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Trophic Magnification Factors: Considerations of Ecology, Ecosystems, and Study Design

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EDITOR'S NOTE

This paper is 1 of 5 articles resulting from the ''Lab-Field Bioaccumulation Workshop'' held in November 2009 in New Orleans, Louisiana, USA. Workshop participants focused on three objectives: 1) compare laboratory and field measurements of bioaccumulation endpoints, 2) evaluate the reasons why laboratory and field bioaccumulation data may not align, and 3) explore the measurement and application of TMFs.

ABSTRACT

Recent reviews by researchers from academia, industry, and government have revealed that the criteria used by the Stockholm Convention on persistent organic pollutants under the United Nations Environment Programme are not always able to identify the actual bioaccumulative capacity of some substances, by use of chemical properties such as the octanol–water partitioning coefficient. Trophic magnification factors (TMFs) were suggested as a more reliable tool for bioaccumulation assessment of chemicals that have been in commerce long enough to be quantitatively measured in environmental samples. TMFs are increasingly used to quantify biomagnification and represent the average diet-to-consumer transfer of a chemical through food webs. They differ from biomagnification factors, which apply to individual species and can be highly variable between predator–prey combinations. The TMF is calculated from the slope of a regression between the chemical concentration and trophic level of organisms in the food web. The trophic level can be determined from stable N isotope ratios ($\delta^{15}N$). In this article, we give the background for the development of TMFs, identify and discuss impacts of ecosystem and ecological variables on their values, and discuss challenges and uncertainties associated with contaminant measurements and the use of $\delta^{15}N$ for trophic level estimations. Recommendations are provided for experimental design, data treatment, and statistical analyses, including advice for users on reporting and interpreting TMF data. Interspecies 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 TMF (i.e., regression slope). Following recommendations herein for study design, empirical TMFs are likely to be useful for understanding the food web biomagnification potential of chemicals, where the target is to definitively identify if chemicals biomagnify (i.e., TMF $>$ or $<$ 1). 1). TMFs may be less useful in species- and site-specific risk assessments, where the goal is to predict absolute contaminant concentrations in organisms in relation to threshold levels. Integr Environ Assess Manag 2012;8:64–84. © 2011 SETAC

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INTRODUCTION

From bioaccumulation to trophic magnification factors

Recent reviews resulting from an international Pellston workshop on bioaccumulation science with scientists from academia, industry, and government revealed that the bioaccumulation (B) criteria used by the Stockholm Convention on Persistent Organic Pollutants (POPs) (UNEP 2001) and many national risk assessment programs (e.g., European Commission 2003) are not always able to correctly identify the actual bioaccumulative capacity of substances by using chemical properties such as the 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 occurring faster than

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elimination (Gobas and Morrison 2000). Biomagnification can lead to concentrations that threaten the health of top predator organisms (Fisk et al. 2005; Letcher et al. 2010).

Environmental risk assessments of chemicals have traditionally 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 factors (e.g., habitat, feeding mode, food quantity and/or quality, trophic transport) (Gobas et al. 2009; Weisbrod et al. 2009; Burkhard et al. 2012a,b; Selck et al. this issue). Although we have a relatively good understanding of the factors controlling bioaccumulation of nonionic 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 always 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. 2012a,b; Selck et al. this issue).

The Pellston workshop concluded that BCF is often 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, whereas 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) (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 time-consuming, costly, and difficult to perform for very poorly water-soluble organic chemicals with high bioaccumulation potential. Trophic magnification factors (TMFs) were suggested as a reliable and conclusive tool for contaminant bioaccumulation assessments of chemicals that have been in commerce long enough to be quantified in environmental samples (Gobas et al. 2009; Weisbrod et al. 2009).

TMFs were earlier called food web magnification factors and food web concentration factors. As described below, TMFs are determined empirically, using field measures of both contaminant concentrations and relative trophic level (TL; estimated from stable N isotope ratios [$\delta^{15}N$] using tissue measures of $^{15}N/^{14}N$) in food webs, and can be viewed as an ''average food web biomagnification factor'' (Fisk, Hobson, et al. 2001; Jardine et al. 2006). Because of dietary absorption occurring faster than elimination (Gobas and Morrison 2000), biomagnification causes TMFs > 1. TMFs above 1 imply a disequilibrium between organisms and abiotic media (water or air) that increases with increasing TL. Chemical properties that enhance or reduce this disequilibrium play a key role in the TMF approach.

Objectives

The objectives of this article are to identify and discuss:

- The effect of ecosystem and ecological variables such as organism properties, food web structure, and spatial and temporal variation in contaminant exposure on TMFs.
- Chemical and environmental properties that affect TMF directly or its baseline conditions, such as exposure concentrations, primary production, and dissolved organic C (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.

Refinement and application of the TMF technique should improve our ability to assess and predict the biomagnification and risk of chemicals to the environment and humans. In the following, we focus primarily on legacy-type organic contaminants (i.e., polychlorinated biphenyls [PCBs] and other POPs) but also include knowledge on Hg and emerging chemicals of concern such as cyclic volatile methylsiloxane (cVMS) materials when appropriate. Readers are referred to a companion article on the regulatory considerations for TMFs (Conder et al. this issue).

TMFs: BACKGROUND

In the 1980s, a long-standing debate about the significance of trophic position on the extent of nonionic 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 > 1, and they hypothesized that this thermodynamically driven increase occurred during dietary uptake. Thomann (1989), using a food chain model, showed optimum log K_{OW} and molecular size for biomagnification, i.e., log K_{OW} 5.0 to 8.0, whereas Clark et al. (1990) developed a fugacity-based food chain model that 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 TL of the organisms. In the early 1990s, biomagnification of POPs (selected polychlorinated dibenzodioxins [PCDDs] and polychlorinated dibenzofurans [PCDFs]) was assessed by an integrated approach using 2 whole food webs in the Northern Baltic Sea (Broman et al. 1992; Rolff et al. 1993) rather than using single predator–prey relationships as in BMFs. The new approach quantified biomagnification by first assessing the organisms' relative positions in the food web based on the biological enrichment or fractionation of the stable isotopes of N (i.e., $\delta^{15}N$) and then regressing the measured contaminant concentration against $\delta^{15}N$ to quantify the rate of trophic transfer of the chemical. This method was soon applied to different compounds and food webs, e.g., Hg and POPs in lakes (Kidd, Hesslein, et al. 1995; Kidd, Schindler, et al. 1995), 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 2 decades, many studies have used $\delta^{15}N$ to assess the trophic transfer of contaminants through marine and freshwater food webs. These studies have built on the

earlier work on Hg and POPs and also included the metalloid Se (Stewart et al. 2004).

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, Hesslein, et al. 1995). Later, the method was refined by calculating integer-based TL (or trophic position [TP]) from $\delta^{15}N$ using enrichment factors (increase in ^{15}N from the diet to the consumer, called Δ15N; Fisk, Hobson, et al. 2001; Eqns. 1–2) and assumptions that the primary producers and primary consumers included in the calculations occupied discrete TLs 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 for an assessment of the average factor change in contaminant concentration per relative TL (rather than per $\delta^{15}N$) in the food web, which is equivalent 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 N from wastewaters or agriculture (e.g., Anderson and Cabana 2005).

The use of a relative TL rather than $\delta^{15}N$ also allows unique enrichment factors for ecosystems, species or groups of animals to be incorporated into Eqns. 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.

Because of differences in biomass and contaminant transfer efficiencies, contaminant concentrations often increase exponentially through the food web (Eqn. 3 and Figure 1). Therefore, the regression is usually, but not always, log-normal (Eqn. 4), and the TMF is calculated as the antilog of the regression slope (''b'' in Figure 1; Eqns. 3–5) with base 10 or e depending on the logarithmic transformation (Eqn. 5). Thus, in their most simple form, TMFs are calculated as:

$$[\text{Contaminant}] = 10^{bTL} \text{ or } e^{bTL} \quad (3)$$

$$\text{Log}\,[\text{Contaminant}] = a + b\text{TL} \quad (4)$$

$$\text{TMF} = 10^{b} \text{ or } e^{b} \quad (5)$$

In the absence of significant metabolism, contaminants with log K_{OW} values < 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 TP (Fisk, Hobson, et al. 2001). Therefore, TMFs can be used to understand whether a chemical biomagnifies through food webs. For TMF = 1 ($b = 0$; Eqn. 5), the chemical does not biomagnify, on average, through the food web. For TMF > 1 ($b > 0$), the chemical biomagnifies through the food web, with an average factor of TMF per TL. For TMF < 1 ($b < 0$), the chemical decreases, with an average factor of TMF, in concentration with each TL in the food web. This is 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 inputs of contaminants to the base of food webs, such as between different lakes, or between ice, pelagic, or benthic food webs, is assumed accounted for by the intercept (a) in the regression (Figure 1 and Eqn. 4), so that the ''rate of increase'' per TL in the food web can be studied independently of the original 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 any interaction between the 2, 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, such as age, size,

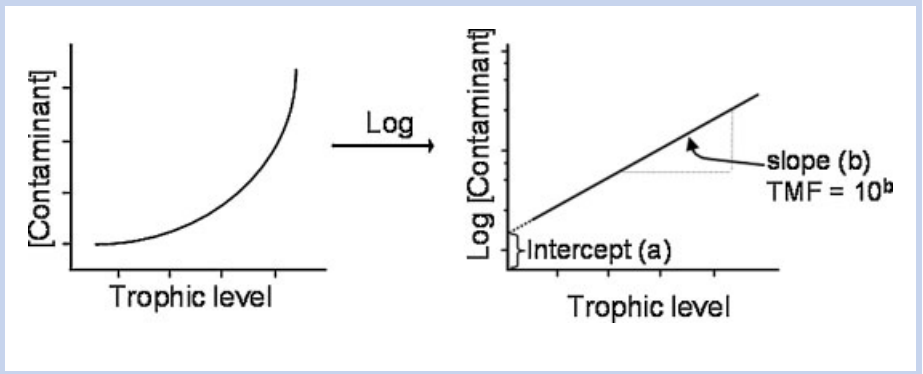

Figure 1. Trophic magnification factors (TMFs) are the change in contaminant concentrations per trophic level. For contaminants that increase exponentially in the food web, a log-normal relationship results in a regression slope (b), from which the TMF is calculated by the antilog (10^b or e^b). The intercept of the regression is represented by (a) and may indicate inputs of the contaminant to the base of the food web.

reproductive status, biotransformation efficiency, and omnivorous feeding, are important for the observed contaminant residue in an organism, the regression of chemical concentration versus TL will become confounded by differences among these factors within and among the species included in the calculation. When estimating the average increase of contaminant concentrations with TL 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. Other factors that influence bioaccumulation in organisms should be acknowledged and accounted for in the regression model, or assumed to be negligible compared to the influence of TP, 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 organisms and TLs (Broman et al. 1992) and that the $\delta^{15}N$ fractionation is similar (or at least known) among TLs. The assumptions introduce different challenges for the estimation and use of TMFs, which will be addressed in later sections.

When assessing the change in contaminant concentration per TL, it is important to ensure that the process quantified is the trophic transfer of contaminants, rather than changes in the cellular medium (i.e., solvent) in which the contaminant is associated (Kidd, Schindler, et al. 1995). Because lipid content and lipophilic 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 few studies have addressed this issue. Alternative approaches for TMF estimations are also presented in a subsequent section on data treatment and statistical analyses (see also Supplemental Data).

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 as discussed here, its diet. The 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). This variation, if not considered in the sampling design, could result in an estimated TL difference of approximately 1.5 in the consumer. Along the same lines, one must ensure that the species included in a food web relationship are actually structurally connected and are representatives of the same food web, e.g., benthic or pelagic (see section on *Properties of Organisms*). 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 (carbon [$\delta^{13}C$] or sulfur [$\delta^{34}S$] isotope ratios). These isotope ratios are conserved or only slightly enriched from the diet to the consumer, 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 TLs 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 are differences in the time needed to obtain a steady state for contaminants versus stable isotopes; these confounding factors will all be addressed in subsequent sections. The challenge of steady state has wide implications, because the estimated TMF is assumed to represent the average biomagnification in a local food web, where the actual food web is represented (see also the section on C*haracterization of Food Webs with Stable Isotopes*).

