2009) and the marine benthipelagic food webs of the Inner and Outer Oslofjord (range 0.3 to 0.7; Powell et al. 2010a) were nearly identical. Moreover, the TMFs were not related to exposure concentrations at the base of the food webs (i.e. the y-intercept of the regression model), which were almost 50 times higher in the Inner Oslofjord relative to Lake Pepin.

TMFs for some POPs in 17 lake trout food webs were affected by physical and chemical characteristics of the systems (Houde et al. 2008). These lakes had lake trout (*Salvelinus namaycush*) as the top predator but varied in their size, mean depth, latitude, longitude, fish communities, and water quality. Houde et al. (2008) found that TMFs for PCB52 and 153 were positively correlated with lake mean depth. Also multiple regression including latitude and mean depth was more strongly related to TMFs for total PCB and PCB52 than mean depth alone. Guildford et al. (2008) used the $\delta^{13}C$ in these lake trout as an indicator of benthic littoral feeding and found a negative correlation between lipid-corrected $\delta^{13}C$ and ΣPCB (lipid corrected),

supporting the hypothesis that increasing access to littoral habitat results in *lower* concentrations in lake trout compared to those that are more restricted to pelagic habitat. Taken together, these result imply that the rate of biomagnification of highly recalcitrant compounds is greater in food webs of deep water lakes that are more dependent upon pelagic carbon, and independent of any effect of "hotspots" due to higher contamination in lakes within the Great Lakes/ St. Lawrence River basin. Although Houde et al. (2008) reported similar TMFs for PCBs across these systems, lowest TMFs for PCB153 were found in the most nutrient impacted lakes, Simcoe (1.5) and Champlain (2.2), compared with a mean TMF of 3.9 for 8 other mid-latitude lakes in their study. Similarly TMFs for *p,p*'-DDE were 1.9 and 2.1 in Simcoe and Champlain, respectively, compared with an average of 4.7 in 8 other lakes. Thus, while TMFs appear to be influenced both by physical and chemical characteristics of the lakes, the degree of influence that these characteristics have on TMFs likely varies from one compound to another.

Whether physical and chemical characteristics of the system affect TMFs in food webs supporting other top predators is not well known. However, it is important to understand in order to assess a contaminant's potential to biomagnify and to prioritize those systems and their top predators that are at greatest risk of contaminant transfer and eventual negative effects of contaminants.

The effects of system characteristics on TMFs may also be examined by comparing tropical and temperate ecosystems. Tropical food webs are more complex than temperate systems because of higher biodiversity, which likely promotes greater diversity of diets in the species (Paine 1966). In addition, higher biomass or tissue turnover in lower latitude systems may decrease TMFs due to higher biomass dilution of contaminants. In contrast, bioavailability in tropical systems may be affected by the higher microbial activity and organic matter. The effects of these factors in concert on TMFs remain unknown and warrant investigation.

When TMFs were compared between Arctic and temperate aquatic food webs, the magnification of contaminants did not differ between systems (Borgå et al. 2004). However, a recent study that compared species specific bioaccumulation factors (BAF) for zooplankton, fish

and seals between the Arctic (Barents Sea) and a more temperate region, the Baltic (Sobek et al. in press), found 5 times higher mean BAFs for the Barents Sea compared to the Baltic. After temperature correction of the BAFs, the systems differed only by a factor 2 (Sobek et al. in press). Thus, ecological or physiological adaptations of organisms to arctic conditions seemed to only marginally affect bioaccumulation, whereas most of the difference in BAFs was due to temperature differences and its effect of direct partitioning to organic matrices.

Most studies of food web biomagnification consider aquatic ecosystems. However, trophic level is also a significant predictor of contaminant concentrations through terrestrial food webs (lichen – caribou – wolf; Kelly et al. 2007). As discussed above in general terms, TMFs in food webs dominated by air breathers are also higher (e.g. for α-HCH) than for food webs dominated by poikilotherms (Kelly et al. 2007). Katz et al. (2009) and Müller et al. (2009) showed that TL (determined by $\delta^{15}N$ of individual samples) was correlated with concentrations of PFOS and C9-C11 perfluoro-carboxylates (PFCAs) in the lichen-caribou-wolf food web. This study showed that the varying diet of the caribou, which is two-thirds lichen in winter but more diverse in summer (Thompson and McCourt 1981; Boertje 1984), needs to be taken into account. Although there is a paucity of data for biomagnifying contaminants in terrestrial food webs, those that exist suggest that TMFs for land-based food webs need to be considered separately from ones containing only aquatic poikilotherms.

Effects of Spatial Variation of Contamination Within and Across Ecosystems

Variable inputs of chemicals into the system of interest are likely to affect the calculation of contaminant accumulation in food webs, and the source of these inputs may include local emissions or biotransport from migratory species. The challenge with the latter is that migrating species accumulate chemicals from locations other than the local food web of interest and, when these organisms are included in a TMF calculation, the estimation of trophic transfer becomes skewed. For example, Fisk et al. (2001a) demonstrated that migrating species did not fit well on the regression of contaminants versus trophic level when compared to more local species. This was explained by the non steady state situation for the migrating species, as they are actually representing $\delta^{15}N$ and/or contaminants levels of a wider region than the local food web under

study. Another confounder is differing inputs of chemicals at one site versus another within a system that would affect concentrations present in lower-trophic-level organisms. The influence of localized hot spots of chemical contamination versus a homogeneous distribution is expected to be reflected in the specific local food webs, and is discussed below in three case studies:

Biomagnification of perfluorinated compounds (PFCs) in dolphin food webs: Houde et al. (2006) compared the biomagnification of PFOS in the food webs of bottlenose dolphins (*Tursiops truncates*) feeding near or in the Charleston, SC, harbor and a population living in Sarasota Bay, FL. The water, sediments, zooplankton, and fish from the Charleston area had about 10-fold higher concentrations of PFCAs and PFOS compared to Sarasota Bay. Calculated TMFs for PFOS (using mean concentrations and TLs) showed similar TMFs in the two locations although variance was high. TMFs were 4.7 ± 4.5 at Charleston and 8.8 ± 4.4 at Sarasota for food webs consisting of planktivorous and forage fish and dolphin plasma. TMFs based on an estimated whole-body concentration of PFOS in dolphins were much lower, 2.0 ± 2.4 at Charleston and 3.3 ± 1.7 at Sarasota. Wastewater treatment plant discharges in the Charleston area may have resulted in non-steady state concentrations of PFCs in the food web. More specifically PFOA, which has generally been reported to not biomagnify, had a TMF of 6.5 ± 4.6. PFOA is a persistent degradation product of many polyfluoro chemicals and increasing levels of it in the food chain may reflect uptake and metabolism of other fluorinated substances which are rapidly transformed to PFOA. The above illustrates that contamination hot spots may influence observed TMFs particularly where there are non-steady state conditions. It also illustrates the challenges of working with protected top predators such as dolphins, for which only plasma was available (for PFC analysis) from capture/release studies.

