Developing a trophic bioaccumulation model for PFOA and PFOS in a marine food web

by

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Abstract

Food web (or trophic) bioaccumulation models are useful tools for estimating the bioaccumulative tendencies of persistent organic pollutants, and are regularly used for regulatory assessment of industrial chemicals. Current models are mostly designed for neutral, lipophilic compounds, yet numerous compounds of concern are ionizable and/or proteinophilic, exhibiting unique bioaccumulation behaviour. In this study, an existing model was modified to evaluate bioaccumulation of two ionizable perfluoroalkyl acids (PFAAs) in a marine food web: perfluorooctanoate (PFOA) and perfluorooctane sulfonate (PFOS). The model was tested against measured concentrations of PFOA and PFOS from a bottlenose dolphin food web in Charleston Harbor, SC. Both compounds were expected to bioaccumulate in this food web. Predicted concentrations of PFOS were in better agreement with empirical measurements compared to PFOA. This study supports the utilization of holistic measures of bioaccumulation (i.e., the trophic magnification factor, or TMF), particularly in food webs containing water- and air-respiring organisms.

Keywords: Trophic magnification; food web model; perfluorinated compounds; ionizable organic compounds; bioaccumulation; policy

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List of Acronyms

BCF	Bioconcentration factor
BMF	Biomagnification factor
BSA	Bovine serum albumin
CEPA	Canadian Environmental Protection Act
D_{BW}	Body-water distribution coefficient
D_{MW}	Membrane-water distribution coefficient
D_{OW}	Octanol-water distribution coefficient
DOC	Dissolved organic carbon
DSL	Domestic Substances List
HSA	Human serum albumin
IOC	Ionogenic organic compound
K_{MW}	Membrane-water partition coefficient
K_{OW}	Octanol-water partition coefficient
K_{OA}	Octanol-air partition coefficient
K_{PW}	Protein-water partition coefficient
OC	Organic carbon
OECD	Organization for Economic Cooperation and Development
PCB	Polychlorinated biphenyl
PFAA	Perfluorinated alkyl acid
PFC	Perfluorinated compound
PFOA	Perfluorooctanoic acid
PFOS	Perfluorooctane sulfonic acid
POC	Particulate organic carbon
POP	Persistent organic pollutant
REACH	Registration, Evaluation, Authorisation, and Restriction of Chemicals
TL	Trophic level
TMF	Trophic magnification factor

Glossary

Acid dissociation constant (pK_a)	Equilibrium constant for the dissociation of an acid; a measure of the strength of an acid in solution (expressed as a negative logarithm)
Bioaccumulation	The process whereby the chemical concentration in an aquatic water-respiring organism exceeds the concentration in the surrounding water medium through all potential routes of exposure under field conditions, including bioconcentration (e.g., respiration and diffusion) and biomagnification (e.g., dietary absorption) (Gobas and Morrison, 2000)
Bioconcentration	The process whereby chemical concentrations in an aquatic water-respiring organism exceeds the concentration in the surrounding water medium via gill respiration or skin absorption under laboratory conditions (Gobas and Morrison, 2000)
Bioconcentration Factor (BCF)	The ratio of the chemical concentration in an organism to the concentration in water. Expressed in L/kg. (Gobas and Morrison, 2000)
Biomagnification	The process whereby chemical concentrations in an organism exceed concentrations of the organism's diet (i.e., prey) considering only dietary absorption as a route of uptake (Gobas and Morrison, 2000)
Biomagnification Factor (BMF)	The ratio of the chemical concentration in an organism to the concentration in the organism's diet (Gobas and Morrison, 2000)
Body-water distribution coefficient (D_{BW})	The ratio of the overall sorption capacity of a chemical in an organism compared to water. A modified approach to measuring chemical partitioning in biota whereby different types of tissues are expressed separately. Expressed in L/kg. (Schmitt, 2008; Armitage et al., 2013)
Ionogenic organic compound (IOC)	A compound able to exist in neutral and ionized (charged) forms in the environment; pH dependent
Membrane-water distribution coefficient (D_{MW})	The ratio of chemical's sorption to phospholipid bilayers and membranes compared to water expressed as a weighted average of the neutral and charged forms of a chemical. Used as a metric to describe chemical partitioning between polar lipids and water phases in aquatic biota (Armitage et al., 2013)
Membrane-water partition coefficient (K_{MW})	The ratio of a chemical's solubility in phospholipid bilayers to a chemical's solubility in water at equilibrium. Used as a metric to describe chemical partitioning of neutral or ionized compounds separately between polar lipids and water phases in aquatic biota. Generally expressed in logarithmic format (log K_{MW}) (Armitage et al., 2013)

Octanol-water distribution coefficient (D_{OW})	The ratio of a chemical's sorption to octanol compared to water expressed as a weighted average of the neutral and charged forms of a chemical. Used as a metric to describe chemical partitioning between non-polar lipids and water phases in aquatic biota (Armitage et al., 2013)
Octanol-water partition coefficient (K_{OW})	The ratio of chemical's solubility in octanol to a chemical's solubility in water at equilibrium. Used as a metric to describe chemical partitioning of neutral or ionized compounds separately between non-polar lipid and water phases in aquatic biota. Generally expressed in logarithmic format (log K_{OW}) (Mackay, 1991)
Octanol-air partition coefficient (K_{OA})	The ratio of chemical's solubility in octanol to a chemical's solubility in air. Used as a metric to describe chemical partitioning between lipids and air in terrestrial biota. Generally expressed in logarithmic format (log K_{OA}) (Mackay, 1991)
Perfluorinated Alkyl Acids (PFAAs)	Anionic form of a group of industrial chemicals known to be highly bioaccumulative and persistent in environment and biota (Houde et al., 2006)
Perfluorinated Alkyl Substances (PFASs)	A group of industrial chemicals known to be highly bioaccumulative and persistent in environment and biota (Krafft and Riess, 2015)
Perfluorinated compounds (PFCs)	A group of anthropogenic organofluorine chemicals widely used for industrial and commercial applications beginning in the mid-1900s, primarily as surfactants (Prevedouros et al., 2006)
Protein-water partition coefficient (K_{PW})	The ratio of chemical's solubility in protein to a chemical's solubility in water. Used as a metric to describe chemical partitioning between protein and water in aquatic biota. Generally expressed in logarithmic format (log K_{PW}) (Hendriks et al., 2005)
Trophic level (TL)	The position of an organism within a food web, according to predation and feeding patterns, and can be calculated using stable nitrogen isotope ratios (δ15N) to measure enrichment of ^{15}N in predators compared to prey (Kidd et al., 1998)
Trophic magnification factor (TMF)	Calculated as the slope of the logarithm of a normalized empirically measured chemical concentration versus trophic levels of organisms in a food web, representing the average increase or decrease in chemical concentrations per unit increase in trophic level (Fisk et al., 2001)

1. Introduction

Chemical pollution caused by environmental contaminants is a major, global-scale problem that scientists and regulators alike have been attempting to mitigate for decades. Widespread application of many industrial and commercial chemicals has resulted in often unintentional adverse impacts on ecosystems, wildlife, and human health. The Stockholm Convention on Persistent Organic Pollutants, an international treaty created to identify and manage persistent, bioaccumulative, and toxic compounds worldwide, assists scientists and governmental agencies in their efforts to categorize and evaluate potential environmental and health impacts of commercial chemicals. To effectively evaluate and categorize the approximately 100,000 existing compounds, in addition to the thousands of new substances developed annually, regulatory agencies often use the persistence, bioaccumulation, and toxicity (PBT) framework for the development of regulatory criteria surrounding environmental contaminants [1-3]. Under the Canadian Environmental Protection Act 1999 (CEPA 1999), all 23,000 substances on the Domestic Substances List (DSL) were assessed for persistence, bioaccumulation, and toxicity [4]. Based on this initial assessment, substances identified as toxic and also likely to persist or bioaccumulate were selected for further evaluation.

Specific criteria are generally established separately for persistence, bioaccumulation, and toxicity. For instance, several metrics are commonly used to establish bioaccumulation thresholds, such as the octanol-water partition coefficient (K_{OW}), bioconcentration factor (BCF), and bioaccumulation factor (BAF). According to the Stockholm Convention on Persistent Organic Pollutants, compounds are considered bioaccumulative if one or more of the following criteria are met:

- the BCF or BAF exceeds 5000 L/kg, or log K_{OW} exceeds 5 (if BCF or BAF measurements are not available);
- other evidence (i.e., observed biomagnification or toxicity) suggests that a compound may cause environmental or health concern; or,

- further evidence justifies consideration of a compound under the Stockholm Convention.

Under many existing regulatory frameworks, only the first criterion is considered when assessing the bioaccumulation and biomagnification of compounds, thus a substance is considered bioaccumulative only when its K_{OW} or BCF values exceed a particular threshold. For instance, under CEPA, substances with K_{OW} ≥ 100,000 or BCF ≥ 5000 L/kg are considered bioaccumulative (Table 1-1; [3,4]).

Table 1-1 Bioaccumulation endpoints for various regulatory agencies (from Gobas et al. 2009)

Regulatory Agency	Bioaccumulation endpoint	Criteria (log values)	Program
Environment Canada	K_{OW}	≥ 100 000 (5)	CEPA (1999)*
Environment Canada	BCF	≥5 000 (3.7)	CEPA (1999)
Environment Canada	BAF	≥5 000 (3.7)	CEPA (1999)
European Union 'bioaccumulative'	BCF	≥2 000 (3.3)	REACh+
European Union 'very bioaccumulative'	BCF	≥5 000 (3.7)	REACh
United States 'bioaccumulative'	BCF	1 000 (3) – 5 000 (3.7)	TSCA, TRI+-
United States 'very bioaccumulative'	BCF	≥5 000 (3.7)	TSCA, TRI
United Nations Environment Programme	K_{OW}	≥ 100 000 (5)	Stockholm Convention #
United Nations Environment Programme	BCF	≥5 000 (3.7)	Stockholm Convention

* CEPA, Canadian Environmental Protection Act, 1999 (Government of Canada 2000).
+ REACh, Registration Evaluation, and Authorization of Chemicals. Anne XII (European Commission 2001).
+-TSCA, Toxic Substances Control Act; TRI, Toxic Release Inventory programs (USEPA, 1976)
Stockholm Convention on Persistent Organic Pollutants.