Challenges and uncertainties with TMFs

Several challenges and uncertainties related to the use of TMFs that are considered in this article include biological factors such as the differences between poikilothermic (cold-blooded) and homeothermic (warm-blooded) organisms 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 present 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 chemical analysis of tissues or organs, rather than the whole body, and the assumption that the subsample is representative of biomagnification in the whole organism. This is a particular problem in the study of mammals and birds, where whole-body samples are generally not available, and for nonlipophilic substances, where normalization practices for the fraction in a particular cellular medium are less standardized. Statistical treatment is also a major challenge, because the TMF is affected by choices made during collection and analysis of samples (i.e., experimental design), data processing, and calculations.

Advantages of TMFs

The main advantage with TMF is that it validates or augments the ''B'' criteria for chemicals, i.e., by quantifying the biomagnification behavior as it occurs in the field. Field-derived TMFs are considered to represent a more conclusive and holistic measure of biomagnification than laboratory-derived metrics and chemical properties, or BMFs between a single predator and its prey (Gobas et al. 2009; Weisbrod et al. 2009). For example, certain high molecular weight phthalate esters are very hydrophobic chemicals with a high K_{OW} ($\log K_{OW} > 5$) and some BCF measurements exceed 1000. However, phthalate esters do not biomagnify in aquatic food chains as demonstrated by their TMF < 1 (Mackintosh et al. 2004). Polyclyclic aromatic hydrocarbons (PAHs) and cVMS are other examples of substances with this bioaccumulation behavior (Wan et al. 2007; Powell et al. 2009, 2010b). In contrast, substances such as perfluorooctane sulfonate (PFOS) exhibit a relatively low BCF (<5000) but are known to biomagnify in some aquatic food webs, as demonstrated by a TMF > 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 TLs. However, this requires an understanding of the ecosystem under study (e.g., sources and transport of the contaminant within the ecosystem), the feeding ecology or trophic structure of the food web, and species-specific factors

that may influence TMF and contaminant accumulation. This is particularly relevant for species where other factors are important for bioaccumulation, such as age, size, reproduction, and biotransformation. In addition, the estimation of concentrations in biota is not the only desired data in risk assessment. Concentrations at the base of the food web 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.

TMFs: WHAT AFFECTS THEM?

When calculating the TMF for a set of species or samples, it is assumed that the relationship between the contaminant concentrations and TL is the same across all species, and that contaminant concentrations are mainly driven by TL (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.

Properties of organisms

Species at the same relative TL may have very different concentrations of contaminants depending on their metabolic rates (e.g., Braune and Norstrom 1989). Lower TMFs have been documented for recalcitrant compounds in aquatic food webs when considering only poikilothermic species, whereas the TMFs were significantly greater when homeothermic species were included (Fisk, Hobson, et al. 2001; Hop et al. 2002). Compared to poikilotherms, homeotherms have higher energy requirements and thus food intake due to higher weight-specific metabolic rates. Thus, homeotherms are exposed to more contaminants through food, resulting in potentially higher biomagnification of recalcitrant contaminants in a bird than in a fish of comparable size and TL (Braune and Norstrom 1989).

Similarly, TMFs may be affected by the biotransformation of contaminants by some species within the food web and be lower in systems where some biotransformation occurs (e.g., Borgå et al. 2004). Food webs that contain an apex predator with the ability to biotransform a compound that is poorly biotransformed by organisms at lower TLs would result in varying BMFs between different predator–prey pairs. This effect has been well documented in the polar bear food web for 4,4′-dichlorodiphenyl dichloroethylene (4,4′-DDE; Letcher et al. 1995). The BMF for 4,4′-DDE between the polar bear and its major food source, the 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 their prey, polar cod, is 39. Similar biotransformation effects were also found for a variety of PCB congeners in this food web (Letcher et al. 1995). Other recent studies that included seabirds in the upper TLs had higher TMFs for most compounds, including metabolites such as oxychlordane (TMF = 9.61), compared to results for the same food web without birds (oxychlordane TMF = 2.12) (Hallanger, Warner, et al. 2011). For less recalcitrant compounds and chemicals susceptible to biotransformation (e.g., *cis*-chlordane), TMF was lower in the food web with birds (1.13) compared to that without birds (2.94), demonstrating 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, whereas the opposite is true for cetaceans (Tuerk et al. 2005). This can 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 ether (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 with TL.

Another property that will influence the TMF is the route of contaminant uptake into the organism. When regressing the contaminant concentrations onto TL, one assumes that the main route of exposure for an organism is from its diet. However, all invertebrates and also fish are, to varying degrees, 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 organisms of lower TLs 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 TLs, size or age affects bioaccumulation of the contaminant (Jardine et al. 2006; Swanson and Kidd 2010). It is well known that larger, slower-growing individuals typically contain higher POP and Hg concentrations than younger, faster-growing conspecifics. Comparisons of TMFs across systems may therefore be confounded if one system is dominated by slow-growing top predators compared to another. A 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 produce estimates of TMFs that are more reflective of trophic transfer by decreasing variability due to confounding factors. In a recent study of Hg in Arctic lake food webs, removing the effects of size and age of the fish decreased the regression slope in some systems (Swanson and Kidd 2010).

Sex is particularly important for POPs, because female mammals can reduce their concentration by transfer due to lactation and via the placenta. Also, 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 dominated by reproductively active females of an apex predator may have lower TMF values for lipophilic POPs

(due to higher maternal elimination rates of contaminants) than a food web dominated by males of the same species.

Disproportionate sampling of the food web or unbalanced replication of samples may significantly influence the slope and thus the TMF. Even if the relationship between contaminant concentrations and TL was very strong (i.e., having a Pearson product moment correlation coefficient > 0.95), the regression may be substantially influenced by the number of samples, the range of TLs on the *x* axis, and the separation that exists between species that occupy adjacent TLs. In other words, not all observations will have an equal contribution to the regression; one or more observations may have substantial influence on conclusions, resulting from a regression analysis that involves a spurious correlation. This was demonstrated for methylmercury (MeHg) in Arctic char food webs in Canadian Arctic lakes (Gantner, Power, et al. 2010) and for organochlorines in the Barents Sea marine pelagic food web (Hop et al. 2002), which showed that the number of TLs included in the regression significantly affected the slope of the regression line.

Characterization of 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 N ($^{15}N/^{14}N$, referred to as $\delta^{15}N$) (e.g., Peterson and Fry 1987). Increases in $\delta^{15}N$ occur because of the preferential retention of the heavier isotope compared to the lighter isotope in the consumer relative to its diet. 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 TL of organisms within a food web. The first, and perhaps most important, is that ^{15}N fractionates in a predictable manner from the diet to the consumer. For aquatic organisms, the fractionation or enrichment factor (Δ15N) used to calculate TL (Eqns. 1 or 2) is generally 3.0‰ to 5.0‰ between TLs, and is often assumed to be approximately 3.4‰ based on a number of feeding experiments and syntheses of the literature (e.g., Hobson and Welch 1992; Vander Zanden and Rasmussen 2001; Post 2002; McCutchan et al. 2003; Jardine et al. 2006). However, there are several organism-level factors (e.g., nutritional status and age) that can affect this enrichment and contribute uncertainty in the application of the trophic enrichment factor within food webs. Such factors contribute to the variability in Δ15N that is observed among individuals in laboratory or field studies and may cause variation in the trophic enrichment factor, i.e., the assumption that approximately 3.4‰ is appropriate for calculations of TL does not always apply. For example, the $\delta^{15}N$ between the bird's diet and its muscle tissue is only 2.4‰ (Mizutani et al. 1991). Nonetheless, an enrichment factor of 3.4‰ per TL step is recommended for constructing food webs without a priori knowledge of Δ15N 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, can have lower enrichment factors than those with slower growth rates (e.g., Hesslein et al. 1993). Starvation, fasting, or higher metabolic rates will also result in some catabolism of body proteins and an enrichment of the ^{15}N of the consumer relative to those with adequate food or lower metabolic rates (e.g., Hobson et al. 1993; Gaye-Siessegger et al. 2004). Several studies that have shown that fractionation of ^{15}N is higher in animals consuming protein-poor than protein-rich foods, particularly for herbivorous mammals consuming low-N feeds (Darr and Hewitt 2008; Robbins et al. 2010). In addition, even at a constant diet, Δ15N from diet to consumer may be affected by age. Although the literature remains unclear, older walleye with 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).

Use of enrichment factors in TL calculations assumes a 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 tissues differ in their metabolic activity and turnover times. For example, liver tissues have higher 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 may reflect feeding habits over shorter (weeks to months) and longer (months to years) periods, respectively (Hobson and Clark 1992; MacNeil et al. 2006). Similarly, if the turnover rates for contaminants and stable isotopes differ in a given tissue, a thorough understanding of the covariation is important to be able to link the 2 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 a slow contaminant turnover.

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 Δ15N 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 organisms (typically those in lower TLs) 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 often used to standardize baselines before any comparisons across systems are 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 ecology. Of the other stable isotopes used to understand food web structure and habitat use in aquatic systems (i.e., isotopes of C, S, H, and O), C and S likely have the most promise in improving how TMFs are calculated. 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 versus aquatic or benthic versus 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) (Hesslein et al. 1991; Swanson et al. 2010). Evidence indicates 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 were sampled and 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 C source on TMFs. To date, this has only been done in systems where the C flow to upper-level consumers is distinct. In temperate and Arctic lakes, estuaries, or oceans, understanding the importance of benthic or pelagic C sources in the biomagnification of chemicals 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 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 to 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 of the $\delta^{13}C$ using C to N ratios was a better approach than lipid extraction 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, and it is beyond the scope of this article to address this in more detail.

To date, C isotopes have mainly been used in the TMF context to look within species at how contaminant concentrations are affected by feeding habits (Eagles-Smith et al. 2008; Guildford et al. 2008) or to identify 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 C. For those that have, TMFs are higher for DDT in organisms relying on pelagic than benthic C (e.g., Kidd et al. 2001; Houde et al. 2008), whereas no difference was found for Hg (Kidd et al. 2003). In Lake Simcoe, Canada, and Lake Champlain, Canada and the United States (see more details below; Houde et al. 2008), the TMFs for *p,p′*-DDE were 0.9 and 1.3, respectively, 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). In contrast, the pelagic food web TMFs for *p,p′*-DDE were 1.9 and 2.1, respectively, for these 2 systems. 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.

Characteristics of ecosystems

Although $\delta^{15}N$ and $\delta^{13}C$ have been used increasingly in biomagnification studies, few systematic comparisons have been performed of how TMFs are affected by ecosystem characteristics such as productivity, species composition, size, and latitude and longitude. Data now exist to start comparing TMFs between freshwater systems (i.e., food webs in streams versus lakes), marine and freshwater food webs, and systems with high and low productivities for a range of biomagnifying compounds. For example, TMFs were similar for 3 cVMS materials in the freshwater benthic food web of Lake Pepin, USA (range 0.3–0.4; Powell et al. 2009) and the marine benthopelagic food webs of the Inner and Outer Oslofjord, Norway (range 0.3 to 0.7; Powell et al. 2010b). 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. In another example, 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 all 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 PCB-52 and PCB-153 were positively correlated with lake mean depth. In addition, multiple regression including latitude and mean depth was more strongly related to TMFs for total PCB and PCB-52 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 total PCBs (ΣPCB; lipid-normalized), supporting the hypothesis that increasing access to littoral habitat results in lower concentrations in lake trout compared to those trout that are more restricted to pelagic habitat. Taken together, these result imply that the biomagnification of highly recalcitrant compounds is greater in food webs of deep-water lakes that are more dependent on pelagic C and independent of any effect of ''hotspots'' due to higher contamination in lakes within the Great Lakes and St. Lawrence River basin. Although Houde et al. (2008) reported similar TMFs for PCBs across these systems, lowest TMFs for PCB-153 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 midlatitude lakes in their study. Similarly, TMFs for *p,p′*-DDE were 1.9 and 2.1 in Simcoe and Champlain lakes, respectively, compared with an average of 4.7 in 8 other lakes. In summary, whereas TMFs appear to be influenced both by physical and chemical characteristics of the systems, the degree of influence that these characteristics have on TMFs likely varies from one compound to another.