Biomagnification of organochlorines in lake trout food webs: Houde et al. (2008) calculated TMFs for selected PCBs and DDE in lake trout food webs of 17 lakes in Canada and the northeastern USA. The mean total PCBs in lake trout in these lakes ranged from 100-5770 ng/g wet wt (whole fish) and were highest in 5 lakes within the Great Lakes/ St. Lawrence River region due to proximity to urban areas and elevated regional atmospheric deposition. Despite the more than 60-fold differences in mean concentrations in lake trout, TMFs for individual PCB congeners (PCB 52, 99, 101, 138, 153, 180) and DDE were not significantly related to lake

location i.e. to latitude and longitude of the lakes. Nor were the TMFs correlated with lake area, dissolved organic carbon (DOC), or % Dinophyta (a mixotrophic protozoa that grazes on picoplankton). Relative standard deviations of the TMFs for PCB congeners and DDE were generally 30-40%. Unlike the highly recalcitrant PCBs and *p,p'*-DDE, TMFs for α-HCH, lindane (γ-HCH) and HCB were positively correlated with latitude and longitude in the same food webs (Houde et al. 2008). TMFs were significantly higher (by about 2 x) for these compounds in more westerly and northern lakes which were more remote and less impacted by human activity. Houde et al. (2008) speculated that the biomagnification of HCH and HCB, which are biotransformed or eliminated more rapidly that PCB congeners or DDE by fish, may be influenced by lower water temperatures and longer ice cover in the northern lakes as a result of lower rates of volatilization, elimination, and/or biotransformation of HCH isomers within the food web; hence, they behave more like recalcitrant POPs in these lakes.

TMFs of methylmercury (MeHg) in Arctic char food webs. Gantner et al. (2010a) compared biomagnification of MeHg in food webs of 18 Arctic lakes in Canada across longitudinal and latitudinal gradients. Lacustrine food webs in the high Arctic typically are short and have low species diversity, with zooplankton communities dominated by pelagic Copepods and benthic invertebrates that are typically limited to a few species of Diptera (Chironomidae). Arctic char (*Salvelinus alpinus*) occupy the top trophic position of these systems and can be cannibalistic. Benthic invertebrates are the main source of nutrients, and thus MeHg, for landlocked (not access to the sea) Arctic char (Chételat et al. 2008; Gantner et al. 2010a). MeHg concentrations in chironomids were commonly higher than in pelagic zooplankton. The strong benthic coupling between chironomids and Arctic char influenced the TMFs for MeHg which ranged from 3.6 to 64.3 among 18 lakes. An unbalanced design, with large numbers of fish and relatively few invertebrates may have influenced TMF values. No relationships between TMF and abiotic factors known to influence Hg inputs to lakes (lake area, catchment area, catchment/lake area ratio, DOC, or chlorophyll a) were found. MeHg TMFs were also not correlated with food chain length. However, log[TMF] x food chain length was weakly correlated with length-adjusted total Hg concentrations in Arctic char (Gantner et al. 2010b). A conclusion from this study is that TMFs were useful to the describe trophic transfer of MeHg in

Arctic char food webs but that they needed to be considered in conjunction with a measure of food web structure, such as food chain length.

Seasonal Variation

If the food web is in steady state, the TMFs may be expected to be constant throughout the year. However, bioaccumulation in lower trophic levels can vary seasonally (Hargrave et al. 2000; Fisk et al. 2001b; Hallanger et al. in press b), as does the $\delta^{15}N$ values that are used to estimate trophic position (Søreide et al. 2006). For example, phytoplankton $\delta^{15}N$ may vary as much as 5‰ depending on bloom stage (Tamelander et al. 2009), which will influence the calculated trophic levels of other longer-lived food web organisms from one time to another. Also the cellular medium, such as lipid reserves for lipid soluble contaminants, varies seasonally depending on species and ecosystem. For example, in eider duck (*Somateria mollissima*), which reduces its body mass up to 50% during breeding, variation in some contaminant concentrations increases during this time (Bustnes et al. 2010). In a recent study of seasonal changes in TMF in a zooplankton-fish-seabird food web, the TMFs differed greatly between seasons, and even varied across the TMF=1 threshold for some halogenated compounds (Hallanger et al. in press a).

Chemical Properties

As both the bioconcentration and the biomagnification processes are driven by chemical diffusion from high fugacity, or activity, to low, there are chemical properties that are likely to influence BCFs, BMFs and TMFs in the same direction. Thus, several physico-chemical properties may be important to consider for all these bioaccumulation metrics, such as the K_{OW}, water solubility, vapor pressure, environmental half-lives, and molecular size/structure of the chemical, although it is still inconclusive if a quantitative structure-activity-relationship may be applied to predict TMFs.

As has been well established for estimation of BCFs, K_{OW} has been suggested as a predictor of TMFs because they are higher for the more lipophilic contaminants in lake trout

food webs (Houde et al. 2008); however, some studies that include air breathing organisms do not show any relationship between K_{OW} and TMF (Borgå et al., in prep). The water solubility and vapor pressure of the chemical affects the rate of elimination in water- and air-breathing organisms, respectively. For air breathing organisms, K_{OW} and BCF in fish are not good predictors of biomagnification for chemicals with log $K_{OA} \geq 6$ and $K_{OW} > 2$ (Kelly et al. 2007). The effects of water solubility and vapor pressure on TMFs may both be particularly important to consider when food webs consist of both air and water breathing organisms at different trophic levels.

Molecular structure also may influence observed TMFs via selective transformation reactions (e.g. biodegradation and biotransformation) of chemicals. While often neglected in early BCF/K_{OW} models, biotransformation and metabolism may be accounted for in BCF estimations by using molecular fragment descriptors (Arnot et al. 2008; 2009). In examining the biomagnification of PCDD/Fs with trophic level in the Baltic Sea marine food web, Broman et al. (1992) recognized the importance of molecular size and structure. They noted, for example, that only 2,3,7,8-TCDD biomagnified while more highly chlorinated PCDD/Fs had negative slopes with trophic level (or TMFs). Bioformation may lead to apparent increases in TMF, as found for certain PBDEs where debromination of BDE183 and other highly brominated BDEs to BDE154 may increase TMFs for BDE154 (Stapleton et al. 2004; Wu et al 2009). Similarly, as noted above, reports of PFOA biomagnification may be due to accumulation and transformation of other PFCs (Houde et al. 2006).

FACTORS AFFECTING REGRESSION BASELINE (INTERCEPT)

Whereas the above sections focused on factors that directly affect the TMF (i.e. the regression slope), the present section discusses the most important factors that affect the baseline of the contaminant-trophic level relationship (i.e. the intercept). The intercept of the log concentration versus TL was first described by Broman et al. (1992) as the background concentration of the system in question and these concentrations should ultimately determine what is present in upper-trophic-levels if TMFs are relatively consistent across systems. As

discussed above, few studies have addressed the importance of ecosystem properties on TMF intercepts.

The background concentration is related to the bioavailable portion of the chemical that has the potential for bioconcentration at the base of the food web and subsequent biomagnification through trophic transfer. Factors affecting the total water column concentration and bioavailability of chemical to the base of the food web therefore affect the intercepts of the contaminant versus TL relationships and are independent of the TMFs. The total concentration of a contaminant in the water column is set by the dynamic interplay between system loading, bioavailability, and removal processes. As such this term is affected by intrinsic properties of the system, and the chemical and physical properties of the compound being biomagnified. Contaminant loading as it relates to the system background is a complicated process driven by numerous variables including the suite of physical and chemical properties of a given compound, likelihood of atmospheric versus water transport, stability, and proximity to sources. Due to these complexities, a thorough description of loading is beyond the scope of this review. Consequently, this section will deal with factors intrinsic to the system that affect the intercept including productivity and differences in metabolism among food web members.

Physico-chemical Properties

The physico-chemical properties of a compound can affect the intercept term in several ways. For hydrophobic compounds, the apparent water concentration or bioavailable fraction is affected by the binding of the compound to sources of carbon in the water column and this is largely driven by its solubility, K_{ow}, and affinity for the organic matter (e.g. Schlautman and Morgan 1993; Burkhard 2000;). Therefore, in principle, lakes could have similar TMFs for hydrophobic organics but different intercept terms due to differences in the bioavailability of a compound in the water column. Houde et al. (2008; unpublished data) found similar TMFs, but intercepts of the log PCB153 vs TL relationships that varied widely for 17 lakes, with highest values in the southern Canadian/northern US lakes (Simcoe, Champlain, Seneca) with legacy sources of PCBs. However, bioavailable concentrations of PCBs in water were not measured and DOC values of these lakes were, in general, not elevated compared to more remote lakes.