Concerns have been raised surrounding the effectiveness of regulatory criteria to adequately protect aquatic and terrestrial food webs in their entirety. For example, the BCF has been labeled as a poor measure of bioaccumulation for certain substances in air-breathing organisms [2,5]. This is particularly true for perfluoroalkyl acids (PFAAs), a group of ionizable chemicals with unique chemical properties compared to many legacy POPs such as dichlorodiphenyltrichloroethane (DDTs) and polychlorinated biphenyls (PCBs). Numerous studies have identified bioaccumulation of various PFAAs within food webs containing air-breathing species (including terrestrial organisms and marine mammals), even though BCF values for most PFAAs are below the threshold of bioaccumulation (in Canada, < 5000 L/kg; investigated further in [2,5,6]). Despite these concerns, the BCF remains the primary indicator of bioaccumulation potential in Canada, the United States, and the European Union [4,7,8].

Failure to implement universally applicable criteria results in 'false negative' categorization of PFASs [6]. This occurs when compounds are not considered to be a bioaccumulative concern based on standard evaluations, but they are shown to bioaccumulate and biomagnify in food webs.

1.1. Perfluorinated Substances

Since the Stockholm Convention came into effect in 2004, additional emerging compounds of concern have been increasingly investigated and added to the list of chemicals flagged for restriction or elimination, including perfluoroalkyl substances (PFASs).

For over 50 years, PFASs have been used in a range of industrial and consumer products, including stain repellents, lubricants, food packaging, firefighting foams, and pesticides [9]. PFASs are highly persistent compounds, due mostly to the presence of the C-F bonds, the strongest in organic chemistry [10]. As a result, many PFASs are environmentally ubiquitous [11,12], having been found in the blood of humans in most populations [13-25], and in pristine ecosystems such as the Arctic and Antarctic [26-37]. In addition to their persistence, Some PFASs are known to accumulate within food webs, and abnormally high concentrations have been found in the blood and tissues of top predators [11,38-43]. PFAS exposure is linked to a range of health issues in both aquatic and terrestrial organisms, such as hepatotoxicity, immunotoxicity, and developmental toxicity (reviewed in [13] and [44]).

Two high-profile PFAAs, perfluorooctanoate (PFOA), and perfluorooctane sulfonate (PFOS), are under particular scrutiny because of their production and use throughout recent decades, resulting in health and environmental concerns. Although the major North American manufacturer of PFOS (3M Co.) announced the phase out of PFOS and related perfluorooctane sulfonyl fluoride-based chemistries in North America between 2000 and 2002, production of PFOS continues elsewhere in the world for use when PFOS substitutes are not available [45,46]. In 2009, the Persistent Organic Pollutants Review Committee added PFOS to the Stockholm Convention on POPs under Annex B (restricted use) [47,48]. Recently, the EU has proposed adding PFOA to

the list of substances for restriction and elimination under the Stockholm Convention and the Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) because of its bioaccumulative tendencies and toxic effects on humans and wildlife [49,50].

1.2. Bioaccumulation Metrics

Another issue with measuring and predicting the bioaccumulation of PFAAs is the selection of inappropriate metrics. The repercussions of assuming a 'one-size-fits-all' framework with regards to bioaccumulation modeling and chemical screening have been identified [3]. For instance, the bioconcentration factor (BCF) is a standardized, laboratory-based measurement used to describe bioaccumulation behaviour within both research and regulatory contexts [3]. However, because the BCF is a measure of the concentration of a substance in an organism compared to the surrounding aquatic environment, this bioaccumulation endpoint is not universally applicable, since it inherently excludes air-breathing organisms [51]. The bioaccumulation factor (BAF) describes the same information as the BCF (i.e., concentration in biota versus concentration in water); however, this is a measurement of bioaccumulation under field conditions, as opposed to laboratory-derived measurements [51]. Like the K_{OW}, the BCF and BAF are also aquatic-based measurements applicable explicitly to water-respiring organisms, and do not consider dietary exposure to chemicals. In order to adequately protect organisms from adverse effects associated with bioaccumulation of contaminants, it is necessary to rectify these inconsistencies between different measurements of bioaccumulation and the actual observed behaviour of PFASs in food webs. The relationship between BCF or BAF and log K_{OW} is typically linear. Recognized exceptions to this rule include readily metabolized substances and syperhydrophobic compounds [51]. The bioconcentration of ionogenic compounds is believed to create a new category of exceptions.

The biomagnification factor (BMF) is also used as a metric of bioaccumulation, and is calculated as a ratio of the concentration of a chemical in an organism compared to the concentration in the organism's diet. Dietary exposure is critical when examining the role of magnification throughout a food web [51]. However, this can only be

calculated for predator-prey relationships, and so this metric fails to provide an assessment of the overall food web magnification [3].

Using the trophic magnification factor (TMF) to measure the magnification of environmentally relevant contaminants throughout a food web has several advantages over the BCF, BAF, and BMF. The TMF is an average measure of biomagnification throughout a food web, evaluating the increase or decrease of a contaminant throughout the trophic levels, or TLs, determined using stable nitrogen isotope ratios ($\delta^{15}N$). A TMF exceeding 1 for normalized concentrations of a contaminant indicates that dietary absorption is occurring faster than elimination, and concentrations are increasing against the thermodynamic gradient with increasing trophic level [3,51-53]. Using the TMF to understand the expected bioaccumulation behaviour throughout an entire food web is useful for determining maximum acceptable concentrations in environmental media (e.g., water and sediment) and lower trophic levels (e.g., benthic invertebrates) in order to protect higher trophic level organisms. Proposed frameworks for identifying bioaccumulation consider the TMF as the most reliable indicator of 'B' (Figure 1-1).

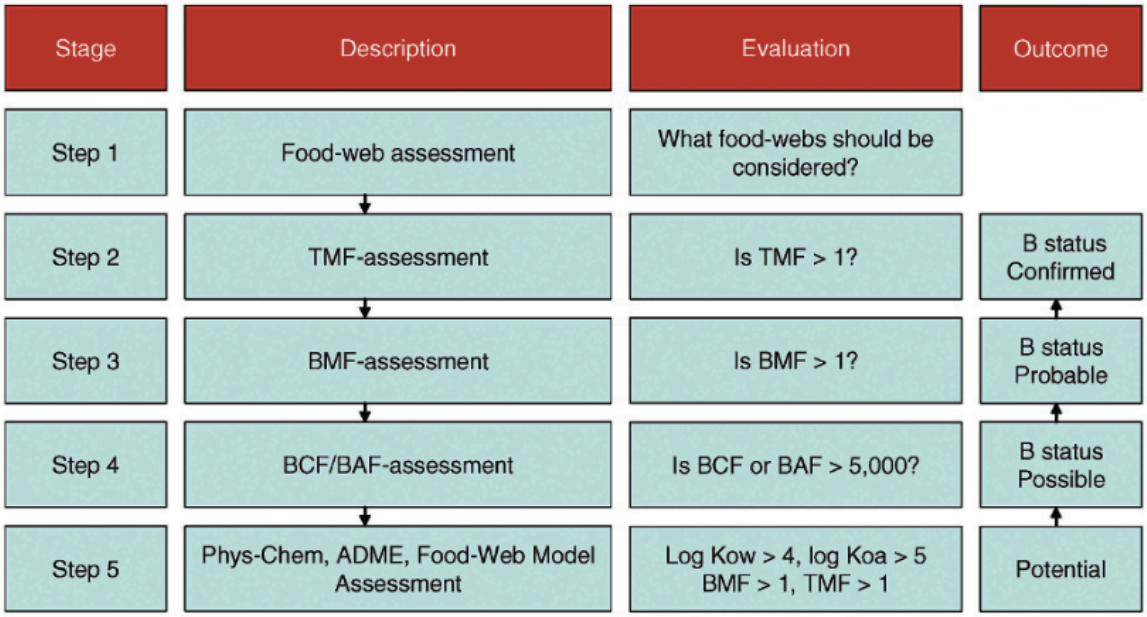


Figure 1-1. Recommendations for a comprehensive framework to identify bioaccumulation ('B') based on field data, laboratory tests, bioaccumulation models, and physicochemical properties (from Gobas et al. 2009).

Recently, BCF, BAF, and TMF values for several PFASs in a benthic-pelagic food web from the Netherlands were compared to determine the ability of these metrics to capture the true bioaccumulative behaviour of these compounds [6]. Results showed multiple 'false negative' results for PFASs. A false negative categorization occurs when substances are not considered bioaccumulative according to BCF or BAF values (< 5000 L/kg); however, TMF values exceed 1, showing evidence that the substance biomagnifies in the food web. Analyses of other environmental contaminants show similar inconsistencies between various measures of bioaccumulation, including both false negatives (i.e., BCF ≤ 5000 L/kg and TMF > 1, indicating trophic magnification), and false positives (i.e., BCF ≥ 5000 L/kg and TMF < 1, indicating trophic dilution) [2,3,6].

1.3. Bioaccumulation Assessment

Predictive modeling tools are often used to evaluate the expected ecological or biological behaviour of potentially persistent, bioaccumulative, and toxic (PBT) substances [54]. Typically, physicochemical and environmental properties are used as input parameters for these models, and in return, the model provides quantitative estimates of the behaviour of specific compounds within ecosystems. Predictive models are beneficial for researchers and regulating bodies. For example, models can help to supplement empirical findings from field research, and can also be used to assist with the development of policies and regulations related to environmental pollutants. Adequate model development is necessary for generating practical estimates of chemical behaviour in environmental media and biota. Specifically, the selection of input parameters ultimately impacts the overall accuracy and usefulness of the model.

Predictive bioaccumulation models have been developed to evaluate the expected bioaccumulation behaviour of legacy persistent organic pollutants (POPs) such as DDTs and PCBs in aquatic ecosystems (e.g., [2,3,55-58]). Some legacy POPs, such as DDT, have been recognized as harmful to ecosystems and biota for several decades [59]. However, new substances – many of which have chemical characteristics that vary from those of legacy POPs – have been developed, manufactured, and released into the environment [2,3,11,60], including PFSAs. As a result, anticipated bioaccumulation

behaviour derived from models developed for legacy POPs may not adequately reflect the true bioaccumulative nature of emerging contaminants of concern [2,55,61,62].