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.

Most studies of biomagnification consider aquatic ecosystems, yet TL 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-hexachlorohexane [HCH]) than for food webs

dominated by poikilotherms (Kelly et al. 2007). Katz et al. (2009) and Müller et al. (2009) showed that concentrations of PFOS and C9–C11 perfluoro-carboxylates (PFCAs) were correlated with TL (determined by $\delta^{15}N$ of individual samples) in lichen–caribou–wolf food webs from 2 remote locations in northern Canada. This study also found that $\Delta 15N$ between lichen and caribou was rather large (7‰–8‰), probably due to the low protein content of lichen (0.4% N), the predominant diet, as discussed earlier. 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.

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 system of interest and, when these organisms are included in a TMF calculation, the estimation of trophic transfer becomes skewed. For example, Fisk, Hobson, et al. (2001) demonstrated that migrating species did not fit well on the regression of contaminants versus TL when compared to the local species. This was explained by the non steady state situation for migrating species, as they are representing $\delta^{15}N$ and/or contaminants levels of a wider region. Another confounding factor is the differing inputs of chemicals at one site versus another within a system that would affect concentrations present in organisms at lower TLs. 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 3 case studies:

Biomagnification of perfluorinated compounds in dolphin food webs. Houde et al. (2006) compared the biomagnification of PFOS in the food webs of bottlenose dolphins (*Tursiops truncatus*) feeding near or in the Charleston, South Carolina, USA, harbor and a population living in Sarasota Bay, Florida, USA. The water, sediments, zooplankton, and fish from the Charleston area had approximately 10-fold higher concentrations of PFCAs and PFOS compared to Sarasota Bay. TMFs for PFOS (using mean concentrations and TLs) were similar in the 2 locations, although variance was high. TMFs were 4.9 at Charleston and 7.9 at Sarasota for food webs consisting of planktivorous and forage fishes and dolphin plasma. TMFs based on an estimated whole-body concentration of PFOS in dolphins were much lower, 1.8 ± 1.2 (standard error) at Charleston and 3.3 ± 1.7 at Sarasota Bay. Wastewater treatment plant discharges in the Charleston area may have resulted in non–steady state concentrations of perfluorinated compounds (PFCs) in the food web. In addition, PFOA, which has generally been reported to not biomagnify, had a TMF of 6.3 ± 6.7. PFOA is a persistent degradation product of many polyfluorinated chemicals and its increasing levels in the food chain may reflect uptake and transformation of other fluorinated substances into PFOA. The above illustrates that contamination ''hot spots'' may influence observed TMFs, particularly where there are non–steady state conditions.

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 United States. Mean total PCBs in lake trout in these systems ranged from 100 to 5770 ng/g wet wt (whole fish) and were highest in 5 lakes within the Great Lakes and St. Lawrence River region due to their proximity to urban areas and elevated regional atmospheric deposition. Despite the more than 60-fold differences in total PCB concentrations in lake trout, TMFs for individual PCB congeners (PCB-52, PCB-99, PCB-101, PCB-138, PCB-153, and PCB-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, DOC, or percent Dinophyta (a mixotrophic protozoa that grazes on picoplankton). Relative standard deviations of the TMFs for PCB congeners and DDE were generally 30% to 40%. Unlike the highly recalcitrant PCBs and *p,p′*-DDE, TMFs for α-HCH, lindane (γ-HCH), and hexachlorobenzene (HCB) were positively correlated with latitude and longitude in the same food webs (Houde et al. 2008). TMFs were significantly higher (by approximately 2 times) for these compounds in more westerly and northern lakes which were more remote from human activity. Houde et al. (2008) speculated that the biomagnification of HCH and HCB, which are biotransformed or eliminated by fish more rapidly than PCB congeners or DDE, 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 MeHg in Arctic char food webs. Gantner, Muir, et al. (2010) compared biomagnification of MeHg in food webs of 18 Arctic lakes in Canada. These food webs are typically 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 TP of these systems and can be cannibalistic. Benthic invertebrates are the main source of nutrients, and thus MeHg, for landlocked (no access to the sea) Arctic char (Chételat et al. 2008; Gantner, Muir, et al. 2010). Within the majority of these lakes, TMFs for MeHg ranged from 3.5 to 15.3 (but 4 of 18 had TMFs from 21.7–64.3). An unbalanced design, with large numbers of fish and relatively few invertebrates, may have influenced these higher TMF values. No relationships between TMF and abiotic factors known to influence Hg inputs to lakes (lake area, catchment area, catchment to lake area ratio, DOC, or chlorophyll *a*) were found.

Seasonal variation in chemical concentrations and stable isotopes

If the food web is at steady state, TMFs can be expected to be constant throughout the year. However, bioaccumulation in lower TLs can vary seasonally (Hargrave et al. 2000; Fisk, Stern, et al. 2001; Hallanger, Ruus, et al. 2011), as do $\delta^{15}N$ values that are used to estimate TL (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 TLs 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 the species and ecosystem. For example, in eider duck (*Somateria mollissima*), which reduces its body mass by up to 50% during breeding, the plasma POP concentrations increase 2- to 8-fold during the incubation period, probably due to remobilization from lipids into blood (Bustnes et al. 2010).

TMFs are dependent on both stable isotope and POP concentrations and seasonal variability in these measures can influence calculations of food web biomagnification. This was illustrated in a recent study of seasonal changes in TMF in a zooplankton-fish-seabird food web; the TMFs differed greatly between seasons (Hallanger, Warner, et al. 2011). The seasonal variation in TMF was caused predominantly by decreasing contaminant levels in zooplankton from May to July and October, in combination with an increase in contaminant concentrations in seabirds, which were the top predators. In addition, the animal used as baseline for TL estimations changed its $\delta^{15}N$ signal relative to the rest of the food web, causing altered TL values of the other organisms between seasons.

Properties of chemicals

Several chemical properties can be expected to influence the TMF. These may include K_{OW}, water solubility, vapor pressure, environmental half-lives, and molecular size and structure of the chemical, although it is still inconclusive if a quantitative structure–activity relationship can be developed for TMFs.

Few studies have examined whether chemical partition coefficients or other properties are predictive of TMFs. Because K_{OW} has been well established for the estimation of BCFs, it has also been suggested as a predictor of TMFs because they appear to be higher for the more lipophilic contaminants in lake trout food webs than those that are less lipophilic (Houde et al. 2008). The water solubility and vapor pressure of the chemical affect the rate of elimination in water- and air-breathing organisms, respectively. As described previously, K_{OW} and BCF in fish are not good predictors of biomagnification in air-breathing organisms 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 are expected to be particularly important in food webs consisting of both air- and water-breathing organisms at different TLs. Comparisons between TMFs and partition coefficients should be made for a wide range of food webs that chemical properties are predictive of TMFs.

Molecular structure also may influence observed TMFs via selective transformation reactions (e.g., biodegradation and biotransformation) of chemicals. The effect of biotransformation on the BCF can in some cases be estimated by using molecular fragment descriptors (Arnot et al. 2008, 2009). In examining the biomagnification of PCDDs/PCDFs with TL 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 whereas more highly chlorinated PCDDs/PCDFs exhibited TMFs < 1. Bioformation may lead to apparent increases in TMF, as found for certain PBDEs where debromination of BDE-183 and other highly brominated BDEs to BDE-154 may increase TMFs for this compound (Stapleton et al. 2004; Wu et al. 2009). Similarly, as noted above, reports of PFOA biomagnification may be due to the accumulation and transformation of other PFC precursors (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 factors that may affect the baseline of the contaminant-TL relationship (i.e., the intercept). The intercept of the chemical concentration regressed onto 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 found in upper TLs 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 to bioconcentrate in organisms at the base of the food web. Factors affecting the total water column concentration and bioavailability of a chemical to the base of the food web should therefore affect the intercepts of the contaminant concentration versus TL relationships and not 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 the regression intercept is affected by intrinsic properties of the system, and of the compound being biomagnified. Contaminant loading is a complicated process driven by numerous variables including the suite of physical and chemical properties of the compound, likelihood of atmospheric versus water transport, and proximity to sources. Because of these complexities, a thorough description of loadings to environments is beyond the scope of this review. Instead, this section will deal with factors intrinsic to the system that likely affect the intercept, including productivity and differences in metabolism among food web members.

Physicochemical properties

The physicochemical properties of a compound can affect the regression intercept in several ways. For hydrophobic compounds, the apparent water concentration or bioavailable fraction is affected by the binding of the chemical to sources of C in the water column and this is largely driven by its solubility, K_{OW}, and affinity for organic matter (e.g., Schlautman and Morgan 1993; Burkhard 2000). Therefore, in principle, lakes could have similar TMFs for hydrophobic organics but different intercepts due to differences in the bioavailability of a compound in the water column. Houde et al. (2008 and Muir et al. 2002) found similar TMFs, but intercepts of the log PCB-153 versus TL relationships varied widely for 17 lakes, with highest values in the southern Canadian and/or northern US lakes (Simcoe, Champlain, Seneca) with legacy sources of PCBs. However, bioavailable concentrations of PCBs in water were not measured and DOC of these lakes were, in general, not different from 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 C partitioning has been shown to affect the bioavailable fraction of neutral hydrophobic organic molecules in sediment and porewater (Akkanen and Kukkonen 2003; Lyytikäinen et al. 2003; Burkhard et al. 2008). Dissolved water concentrations are expected to be reduced when black C is present in sediments and in suspended solids because of the exceptional affinity of some chemicals for this phase (Burkhard et al. 2008; Gustaffson et al. 1997). Recent modeling work suggests that including soot-derived black C 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 affected by temperature (Kucklick et al. 1991) such that cooler waters tend to have higher dissolved contaminant concentrations than warmer waters. Therefore, a lake with a cooler average water column temperature could have a higher intercept than a lake with a higher average temperature. In line with this, Sobek et al. (2010) 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 pK_a—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). However, this effect has only been examined in a few aquatic organisms. In surface water, ambient pH can also affect speciation such that anionic compounds may be more bioavailable in low pH 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 its 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) because of their influence on microbial metabolism (Munthe et al. 2007). Acidic waters and reducing conditions associated with low dissolved O_2 favor Hg methylation. In tropical climates, black waters, which are systems with high DOC because they drain soils rich in organics, have higher temperatures that 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 protects MeHg in such aquatic systems. In the case of tropical rivers and reservoirs that have high DOC, low dissolved oxygen (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, because it presents perfect conditions for Hg mobility and methylation (da Silva et al. 2006), and it was hypothesized that Hg is being accumulated in local soils and then by the local aquatic food chain. There are high MeHg values in biota, even with no specific Hg sources (Barbosa et al. 2003; Dórea et al. 2006; Dórea and Barbosa 2007). These same conditions observed in the Negro River (high DOC and low DO and pH) were sometimes also seen in some new, artificial reservoirs in deep areas just upstream and downstream of the dam (Malm et al. 2004; Palermo et al. 2004; Kehrig et al. 2004). It is possible that these elements can influence the intercept of MeHg versus TL relationships although, to our knowledge, this has not been specifically investigated.

Productivity of the system

Eutrophication affects bioavailability by increasing particulate organic C, DOC and sedimentation rates of contaminants in the water. Negative relationships between trophic status and/or productivity and organochlorines in organisms have been observed in lakes. Proposed mechanisms include 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 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 local uptake and decrease downstream transport of lipophilic compounds. In addition to the effects above, increased biomass of primary producers has also been correlated both to increased air-water exchange and particle sedimentation (Dachs et al. 2000).