However, DOC has been suggested as a variable explaining lower PCB bioconcentration observed in fish in Lake Winnipeg compared with the Great Lakes (Gewurtz et al. 2006).

Organic carbon partitioning has been shown to affect the bioavailable fraction of neutral hydrophobic organic molecules in sediment and pore water (Akkanen and Kukkonen 2003; Lyytikäinen et al. 2003; Burkhard et al. 2008). Dissolved water concentrations are expected to be significantly reduced when black carbon is present in sediments and in suspended solids because of the exceptional affinity of some chemicals for the black carbon phase (Burkhard et al. 2008; Gustaffson et al. 1997). Recent modeling work suggests that including soot-derived black carbon reduced the fraction of PBDE 47 in water and biota and resulted in improved prediction of PBDEs in Baltic Sea fish (Mattila and Verta 2008).

The intercept may also be affected by the air-water partitioning of a chemical (K_{AW}), calculated using Henry's law constant (HLC; the partition coefficient for equilibrium between air and water). HLC is greatly affected by temperature (Kucklick et al. 1991) such that cooler waters tend to have higher concentrations than warmer waters. Therefore, a lake with a cooler average water column temperature could have a higher intercept term than a lake with an warmer average temperature. In line with this, Sobek et al. (in press) found that BAFs for PCBs in the Arctic marine food web were 5 times higher than for a temperate food web, and that this difference was reduced to 2 times after temperature and salinity corrections were included.

Ionizable organic pollutants represent a special class of compounds where the speciation of the compound may have effects on the intercept of the TMF relationship. For this class of compounds, pH - primarily through the pKa - becomes an important driver that directly affects the bioavailability of the compound (Fu et al. 2009). The bioavailability of anionic compounds, for example, is greater for the associated form which increases with declining pH. The low pH microenvironment of the fish gill has been suggested to enhance the accumulation of anionic compounds (Erickson et al. 2006). This effect has only been examined in a few aquatic organisms. In surface water, ambient pH can also affect speciation and bioavailability such that anionic compounds may be more bioavailable in low pH waters than in higher pH waters (Kah and Brown 2008; Shiu et al. 1994). In addition, pH can also affect the air-water exchange of

ionizable compounds, such as chlorophenols, and therefore affect the background concentration in the system and the intercept of the TMF relationship (Shiu et al. 1994).

The bioavailability of MeHg to plankton and benthic invertebrates at the bottom of food webs is affected by MeHg partitioning and complexation (Munthe et al. 2007). Aquatic system characteristics such as pH and DOC have important influences on Hg and MeHg cycling (Morel et al 1998). These conditions affect Hg methylation rates, which also depend to some extent on the availability of electron acceptors such as oxygen, nitrate, sulfate, or Fe(III) and their influence on microbial metabolism (Munthe et al. 2007). Acidic waters and reducing conditions associated with low dissolved oxygen favor Hg methylation. In tropical climates, black waters (with high DOC) have higher temperatures that also favor microbial processes. Photoreactions (such as the conversion of organic to inorganic Hg) are stronger in the tropics because of higher solar radiation but high DOC protect MeHg in such aquatic systems. In the case of tropical rivers and reservoirs with high DOC, low DO and acidic conditions, higher MeHg is usually found at the base of the food web. An example, is the Negro river in the Brazilian Amazon which is considered a sink for Hg since it presents perfect conditions for Hg mobility and methylation (da Silva et al. 2006). There are high MeHg values in biota even with no specific Hg sources (Barbosa et al. 2003; Dórea et al. 2006; 2007). These same conditions observed in the Negro river were sometimes also seen in some new manmade reservoirs (Malm et al. 2004; Palermo et al. 2004; Kehrig et al. 2004) in areas just upstream and downstream of the dam. It is possible that these elements can influence the intercept of MeHg vs TL relationships although, to our knowledge, this has not been specifically investigated.

System Productivity

Eutrophication affects bioavailability in water by increasing POC, DOC and sedimentation rates of contaminants in the system. Negative relationships between trophic status/productivity and organochlorines in organisms have been observed in lakes. Proposed mechanisms have been linked with the changes in primary producer biomass or composition (i.e. changes in lipid content; Berglund et al. 2001a; 2001b). Increased primary producer biomass may “dilute” organochlorines or withdraw the compounds from the water column to the

sediment through increased sedimentation rates (Berglund et al. 2001b). Thus, bioavailability would decrease for the pelagic food webs but may increase for the benthically-linked organisms. In lotic environments however, the opposite relationships have been observed, with increasing concentrations in biota with increasing primary producer biomass. Periphyton density influences organochlorine accumulation in rivers (Berglund 2003). Here, primary producers are mainly attached benthic periphyton and an increased biomass will increase the probability of uptake and decrease downstream transport of lipophilic compounds. In addition to the effects above, increasing primary production has also been correlated both to increased air-water exchange and particle sedimentation (Dachs et al. 2000).

Choice of Organism for $\delta^{15}N$ Baseline Affects the Intercept

Intercepts of the regressions of contaminants versus TL are affected not only by chemical inputs and bioavailability, but also by enrichment of $\delta^{15}N$ at the base of the system and by the choice of organism(s) used to calculate the TL of other members of the food web. It is well known that short-lived organisms are more temporally variable in their $\delta^{15}N$ than longer-lived organisms. Although some studies have used net plankton (Houde et al. 2008) or copepods (Fisk et al. 2001a; 2003, Campbell et al. 2005), primary consumers such as mussels (Post 2002; Vander Zanden and Rasmussen 1999), clams (Fry 1999; Swanson et al. 2003) or gastropods (Kidd et al. 1998; Post 2002) are preferred because there is less likelihood of over or underestimating the longer term baseline $\delta^{15}N$ of the system. This issue is discussed in greater detail above (see section Characterizing Food Webs with Stable Isotopes).

PRACTICAL CONSIDERATIONS FOR DERIVING AND USING TMF VALUES

In addition to the previously-discussed elements and their effects on the derivation of TMFs, other practical concerns should be considered to maximize the usefulness of food web data. The following discussion will focus on these considerations.

Analytical Considerations

The TMF value is currently derived from measured chemical concentrations and trophic levels estimated from ratios of element concentrations (stable nitrogen isotopes). More specifically, the components of the TMF calculation include the chemical mass per amount of sample in a given trophic level, the ratio of $^{15}N/^{14}N$ in the sample relative to the $^{15}N/^{14}N$ in the standard (typically atmospheric nitrogen), and the enrichment factor per trophic level ($\Delta^{15}N$; typically 3.4‰). For lower trophic levels with small individual organism size, such as plankton, there may be a challenge to obtain parallel samples for both contaminant and $\delta^{15}N$ analyses. In some studies the samples are collected simultaneously, but separately, making the pairing of contaminant and $\delta^{15}N$ samples difficult. Thus, rather than splitting one homogenized sample in the laboratory to obtain matching sub-samples, these studies use average $\delta^{15}N$ for (species-specific) zooplankton at a given time, and match these data with individual samples of contaminants. However for samples that require pooling of individuals, it is recommended that they are pooled during sampling and split into sub-samples in the lab after homogenization. Uncertainty with respect to $\delta^{15}N$ fractionation and other aspects of trophic assignment are detailed in Jardine et al. (2006) as well as earlier in the text and will not be further considered here. The following discussion focuses on the measurement of chemical concentrations.