Historically, predictive modeling of the bioaccumulation of hydrophobic organic contaminants in fish and other aquatic organisms has worked under the assumption that lipid-water partitioning is an underlying mechanism causing bioaccumulation. This is evaluated using the octanol-water partition coefficient (K_{OW}) as the key physiochemical property in estimating the bioaccumulative tendencies of a compound (e.g., [56,63,64]). The K_{OW} describes the ratio of a chemical concentration in 1-octanol (a surrogate for neutral lipids such as adipose tissue) versus the concentration in a surrounding water environment. K_{OW} values typically correlate with an increased likelihood of bioaccumulation [65,66]. The K_{OW} serves as an adequate metric for chemicals, such as DDT and PCBs, which are lipophilic and accumulate in the lipids of organisms [1-3,56,58,62,63,67-70]. The K_{OW}, however, has several limitations when applied indiscriminately to many substances. Firstly, because K_{OW} evaluates the partitioning of chemicals from an aquatic environment, K_{OW} alone may not be an appropriate metric for predicting the expected bioaccumulation within air-breathing species, such as marine mammals and terrestrial organisms [2,3,57,71-73]. Secondly, K_{OW} is applied to modeling applications assuming that bioaccumulation occurs exclusively in neutral lipids. This assumption may not hold true for all substances, including some PFAAs. Shorter-chain PFAAs generally have lower K_{OW} values than legacy compounds, yet are known to bioaccumulate and biomagnify in various food webs, including Arctic terrestrial ecosystems [74], Arctic marine food webs [71,73], temperate lake ecosystems [72,75,76], and temperate marine ecosystems, such as the Charleston Harbor bottlenose dolphin food web [77]. Modeling expected concentrations of PFAAs in these food webs based on K_{OW} values may underestimate the degree of bioaccumulation that occurs in real food webs.

Well-documented legacy POPs are typically neutral, lipophilic substances with predictable bioaccumulation behaviour: a high affinity for non-polar (i.e., neutral) lipids, and a tendency to accumulate in tissues with a high proportion of neutral lipids, such as adipose muscle tissue and blubber [2,3]. In contrast, PFAAs bind preferentially to protein [11,47,78], resulting in high PFAA concentrations in tissues with high fractions of protein such as blood plasma and liver [73,74,79-89].

Additionally, some PFAAs of current interest (e.g., PFOA, PFOS) are those substances which are acids, bases, or zwitterionics at relevant pH (i.e., ionogenic organic compounds, or IOCs). Therefore, their bioaccumulation behaviour is pH-dependent and can differ based on whether the substance is in a neutral or ionized state [55,90-92]. Whereas many POPs exist in a chemically neutral state under typical environmental and biological conditions, reported acid dissociation constant (pK_a) values of PFOA and PFOS can be extremely low (i.e., < 1), resulting in almost completely ionized substances at physiologically and environmentally relevant pH [93,94]. Model development, however, has largely assumed neutrality without accommodating for ionization [55,95]. Therefore, there is reason to suspect that many existing bioaccumulation models are failing to adequately account for the observed bioaccumulation behaviour of PFAAs.

1.4. Objectives.

In this study, an existing food web model is modified to predict the trophic magnification of PFOA and PFOS in the bottlenose dolphin (*Tursiops truncatus*) food web from Charleston Harbor, South Carolina. The bottlenose dolphin food web was specifically selected because several research efforts have focused on this ecosystem, and sufficient information regarding PFAA levels exists for abiotic and biotic media, which are used in this study for model input and verification [39,77,87,96-101]. Additionally, extensive information regarding dietary intake, physiology, and life history of the bottlenose dolphin food web is available [102,103]. Furthermore, the presence of an air-breathing organism occupying a high trophic position (i.e., bottlenose dolphin; trophic level 4.4) in a marine food web can considerably influence the expected food web magnification of substances such as PFAAs [71,73,77].

The purpose of this project is to develop a predictive modeling tool capable of adequately estimating the bioaccumulative behaviour of PFOA and PFOS in a marine food web. The objectives of this research project are three-fold:

- *modify* an existing bioaccumulation model originally designed for neutral, lipophilic compounds such that it is suitable for analysis of PFOA and PFOS by

- accounting for the ionizable nature of PFOA and PFOS, and
- accounting for partitioning of PFAAs into multiple tissues, including protein-rich media such as blood plasma;

- *evaluate* the modified model in terms of predicted bioaccumulation estimates, as well as the effectiveness of indicators typically used to assess bioaccumulation in regulatory frameworks (e.g., K_{OW}, BCF, BAF) with indicators more inclusive of whole food webs (e.g., TMF) in their ability to adequately describe patterns estimated bioaccumulation, particularly for apex predators and high trophic level organisms; and

- *test* the modified model through comparison of calculated bioaccumulation to
  - the existing Aquaweb model, and
  - empirical data

in order to evaluate whether the modified model better accounts for the behaviour of PFAAs.

Bottlenose dolphins are at risk of accumulating high levels of PFAAs and other contaminants as a result of biomagnification, potentially leading to adverse health effects. For instance, studies have identified links between PFOS exposure in bottlenose dolphins and immune system complications, hepatotoxicity, developmental toxicity, and interference with endocrine function (reviewed in [13,44]). Young bottlenose dolphins are reported to have higher concentrations of PFAAs than their mothers, which is thought to be the result of maternal transfer of PFAAs through milk [104]. Because compounds with higher K_{OA} values (compared to legacy POPs) are not readily eliminated from air-breathing species such as bottlenose dolphins, despite residing in water [77], it was practical to modify a marine food web with an air-breathing organism to examine the patterns of expected bioaccumulation behaviour for species within the same food web utilizing different means of respiration (i.e., gills versus lungs).

1.5. Models for Ionogenic Compounds.

To mitigate complications resulting from the use of insufficient physicochemical properties for predicting bioaccumulation behaviour of environmental contaminants, it

has been suggested that three specific modifications be made to the partition coefficients used in modeling efforts. Firstly, the octanol-air partition coefficient (K_{OA}) can be used in evaluating the bioaccumulation potential for air-breathing organisms and food webs with air-breathing organisms in order to account for respiratory uptake and elimination from air [2,3,73,105]. This is useful because PFAAs have higher K_{OA} values compared to many legacy POPs, inhibiting respiratory elimination from air-breathing organisms, such as marine mammals [2,41,71-74,77]. It has been shown that high K_{OA} substances are eliminated slowly in air-breathing organisms [2,57]). Simultaneously, PFAAs are expected to have lower K_{OW} values than many POPs. Water-respiring species, such as fish, can more readily eliminate less hydrophobic substances via gill elimination. Similarly, the protein-water partition coefficient (K_{PW}) can also be applied in bioaccumulation modeling to allow for the measurement of chemical partitioning into protein, specifically serum albumin, fatty acid binding proteins (FABPs) and organic anion transporters (OATs) [73,79,80,84,85,106-108]. Lastly, since PFAAs maintain a level of hydrophobicity due to the presence of the perfluoroalkyl tail [42,82]), some degree of bioaccumulation is still likely to occur in neutral lipids, thus lipophilicity should not be fully dismissed from the model. Rather, a modified approach can be applied for ionizable substances. Log *D*, or distribution ratios, evaluates octanol-water partitioning, but accounts for both the neutral and ionized portion of a substance [95,109]. There is another type of tissue often neglected in bioaccumulation modeling: polar lipids in the form of phospholipid bilayers [62,109,110]. Despite accounting for a small fraction of overall tissue, the polar head of the bilayer will interact with polar molecules, such as PFAAs, potentially contributing to bioaccumulation [62,69,110]. The inclusion of log *D* in food web models, in addition to K_{OA} and K_{PW}, is expected to improve the applicability of bioaccumulation modeling to ionogenic POPs, such as PFAAs.

This research responds to the need for more inclusive food web bioaccumulation modeling to better evaluate the risks associated with emerging environmental contaminants. The model used in this study is a more comprehensive tool to evaluate bioaccumulation for a range of environmentally relevant compounds in a range of food webs. Furthermore, this model can assist with the development of regulatory frameworks and environmental quality thresholds that better protect species and ecosystems from emerging ionogenic substances not captured using conventional approaches.

2. Modeling Theory

2.1. Overview

Theories underlying the fundamental bioconcentration model (i.e., on an individual organism scale) and the existing marine food web model [111] are addressed in this section. In particular, adjustments required to allow for full applicability to ionizable compounds are noted. These adjustments are divided into three major areas of modification: ionization, tissue partitioning, and respiratory physiology. Relevant routes of uptake and elimination in the bioaccumulation of PFOA and PFOS are described.

2.2. Bioaccumulation Theory

2.2.1. Bioconcentration

Bioconcentration is the ratio of the concentration of a chemical in biota compared to its surrounding (aquatic) environment, without any influence from dietary uptake. When a system is at steady-state, the BCF for aquatic organisms can be calculated:

$$BCF = C_B/C_{WD} = k_1/(k_2 + k_E + k_M + k_G) \quad (1)$$

where C_B is the concentration in biota normalized to total protein (ng/kg), C_{WD} is the dissolved chemical concentration in water (ng/L), k_1 is the gill uptake rate constant ($kg \cdot day^{-1}$), k_2 is the gill elimination rate constant (day^{-1}), k_M is the biotransformation rate (day^{-1}), and k_G is the growth rate constant (day^{-1}).

2.2.2. Biomagnification

Biomagnification refers to the increase in concentration of a chemical in a predator over that in the prey of the organism [51]. Biomagnification accounts for food uptake (bioconcentration does not); however, this metric is limited only to a predator-prey interaction (between two species). At steady state, BMF can be modeled as:

$$BMF = C_B/C_D = k_D/(k_2 + k_E + k_M + k_G) \quad (2)$$

The general equation for calculating the biomagnification factor (BMF) is:

$$BMF = C_{PREDATOR}/C_{PREY} \quad (3)$$

where $C_{PREDATOR}$ is the chemical concentration in the consumer normalized to total protein (ng/kg), and C_{PREY} is the chemical concentration in the prey normalized to total protein (ng/kg).