Choice of organism for the baseline of the food web

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 single or multiple organisms 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, Hobson, et al. 2001; Fisk et al. 2003; Campbell et al. 2005), primary consumers such as mussels (Vander Zanden and Rasmussen 1999; Post 2002), clams (Fry 1999; Swanson et al. 2003), or gastropods (Kidd et al. 1998; Post 2002) are preferred, because there is less likelihood of overestimating or underestimating the longer term baseline $\delta^{15}N$ of the system. This issue is discussed in greater detail above (see section *Characterization of 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 issues should be considered to maximize the usefulness of food web

data to derive a TMF. The following discussion focuses on these considerations.

Analytical considerations

The TMF is currently derived from measured chemical concentrations and TLs estimated from ratios of element concentrations (stable N isotopes). More specifically, the components of the TMF estimation include the chemical mass per amount of sample, the ratio of ^{15}N-to-^{14}N in the sample relative to the ^{15}N to ^{14}N ratio in the standard (typically atmospheric N), and the enrichment factor per TL (Δ15N; typically 3.4‰). For lower TLs with small individual organism size, such as plankton, there may be a challenge to obtain parallel samples for both contaminant and δ^{15}N analyses. In some studies, the samples are collected simultaneously but separately making the pairing of contaminant and δ^{15}N measurements problematic. They use average δ^{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 multiple composite samples be collected in the field and that each of these pooled samples be split into subsamples for contaminant and δ^{15}N analyses in the laboratory after homogenization. Uncertainty with respect to δ^{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.

Typically, the analytical variability associated with the measurement of bioaccumulative pollutants is less than the ecological 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 TLs. 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 organisms at higher TLs, whereas lower sample masses are available at lower TLs, primarily due to the difficulty in obtaining the latter samples. The sample mass used for analysis must be scaled to provide the appropriate analyte mass for proper detection. The instrumental calibration curve must bracket the observed concentrations; in other words, the method must be set up to have adequate sensitivity. 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 Environmental Protection Agency (Gomez-Taylor et al. 2003), International Organization for Standardization and International Union of Pure and Applied Chemistry (ISO/IUPAC) (Currie 1995), and in many other publications, and thus they will not be defined further here.

For field monitoring, a measurement that is less than a specified detection limit (DL) may be reported as: 1) ''below detection'', 2) zero, 3) less than the value of the DL, 4) some value between zero and the DL, for example one-half the DL, or 5) the actual value (positive or negative), whether it is below the DL. The last option, the reporting of the actual value (i.e., uncensored value), is generally recommended in preference to 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. The types of samples from a food web could potentially range from blubber to a pooled plankton sample, and multiple analytical schemes will likely be needed to remove interferences that may be specific to that TL (e.g., high lipid in blubber samples). Contamination of samples during collection in the field and from laboratory sources during processing and analysis are also a potential source of interference and uncertainty for the compounds under 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; Powell et al. 2010a). Care must be exercised through the running of appropriate blanks and removing sources of contaminants from the analytical stream.

Other considerations include the use of natural matrix reference materials, which are important for reducing measurement uncertainty. Numerous reference materials 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 the analyses, 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 because contaminant concentrations generally relate to lipid content across TLs. The determination of lipid in samples with low lipid content, such as for some samples from organisms at lower TLs 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. Gravimetric, colorimetric, and enzymatic techniques are also available for the estimation of lipid content in blood (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 certified lipid values (Wise et al. 2006).

The TMF is best derived from measurements that involve whole organisms. However, for top-level consumers such as marine mammals and birds, chemical measurements in whole organisms are 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 TLs. 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 from live animals 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 methods 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 *Study Design* below). To adequately characterize the food web, sufficient numbers of key organisms from each TL must be obtained. For most studies, organisms in upper TLs, such as fish, marine mammals, or birds, are typically analyzed individually for stable isotopes and bioaccumulative pollutants because 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 TLs. Therefore, the number of individuals required to achieve sufficient power should be estimated prior to sampling (Keith et al. 1983). For lower TLs, pooling or compositing of samples is often 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 TLs. If the entire composite is not used for analysis, the homogeneity of the pooled sample should be assessed through analysis of multiple subsamples. If composite samples are required, it is recommended that multiple composite samples be collected in the field and analyzed as separate samples in order to minimize pseudoreplication. In addition, use of a single composite sample will contribute to the unbalance of the design and will result in considerable leverage of the single data point, both of which may significantly impact the TMF calculation.

Quality control and quality assurance

Numerous studies have reported the high biomagnification potential of PCB-153 (Fisk, Hobson, et al. 2001; 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 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 regression modeling across a study design that incorporates multiple species. A full review of regression modeling and study design is beyond the scope of this article; see, for example, Zar (1999) and Sokal and Rohlf (1999). The following sections review several of the most commonly encountered challenges related to the experimental design of food web biomagnification 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 nondetect 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 nondetects 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 data sets. In particular, it can create a systematic error in the data when the LOD varies with species. Therefore, it has been suggested that the actual measured values be used for concentrations (C) 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 negative values or for concentrations less than the LOD (Clarke 1998; Antweiler and Taylor 2008).

In cases where the measured concentrations are less than the MDL but greater than the LOD and unavailable, there are several other more statistically robust methods for using left-censored data (Helsel 2005). Figure 2 depicts the effects of 2 treatments of nondetect data on calculations of food web biomagnification: 1) substitution of values one-half the detection limit, and 2) substitution of values derived from regression order statistics (ROS), one of the approaches for substitution discussed in Clarke (1998), Helsel (2005), and Antweiler and Taylor (2008). Details on the substitution methods can be found in the Supplemental Data. Both substitution approaches yielded overall conclusions that corresponded to those derived from the uncensored data. The uncensored data sets yielded TMFs (95% confidence interval [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 (Figure 2a and d). The method of substituting one-half the detection limit yielded the same overall conclusion regarding TMFs significantly greater than (Figure 2b) and less than (Figure 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 nondetect with ROS-generated values were 2.9 for PCB-153 and 1.1 for dieldrin (Figure 2c and f). All

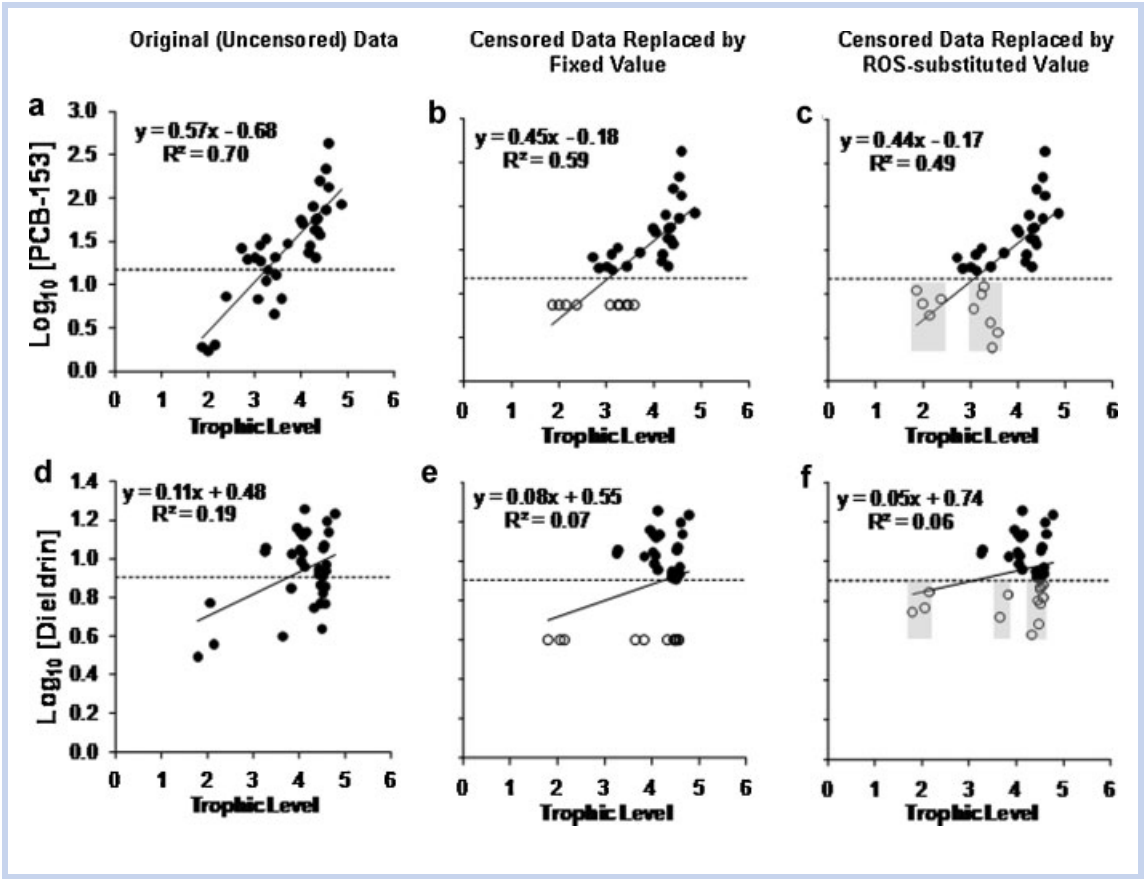

Figure 2. Effects of 2 substitution approaches to incorporate nondetect data into food web magnification studies of PCB-153 and dieldrin to which hypothetical detection limits for chemical concentration data (dashed line) have been applied. Original uncensored data and regression models are presented in (**a**) and (**d**). Regression analyses where hypothetical nondetect 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 regression order statistics (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 Data.

randomly generated TMFs (including 95% CI for the ROS-substituted PCB-153 data sets) were greater than one, suggesting significant biomagnification as observed in the original uncensored data set. A total of 93% of the randomly generated TMFs for the ROS-substituted dieldrin data sets were < 1, corresponding to the overall conclusions of the original uncensored data set.

Although both substitution methods (one-half the detection limit and ROS) performed relatively well in the examples shown (Figure 2), substitution of nondetect values with a fixed value (one-half the detection limit, the detection limit, zero, and so forth) would likely violate assumptions of regression analysis and would result in a distortion of the TMF value. This would likely be observed in data sets that were more balanced among TLs 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 (Figure 2c and f) or the approaches outlined by Helsel (2005) should be employed.

Another method to deal with several values below the LOD in a contaminant concentration-TL regression is the maximum likelihood estimation technique (Frome and Wambach 2005). As the range within which 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 data set. Instead of estimating a mean and uncertainty for a species based on the replicates, this method uses estimated values in a regression, assumes a parametric relationship such as $\log[C] = a + b\mathrm{TL}$, and estimates a, b, and residual standard deviation by maximizing cumulative probabilities for the whole data set. 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 nondetect 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 nondetects using advanced substitution methods such as ROS-generated values.

Statistical power and sample size

The question often posed by stakeholders and policy makers is whether a TMF exceeds the biomagnification threshold of 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 data sets to be used in evaluating biomagnification potential. The key

evaluation is the statistical significance of the slope of the regression of the concentration of a chemical in biota onto TL, which evaluates the null hypothesis that the slope of the regression model is equal to zero (i.e., TMF = 1).