Typically, the variability associated with the measurement of bioaccumulative pollutants is lower than the variability among individuals within a species. However, there are several practical considerations that will allow for better comparability of data among studies and better control of variance among trophic levels. Two major factors in food web analysis are the impacts of sample size (mass) and concentration. Typically the greatest mass of sample available for analysis is for the higher trophic level organisms and lower sample masses are available at lower trophic levels, primarily due to the difficulty in obtaining the latter samples. The analytical protocol must take this into account. The sample mass used for analysis must be scaled to provide the appropriate analyte mass for proper detection. A calibration curve must be used during the analysis for food web samples and the curve must bracket the observed concentrations. The uncertainty associated with measuring analytes close to the detection limit can be an important component in the overall population variance and this should be estimated through repeated analysis of low-concentration samples.

Of the different detection levels that are defined, the limit of detection (LOD) and the method detection level (MDL) are the most valuable for field monitoring studies. Methods for determining them are available from the American Chemical Society (MacDougall et al. 1980), US EPA (Gomez-Taylor et al. 2003) and ISO/IUPAC (Currie 1995) and in many other publications, and will not be defined further here.

For field monitoring, a measurement that is less than a specified detection limit (DL) may be: 1) reported as "below detection", 2) reported as zero, 3) reported as less than (<) the value of the DL, 4) reported as some value between zero and the DL, for example one-half the DL, or 5) reported as the actual value (positive or negative), whether or not it is below the DL. The last option, the reporting of the actual value (i.e. uncensored value), is generally recommended over reporting left-censored values (Clarke 1998; Antweiler and Taylor 2008) and is discussed further under Data Analysis and Study Design.

Matrix effects and interferences are also a potential source of measurement uncertainty. Given the range of sample types present in a food web potentially ranging from blubber to a pooled plankton sample, multiple analytical schemes will likely be needed to remove interferences that may be specific to that trophic level (e.g. high lipid in blubber samples). Contamination of samples from laboratory sources are also a potential source of interference and uncertainty for the compounds unders study. This has especially been a problem for the brominated flame retardants (Thomsen et al. 2001), PFCs (Martin et al. 2004) and siloxanes (Varaprath et al. 2006). Care must be exercised through the running of appropriate blanks and removing sources of contaminant from the analytical stream.

Other considerations include the use of natural matrix reference materials, which are an important tool that can help to reduce measurement uncertainty. There are numerous reference materials that are commercially available (Wise et al. 2006), many of which are value-assigned for compounds that are currently under study or include the species in TMF investigations (Kucklick et al. 2010). Along with the inclusion of control materials in analysis, the participation in interlaboratory studies especially for the compounds of emerging interest will also help to reduce measurement uncertainty in TMFs.

For lipophilic compounds, lipid is a key parameter that is determined on samples since contaminant concentrations generally relate to % lipid across trophic levels. The determination of lipid on lower trophic-level samples or in blood is potentially a large source of variability. For instance, lipid in bivalve tissue or blood is typically <2% of the mass fraction. For blood, there are also different techniques available for the estimation of lipid content including gravimetric, colorimetric, and enzymatic techniques (Muir and Sverko 2006). This source of error should be recognized in TMF studies and the lipid determination methods should be assessed for variability through the use of appropriate analytical reference materials, many of which have lipid values (Wise et al. 2006).

The TMF is best derived from measurements done on whole organisms. However, for top-level consumers such as marine mammals and birds, chemical measurement in whole organisms is impractical or impossible due to wildlife protection laws. Blood or blubber, in the case of marine mammals, is often used instead of whole animals. Blubber of marine mammals contains the majority of lipophilic pollutants. For instance, approximately 90% of lipophilic pollutants occur in the blubber of bottlenose dolphins (Yordy et al. 2010). Ideally for a TMF study, the average body concentration should be estimated based on blubber concentrations and a blubber to total body mass conversion factor (Yordy et al. 2010). Proteinophilic compounds such as PFCs are generally most abundant in blood or liver hence these tissues should be sampled for upper trophic levels. As above, the concentration of the whole animal should be estimated based on blood to body mass conversion factors if available. Houde et al. (2005) calculated whole animal concentrations of PFOS and PFCs using plasma and liver tissue distribution factors determined by analysis of tissues in dead dolphins. They showed that TMFs for PFOS based on the whole animal concentrations were much lower than calculated with blood values and closer to other measurements e.g. with poikilotherms. Alternatively, for proteinophilic metals such as MeHg, muscle may be a better indicator if it is available by biopsy or from dead or hunted animals (Loseto et al. 2008).

TMFs may not be possible to calculate for some chemicals if analytical methods are not yet available for environmental samples. Thus TMF studies that intend to examine a wide range

of potentially "B" chemicals in commerce (e.g. see Howard and Muir 2010) need, as a first organizational step, to be coordinated with analytical laboratories capable of developing or refining methodology to fulfill study requirements.

Sampling Considerations

The sampling of aquatic food webs is generally the most important and challenging aspect of a field biomagnification study (see also Data Analysis and Experimental Design below). To adequately characterize the food web, sufficient numbers of key organisms from each trophic level must be obtained. For most studies, samples from upper trophic levels such as fish, marine mammals, or birds are comprised of animals that are typically analyzed for stable isotopes and bioaccumulative pollutants as individuals since this provides information on individual variability. However, this is frequently done without regard to the statistical power needed to adequately describe variability and provide statistical separation between adjacent trophic levels. Therefore, prior to sampling, the number of individuals required to achieve statistical separation from adjacent trophic levels should be estimated (Keith et al. 1983). For lower trophic levels, typically at the primary producer and primary consumer levels, pooling or compositing of samples is needed to provide adequate sample mass for analysis. In this case, determining chemical variability among individuals is impractical due to the small mass of the organisms and low concentrations of target compounds in lower trophic levels. If the entire composite is not used for analysis, the homogeneity of the pooled sample should be assessed through analysis of multiple subsamples.

Quality Control/Quality Assurance in Food Web Biomagnification Studies

Numerous studies have reported the high biomagnification potential of PCB-153 (Fisk et al. 2001a; Hop et al. 2002; Hoekstra et al. 2003; Borgå et al. 2004; Houde et al. 2008; Kelly et al. 2008). Given that TMF values for PCB-153 are consistently significantly greater than 1 among almost all food webs that have been characterized, quantification of the TMF for PCB-153 should be included in food web magnification study designs as a "positive control" for the evaluation of the biomagnification potential for other chemicals. Assuming PCB-153 is present

at detectable concentrations in all biota within a given study, an inability to detect statistically-significant TMF values for PCB-153 may reveal an insufficient study design that could be due to inadequate number of samples, poor characterization of TL values via stable isotope analysis (Jardine et al. 2006), or other functional issues identified above.

Data Analysis and Study Design

Some of the challenges identified and discussed above are examined more closely in this section to determine their quantitative effects on TMFs. In contrast to other measures of bioaccumulation potential (e.g. BCF and BAF values), TMFs are statistically more complex, as values are derived from linear regression modeling across an experimental design that incorporates multiple species. A full review of regression modeling and study design is beyond the scope of this paper; see e.g. Zar (1999) and Sokal and Rohlf (1999) prior to study design for a more extensive understanding of regression and experimental design. The following sections review several of the most-commonly encountered challenges related to the experimental design of food web magnification studies and the analysis of their data to understand biomagnification potential. It should be noted that by using the TMF method, we are applying a very simplistic approach and model to capture a process that is much more complex in nature due to the factors discussed above.