2.2.3. Trophic Magnification

Trophic magnification refers to the average change in concentration of a chemical throughout a food web [2,3,73,112-114]. The trophic magnification factor (TMF) measures the factor by which chemical concentration increases per trophic level [3].

Trophic positions described in [77,113] were used to evaluate trophic magnification of PFAAs. To calculate the TMF:

$$TMF = e^b \quad (4)$$

where b is the slope of the concentrations for each species in the food web plotted against trophic level (Figure 2-1).

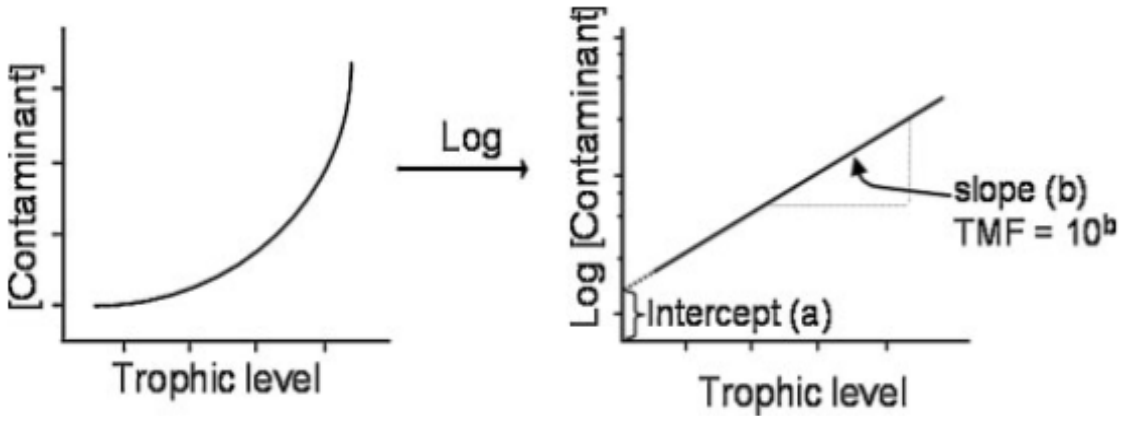


Figure 2-1. Calculation of the trophic magnification factor (TMF), which evaluates the change in contaminant concentration per trophic level throughout the food web. (Image from Borga et al., 2012).

2.3. Bioaccumulation Metrics

Various bioaccumulation metrics (i.e., BMF and TMF) were calculated for model-estimated PFAA concentrations and concentrations of PFAAs measured in the Charleston Harbor food web from [77]. BCFs, BMFs, and TMFs estimated from the modified model were compared to estimates provided by the original model, as well as empirical data in order to determine the degree to which model-estimated calculations agreed with observed measurements of bioaccumulation.

2.3.1. Bioconcentration

Bioconcentration factors (BCFs) of PFOA and PFOS were calculated for each species in the food web. BCF calculations were determined based on uptake and elimination from gill respiration for the modeled food web (see Section 2.4.1 for detailed equations).

BCFs for PFOA and PFOS were evaluated for aquatic organisms based on the regulatory threshold in Canada under CEPA (i.e, if BCF ≥ 5000, substance is bioaccumulative). Despite the recognized inapplicability of BCFs to air-breathing organisms (since they do not utilize gill uptake and elimination), the BCF was still calculated for the bottlenose dolphin. This was done in order to evaluate the usefulness

of the BCF to describe estimated food web bioaccumulation of PFOA and PFOS, regardless of inherent technical limitations. Calculated dolphin BCFs may align with the observed bioaccumulation behaviour of these compounds, or conversely, BCFs could fail to reflect patterns of observed bioaccumulation. The outcome of this relationship could influence policy recommendations concerning whether a metric technically applicable only to water-respiring organisms is still an adequate indication of bioaccumulation for air-breathing animals.

2.3.2. Biomagnification

Biomagnification occurs when the chemical concentration in an organism exceeds that in the diet as a result of dietary absorption [51]. BMFs were calculated for direct predator-prey relationships according to adjacent trophic levels in the model (refer to Section 2.4.2 for model theory and calculation). This method allows for insight as to patterns of biomagnification throughout the food web.

2.3.3. Trophic Magnification

Trophic magnification of PFOA and PFOS was calculated for four overall scenarios with two versions of the bottlenose dolphin food web (Table 2-1):

- *Full food web developed for this study (plankton + invertebrates + fish; with and without marine mammal)*
- *Food web evaluated in* [77] *(fish; with and without marine mammal)*

The first scenario evaluates TMFs of PFOA and PFOS in a food web that includes fish and marine mammal species evaluated in the original field study, in addition to phytoplankton, zooplankton, and marine invertebrates (from TLs 1 through 2.8) not evaluated in the original study. Under this scenario, trophic magnification can be evaluated for an inclusive complete food web.

Because the TMFs of PFAAs calculated by [77] only considered fish and marine mammal species, modeled TMFs in the second scenario was calculated for fish and marine mammal. Genuine comparisons between observed and calculated trophic

magnification can only be made for TMF calculations including the same number and type of species in the model and in the observed food web.

TMFs were calculated with and without marine mammals for both versions of the food web explored in this study. This was done in order to evaluate the influence of air-breathing organisms on the degree of trophic magnification that occurs in food webs containing both aquatic and mammalian species.

Table 2-1 Species included in estimates of trophic magnification in this study. The full food web considered the full range of species in the model, whereas the range of species evaluated in Houde et al. (2006) was limited to fish and marine mammals. Furthermore, trophic magnification in each food web was evaluated with and without the marine mammal to investigate the role of air-breathing organisms on food web bioaccumulation.

		Full Food Web		Food Web Evaluated in Houde et al. 2006	
Species Type	**TLs**	**Including Marine Mammal**	**Excluding Marine Mammal**	**Including Marine Mammal**	**Excluding Marine Mammal**
Phytoplankton	1	✓	✓		
Zooplankton	2	✓	✓		
Marine Invertebrate	2.1-2.8	✓	✓		
Fish	3.4-4.3	✓	✓	✓	✓
Marine Mammal	4.4	✓		✓	

Trophic positions described in Alava et al. (2012) and SeaLifeBase/FishBase (for phytoplankton, zooplankton, and marine invertebrates) and [77,111,113] (for fish and marine mammals) were used in calculations of trophic magnification for PFAAs.

Several inherent assumptions exist for TMF analysis, including the assumption that all biota in the food web are subject to the same environmental conditions throughout the study area [112,115]. Study design should either attempt to eliminate or account for factors impacting the overall effectiveness of TMF values (as identified by [115]), including spatial concentration differences. For instance, when spatial differences in concentrations exist, TMF values for substances that are likely to undergo slight

trophic dilution have a 13% to 47% probability of erroneously being calculated as having a TMF > 1 [115].

2.4. Bioconcentration Model

The ionogenic bioconcentration model applies equilibrium partitioning to estimate the ratio of chemical concentrations in an organism compared to its surrounding water environment (i.e., does not consider dietary intake). Bioconcentration refers to the distribution of a chemical between water and biota, that is, the loss or elimination of chemicals via the respiratory surface, fecal elimination, and biotransformation, and growth (as dilution), but does not consider dietary uptake [51]. Rates of uptake (k_1) and elimination (k_2) of non-metabolizing organic chemicals in fish from water via the respiratory route are included within the bioconcentration model [57,64,116]. The model views the uptake and elimination rates resulting from gill ventilation, transportation through aqueous boundary layers, parallel transport through the membrane bilayers, and pore transport.

The rate at which organic chemicals are absorbed by the water through the gills is the uptake rate constant, k_1 (d^{-1}), and is a function of the individual's mass and lipid-water partition coefficient(s), typically represented by K_{OW}. Conversely, k_2 (d^{-1}) is the rate at which compounds are eliminated at the respiratory surface, which is usually a function of the relative tissue fractions of the organism and their respective partition coefficients [57].

The overall resistance encountered by bioconcentrating chemicals can be expressed as described below (assuming steady-state conditions; i.e., $dC_B/dt = 0$, where C_B is the concentration of chemical in biota):

$$R_{total} = R_{ventilation} + (1/R_{membrane} + 1/R_{pore})^{-1} + R_{internal} \quad (5)$$

where R_{total} is the total resistance for uptake or elimination, and the respective remaining variables represent resistance due to gill ventilation ($R_{ventilation}$), water phase diffusive transport and lipid phase diffusive transport ($R_{membrane}$), pore or joint gap transport (R_{pore}), and internal transport ($R_{internal}$) (Figure 2-2). Resistance refers to factors

that affect the ability of a compound to enter and exit an organism. If uptake is greater than elimination, this can lead to bioconcentration of molecules. These terms can be described by the transport parameter (Q; units m^3/s), combining all diffusion processes involved in solute transport for each phase, along with the fugacity capacity of water, Z_W; as in Equation 2 for instance (see Appendix A for full derivation):

$$D_{ventilation} = Q_{ventilation}.Z_w \quad (6)$$

Parameters associated with the resistance encountered by bioconcentrating compounds include:

Gill ventilation: the amount of water passing through the gills per day in units of L/day;

Water phase diffusive transport: a function of hydrophobicity, such that more hydrophobic compounds will experience less diffusive transport in aqueous spaces;

Lipid phase diffusive transport: passive diffusion across a cell membrane occurs when the solubility of a compound in the lipid bilayer or membrane channels is high, and the concentration of the substance outside the cell is high (applicable to unionized species only);

Pore transport: applicable to ionized species passing through cell membranes. [56,64,117]; and

Internal transport: relates to mechanisms responsible for the mobility of compounds inside an organism.