The statistical power associated with past studies on trophic magnification was evaluated using regression slopes obtained from approximately 80 TMF values for a suite of contaminants. These values were compiled from several studies conducted in aquatic ecosystems primarily in North America (Fisk, Hobson, et al. 2001; 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, Power, et al. 2010; Powell et al. 2010b) (Supplemental Table S1). Details on study selection and variability statistics can be found in the Supplemental Data. Variability of the 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 (Figure 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 less than the range of 0.3–0.5 or greater than the range of 2.0–3.2) as being statistically different from a slope of 0 (Figure 4). Conversely, these study designs would likely have failed to detect significant (i.e., $p < 0.05$) regression slopes for contaminants with apparent TMF values in the range of 0.5 to 2.0. 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 TMFs 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

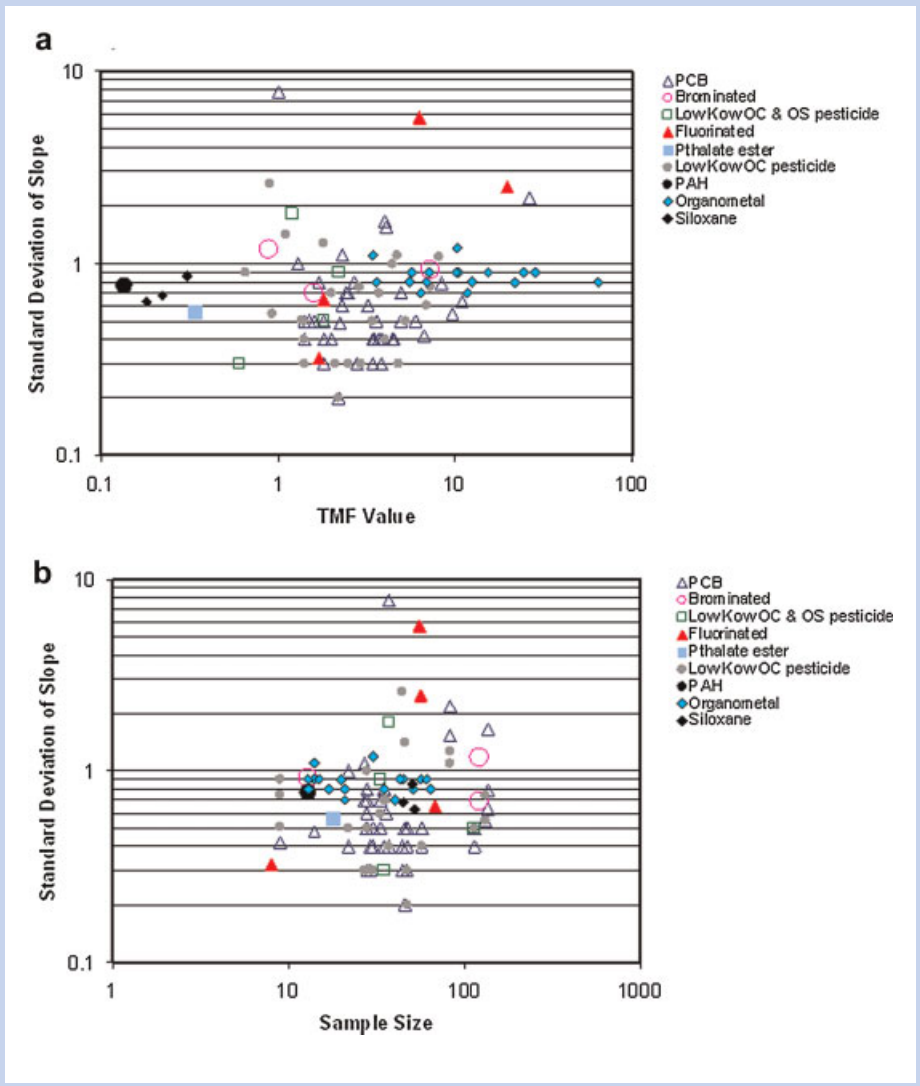

Figure 3. Trophic magnification factor (TMF) variability (standard deviation [SD] of the untransformed values for the slopes of the regressions of log-transformed concentrations on trophic level) compared to TMFs (**a**) and sample size (**b**) in the selected food web biomagnification studies described in the text. OC = organochlorine; OS = organosulfur; PAH = polycyclic aromatic hydrocarbon; PCB = polychlorinated biphenyl.

Table 1. Statistical summary of trophic magnification factors (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[a]	Absolute value of the base 10 SE of original regression slope[a]	Absolute value of the base 10 SD of original regression slope[a]
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

[a]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 (SD) 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. SE = standard error.

significant regression slopes for contaminants having apparent TMFs 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 TMFs 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 (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 approximately 0.7, which is equivalent to an apparent TMFs of 5.0 (Figure 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 TMFs as low as 2.0 (Figure 4).

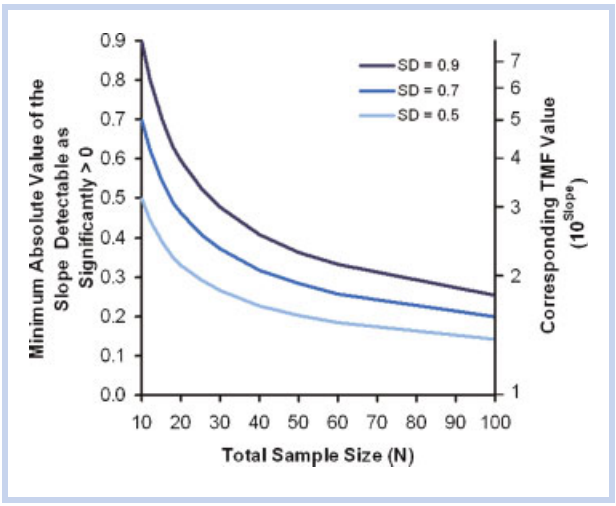

Figure 4. Minimum food web magnification slope (b; absolute value) able to be detected as significantly greater than 1 ($\alpha = 0.05$, $\beta = 0.8$) with the range of variability (standard deviation [SD] of the slope 0.5–0.9) commonly observed in food web biomagnification studies. The corresponding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

The issues of high variability associated with the 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, and so forth) and may be better controlled by improved experimental designs. Thus the power analysis presented here 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.

Use of raw data versus average data

Unless the purpose is to explore the effects of a potentially overrepresented 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 TLs and mean chemical concentrations for each species. In general, using species means results in a reduction in total sample size, which usually results in a loss of statistical power, and thus, the ability to detect TMFs significantly greater than 1. This is illustrated in Figure 5, in which TMFs 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 TL and mean log-transformed, lipid-normalized concentrations for each species. In general, TMFs are similar between the 2 approaches, falling close to the 1:1 line (Figure 4). However, the lack of statistical power for the TMFs developed using the mean data is noted by their much wider 95% CIs. With the exception of the 2 extremely wide 95% CIs (17 and 35), 95% CIs for TMFs generated by regressions based on mean data

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)			
Lake	Raw data TMF values	Mean	RPD[a] (%)	Median	RPD[a] (%)
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

[a]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.

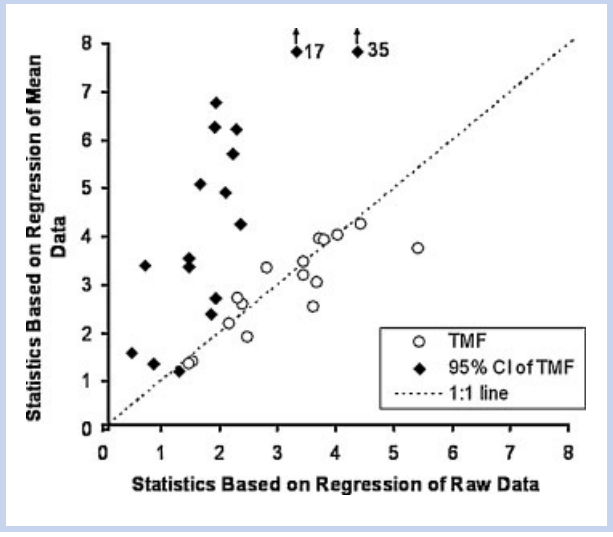

Figure 5. Trophic magnification factor (TMF) and 95% confidence intervals (CI) of TMF for regressions based on the means of chemical concentration and trophic level versus those calculated from regressions, using the raw data.

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, the use of 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.

Study design

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

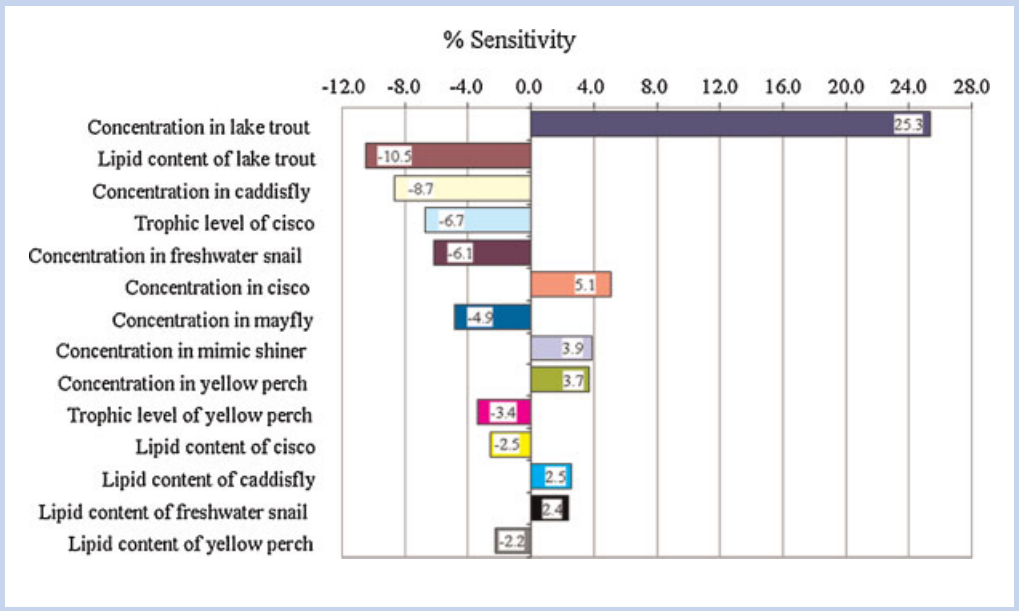

Figure 6. Sensitivity evaluation for the slope of the regression line used to calculate the TMF of PCB-153 for Lake Paguchi (data from Houde et al. 2008). The percent sensitivity is the percentage contribution of each factor on the left side of the figure to the overall variance associated with the slope of the regression line. The percent contribution to the overall variance was calculated by squaring the rank correlation coefficients between each factor and the slope, and normalizing them to 100%. The sign for the percent contribution indicates either that there was an increase (i.e., the rank correlation coefficient was positive) or a decrease (i.e., the rank correlation coefficient was negative) in the slope.

results, 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) 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 Data for details on Monte Carlo simulation; Supplemental Table S2). TMFs calculated using raw data across all samples and species and the MC-TMFs (mean and median values) differed due to the unbalanced sample collection and analysis across species (Supplemental Table S3), with the raw data TMFs subject to a large influence from the larger number of higher TL fish (lake trout, in this case) present in the data set (Figure 6). In most cases, however, TMFs generated from the raw data and the MC-TMFs were similar and ultimately came to the same general conclusion regarding biomagnification (i.e., TMFs > 1) (Supplemental Table S3). In summary, the Monte Carlo analysis provided in this example (or a similar approach using statistical software to identify the effects of unbalanced regression models) should be used in cases where unbalanced study designs may be suspected. For example, in the Lake Paguchi, Canada, data set from Houde et al. (2008), lake trout represent 50% of the samples, suggesting that TMFs 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) (Figure 6).

As discussed previously, the properties of organisms (i.e., size, physiology, reproductive status, age, and ability to biotransform chemicals) have the potential to affect contaminant accumulation and, thus, TMFs. In studies by Fisk, Hobson, et al. (2001) and Hop et al. (2002), TMFs of recalcitrant POPs for a food web consisting of both poikilotherms and homeotherms may overestimate biomagnification for poikilotherms and underestimate it for homeotherms because of their different metabolism. In these situations, TMFs should be calculated both with and without homeotherms to assess the effects of including this group (Gobas et al. 2009). Another useful approach is to identify thermoregulation in the regression by using an interaction term (Hop et al. 2002; Hallanger, Warner, et al. 2011).

Additional analysis (multivariate regression, analysis of covariance, and so forth) may be useful in identifying and quantifying the effects of ecological variables on TMFs. Such ancillary analyses will provide a more complete picture of food web biomagnification and can be used to further understand and identify potential artifacts related to migration of homeotherms, species-specific differences in adsorption, metabolism, excretion, differing C 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 chemical concentrations separately and recommendations for statistical reporting on TMF calculations are provided in Supplemental Data.

KNOWLEDGE GAPS AND RECOMMENDATIONS

Although the integrated approach of using TMFs to assess contaminant bioaccumulation through food webs was initiated 2 decades ago (Broman et al. 1992), and the method has been further developed and used in several recent studies, few 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.