Use of Non-Detect Data

The common practice for incorporating data with chemical concentrations below detection or reporting limits ("left-censored" data) in food web biomagnification analyses has been to substitute non-detects with a value equal to one-half the detection or reporting limit (e.g. Hop et al. 2002; Houde et al. 2008). This practice can lead to a violation of linear regression assumptions and is not recommended for environmental datasets. In particular it can create a systematic error in the data when the LOD varies with species. Therefore, it is recommended that the actual measured values be used for concentrations that are less than the previously described MDL but greater than the LOD (i.e. MDL > C > LOD), and that censored values be used for concentrations that are less than the LOD (Clarke 1998; Antweiler and Taylor 2008).

In cases where the actual measured values that are less than the previously described MDL but greater than the LOD are unavailable, there are several other more statistically-robust methods for using left-censored data (Helsel 2005). Figure 2 depicts the effects of two different treatments of non-detect data on calculations of food web biomagnification: 1) substitution of one-half the detection limit, and 2) substitution of values derived from Regression Order Statistics (ROS), one of the approaches for substitution discussed in Clark (1998), Helsel (2005), and Antweiler and Taylor (2008). Details on the substitution methods can be found in the Supplemental Information. Both substitution approaches yielded overall conclusions that corresponded to those derived from the uncensored data, although it should be noted that this exercise is more for illustrative purposes rather than a rigorous analysis of approaches to incorporate non-detect data. The uncensored datasets yielded TMFs (95% CI) for PCB-153 of 3.7 (2.8-5.1) and for dieldrin of 1.2 (0.97-1.6), suggesting significant biomagnification was observed for PCB-153, but not for dieldrin (Fig. 2a and d). The method of substituting one-half the detection limit yielded the same overall conclusion regarding TMFs significantly greater than (Fig. 2b) and less than (Fig. 2e) one, with TMFs (95% CI) for PCB-153 of 2.8 (2.1-3.8) and for dieldrin of 1.1 (0.8-1.5). Median TMFs generated by the substitutions of non-detect with ROS-generated values were 2.9 for PCB-153 and 1.1 for dieldrin (Fig. 2 c and f). All randomly-generated TMFs (including 95% CI for the ROS-substituted PCB-153 datasets) were greater than one, suggesting significant biomagnification as observed in the original uncensored dataset. Ninety-three % of the randomly-generated TMFs for the ROS-substituted dieldrin datasets were less than one, corresponding to the overall conclusions of the original uncensored dataset.

Although both methods (one-half the detection limit and ROS) performed relatively well in the examples shown (Fig. 2), substitution of non-detect values with a fixed value (one-half the detection limit, the detection limit, zero, etc.) would likely violate assumptions of regression analysis and would result in a distortion of the TMF value. This would likely be observed in datasets that were more balanced among trophic levels than the ones shown in Figure 2, and would be an issue when the proportion of left censored data increased. In these cases, more advanced methods, such as substitution of values with ROS-generated values (Fig. 2c and 2f) or the approaches outlined by Helsel (2005) should be employed.

Another method to deal with several values below LOD in a contaminant-TL regression is the maximum likelihood estimation technique (Frome and Wambach 2005). As the range where the value lies is known, the maximum likelihood can be used to estimate the most likely values within the ranges using the rest of the dataset. Instead of estimating a mean and uncertainties for a species based on the replicates, one uses estimated values in a regression and assumes a parametric relationship such as log[C] = a+bTL, and estimates a, b, and residual standard deviation by maximizing cumulative probabilities for the whole dataset. The uncertainties in the estimated parameters can also be determined. The probabilities are calculated as a function of the observed value and parameterized mean and standard deviation for the normal or lognormal distribution.

In summary, it is recommended that in all cases in which non-detect data are present, more than one method of deriving a regression model should be examined, especially in cases where substitution with a fixed value is considered. The use of uncensored data is preferable, followed by substitution of non-detects using advanced substitution methods such as ROS-generated values.

Statistical Power and Sample Size

Although TMF values developed from datasets are continuous variables and, as such, may be useful for risk assessments for specific trophic levels, the ultimate question often posed by stakeholders and policy makers concerns the binary condition of food web biomagnification, which is indicated by a TMF value > 1. Although decision-making frameworks for chemicals should not always be rigidly bound to tests of statistical significance, statistical hypothesis testing can be useful in characterizing the uncertainty and power of datasets to be used in evaluating biomagnification potential. The key evaluation is the statistical significance of the slope of the regression of the log-transformed concentration of a chemical in biota vs. TL, which evaluates the null hypothesis that the slope of the regression model is equal to zero (i.e. a TMF = 1).

The statistical power associated with past studies on trophic magnification was evaluated using regression slopes obtained from approximately 80 TMF values for BEHP, PFOS, β-HCH, γ-HCH, HCB, endosulfan, BDE-47, BDE-153, PCB-52, PCB-153, PCB-209, pyrene, MeHg, and decamethylcyclopentasiloxane. These values were compiled from several studies conducted in aquatic ecosystems primarily in North America (Fisk et al. 2001a; Hop et al. 2002; Hoekstra et al. 2003; Mackintosh et al. 2004; Houde et al. 2006; Wan et al. 2007; Houde et al. 2008; Kelly et al. 2008; Wan et al. 2008; Powell et al. 2009; Tomy et al. 2009; Gantner et al. 2010b; Powell et al. 2010b) (Table S1). Details on study selection and variability statistics can be found in Supporting Information. Variability of the TMF regression slopes from these past studies, as expressed by the standard deviation (SD) for each regression slope, was not consistently related to sample size, TMF, or chemical class (Fig. 3). Assuming that the 25th, 50th, and 75th percentiles of the slope SD shown in Table 1 reasonably represent the range of variability associated with past trophic magnification studies, most study designs having 30 to 40 samples would only have been able to detect regression slopes with an absolute value greater than 0.3 to 0.5 (equivalent to TMF values greater than 2-3) as being statistically different from a slope of 0 (Fig. 4). Conversely, these study designs would likely have failed to detect significant (i.e. $P < 0.05$) regression slopes for log-transformed biotic concentrations vs. TL for contaminants with apparent TMFs < 2. Results indicate that with the level of variability associated with past experimental designs, only very large sample sizes ($n \geq 60$-100) would have been expected to consistently detect significant regression slopes for contaminants with apparent TMF values in the range of approximately 1.5 to 2.0.

Results from the power analysis showed that a minimum of 30 to 40 samples are likely needed to conduct a trophic magnification study following experimental designs similar to those previously used. Within the range of variability depicted in Figure 4, most experimental designs with fewer than 30 to 40 samples are unlikely to detect statistically significant regression slopes for contaminants having apparent TMF values that may be near the lower limits of potential relevance. Moreover, this lack of sensitivity increases when variability is high. For example, statistically significant regression slopes for contaminants having apparent TMF values as low as 1.4 to 1.6 can be detected with sample sizes of 20 to 30 if very low variability is associated with the regression slope (i.e. 10th percentile of the SD values in Table 1, SD = 0.3). However, as

variability associated with the regression slope increases, such as that observed at the 90th percentile of SD values from the example studies (SD = 1.2), the minimum value of a slope that can be identified as being statistically > 0 approaches a value of about 0.7, which is equivalent to an apparent TMF value of 5.0 (Fig. 4). At such high levels of variability (e.g. SD = 1.2), approximately 100 to 150 samples would be required to obtain a statistically significant regression slope for contaminants having apparent TMF values as low as 2.0 (Fig. 4).