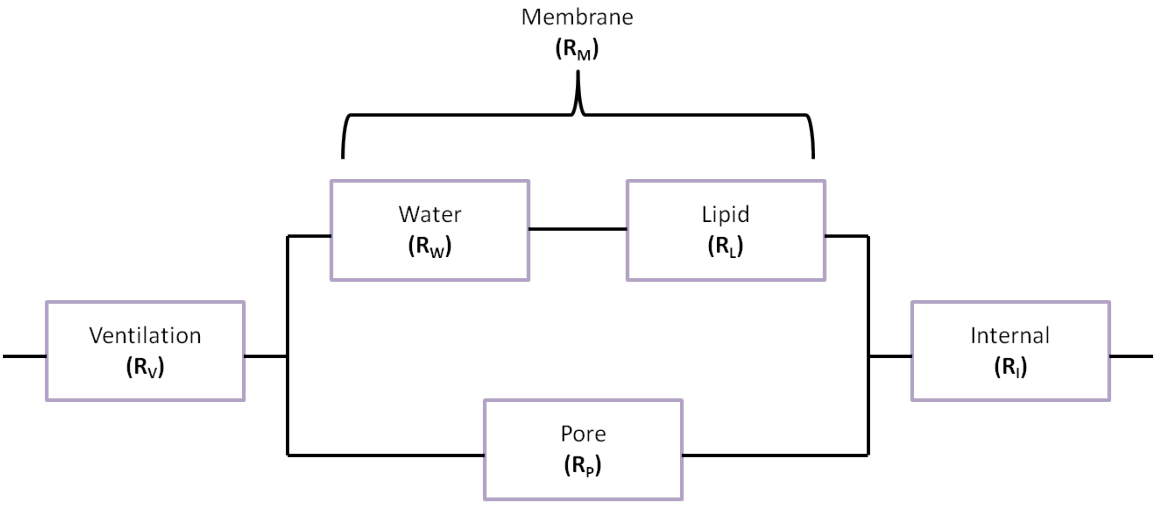


Figure 2-2. Resistance (R) encountered by bioconcentrating chemicals in aquatic organisms under steady-state conditions.

2.4.1. Modifications to Bioconcentration Model for Ionogenic Substances

Historically, bioconcentration model parameters have been calculated under the assumption that partitioning of a chemical into non-polar lipids is the most relevant form of chemical sorption, whereas that did not hold true for this study. (It is important to note that dietary uptake and elimination is not included in the bioconcentration model, but is incorporated into the food web analysis to evaluate biomagnification and trophic magnification).

Previously, this model was derived from the hydrophobic organic chemical bioconcentration model described in [116]**,** originally designed for neutral compounds and dependent on the mechanism of chemical partitioning into lipids. Here, this model is modified to account for the ionizable nature of compounds such as PFAAs, which have ionizable or neutral moieties and show differences in terms of physiological interactions with organisms (i.e., they do not have a high affinity for fat tissue) [118]. Therefore, a bioconcentration model based on lipophilicity is not necessarily appropriate for IOCs such as PFAAs. Modifications to the existing model (i.e., ionization, tissue partitioning, respiratory physiology) are outlined here.

Ionization

By considering a substance's acid dissociation constant, or pK_a, the model calculated the fraction of the compound ionized at the pH of Charleston Harbor water assuming pH = 7.86 [93,119]:

$$f_I = 1/(1+(1/10^{pH}\text{-}pK_a) \quad (7)$$

Various pK_a values for PFAAs exist in the literature (e.g., [93,120-122] and much discussion has ensued over the accuracy of these reported values values. PFOA and PFOS pK_a estimates range from approximately -4 to +4 [46,93,121,123]. Most discussion arises regarding the tools or software used to estimate pK_a values, and whether the formulas used are adequate.

Some of the highest reported pK_a values were used in the model (3.4 and 4.0 for PFOA and PFOS, respectively) in order to account for any potential neutral fractions that may exist in the environment or biota, even though these estimates are higher than commonly used pKa values [93,120,122,124]. Because the pK_a values are considerably lower than the pH of the organisms in the food web and the surrounding environmental media, PFOA and PFOS are expected to be almost completely ionized in the Charleston Harbor ecosystem and under standard conditions elsewhere.

Whereas the shake-flask method is commonly used to experimentally determine partition coefficients between octanol and water, this is not feasible for perfluorinated compounds because the surfactant nature of these substances causes them to aggregate at the interface of a liquid-liquid system, creating 3 separate layers [125,126]. Therefore, a calculated log K_{OW} value for neutral substances (i.e., log $K_{OW,N}$) was determined using EPI Suite, a computer software program capable of applying computational algorithms to estimate physicochemical properties based on the molecular and chemical structure of compounds. Once $K_{OW,N}$ is known, the octanol-water partition coefficient can also be calculated for the ionized form of a compound (i.e., log $K_{OW,I}$):

$$\log K_{OW,I} = \log K_{OW,N} - \Delta_{OW} \quad (8)$$

where Δ_{OW} is a scaling factor relating the neutral and ionized partition coefficients, and is equal to -3.1 for carboxylic acids and sulfonic acids [62,127,128].

It is critical to also consider partitioning of PFAAs to membranes (i.e., phospholipid bilayers), as models omitting this behaviour may underestimate the expected bioconcentration for substances that are largely or completely ionized and have a heightened affinity for polar lipids [62]. To determine the neutral and polar membrane-water partition coefficients ($K_{MW,N}$ and $K_{MW,I}$, respectively), we first calculate the value for the neutral form (K_{MW}) using a simple regression equation [118,128-130].

$$\log K_{MW} = a \cdot \log K_{OW,N} + b \quad (9)$$

where a = 1.01 and b = 0.12 (see [118]). This is a single-parameter linear free energy relationship (sp-LFER) equation (see [62] for detailed methodology).

Once $K_{MW,N}$ is known, the membrane-water partition coefficient for the ionized form of a substance ($\log K_{MW,I}$) can be determined:

$$\log K_{MW,I} = \log K_{MW,N} - \Delta_{MW} \quad (10)$$

where Δ_{MW} is a scaling factor to account for increased solubility in the aqueous phase against the interactions with polar membranes [62]. The value of Δ_{MW} used is -2.0, consistent with previous studies [62,128].

Tissue Partitioning

Historically, temperature-corrected K_{OW} and K_{OA} have been included in the food web model to determine the amount of a substance expected to partition from aqueous phases to lipids. Though appropriate for neutral compounds, additional partition coefficients need to be integrated into the model to calculate the chemical affinity of PFAAs for various tissues.

Because PFAAs are not lipophilic compounds, K_{OW} values measured or calculated for neutral substances are not expected to adequately describe the partitioning behaviour of PFAAs from water to biota. Therefore, this study utilized a

similar approach as described in [62], whereby partitioning of PFAAs into non-polar (neutral) lipids, polar lipids, and protein are considered.

The body-water distribution coefficient (D_{BW}) describes the distribution of a compound throughout the whole organism, combining the relative neutral and ionized contributions of non-polar lipid (neutral, storage lipids), polar lipid (phospholipid bilayer membranes), protein, and water. This determines the overall partition coefficient that accounts for all tissue types and their associated individual partition coefficients. Non-polar lipids are hydrophobic storage lipids (adipose tissue such as muscle or blubber), whereas polar lipids consist of membrane lipids (phospholipids), which are negatively charged due to the presence of a polar head group. Neutral compounds typically do not interact with polar lipids to an extent that is relevant for bioaccumulation. Ionized compounds, on the other hand, will sorb to polar lipids because of electrostatic interactions between charges of the molecules [62]. Additionally, IOCs may sorb to protein, such as albumin, FABPs, or OATs, which are also often polar molecules [85]. The following equation describes the sorption capacity of an organism in a water environment (D_{BW}, in L/kg) applicable to IOCs (modified from [62,131]):

$$D_{BW} = f_{NPL} \cdot D_{OW} + f_{PL} \cdot D_{MW} + f_{P} \cdot K_{PW} + f_{W} \quad (11)$$

where f_{NPL}, f_{PL}, and f_{P} are the fractions of non-polar lipid, polar lipid, and protein by weight, respectively, that makes up an individual organism. This modified equation now allows for the calculation of the approximate fraction of each tissue component and its respective affinity for a substance.

With values for the neutral and ionic forms of K_{OW} and K_{MW} available (see previous section describing ionization), the octanol-water distribution ratio (D_{OW}; similar to log$D$, see [109]) and membrane-water distribution ratio (D_{MW}) can be determined. They were estimated according to the equations below [62]:

$$D_{OW} = f_{N} \cdot K_{OW,N} + f_{I} \cdot K_{OW,I} \quad (12)$$

$$D_{MW} = f_{N} \cdot K_{MW,N} + f_{I} \cdot K_{MW,I} \quad (13)$$

where f_N is the neutral fraction of the compound and f_I is the ionized fraction of the compound.

In order to calculate D_{BW} for PFAAs in this food web, it is also necessary to include a coefficient for protein-water partitioning (K_{PW}). Protein binding behaviour is observed for PFAAs in plasma albumin [132-134]. Experiments to explore the affinity of PFAAs for protein have primarily been done using human serum albumin (HSA; [83,132]), bovine serum albumin (BSA; [80,82,83]), fatty acid binding protein [84], and soy albumin [80]. Empirical data on PFAA binding is largely absent from the literature. However, several studies have measured protein binding of perfluorinated compounds. For example, K_{PW} values for several PFAAs were measured in BSA of *Daphnia magna* [80], and K_{PW} measurements for PFOA and PFOS were measured in BSA [82]. High log K_{PW} values (>4) were reported for PFOA and PFOS. The authors also found no change in binding affinity for PFOA and PFOS with varying degrees of ionization; Experimentally determined K_{PW} values were the same for both the neutral and ionized forms of PFAAs [82]. Because these values were measured experimentally, the measured K_{PW} were implemented into the model without having to calculate separate partition coefficients for neutral and ionized fractions. The measured log K_{PW} value for PFOA was 4.14 (±0.04), and the value for PFOS was 4.1 (±0.1) with a BSA concentration of 4μM. The equation for determining the log K_{PW} is [82]:

$$K_{PW} = C_P/C_W = (f_{bound}/\rho_{alumbin})\cdot[P](1-f_{bound}) \quad (14)$$

where f_{bound} is the fraction of chemical bound to protein, [P] is the concentration of protein (g/mL), and $\rho_{alumbin}$ is the partial specific volume of protein in aqueous solution (0.733 mL/g). These log K_{PW} values were selected over other available values, as non-experimental protein-water partition coefficient values for PFAAs are typically derived based on a general relationship with the K_{OW} (e.g., $K_{PW} = 0.05\cdot K_{OW}$; [79]), where the relationship established between K_{OW} and K_{PW} is relevant only to neutral compounds). This generalized relationship, for instance, cannot account for observed decreases in K_{PW} values as PFAA molecules exceed a certain chain length (e.g., PFCAs with chain lengths of six fluorinated carbons or longer, including PFOA). Decreasing affinity for protein occurs when the chain length is long enough that hydrophobicity of the molecule increases due to increased steric hindrance with longer fluorocarbon tails, and there is a

decrease in affinity of longer-chain PFAAs for BSA [82]. Therefore, caution should be taken when assuming linear relationships for PFAAs of different chain lengths. As more empirical and experimental values of protein-water partitioning become available, these measured values can be used in bioaccumulation models, while being wary of assuming linear relationships for PFAAs of all chain lengths.