Knowledge gaps that have been identified include the:

- Lack of well-designed studies examining the influence of ecosystem characteristics on TMFs

- 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:

- The study ideally includes species and individual organisms that range over at least 3 TLs to achieve the objective of quantifying biomagnification potential of a chemical.
- Appropriate sample sizes are needed to achieve sufficient statistical power to evaluate whether TMF is less than or greater than 1 for ''B'' assessment. Required sample sizes are affected by the design of the trophic transfer study, which improves with an advanced ecological understanding of trophic relationships.
- The use of independent individual and uncensored data is recommended in well-balanced studies. The use of left-censored (i.e., nondetect data) should be clearly identified in graphs and tables, and should be treated with care during TMF calculations, employing additional analyses beyond a simple substitution of left-censored data with one-half the detection limit to determine the possible effect of the data treatment on the TMF.
- For samples that require pooling of individuals for contaminant and $\delta^{15}N$ analysis, it is recommended that multiple composite samples be collected in the field and that each of these pooled sample be split into subsamples in the laboratory after homogenization.
- Report slope and intercept with error estimates (SE, SD, and/or 95% CI) for the chemical versus TL (and $\delta^{15}N$) relationships, as well as relevant significance levels associated with the application of regression models.
- Include information on how TL is calculated, e.g., enrichment factors and baseline organisms used.
- Include the chemical, physical, and biological characteristics of the system or systems studied to facilitate a broader understanding of how TMFs may be 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 versus homeothermic), and eliminate nonsignificant factors.
- Planning for TMF studies needs to consider whether appropriate analytical methods are available to determine contaminant concentrations in biota and, if necessary, include coordination with analytical laboratories capable of developing or refining the analytical methods to fulfill study requirements.
- The accuracy and representativeness of ancillary data such as the lipid content, and stable isotope measurements (e.g., tissues selected, seasonal effects) needs to be assessed.

SUPPLEMENTAL DATA

Supplemental Info.

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REFERENCES

Akkanen J, Kukkonen JVK. 2003. Measuring the bioavailability of two hydrophobic organic compounds in the presence of dissolved organic matter. *Environ Toxicol Chem* 22:518–524.

Anderson C, Cabana G. 2005. $\delta^{15}N$ in riverine food webs: Effects of N inputs from agricultural watersheds. *Can J Fish Aquat Sci* 62:333–340.

Antweiler RC, Taylor HE. 2008. Evaluation of statistical treatments of left-censored environmental data using coincident uncensored data sets: I. Summary statistics. *Environ Sci Technol* 42:3732–3738.

Arnot JA, Gobas FAPC. 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. *Environ Rev* 14:257–297.

Arnot JA, Mackay D, Parkerton TF, Bonnell M. 2008. A database of fish biotransformation rates for organic chemicals. *Environ Toxicol Chem* 27: 2263–2270.

Arnot JA, Meylan W, Tunkel J, Howard PH, Mackay D, Bonnell M, Boethling RS. 2009. A quantitative structure-activity relationship for predicting metabolic biotransformation rates for organic chemicals in fish. *Environ Toxicol Chem* 28:1168–1177.

Barbosa AC, de Souza J, Dórea JG, Jardim WF, Fadini PS. 2003. Mercury biomagnification in a tropical black water, Rio Negro, Brazil. *Arch Environ Contam Toxicol* 45:235–246.

Berglund O, Larsson P, Ewald G, Okla L. 2001a. The effect of lake trophy on lipid content and PCB concentrations in planktonic food webs. *Ecology* 82:1078–1088.

Berglund O, Larsson P, Ewald G, Okla L. 2001b. Influence of trophic status on PCB distribution in lake sediments and biota. *Environ Pollut* 113:199–210.

Berglund O. 2003. Periphyton density influences organochlorine accumulation in rivers. *Limnol Oceanogr* 48:2106–2116.

Boertje RD. 1984. Seasonal diets of the Denali caribou herd, Alaska. *Arctic* 37:161–165.

Borgå K, Fisk AT, Hoekstra PF, Muir DCG. 2004. Biological and chemical factors of importance in the bioaccumulation and trophic transfer of persistent organochlorine contaminants in arctic marine food webs. *Environ Toxicol Chem* 23:2367–2385.

Borgå K, Fisk AT, Hargrave B, Hoekstra PF, Swackhamer D, Muir DCG. 2005. Bioaccumulation factors for PCBs revisited. *Environ Sci Technol* 39:4523–4532.

Braune BM, Norstrom RJ. 1989. Dynamics of organochlorine compounds in herring gulls: III. Tissue distribution and bioaccumulation in Lake Ontario gulls. *Environ Toxicol Chem* 8:957–968.

Broman D, Rolf C, Näf C, Zebühr Y, Fry B, Hobbie J. 1992. Using ratios of stable nitrogen isotopes to estimate bioaccumulation and flux of polychlorinated dibenzo-*p*-dioxins (PCDDs) and dibenzofurans (PCDFs) in two food chains from the Northern Baltic. *Environ Toxicol Chem* 11:331–345.

Burkhard LP. 2000. Estimating dissolved organic carbon partition coefficients for nonionic organic chemicals. *Environ Sci Technol* 34:4663–4668.

Burkhard LP, Cook PM, Lukasewycz MT. 2008. Organic carbon-water concentration quotients ($\prod_{soc}$s and π_{poc}s): Measuring apparent chemical disequilibria and exploring the impact of black carbon in Lake Michigan. *Environ Sci Technol* 42:3615–3621.

Burkhard LP, Arnot JA, Embry MR, Farley KJ, Hoke RA, Kitano M, Leslie HA, Lotufo GR, Parkerton TF, Sappington KG, Tomy GT, Woodburn KB. 2012a. Comparing laboratory and field measured bioaccumulation endpoints. *Integr Environ Assess Manag* 8:17–31.

Burkhard LP, Arnot JA, Embry MR, Farley KJ, Hoke RA, Kitano M, Leslie HA, Lotufo G, Parkerton TF, Sappington KG, Tomy GT, Woodburn KB. 2012b. Comparing laboratory- and field-measured biota–sediment accumulation factors. *Integr Environ Assess Manag* 8:32–41.

Bustnes JO, Moe B, Herzke D, Hanssen SA, Nordstad T, Sagerup K, Gabrielsen GW, Borgå K. 2010. Strongly increasing blood concentrations of lipid-soluble organochlorines in High Arctic common eiders during incubation fast. *Chemosphere* 79:320–325.

Campbell LM, Norstrom RJ, Hobson KA, Muir DCG, Backus S, Fisk AT. 2005. Mercury and other trace elements in a pelagic Arctic marine food web (Northwater Polynya, Baffin Bay). *Sci Total Environ* 351-352:247–263.

Chételat J, Amyot M, Cloutier L, Poulain A. 2008. Metamorphosis in chironomids, more than mercury supply, controls methylmercury transfer to fish in high Arctic lakes. *Environ Sci Technol* 42:9110–9115.

Clark KE, Gobas FAPC, Mackay D. 1990. Model of organic chemical uptake and clearance by fish from food and water. *Environ Sci Technol* 24:1203–1213.

Clarke JU. 1998. Evaluation of censored data methods to allow statistical comparisons among very small samples with below detection limit observations. *Environ Sci Technol* 32:177–183.

Conder JM, Gobas FAPC, Borgå K, Muir DCG, Powell D. 2012. Characterizing bioaccumulation potential of chemicals using trophic magnification factors and related measures. *Integr Environ Assess Manag* 8:85–97.

Connolly JP, Pedersen CJ. 1988. A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms. *Environ Sci Technol* 22:99–103.

Croisetière L, Hare L, Tessier A, Cabana G. 2009. Sulphur stable isotopes can distinguish trophic dependence on sediments and plankton in boreal lakes. *Freshw Biol* 54:1006–1015.

Currie LA. 1995. Nomenclature in evaluation of analytical methods including detection and quantification capabilities. *Pure Appl Chem* 67: 1699–1722.

Dachs J, Eisenreich SJ, Hoff RM. 2000. Influence of eutrophication on air-water exchange, vertical fluxes, and phytoplankton concentrations of persistent organic pollutants. *Environ Sci Technol* 34:1095–1102.

Darr RL, Hewitt DG. 2008. Stable isotope trophic shifts in white-tailed deer. *J Wildl Manag* 72:1525–1531.

da Silva GS, Jardim WF, Fadini PS. 2006. Elemental gaseous mercury flux at the water/air interface over the Negro River basin, Amazon, Brazil. *Sci Total Environ* 368:189–198.

Dórea JG, Barbosa AC, Silva GS. 2006. Fish mercury bioaccumulation as a function of feeding behavior and hydrological cycles of the Rio Negro, Amazon. *Comp Biochem Physiol C Toxicol Pharmacol* 142:275–283.

Dórea JG, Barbosa AC. 2007. Anthropogenic impact of mercury accumulation in fish from the Rio Madeira and Rio Negro Rivers (Amazonia). *Biol Trace Elem Res* 115:243–254.

Eagles-Smith CA, Suchanek TH, Colwell AE, Anderson NL. 2008. Mercury trophic transfer in a eutrophic lake: The importance of habitat-specific foraging. *Ecol Appl* 18 (8 Suppl): A196–A212.

Erickson RJ, McKim JM, Lien GJ, Hoffman AD, Batterman SL. 2006. Uptake and elimination of ionizable organic chemicals at fish gills: II. Observed and predicted effects of pH, alkalinity, and chemical properties. *Environ Toxicol Chem* 25:1522–1532.

European Commission. 2003. Technical guidance document on risk assessment EUR 20418 EN/3. Ispra (IT): Joint Research Centre, Institute for Health and Consumer Protection, European Chemicals Bureau. 112 p.

Fisk AT, Hobson KA, Norstrom RJ. 2001. Influence of chemical and biological factors on trophic transfer of persistent organic pollutants in the northwater polynya marine food web. *Environ Sci Technol* 35:732–738.

Fisk AT, Stern GA, Hobson KA, Strachan WJ, Loewen MD, Norstrom RJ. 2001. Persistent organic pollutants (POPs) in a small, herbivorous, Arctic marine zooplankton (*Calanus hyperboreus*): Trends from April to July and the influence of lipids and trophic transfer. *Mar Pollut Bull* 43:93–101.

Fisk AT, Hoekstra PF, Gagnon JM, Duffe J, Norstrom RJ, Hobson KA, Kwan M, Muir DCG. 2003. Influence of habitat, trophic ecology and lipids on, and spatial trends of, organochlorine contaminants in Arctic marine invertebrates. *Mar Ecol Prog Ser* 262:201–214.

Fisk AT, De Wit CA, Wayland M, Kuzyk ZZ, Burgess N, Letcher R, Braune B, Norstrom R, Blum SP, Sandau C. and others. 2005. An assessment of the toxicological significance of anthropogenic contaminants in Canadian arctic wildlife. *Sci Total Environ* 351-352:57–93.

Frome EL, Wambach PF. 2005. Statistical methods and software for the analysis of occupational exposure data with non-detectable values. Oak Ridge National Laboratory Technical Report ORNL/TM-2005/52. Oak Ridge (TN): Oak Ridge National Laboratory. 47 p.

Fry B. 1999. Using stable isotopes to monitor watershed influences on aquatic trophodynamics. *Can J Fish Aquat Sci* 56:2167–2171.

Fu W, Franco A, Trapp S. 2009. Methods for estimating the bioconcentration factor of ionizable organic chemicals. *Environ Toxicol Chem* 28:1372–1379.

Gantner N, Muir DCG, Power M, Iqaluk D, Reist JD, Babaluk J, Meili M, Borg H, Hammar J, Michaud W, et al. 2010. Mercury concentrations in landlocked Arctic char (*Salvelinus alpinus*) in the Canadian Arctic. Part II: Spatial comparison of 27 populations. *Environ Toxicol Chem* 29:633–643.

Gantner N, Power M, Iqaluk D, Meili M, Borg H, Sundbom M, Solomon KR, Lawson G, Muir DCG. 2010. Mercury concentrations in landlocked Arctic char (*Salvelinus alpinus*) in the Canadian Arctic. Part I: Insights from trophic relationships in 18 lakes. *Environ Toxicol Chem* 29:621–632.