The issues of high variability associated with the TMF regression slopes from past studies are likely related to the previously discussed parameters that impact TMF (e.g. biological factors, chemical factors, trophic dynamics, confounded food chains, etc.), and may be better controlled by improved experimental designs. Thus the power analysis may be biased by the limitations of the design of previous TMF studies. Therefore, rather than increasing sample sizes for all studies, improved experimental designs are recommended to reduce the variability and the number of samples needed for the regression analysis. Having a good understanding of the ecology of the system being studied is likely to have the greatest impact on decreasing uncertainty and the number of samples needed for the regression analysis.

Use of Raw Data Versus Average Data

Unless the purpose is to explore the effects of a potentially over-represented species in an unbalanced experimental design (see next section below), it is recommended that regression models used to estimate TMFs should be based on the raw data rather than reducing the raw data to mean trophic levels and mean chemical concentrations for each species. In general, reducing raw data to means results in a reduction in total sample size, which usually results in a loss of statistical power, and thus, the ability to detect TMF values significantly greater than one. This is illustrated in Figure 5, in which TMF values for PCB-153 from Houde et al. (2008) were generated via regression with the raw data (as in Houde et al. 2008) or via mean trophic level and mean log-transformed, lipid-normalized concentrations for each species. In general, TMFs are similar between the two approaches, falling close to the 1:1 line (Fig. 4). However, the lack of statistical power for the TMF values developed using the mean data is noted by the much wider 95% CIs. With the exception of the two extremely wide 95% CIs (17 and 35), 95% CIs for TMF

values generated by regressions based on mean data were approximately twice that of 95% CIs based on raw data. With respect to the presence of statistically significant biomagnification, slopes of regressions based on raw data were all significant, whereas 4 of 17 (nearly 25%) regressions based on mean data failed to show statistical significance.

In summary, reduction of data to mean values prior to regression is not advised in most cases, and will result in a loss of statistical power to detect TMF values >1. We recommend using individual samples and dealing with lack of balance in the study design as described below. In addition to statistical considerations of power, using mean values is ecologically unsound when species exhibit a large degree of omnivory or there are other reasons for a large spread in trophic positions and/or contaminant concentrations.

Balancing Study Design

In general, individual samples of tissues from higher trophic levels (fish, mammals) are more easily collected than pelagic or benthic invertebrates. As a result, datasets used for regression analysis are usually heavily weighted with samples from higher trophic levels. In extreme cases of unbalanced designs, TMF values derived from these datasets can be more reflective of biomagnification among these higher trophic levels rather than the full food web.

To illustrate the effects of study balance, TMFs for PCB-153 from Houde et al. (2008) were compared to a Monte Carlo simulation with Bootstrap analysis (using Crystal Ball predictive modeling software (www.Oracle.com)) of regression models which were balanced by species. Monte Carlo-derived TMFs (MC-TMF) were calculated from "forecasts" using variables or "assumptions" that were defined as probability distributions (See Supplemental Information for details on Monte Carlo simulation, Table S2). The MC-TMFs were log-normally distributed and not significantly different when calculated using the mean lipid-normalized concentrations (ng/g lw) across individuals or the mean PCB-153 concentration (ng/g ww) divided by the mean lipid content (g/g ww) across species. Therefore, the reported MC-TMF values were based on the mean PCB-153 concentration (ng/g ww) divided by the mean lipid content (g/g ww) across species so that subsequent sensitivity analyses could address the

impact of lipid content on the resulting TMF values (see Supplemental Information for details for Alternate Approaches for Understanding Biomagnification).

TMFs calculated using the raw data across all samples and species and the MC-TMF values (mean and median values) differed due to the unbalanced sample collection and analysis across species (Table S3), with the raw data TMFs subject to a large influence from the large number of higher trophic level fish (herein lake trout) present in the dataset (Fig. 6). In most cases, however, TMF values generated from the raw data and the MC-TMFs were similar, and ultimately came to the same general conclusion regarding biomagnification (i.e. TMF values > 1) (Table S3).

In summary, the Monte Carlo analysis provided in this example (or a similar approach using statistical software to identify the affects of unbalanced regression models) should be used in cases where unbalanced study designs may be suspected. For example, in the Lake Paguchi dataset from Houde et al. (2008), lake trout represent 50% of the samples, suggesting that TMF values would be highly affected by this element of the experimental design. Sensitivity analysis with the Monte Carlo simulation suggested that the concentration of PCB-153 in lake trout was the variable with highest influence on the regression slope (and therefore TMF) (Fig. 6).

Aside from the study design and sample size aspects, many intrinsic ecological variables such as organism size, reproductive status, gender, age, ability to biotransform chemicals, and other factors have the potential to affect the TMFs, as discussed above. For example, thermoregulation strategies of the species can increase the variability in a dataset characterized by a high number of poikilotherms. As discussed in Fisk et al. (2001a) and Hop et al. (2002), TMFs calculated from regressions that include an entire food web consisting of both poikilotherms and homeotherms may overestimate biomagnification for poikilotherms and underestimate biomagnification for homeotherms. In these situations, TMF values should be calculated separately for the two thermal groups in cases where regression models incorporating the entire food web yield insignificant models or highly-variable slope/TMF values. Where the number of homeotherm samples is limited, or TL separation of homeotherms is small (i.e. differences in TL of homeotherms are approximately less than 1), TMFs for the dataset should be

evaluated with, and without, homeotherms to evaluate the effects of including this group (Gobas et al. 2009). Identification of thermoregulation in the regression by inclusion of an interaction term is also a useful approach (Hop et al. 2002; Hallanger et al. in press a).

Additional analysis (multivariate regression, ANCOVA, etc.) may be useful in identifying and quantifying the effects of ecological variables in TMF analyses. Such ancillary analyses will aid in a more complete analysis of food web biomagnification potential, and can be used to further understand and identify potential artifacts related to migration of homeotherms, species-specific differences in adsorption, metabolism, excretion, differing carbon sources for benthic and pelagic feeding guilds, and the other factors that may affect TMF values. Suggestions for additional analyses, such as examining TMFs calculated with wet weight and lipid normalized concentrations separately, and recommendations for statistical reporting on TMF calculations are provided in Supporting Information.

SUMMARY AND RECOMMENDATIONS

Although this integrated approach of using TMF to assess contaminant bioaccumulation through food webs was initiated almost two decades ago (Broman et al. 1992), and the method has been further developed and used in several studies in recent years, few studies have evaluated the usefulness of the approach. The present review has discussed different factors affecting food web magnification, and illustrates various considerations that must be taken into account when designing and interpreting TMF studies. A sensitivity analysis for PCB-153 data showed that concentrations measured in high trophic level species had the largest leverage on TMF, whereas contaminant concentrations and $\delta^{15}N$ in lower-trophic-level organisms did not. However the selection of lower-trophic-level organisms is important for the intercept and thus the baseline. Knowledge gaps that have been identified include:

- Lack of well designed studies examining the influence of ecosystem characteristics on TMF
- Limited interpretation of the regression intercept. Does this “only” account for baseline conditions or may there also be an interaction with the slope, and thus TMF.
- Limited application of TMFs in terrestrial ecosystems

Our main recommendations for future TMF studies are as follows:

- The study must include species and individual organisms that range over at least 3 trophic levels to achieve the objective of quantifying biomagnification potential.
- In some cases, sample sizes of at least 30-60 are needed to achieve sufficient statistical power to evaluate whether TMF is less than or greater than one for "B" assessment. Sample sizes can be reduced without loss of statistical power by advanced ecological understanding of trophic relationships.
- Use individual samples and uncensored data when available. Any use of left-censored (non-detect data) should be clearly identified in graphs and tables, and should treated with care during TMF calculations, employing additional analyses beyond a simple substitution of left-censored data with one-half the detection limit.
- For samples that require pooling of individual samples for contaminant and $\delta^{15}N$ analysis, it is recommended that they are pooled during sampling and split into sub-samples in the laboratory after homogenization.
- Report slope and intercept with error estimates (SE, SD, and/or 95% CI), as well as significance level and fit of the model.
- Include information on how TL is calculated, including enrichment factors and baseline organisms used.
- Report, whenever possible, chemical, physical and biological characteristics of the system(s) to facilitate a broader understanding of how TMFs are affected by ecosystem characteristics.
- Start with a full regression model and include factors that may influence the TMF, such as organism size, age, and physiology (poikilothermic vs. homeothermic), and eliminate the non significant factors. Use uncensored data for the estimation of TMFs, if possible.
- Planning for TMF studies needs to consider if analytical methods are available and, if necessary, include coordination with analytical laboratories capable of developing or refining methodology to fulfill study requirements
- The accuracy and representativeness of ancillary data such as % lipid, and stable isotope measurements (e.g. tissues selected, seasonal effects) needs to be assessed

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List of Figures

Figure 1. Trophic magnification factors are the change in contaminant concentrations per trophic level. As many contaminants increase exponentially in the food web, a log-normal relationship results in a regression slope (b), from which the TMF is calculated by the antilog (10^b or e^b).

Figure 2. Effects of two substitution approaches to incorporate non-detect data into food web magnification studies of PCB-153 and dieldrin to which hypothetical detection limits for chemical concentration data (dashed line) have been applied. Original uncensored data and regression models are presented in (a) and (d). Regression analyses where hypothetical non-detect data (open symbols) have been replaced by one-half of the hypothetical detection limit are shown in (b) and (e). Examples of regressions using one of the randomly-selected ROS-substituted data (open symbols) are shown in (c) and (f). The shaded boxes in (c) and (f) represent the domain of the randomly allocated ROS-substituted values. Sample data are from Houde et al. (2008). Details on substitution methods and data set can be found in the Supplemental Information.

Figure 3. Trophic magnification factor (TMF) variability (standard deviation (SD) of the untransformed values for the slopes of the regressions of log-transformed concentrations on trophic level) compared to TMFs (a) and sample size (b) in the selected food web biomagnification studies described in the text.

Figure 4. Minimum food web magnification slope (absolute value) able to be detected as significantly greater than 1 ($\alpha = 0.05$, $\beta = 0.8$) with the range of variability (standard deviation (SD) of the slope 0.5 to 0.9) commonly observed in food web biomagnification studies. The corresponding trophic magnification factor (TMF) value, calculated as 10^{Slope}, is shown for reference on the secondary y-axis.

Figure 5. Trophic magnification factor (TMF) and 95% confidence intervals (CI) of TMF for regressions based on the means of chemical concentration and trophic level versus those calculated from regressions using the raw data.

Figure 6. Sensitivity evaluation for the a) slope and b) intercept of the regression line used to calculate the TMF of PCB-153 for Lake Paguchi (data from Houde et al. 2008).

Tables

Table 1. Statistical summary of TMFs, total study sample sizes (n), and untransformed regression values for the selected food web biomagnification studies.

Statistic	TMF	N	Original Base 10 Regression Slope[1]	Absolute Value of the Base 10 SE of Original Regression Slope[1]	Absolute Value of the Base 10 SD of Original Regression Slope[1]
Minimum	0.14	8	-0.9	0.03	0.20
10th Percentile	1.0	14	0.0	0.05	0.30
25th Percentile	1.8	28	0.3	0.07	0.49
Median	3.4	36	0.5	0.11	0.70
75th Percentile	6.2	56	0.8	0.16	0.90
90th Percentile	10.5	113	1.0	0.24	1.2
Maximum	64	136	1.8	1.28	8
SD	7.9	34	0.5	0.15	1.0

[1]All regression statistics are expressed on a base 10 scale (i.e. Log_{10} of concentrations versus trophic level). Values expressed on a base e scale (as reported in the literature) were standardized to a base 10 scale by expressing the standard deviation of the original base e regression relationship as percent coefficient of variation, which was then multiplied by the antilog of the TMF value (original regression slope) to obtain the base 10 SD of the slope.

Table 2. Trophic magnification factors (TMF) for PCB-153 among Canadian lakes (Houde et al. 2008) based on regressions with raw data or a Monte Carlo (MC) balanced study simulation.

		Monte Carlo TMF Values (MC-TMF)			
	Raw Data				
Lake	TMF Values	Mean	RPD[1] (%)	Median	RPD[1](%)
Athabasca	5.4	4.8	12.7	4.3	23.5
Cayuga	2.1	3.2	-40.8	2.3	-9.7
Champlain	2.2	2.2	0.3	2.1	3.8
Cold	2.5	2.0	23.9	1.9	27.6
Eva	4.4	4.0	11.1	3.8	14.3
Grist	3.5	3.6	-2.6	3.4	1.4
Kingsmere	1.5	1.4	7.4	1.4	10.3
Lac la Ronge	3.7	4.5	-18.4	4.0	-6.3
Namur	2.4	2.7	-13.2	2.6	-8.7
Opeongo	2.8	3.2	-10.5	2.9	-2.4
Paguchi	3.6	2.5	37.8	2.4	40.9
Reindeer	3.7	2.9	23.2	2.8	28.7
Sandybeach	3.8	3.7	1.7	3.6	4.3
Seneca	3.5	3.2	9.2	3.0	14.4
Simcoe	1.5	1.4	5.9	1.3	9.8
Superior	6.0	7.4	-20.0	5.7	5.0
Thunder	4.0	4.4	-8.6	4.3	-6.4
Wollaston	2.3	3.0	-25.8	2.7	-16.3

1: Relative Percent Differences (RPD) between the TMF values derived from raw data and median or mean CB-TMF values derived from the Monte Carlo simulation using Crystal Ball. Negative values indicate that that MC-TMF are larger than TMF.

Figure 1

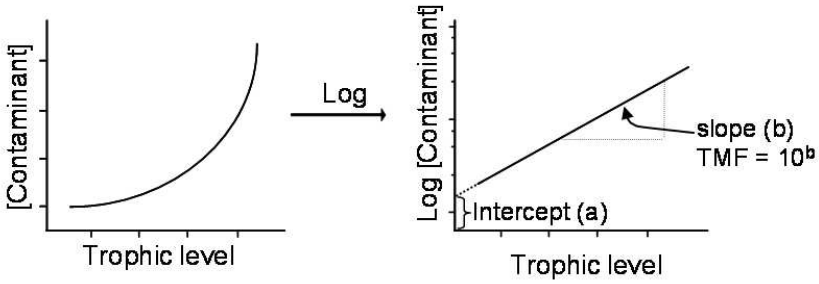

Figure 1
254x190mm (96 x 96 DPI)