Because PFAAs have relatively high K_{OA} values, partitioning into the air phase is not considered significant within the context of this research (i.e., estimated air concentration = 0), as they are largely non-volatile compounds. K_{OA} values were calculated using SPARC. Neutral and ionized fractions of PFOA and PFOS were not considered separately for K_{OA} due to a lack of data regarding this differentiation.

In order to expand the applicability of this model to ionizable compounds, additional partition coefficients were integrated into the equation. Membrane-water partitioning (D_{MW}) is integrated into the model to account for interactions of ionized compounds with lipid bilayers. Because the sorption of a substance to internal tissues affects the rate of elimination from fish, the $D_{OW,}$ D_{MW}, and K_{PW} were all incorporated into the model, thereby accounting for all relevant methods of chemical partitioning into biota (i.e., the biota-water partition coefficient, or D_{BW}). Model calculations used log K_{OW} values calculated using SPARC to derive a body-water distribution coefficient (log D_{BW}) for PFOA and PFOS that encompasses all four possible combinations of ionization and charge (i.e., neutral and non-polar; ionized and non-polar; neutral and polar; ionized and polar). Partition coefficients were also calculated for PCB 153 to compare tissue affinity between non-ionizable compounds and IOCs .

Respiratory Physiology

Bioconcentration and bioaccumulation for marine mammals was adjusted to account for the air-breathing nature of these organisms. Because PFOA and PFOS have high K_{OA}s compared to many POPs, they are considered involatile substances [2]. As such, the concentration of these compounds in air is negligible (i.e., C_{AIR} = 0), and therefore respiratory inhalation of PFOA and PFOS for mammals is also assumed to be zero. Additionally, respiratory exhalation is not expected to be an effective route of elimination for PFAAs, as chemicals with high K_{OA} values experience slow exchange

from organism to air [2]. It was therefore important to account for the role of physiology in varying bioaccumulation behaviour of PFOA and PFOS between water- and air-respiring organisms. It should be noted that PFAA precursor compounds were not considered as potential sources of PFOA and PFOS in this model. For example, 8:2 fluorotelomer alcohol (8:2 FtOHs) and perfluorooctane sulfonamidoalcohol (EtFOSE) are known PFOA- and PFOS-precursors, respectively, which may contribute to human and wildlife exposure [135]. Some of these precursors are semi-volatile, with relatively high Henry's Law Constant (HLC) values; the HLC for 8:2 FtOH is $9.7 \cdot 10^3$ Pa·m^3/mol, and for EtFOSE is $1.9 \cdot 10^3$ Pa·m^3/mol [136]. This may represent a significant indirect source of PFAAs via the air, challenging the negligible concentration in air applied in this model [12]. If the majority of exposure were to occur via air, the model can be expected to underpredict body burden, as exposure to precursors via this route of exposure is not explicitly integrated into the model.

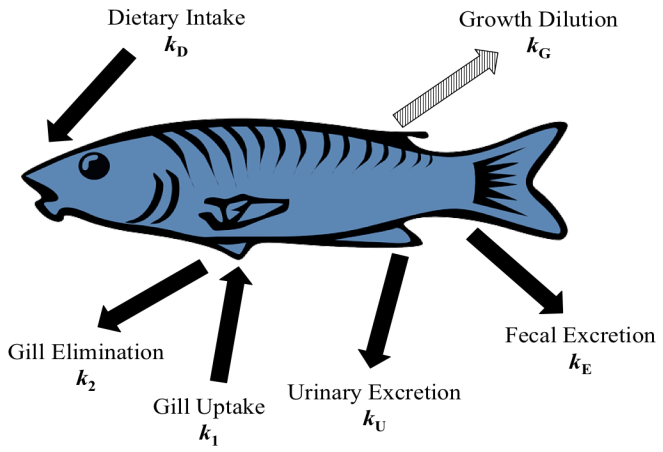
2.5. Food Web Accumulation Model

The food web model evaluates the collective bioconcentration and biomagnification of a chemical in all species of a food web. Following adjustment of the ionogenic bioconcentration model for IOCs, the food web accumulation model also required modification to account for relevant partition coefficients, animal tissue composition (i.e., lipid, protein, and water content), and protein normalization. Note that the food web model does not consider specific binding (discussed further in the next section).

2.5.1. Chemical Uptake and Elimination

The routes of chemical uptake and elimination that are considered in this model are demonstrated by fish and bottlenose dolphin in Figure 2-3**.** This section describes the parameters used to calculate predicted concentrations, BCFs, and BMFs under steady state conditions (i.e., $dC_B/dt = 0$) in the modified food web model (Table 2-2**;** see [64] for full model description and theory).

(a) Fish



(b) Bottlenose Dolphin

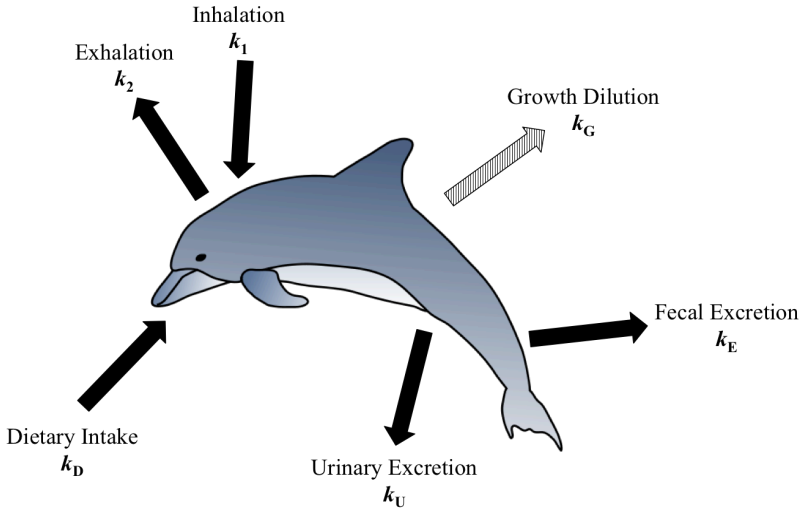


Figure 2-3. Conceptual diagram of uptake and elimination processes for PFOA and PFOS in the (a) fish and (b) bottlenose dolphin, as well as associated rate constants. The dashed arrow for growth dilution (k_G) represents apparent elimination. Note that metabolic biotransformation (k_M) is not evaluated in this study, as metabolic biotransformation is assumed to be negligible for PFAAs (i.e., k_M = 0).

Fish gill uptake efficiency **(E_W, %)**. This is the amount of chemical absorbed through the respiratory surface per unit time relative to the amount of chemical in contact

with the respiratory surface through gill ventilation. With hydrophobic substances, there is a relationship between E_W and the K_{OW}, but this relationship is thought to not hold true for higher K_{OA} substances, even though gill uptake efficiency may still be high. In this study, E_W was calculated as:

$$E_W = 1/(G_V \cdot (1/(Q_W + 1/(0.001 \cdot Q_W \cdot D_{BW} + Q_P))) \quad (15)$$

where G_V is the gill ventilation rate (L/day), Q_W is the transport rate in the aqueous phase of the organism (1/day) [56,116], Q_P is the transport rate in pores (i.e., $Q_P = 0.001 \cdot Q_W$ when adequate data is not available for Q_P) and D_{BW} is the body-water distribution coefficient, representing whole body partitioning behaviour of the neutral and ionized form of PFOA and PFOS; discussed later in this section) [62]. Based on derivations from experimental data [116], the equation for Q_W, in relation to organism weight, is:

$$Q_W = 88.3 \cdot W_B^{0.6\ (\pm 0.2)} \quad (16)$$

where W_B is weight of the organism (kg).

Recent studies examining the toxicokinetics of PFOA in rainbow trout have measured the specific gill uptake efficiency value in rainbow trout (*Oncorhynchus mykiss)*. Reported experimental E_W values were applied to all fish (uptake efficiency of PFOS = 0.36 ± 0.18% and uptake efficiency of PFOA = 0.1 ± 0.07%) [137,138].

Gill uptake rate constant **(k_1, L/kg·day)**. The rate at which chemicals are absorbed from the water via the respiratory surface (i.e., gills) [56,64,139].

$$k_1 = E_W \cdot G_V / W_B \quad (17)$$

Gill elimination rate constant **(k_2, 1/day)**. Compounds within a fish will be transported to the gills and eliminated during gill ventilation. Gill elimination tends to decrease with increasing lipophilicity [51,56,64]. Since chemical sequestration of PFAAs occurs in multiple tissues, the calculation was modified to include neutral lipids, polar lipids, protein, and water, which the model has been redesigned to consider. If there is low accumulation in neutral lipids, but high accumulation in protein, an equation for

respiratory elimination that considers only binding to lipids will not capture the true behaviour of PFAAs. The equation for k_2 is:

$$k_2 = k_1 / D_{BW} \quad (18)$$

where D_{BW} is the body-water distribution coefficient, which accounts for the proportion of chemical in each tissue.