Gaye-Siessegger J, Focken U, Muetzel S, Abel H, Becker K. 2004. Feeding level and individual metabolic rate affect $\delta^{13}C$ and $\delta^{15}N$ values in carp: implications for food web studies. *Oecologia* 138:175–183.

Gewurtz SB, Gandhi N, Stern GA, Franzin WG, Rosenberg B, Diamond ML. 2006. Dynamics of PCBs in the food web of Lake Winnipeg. *J Great Lakes Res* 32:712–727.

Gobas FAPC, Wilcockson JB, Russell RW, Haffner GD. 1999. Mechanism of biomagnification in fish under laboratory and field conditions. *Environ Sci Technol* 33:133–141.

Gobas FA, Morrison HA. 2000. Bioconcentration and biomagnification in the aquatic environment. In: Boethling RS, Mackay D, editors. Handbook of property estimation methods for chemicals, environmental and health sciences. Boca Raton (FL): CRC. p 189–231.

Gobas FAPC, de Wolf W, Burkhard LP, Verbruggen E, Plotzke K. 2009. Revisiting bioaccumulation criteria for POPs and PBT assessments. *Integr Environ Assess Manag* 5:624–637.

Gomez-Taylor M, Kahn HD, Telliard WA, Ditthavong K, Kopylev L, McCarty H, Riddick L, Miller K, Cuddeback J, Rushneck D, et al. 2003. Technical support document for the assessment of detection and quantitation approaches. Washington (DC): US Environmental Protection Agency. 124 p.

Guildford S, Muir DCG, Houde M, Evans MS, Kidd KA, Whittle DM, Drouillard K, Wang X, Anderson MR, Bronte CR, et al. 2008. PCB concentrations in lake trout (*Salvelinus namaycush*) are correlated to carbon stable isotope signature and lake characteristics. *Environ Sci Technol* 42:8239–8244.

Gustaffson O, Haghseta F, Chan C, MacFarlane J, Gschwend PM. 1997. Quantification of the dilute sedimentary soot phase: Implications of PAH speciation and bioavailability. *Environ Sci Technol* 31:203–209.

Hallanger IG, Warner NA, Ruus A, Evenset A, Christensen G, Herzke D, Gabrielsen GW, Borgå K. 2011. Seasonality in contaminant accumulation in Arctic marine pelagic food webs using trophic magnification factor as a measure of bioaccumulation. *Environ Toxicol Chem* 30:1026–1035.

Hallanger IG, Ruus A, Herzke D, Warner NA, Evenset A, Heimstad ES, Gabrielsen GW, Borgå K. 2011. Influence of season, location, and feeding strategy on bioaccumulation of halogenated organic contaminants in Arctic marine zooplankton. *Environ Toxicol Chem* 30:77–87.

Hargrave BT, Phillips GA, Vass WP, Bruecker P, Welch HE, Siferd TD. 2000. Seasonality in bioaccumulation of organochlorines in lower trophic level arctic marine biota. *Environ Sci Technol* 34:980–987.

Hecky RE, Hesslein RH. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. *J N Am Benthol Soc* 14:631–653.

Helsel DR. 2005. More than obvious: Better methods for interpreting nondetect data. *Environ Sci Technol* 39:419A–423A.

Hesslein RH, Capel MJ, Fox DE, Hallard KA. 1991. Stable isotopes of sulfur, carbon, and nitrogen as indicators of trophic level and fish migration in the lower Mackenzie River basin, Canada. *Can J Fish Aquat Sci* 48:2258–2265.

Hesslein RH, Hallard KA, Ramlal P. 1993. Replacement of sulfur, carbon, and nitrogen in tissue of growing broad whitefish (*Coregonus nasus*) in response to a change in diet traced by delta^{34}S, delta^{13}C, and delta^{15}N. *Can J Fish Aquat Sci* 50:2071–2076.

Hobson KA, Clark RG. 1992. Assessing avian diets using stable isotopes II: Factors influencing diet-tissue fractionation. *Condor* 94:189–197.

Hobson KA, Welch HE. 1992. Determination of trophic relationships within a High Arctic marine food web using delta-C-13 and delta-N-15 analysis. *Mar Ecol Prog Ser* 84:9–18.

Hobson KA, Alisauskas RT, Clark RG. 1993. Stable-nitrogen iostope enrichment in avain tissues due to fasting and nutritional stress: Implications for isotopic analyses of diet. *Condor* 95:388–394.

Hoekstra PF, O'Hara TM, Fisk AT, Borga K, Solomon KR, Muir DC. 2003. Trophic transfer of persistent organochlorine contaminants (OCs) within an Arctic marine food web from the southern Beaufort-Chukchi Seas. *Environ Pollut* 124:509–522.

Hop H, Borga K, Gabrielsen GW, Kleivane L, Skaare JU. 2002. Food web magnification of persistent organic pollutants in poikilotherms and homeotherms from the Barents Sea. *Environ Sci Technol* 36:2589–2597.

Houde M, Wells RS, Fair PA, Bossart GD, Hohn AA, Rowles TK, Sweeney JC, Solomon KR, Muir DCG. 2005. Polyfluoroalkyl compounds in free-ranging bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Mexico and the Atlantic Ocean. *Environ Sci Technol* 39:6591–6598.

Houde M, Bujas TAD, Small J, Wells RS, Fair PA, Bossart GD, Solomon KR, Muir DCG. 2006. Biomagnification of perfluoroalkyl compounds in the bottlenose dolphin (*Tursiops truncatus*) food web. *Environ Sci Technol* 40:4138–4144.

Houde M, Muir DCG, Kidd K, Guildford S, Drouillard K, Evans M, Wang X, Whittle M, Haffner D, Kling H. 2008. Influence of lake characteristics on the biomagnification of persistent organic pollutants in lake trout and their food webs. *Environ Toxicol Chem* 27:2169–2178.

Howard PH, Muir DCG. 2010. Identifying new persistent and bioaccumulative organics among chemicals in commerce. *Environ Sci Technol* 44:2277–2285.

Jardine TD, Kidd KA, Fisk AT. 2006. Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. *Environ Sci Technol* 40:7501–7511.

Kah M, Brown CD. 2008. Log D: Lipophilicity for ionisable compounds. *Chemosphere* 72:1401–1408.

Katz S, Muir D, Gamberg M, Chan L, André A, Trimble A, Mueller CE, DeSilva AO, Wang X, Small J. 2009. Bioaccumulation of perfluorinated compounds in the vegetation-caribou-wolf food chain. In: Smith SL, Stow J, Edwards J, editors. Synopsis of research conducted under the 2008-2009, Northern Contaminants Program. Ottawa (ON): Indian and Northern Affairs Canada.

Kehrig HA, Palermo EFA, Malm O. 2004. Inorganic and methyl mercury in food chain from a Brazilian reservoir. *RMZ Mater Geoenviron* 51:1103–1106.

Keith LH, Crummett W, Deegan J, Libby R, Taylor JK, Wentler G. 1983. Principles of environmental analysis. *Anal Chem* 55:2210–2218.

Kelly BC, Ikonomou MG, Blair JD, Morin AE, Gobas FAPC. 2007. Food web-specific biomagnification of persistent organic pollutants. *Science* 317:236–239.

Kelly BC, Ikonomou MG, Blair JD, Gobas FAPC. 2008. Bioaccumulation behaviour of polybrominated diphenyl ethers (PBDEs) in a Canadian Arctic marine food web. *Sci Total Environ* 401:60–72.

Kelly BC, Ikonomou MG, Blair JD, Surridge B, Hoover D, Grace R, Gobas FAPC. 2009. Perfluoroalkyl contaminants in an arctic marine food web: Trophic magnification and wildlife exposure. *Environ Sci Technol* 43:4037–4043.

Kemper RA, Jepson GW. 2003. Pharmacokinetics of perfluorooctanoic acid in male and female rats. *Toxicologist* 72:148.

Kidd KA, Hesslein RH, Fudge RJP, Hallard KA. 1995a. The influence of trophic level as measured by $\delta^{15}N$ on mercury concentrations in freshwater organisms. *Water Air Soil Pollut* 80:1011–1015.

Kidd KA, Schindler DW, Hesslein RH, Muir DCG. 1995b. Correlation between stable nitrogen isotope ratios and concentrations of organochlorines in biota from a freshwater food web. *Sci Total Environ* 160-161:381–390.

Kidd KA, Schindler DW, Hesslein RH, Muir DCG. 1998. Effects of trophic position and lipid on organochlorine concentrations in fishes from subarctic lakes in Yukon Territory. *Can J Fish Aquat Sci* 55:869–881.

Kidd KA, Bootsma HA, Hesslein RH, Muir DC, Hecky RE. 2001. Biomagnification of DDT through the benthic and pelagic food webs of Lake Malawi, East Africa: importance of trophic level and carbon source. *Environ Sci Technol* 35:14–20.

Kidd KA, Bootsma HA, Hesslein RH, Lockhart WL, Hecky RE. 2003. Mercury concentrations in the food web of Lake Malawi, East Africa. *J Great Lakes Res* 29:258–266.

Kucklick JR, Hinckley DA, Bidleman TF. 1991. Determination of Henry's Law constants for hexachlorocyclohexanes in distilled and artifical seawater as a function of temperature. *Mar Chem* 34:197–209.

Kucklick JR, Schantz MM, Pugh RS, Porter BJ, Poster DL, Becker PR, Rowles TK, Leigh S, Wise SA. 2010. Marine mammal blubber reference and control materials for use in the determination of halogenated organic compounds and fatty acids. *Anal Bioanal Chem* 397:423–432.

Lee JJ, Schultz IR. 2009. Sex differences in the uptake and disposition of perfluorooctanoic acid in fathead minnows after oral dosing. *Environ Sci Technol* 44:491–496.

Letcher RJ, Norstrom RJ, Bergman A. 1995. Geographical distribution and identification of methyl sulphone PCB and DDE metabolites in pooled polar bear (*Ursus maritimus*) adipose tissue from western hemisphere arctic and subarctic regions. *Sci Total Environ* 160-161:409–420.

Letcher RJ, Bustnes JO, Dietz R, Jenssen BM, Jorgensen EH, Sonne C, Verreault J, Vijayan MM, Gabrielsen GW. 2010. Exposure and effects assessment of persistent organohalogen contaminants in arctic wildlife and fish. *Sci Total Environ* 408:2995–3043.

Loseto LL, Stern GA, Ferguson SH. 2008. Size and biomagnification: How habitat selection eplains beluga mercury levels. *Environ Sci Technol* 42:3982–3988.

Lyytikäinen M, Hirva P, Minkkinen P, Hämäläinen H, Rantalainen AL, Mikkelson P, Paasivirta J, Kukkonen JVK. 2003. Bioavailability of sediment-associated PCDD/Fs and PCDEs: Relative importance of contaminant and sediment characteristics and biological factors. *Environ Sci Technol* 37:3926–3934.

MacDougall D, Crummett WB, et al. 1980. Guidelines for data acquisition and data quality evaluation in environmental chemistry. *Anal Chem* 52:2242–2249.

Mackintosh CE, Maldonado J, Hongwu J, Hoover N, Chong A, Ikonomou MG, Gobas FAPC. 2004. Distribution of phthalate esters in a marine aquatic food web: Comparison to polychlorinated biphenyls. *Environ Sci Technol* 38:2011–2020.

MacNeil MA, Drouillard KG, Fisk AT. 2006. Variable uptake and elimination of stable nitrogen isotopes between tissues in fish. *Can J Fish Aquat Sci* 63:345–353.

Malm O, Palermo EFA, Santos HSB. 2004. Transport and cycling of mercury in Tucuruí reservoir, Amazon, Brazil: 20 years after fulfillment. *RMZ Mater Geoenviron* 51:1195–1198.

Martin JW, Kannan K, Berger U, De Voogt P, Field J, Franklin J, Giesy JP, Harner T, Muir DCG, Scott B, et al. 2004. Analytical challenges hamper perfluoroalkyl research. *Environ Sci Technol* 38:248A–255A.