Figure 2

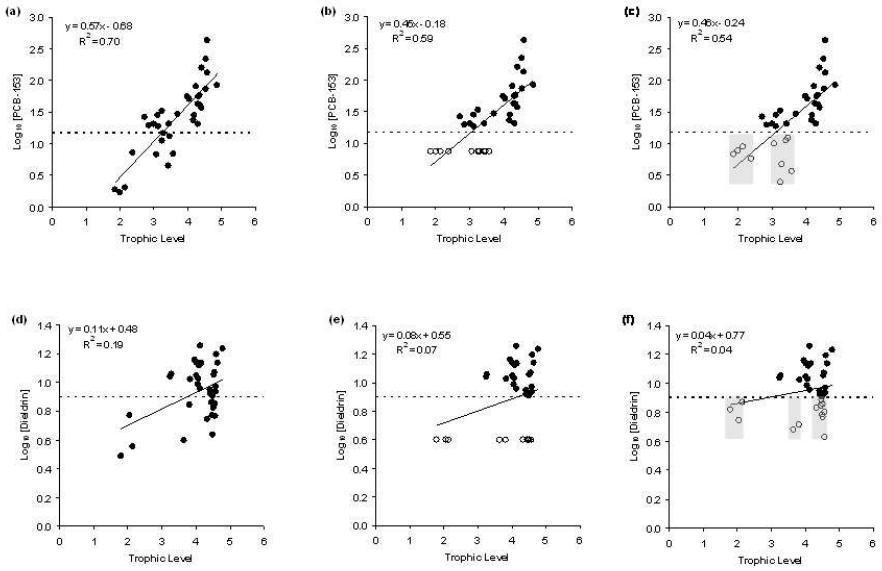

Figure 2
254x190mm (96 x 96 DPI)

Figure 3

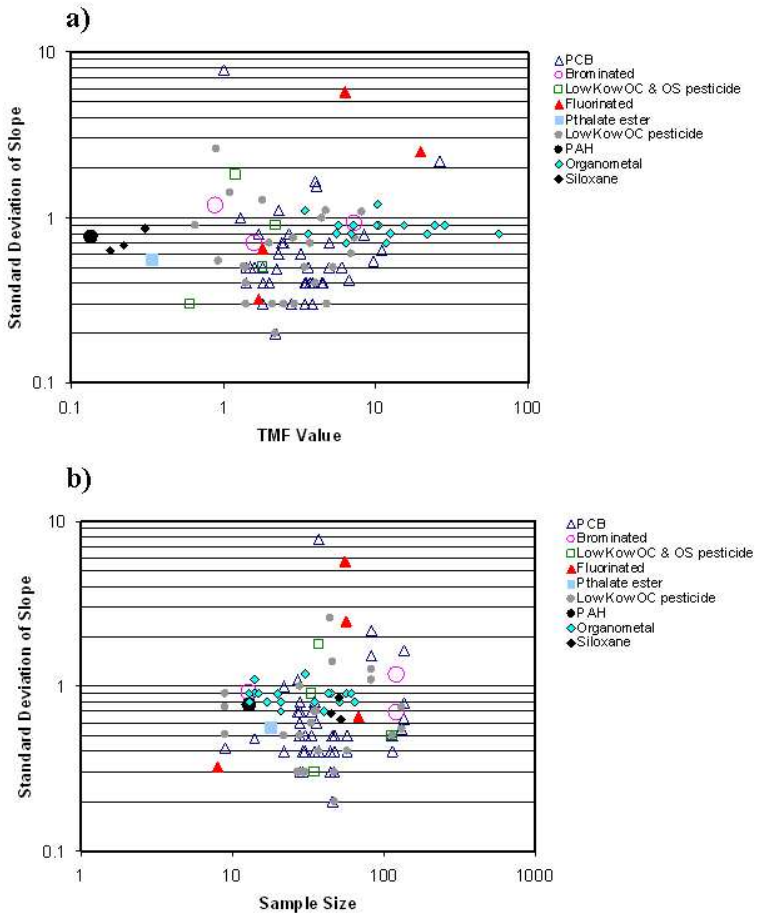

Figure 3
190x254mm (96 x 96 DPI)

Figure 4

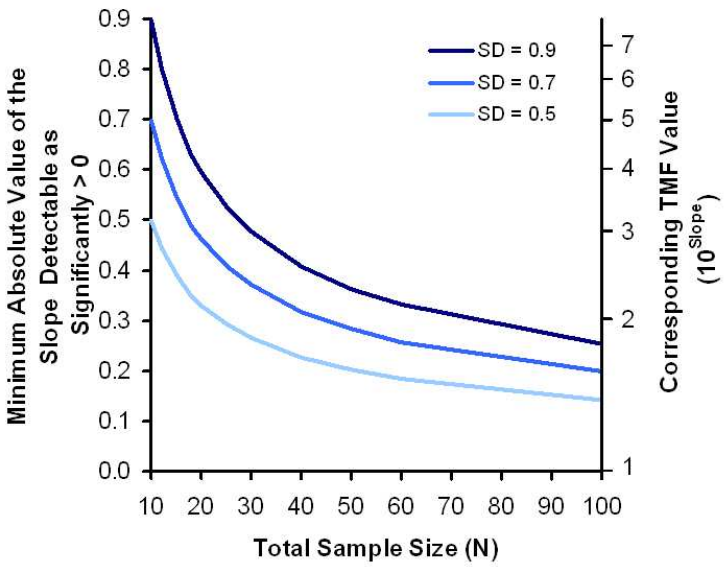

Figure 4
254x190mm (96 x 96 DPI)

Figure 5

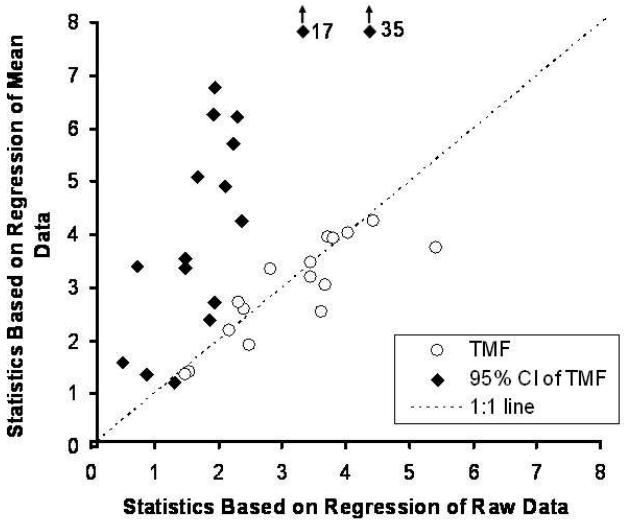

Figure 5
254x190mm (96 x 96 DPI)

Figure 6

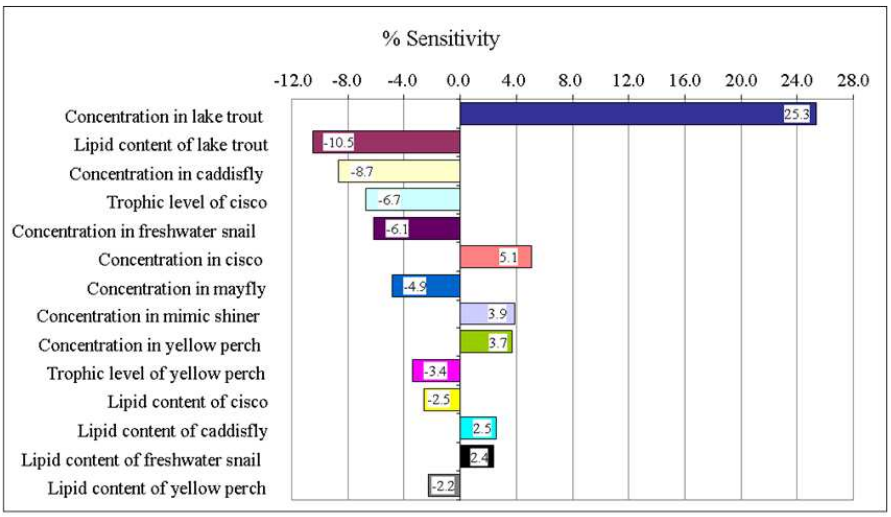

Figure 6
254x190mm (96 x 96 DPI)