Dietary uptake efficiency **(E_D, %)**. Typically, E_D is a relationship between dietary chemical absorption efficiencies and K_{OW}, describing the fraction of ingested chemical actually absorbed by the organism via the gastro-intestinal tract [51,64]. Here, the D_{BW} is used in replacement of K_{OW} to account for whole body distribution:

$$E_D = 1/(E_{D,A} \cdot D_{BW} + E_{D,B}) \quad (19)$$

where $E_{D,A}$ and $E_{D,B}$ are species-dependent feeding rate constants.

Dietary uptake rate constant **(k_D, kg/kg·day)**. This is the clearance rate constant for chemical uptake via ingestion of food and water, with the exception of phytoplankton, for which k_D is zero due to a lack of food uptake rates [64]:

$$k_D = E_D \cdot G_D/W_B \quad (20)$$

where G_D is the food ingestion rate in kg·food/day.

Fecal elimination rate constant **(k_E, 1/day).** The rate constant for chemical elimination via excretion into egested feces. The values for k_E typically remain relatively constant regardless of hydrophobicity and lipophilicity, except for superhydrophobic compounds [129].

$$k_E = K_{GB} / (W_B \cdot E_D \cdot G_F) \quad (21)$$

where K_{GB} is the ratio of Z_{GUT} to $Z_{ORGANISM}$ (i.e., $K_{GB} = Z_{GUT}/Z_{ORGANISM}$ and Z is the fugacity capacity), and G_F is the fecal elimination rate in kg/day.

Growth dilution rate constant (**k_G, 1/day**). The growth dilution rate constant evaluates the dilution effect of growth on chemical concentration.

$$k_G = \text{Growth rate factor} \cdot W_B^{-0.2} \quad (22)$$

where the growth rate factor is $3.5 \cdot 10^{-4}$ 1/day for invertebrates and $7.0 \cdot 10^{-4}$ 1/day for fish [111]. For marine mammals, the growth rate for killer whales were used as the best available data [111,140]:

$$k_G = 0.65/W_B \quad (23)$$

Metabolic transformation rate constant (**k_M, 1/day**). PFOA and PFOS are particularly persistent in biota such that biotransformation is considered negligible [62]. Therefore, this model assumes no biotransformation of PFAAs.

$$k_M = 0 \text{ day}^{-1} \quad (24)$$

Prey concentration (**C_D, ng/kg**). Concentration of chemical in diet.

Lung uptake efficiency (**E_L, %**). Lung uptake efficiencies in marine mammals. In this model, E_L is equal to $7.0 \cdot 10^{-1}$ for bottlenose dolphin [111].

Table 2-2. Model parameterization and methods for calculating food web bioaccumulation for the modified food web model.

Symbol	Parameter	Value	Units
	Model Parameterization		
T	Mean water temperature[a]	16.5	ºC
pH	pH of water	7.86	Unitless
W_B	Weight of organism	Variable	kg
χ_{POC}	Concentration of particulate organic carbon	$1.3 \cdot 10^{-6}$	kg/L
χ_{DOC}	Concentration of dissolved organic carbon	$4.7 \cdot 10^{-6}$	kg/L
pK_a	Acid dissociation constant	Chemical dependent	Unitless
DO	Dissolved oxygen	7.7	mg O_2/L

Methods for Calculating Food Web Accumulation			
f_{NPL}	Non-polar lipid content of organism[b]	2% (invertebrates) 4% (fish) 9% (mammals)	Unitless
f_{PL}	Polar lipid (phospholipid) content of organism	1%	Unitless
f_{PR}	Total protein content of organism	5% (plants) 13% (anthropods) 10% (other invertebrates) 18% (fish) 21% (mammals)	Unitless
f_W	Water fraction of organism	1- f_{NPL}- f_{PL}- f_{PR}	Unitless
χ_N	Neutral fraction of compound	1/(1+10^(pH-pK_a))	Unitless
χ_I	Ionized fraction of compound	1-χ_N	Unitless
D_{OW}	Octanol-water distribution coefficient	$f_N \cdot K_{OW,N} + f_I \cdot K_{OW,I}$	Unitless
D_{MW}	Membrane-water distribution coefficient	$f_N \cdot K_{MW,N} + f_I \cdot K_{MW,I}$	Unitless
K_{PW}	Protein-water distribution coefficient	Measured	Unitless
D_{BW}	Body-water distribution coefficient	$(f_{NPL} \cdot D_{OW}) + (f_{PL} \cdot D_{MW}) + (f_P \cdot K_{PW}) + f_W$	L/kg
G_V	Gill ventilation rate	$(1400 \cdot (W_B^{0.65}))/DO$	L/day
G_D	Food ingestion rate	$0.022 * W_B^{0.85(0.06 \cdot T)}$	kg_{food}/day
G_F	Fecal egestion rate	$((1-\varepsilon_{NPL}) \cdot \upsilon_{NPL}) + (1-\varepsilon_{PL}) \cdot \upsilon_{PL} + (1-\varepsilon_{PR}) \cdot \upsilon_{PR}) + (1-\varepsilon_W) \cdot \upsilon_W) \cdot G_D$	kg_{feces}/day
E_W	Gill uptake efficiency[d]	$1/(W_B \cdot (G_V^{-1} + Q_i^{-1} + (1/(Q_{mem} \cdot D_{MW} + Q_p))))$	Unitless
E_L	Lung uptake efficiency[c]	$7.0 \cdot 10^{-1}$	Unitless
E_D	Gut uptake efficiency	$1/(E_{D,A} \cdot D_{BW} + E_{D,B})$	Unitless
k_1	Uptake rate constant[e]	$E_W \cdot G_V / W_B$	Unitless
k_2	Elimination rate constant[f]	k_1 / D_{BW}	Unitless
k_M	Metabolic transformation rate constant	0 day^{-1}	Unitless
k_D	Dietary uptake rate constant	$(1/((8.5 \cdot 10^{-8}) \cdot D_{BW} + 2)) * G_D / W_B)$	kg/kg·day
k_E	Fecal egestion rate constant	$(Z_{GUT}/Z_{ORGANISM})/(W_B \cdot E_D \cdot G_F)$	day^{-1}
k_G	Growth rate constant	Growth rate factor$\cdot W^{-0.2}$	day^{-1}
IGR	Growth rate factor (invertebrate)	$3.5 \cdot 10^{-4}$	Unitless
PGR	Growth rate factor (fish)	$1.4 \cdot 10^{-3}$	Unitless

[a] Based on 2012 sampling study provided by [119].
[b] Neutral lipid fraction of all invertebrates not given in [69]; applied value assigned to arthropods.
[c] Value for bottlenose dolphin [111].
[d] Replace E_W with E_L for air-breathing species.

[e] Replace with lung uptake rate constant for air-breathing species.
[f] Replace with lung elimination rate constant for air-breathing species.

3. Methodology

3.1. Overview

To adequately evaluate the expected bioaccumulation and food web magnification behaviour of PFOA and PFOS, the parameterized model was subjected to a sensitivity analysis, and tested against other model-derived and empirical data [77]. The objective of model parameterization and testing was to examine estimates of expected bioaccumulation of PFOA and PFOS in biota throughout the food web.

Input concentrations of PFOA and PFOS were sampled in water and sediment from Charleston Harbor in a separate study. Anticipated concentrations of these compounds in biota from a marine food web were calculated by the model based on these environmental inputs, and were then compared to measured concentrations in biota from [77].

3.2. Model Testing

3.2.1. Study Area

The environmental and food web input data used within this model is from Charleston Harbor, South Carolina, USA (Figure 3-1). Charleston Harbor is adjacent to a heavily industrialized area, where an abundance of PFASs have been measured in the environment and biota, typically at higher concentrations than nearby areas (e.g., [87,96,101], and in one study, higher than any other U.S urban area examined [98]. Several studies have measured concentrations of PFASs in bottlenose dolphin from Charleston Harbor. Higher PFAS concentrations were observed in Charleston Harbor dolphins than in wildlife from other locations [39,96,98], so it is of particular interest to model accumulation patterns for these compounds within this food web. Concentration

data analyzed in this study were collected from Charleston Harbor, as well as the tributaries of Cooper, Ashley, and Wando Rivers and the Stono River estuary [77].

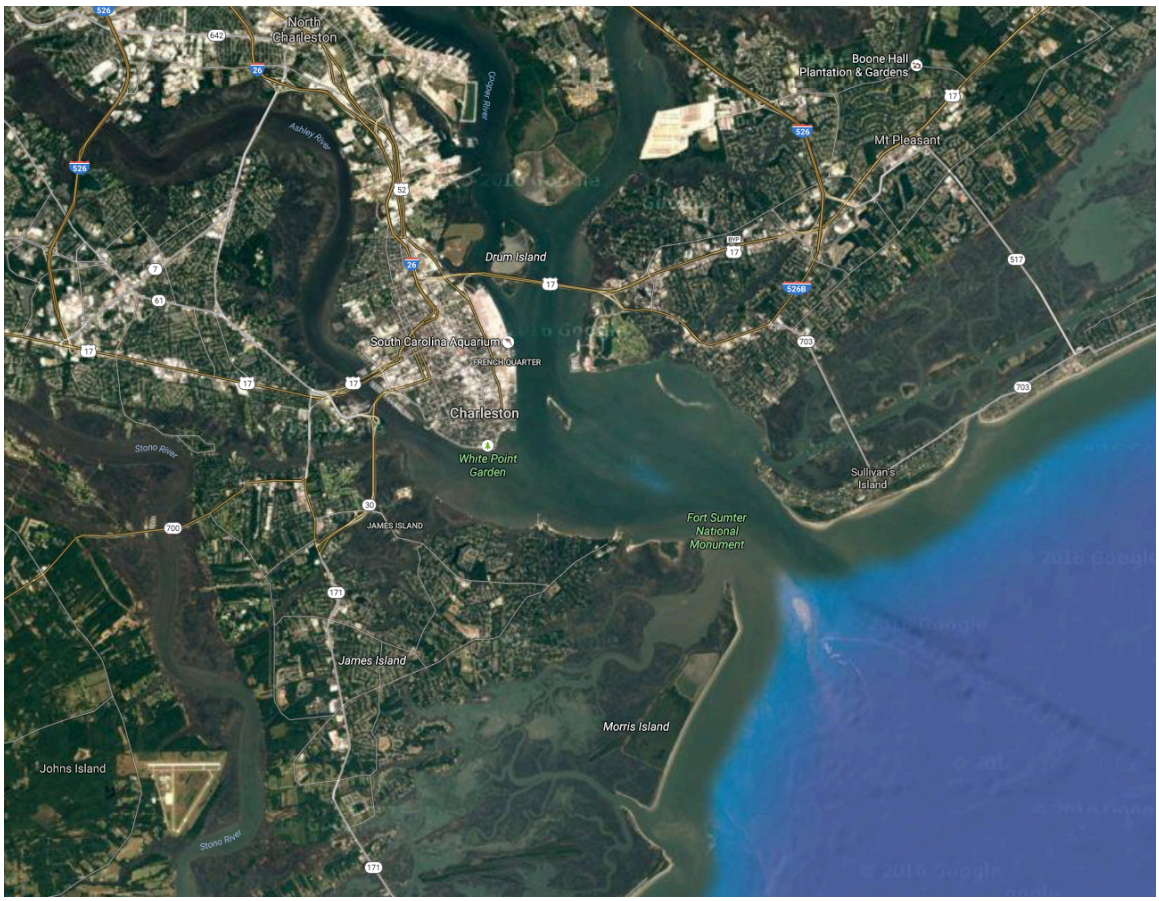


Figure 3-1. Charleston Harbor study area from Houde et al. (2006) study (Google Maps).