Mattila TJ, Verta M. 2008. Modeling the importance of biota and black carbon as vectors of polybrominated diphenyl ethers (PBDEs) in the Baltic Sea ecosystem. *Environ Sci Technol* 42:4831–4836.

McConnaughey T, McRoy CP. 1979. Food-web structure and the fractionation of carbon isotopes in the Bering Sea. *Mar Biol* 53:257–262.

McCutchan JH, Lewis WM, Kendall C, McGrath CC. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 102:378–390.

Mizutani H, Kabaya Y, Wada E. 1991. Nitrogen and carbon isotope compositions related linearly in cormorant tissues and its diet. *Isotopenpraxis* 27:166–168.

Morel FMM, Kraepiel AML, Amyot M. 1998. The chemical cycle and bioaccumulation of mercury. *Annu Rev Ecol Syst* 29:543–566.

Muir D, Anderson R, Droulliard K, Evans M, Fairchild W, Guildford S, Haffner D, Kidd K, Payne J, Whittle M. 2002. Biomagnification of Persistent Organic Pollutants and Mercury in Canadian freshwater subsistence fisheries and food webs. TSRI Project #236 Final Report. Ottawa: Health Canada. 65 p.

Muir D, Savinova T, Savinov V, Alexeeva L, Potelov V, Svetochev V. 2003. Bioaccumulation of PCBs and chlorinated pesticides in seals, fishes and invertebrates from the White Sea, Russia. *Sci Total Environ* 306:111–131.

Muir D, Sverko E. 2006. Analytical methods for PCBs and organochlorine pesticides in environmental monitoring and surveillance: A critical appraisal. *Anal Bioanal Chem* 386:769–789.

Müller CE, DeSilva AO, Small J, Wang X, Muir D, Gamberg M, Katz S. 2009. Enrichment of perfluorinated compounds along a remote terrestrial food chain: From lichen to wolves. Presented at the Society of Environmental Toxicology

and Chemistry (SETAC) Europe 19th Annual Meeting; Goteborg, Sweden; 2009 May 31 to June 4. Pensacola (FL): SETAC.

Munthe J, Bodaly RA, Branfireun BA, Driscoll CT, Gilmour CC, Harris R, Horvat M, Lucotte M, Malm O. 2007. Recovery of mercury-contaminated fisheries. *Ambio* 36:33–44.

Overman NC, Parrish DL. 2001. Stable isotope composition of walleye: ^{15}N accumulation with age and area-specific differences in $\delta^{13}C$. *Can J Fish Aquat Sci* 58:1253–1260.

Paine RT. 1966. Food web complexity and species diversity. *Am Nat* 100:65–75.

Palermo EFA, Kasper D, Reis TA, Nogueira S, Branco CWC, Malm O. 2004. Mercury level increase in fish downstream the Tucuruí reservoir, Brazil. *RMZ Mater Geoenviron* 51:1292–1294.

Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annu Rev Ecol Syst* 18:293–320.

Post DM. 2002. Using stable isotopes to estimate trophic position: Models, methods, and assumptions. *Ecology* 83:703–718.

Post DM, Layman CA, Arrington DA, Takimoto G, Quattrochi J, Montanã CG. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia* 152:179–189.

Powell DE, Woodburn KB, Drottar KR, Durham J, Huff DW. 2009. Trophic dilution of cyclic volatile methylsiloxane (cVMS) materials in a temperate freshwater lake. Final project report submitted to the Centre Europeén des Silicones (CES), Brussels, Belgium. Midland (MI): Dow Corning.

Powell DE, Durham J, Huff DW. 2010a. Preliminary assessment of cyclic volatile methylsiloxane (cVMS) materials in surface sediments, cores, zooplankton, and fish of Lake Opeongo, Ontario, Canada. Final project report submitted to the Centre Europeén des Silicones (CES), Brussels, Belgium. Midland (MI): Dow Corning.

Powell DE, Durham J, Huff DW, Böhmer T, Gerhards R, Koerner M. 2010b. Bioaccumulation and trophic transfer of cyclic volatile methylsiloxane (cVMS) materials in the aquatic marine food webs of the inner and outer Oslofjord, Norway. Final project report submitted to the Centre Europeén des Silicones (CES), Brussels, Belgium. Midland (MI): Dow Corning.

Robbins CT, Felicetti LA, Florin ST. 2010. The impact of protein quality on stable nitrogen isotope ratio discrimination and assimilated diet estimation. *Oecologia* 162:571–579.

Rolff C, Broman D, Naf C, Zebuhr Y. 1993. Potential biomagnification of PCDD/Fs--New possibilities for quantitative assessment using stable isotope trophic position. *Chemosphere* 27:461–468.

Schlautman MA, Morgan JJ. 1993. Effects of aqueous chemistry on the binding of polycyclic aromatic hydrocarbons by dissolved humic materials. *Environ Sci Technol* 27:961–970.

Selck H, Drouillard K, Eisenreich K, Koelmans AA, Palmqvist A, Ruus A, Salvito D, Schultz I, Stewart R, Weisbrod A, van den Brink NW, van den Heuvel-Greve M. 2012. Explaining variability of bioaccumulation measurements between laboratory and field. *Integr Environ Assess Manag* 8:42–63.

Shiu W-Y, Ma K-C, Varhanícková D, Mackay D. 1994. Chlorophenols and alkylphenols: A review and correlation of environmentally relevant properties and fate in an evaluative environment. *Chemosphere* 29:1155–1224.

Sobek A, McLachlan MS, Borgå K, Asplund L, Lundstedt-Enkel K, Polder A, Gustafsson O. 2010. A comparison of PCB bioaccumulation factors between an arctic and a temperate marine food web. *Sci Total Environ* 408:2753–2760.

Sokal RR, Rohlf FJ. 1999. Biometry. New York (NY): W.H. Freeman.

Søreide JE, Hop H, Carroll ML, Falk-Petersen S, Hegseth EN. 2006. Seasonal food web structures and sympagic-pelagic coupling in the European Arctic revealed by stable isotopes and a two-source food web model. *Prog Oceanogr* 71: 59–87.

Stapleton HM, Letcher RJ, Baker JE. 2001. Metabolism of PCBs by the deepwater sculpin (*Myoxocephalus thompsoni*). *Environ Sci Technol* 35:4747–4752.

Stapleton HM, Letcher RJ, Li J, Baker JE. 2004. Dietary accumulation and metabolism of polybrominated diphenyl ethers by juvenile carp (*Cyprinus carpio*). *Environ Toxicol Chem* 23:1939–1946.

Stapleton HM, Brazil B, Holbrook RD, Mitchelmore CL, Benedict R, Konstantinov A, Potter D. 2006. In vivo and in vitro debromination of decabromodiphenyl ether (BDE 209) by juvenile rainbow trout and common carp. *Environ Sci Technol* 40:4653–4658.

Stewart AR, Luoma SN, Schlekat CE, Doblin MA, Hieb KA. 2004. Food web pathway determines how selenium affects aquatic ecosystems: A San Francisco Bay case study. *Environ Sci Technol* 38:4519–4526.

Swanson HK, Johnston TA, Leggett WC, Bodaly RA, Doucett RR, Cunjak RA. 2003. Trophic positions and mercury bioaccumulation in rainbow smelt (*Osmerus mordax*) and native forage fishes in northwestern Ontario lakes. *Ecosystems* 6:289–299.

Swanson HK, Kidd KA. 2010. Mercury concentrations in Arctic food fishes reflect presence of anadromous Arctic charr (*Salvelinus alpinus*), species, and life history. *Environ Sci Technol* 44:3286–3292.

Swanson HK, Kidd KA, Babaluk JA, Wastle RJ, Yang PP, Halden NN, Reist JD. 2010. Annual marine migrations in lake trout (*Salvelinus namaycush*) from the central Canadian Arctic: insights from otolith microchemistry, stable isotope ratios, and comparisons to Arctic charr (*S. alpinus*). *Can J Fish Aquat Sci* 67:842–853.

Tamelander T, Kivimäe C, Bellerby RGJ, Renaud PE, Kristiansen S. 2009. Base-line variations in stable isotope values in an Arctic marine ecosystem: Effects of carbon and nitrogen uptake by phytoplankton. *Hydrobiologia* 630:63–73.

Thomann RV. 1989. Bioaccumulation model of organic chemical distribution in aquatic food chains. *Environ Sci Technol* 23:699–707.

Thompson DC, McCourt KH. 1981. Seasonal diets of the Porcupine Caribou herd. *Am Midland Nat* 105:70–76.

Thomsen C, Leknes H, Lundanes E, Becher G. 2001. Brominated flame retardants in laboratory air. *J Chromatogr A* 923:299–304.

Tomy GT, Pleskatch K, Ferguson SH, Hare J, Stern G, Macinnis G, Marvin CH, Loseto L. 2009. Trophodynamics of some PFCs and BFRs in a western Canadian arctic marine food web. *Environ Sci Technol* 43:4076–4081.

Tuerk KJ, Kucklick JR, McFee WE, Pugh RS, Becker PR. 2005. Factors influencing persistent organic pollutant concentrations in the Atlantic white-sided dolphin (*Lagenorhynchus acutus*). *Environ Toxicol Chem* 24:1079–1087.

[UNEP] United Nations Environment Programme. 2001. Final act of the plenipotentiaries on the Stockholm Convention on persistent organic pollutants UNEP/POPS/CONF/4. Geneva (CH): UNEP. 44 p.

van Wijk D, Chénier R, Henry T, Hernando M, Schulte C. 2009. Integrated approach to PBT and POP prioritization and risk assessment. *Integr Environ Assess Manag* 5:697–711.

Vander Zanden MJ, Rasmussen JB. 1999. Primary consumer $\delta^{13}C$ and $\delta^{15}N$ and the trophic position of aquatic consumers. *Ecology* 80:1395–1404.

Vander Zanden MJ, Rasmussen JB. 2001. Variation in $\delta^{15}N$ and $\delta^{13}C$ trophic fractionation: Implications for aquatic food web studies. *Limnol Oceanogr* 46:2061–2066.

Varaprath S, Stutts DH, Kozerski GE. 2006. A primer on the analytical aspects of silicones at trace levels-challenges and artifacts. A review. *Silicon Chem* 3:79–102.

Wan Y, Jin X, Hu J, Jin F. 2007. Trophic dilution of polycyclic aromatic hydrocarbons (PAHs) in a marine food web from Bohai Bay, North China. *Environ Sci Technol* 41:3109–3114.

Wan Y, Hu J, Zhang K, An L. 2008. Trophodynamics of polybrominated diphenyl ethers in the marine food web of Bohai Bay, North China. *Environ Sci Technol* 42:1078–1083.

Weisbrod AV, Woodburn KB, Koelmans AA, Parkerton TF, McElroy AE, Borgå K. 2009. Evaluation of bioaccumulation using in vivo laboratory and field studies. *Integr Environ Assess Manag* 5:598–623.

Wise SA, Poster DL, Kucklick JR, Keller JM, VanderPol SS, Sander LC, Schantz MM. 2006. Standard reference materials (SRMs) for determination of organic contaminants in environmental samples. *Anal Bioanal Chem* 386:1153–1190.

Wu J-P, Luo X-J, Zhang Y, Yu M, Chen S-J, Mai B-X, Yang Z-Y. 2009. Biomagnification of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls in a highly contaminated freshwater food web from South China. *Environ Pollut* 157:904–909.

Wyn B, Kidd KA, Burgess NM, Curry RA. 2009. Mercury biomagnification in the food webs of acidic lakes in Kejimkujik National Park and National Historic Site, Nova Scotia. *Can J Fish Aquat Sci* 66:1532–1545.

Yordy JE, Pabst DA, McLellan WA, Wells RS, Rowles TK, Kucklick JR. 2010. Tissue-specific distribution and whole body burden estimates of persistent organic pollutants in the bottlenose dolphin (*Tursiops truncatus*). *Environ Toxicol Chem* 29:1263–1273.

Zar JH. 1999. Biostatistical analysis. Upper Saddle River (NJ): Prentice-Hall.