3.2.2. Food Web Composition

Measured concentrations of PFOA and PFOS used in this study were obtained from previous research investigating the bioaccumulation and trophic magnification in the Charleston Harbor bottlenose dolphin food web [77], where PFAS concentration data for marine water, wastewater treatment plant effluent (WWTP), sediment, striped mullet (*Mugil cephalus*), red drum (*Sciaenops ocellatus*), Atlantic croaker (*Micropogonias undulates*), spotfish (*Leiostomus xanthurus*), pinfish (*Lagodon rhomboids*), spotted seatrout (*Cynoscion nebulosus*), and bottlenose dolphin (*Tursiops truncates*) were reported. These data were used within this research to predict the measured bioaccumulation and food web magnification of PFOA and PFOS, with the exception of

WWTP, which was not considered in this modeling study. (See Houde et al. (2006) for detailed information regarding the sampling methodology used to obtain measured PFAS concentration data).

Bottlenose dolphins and all fish species examined in Charleston Harbor [77] made up part of the food web examined in this study (TLs 3.4-4.4). Although concentration data for lower trophic species (i.e., phytoplankton, zooplankton, and marine invertebrates) were not provided in [77], these species were still included in the food web model in order to calculate estimates of BCF, BMF, and TMF values. Additional species include phytoplankton (species not applicable), zooplankton (*Copepoda sp.*), oligochaete (*Monopylephorus rubroniveus*), grass shrimp (*Palamonetes pugio*), hard clam (*Mercenaria mercenaria*), Eastern oyster (*Crassostrea virginica*), and blue crab (*Callinectes sapidus*).

Analyzing and modeling the bioaccumulation of persistent environmental contaminants such as PFAAs in bottlenose dolphins is valuable, as bottlenose dolphins occupy the top trophic position in this food web (trophic level 4.4) [77]. In addition, these dolphins reside in the harbor year-round, and concentration data surrounding the dolphins' physiology and life history trends are available (as described in [77]). PFAA concentrations in Charleston Harbor bottlenose dolphins can provide insight into trophic magnification occurring throughout the food web [77,141]. Furthermore, obtaining empirical concentration measurements can be difficult for dolphins and other cetaceans because of their size, and often their status as a protected species, in addition to the time and financial investment required to carry out experiments for an entire food web [96,142].

3.2.3. Diet

Unless gut content analyses are conducted or feeding behaviours are evaluated over a period of time, diet compositions in a specific food web remain largely unknown. Dietary consumption was not included as part of [143]. To assemble suitable food web diets for marine invertebrates and fish, dietary information was obtained and modified from a previous investigation of PCBs on marine mammals [111]. When diet information did not apply to species considered within this study, diet consumption patterns were

based on available resources according to SeaLifeBase and FishBase, global databases of marine and fish species, respectively. Here, qualitative descriptions of feeding behaviour from the databases were converted into quantitative values based on the descriptions provided. For example, a 'mainly' detritivorous species was assigned a diet composition composed of >50% sediment consumption). Where sources documenting more detailed diet composition were available, this information was integrated into the model.

Information regarding the Charleston Harbor bottlenose dolphin diet was obtained from [144] (Appendix B). The model assumes a feeding rate of 6.5 kg/day/dolphin, the maximum rate in the average range given by [102,144]. Diet composition is based on feeding behaviours of adult species.

3.2.4. Environmental Input Parameters

The environmental input parameters used in the model were selected to reflect the conditions of Charleston Harbor (Table 3-1). Average measurements of water temperature, pH, dissolved oxygen, and salinity collected during sampling for a study of the concentration of PFASs in Charleston Harbor sediment were provided [98,119]. Values for dissolved organic carbon (DOC) and particulate organic carbon (POC) were obtained from the South Carolina Wildlife and Marine Resources Department [145].

Table 3-1. Environmental input parameters for Charleston Harbor, South Carolina.

Parameter	Value	Variability (+/-)	Unit	Reference
Mean Water Temperature	16.5	0.74	(°C)	Average from 2012 sampling study [119]
Mean Air Temperature	17.2	n/a	(ºC)	The Southeast Regional Climate Center - Average Coastal Water Temperature for the Southeast [146].
Mean Homeothermic Biota Temperature	37.5	1.00	(ºC)	[111]
pH of Water	7.86	0.18	Unitless	Average from 2012 sampling study [119]
Practical Salinity Units (PSU)	22.3	8.16	(g/kg)	Average from 2012 sampling study [119]
Dissolved Oxygen Concentration @ 90% Saturation (DO)	7.70	0.39	(mg O_2/L)	Average from 2012 sampling study [119]
Dissolved Organic Carbon Content - Water (OCwater)	4.70E-06	0.00	(kg/L)	South Carolina Wildlife and Marine Resources Department [147]
Particulate Organic Carbon Content - Water (POC)	1.30E-06	0.00	(kg/L)	South Carolina Wildlife and Marine Resources Department [147]
Concentration of Suspended Solids (Vss)	8.72E-05	0.00	(kg/L)	Calculated (POC/Ocsed)
Percentage of Organic Carbon - Sediment (OCsed)	1.49%	1.30	(%)	Average from [148]
Density of Organic Carbon - Sediment (Docsed)	0.9	-	(kg/L)	Mackay, 1991 [149]
Setschenow Proportionality Constant (SPC)	0.0018	-	(L/cm^3)	Xie et al., 1997 [150]
Molar Concentration of Seawater @ 35 ppt (MCS)	0.5	-	(mol/L)	Xie et al., 1997 [150]
Absolute Temperature (K)	273.16	-	K	
Ideal Gas Law Constant	8.314	-	($Pa.m^3$/ mol.K)	

3.3. Concentration Normalization

Data from measured concentrations and model predictions were expressed in terms of multiple tissues and normalized to total protein. All concentrations of PFOA and PFOS in the food web were compared based on protein-normalized (pw) values. Overall, the process considers the relative solubility of chemicals in neutral lipids, polar lipids (i.e., phospholipids), and protein, but is expressed as a protein-equivalent concentration (ng chemical/kg equivalent protein).

PFAAs have a higher affinity for protein than do many lipophilic POPs such as DDT and PCBs. PFAAs are primarily protein-binding compounds [80,112]. For this reason, it is necessary to normalize the measured and predicted concentrations to the fraction of protein within organisms [73]. Expressing concentrations according to the fraction of protein (serum albumin, specifically, as a model protein) in an organism enables concentrations to be compared similarly between different sources (here, the reported Charleston Harbor values and estimated concentrations from the food web model). This process ensures a uniform evaluation of accumulation and magnification by expressing the concentration according to the tissue with the highest affinity for the compound.

Serum albumin, an abundant blood protein in many species, plays an important role in the transport of organic ligands, both natural and xenogenous. For instance, it is the most likely candidate for PFCA interactions in human blood [151]. Because fatty acids are a major albumin ligand, and PFCAs resemble fatty acid structure, potential interaction between serum albumin and PFAAs is important (i.e., investigating interference with endogenous ligands), particularly given the ubiquity of PFAAs [151]. In fish, albumin can compose between one-third and one-half of total protein in blood [152]. In their bioconcentration model, Ng and Hungerbuhler (2013) determined that the concentration of albumin in the blood and liver interstitial fluid was one of the most important factors affecting BCFs values for PFAAs in blood and liver, respectfully [84]. Xia et al. (2013) also found that partition coefficients of PFASs were higher for bovine albumin than soy peptone [80].

It is acknowledged that serum albumin is not the only protein of concern for chemical sorption within organisms. Research has also evaluated sorption of IOCs to structural proteins such as muscle protein, as they are often present in high quantities and are polar, with the potential for interaction with ionic compounds, including anionic PFAAs. It was found that the muscle protein-water partition coefficient was low for ions, and that anionic chemicals sorb preferentially to BSA over muscle protein by approximately 3.5 orders of magnitude [153]. Often, serum albumin is the only type of protein for which sorption data are available, so all protein will be represented as albumin within modeling studies, despite suggestions that serum albumin and muscle protein should be considered separately due to differences in sorption of IOCs between the protein types [153]. Therefore, even though partitioning to serum albumin does not account for all protein within an organism, understanding the behaviour of PFAAs with respect to serum albumin remains a vital component of better understanding bioaccumulation of PFAAs in food webs.

To evaluate the difference in sorption capacities between different tissues, the goal was to calculate how many times more or less sorptive each tissue is relative to other tissues. Therefore, concentrations of PFAAs in non-polar lipid, phospholipids, and water are expressed in terms of concentration in serum albumin. PFAA concentrations were normalized to protein, while also accounting for the relative sorption to non-polar lipids, phospholipids, and protein. This is done by calculating the concentrations in these media in relation to their corresponding concentrations in the protein fraction of the organism:

$$C_{NPL} = K_{NPL\text{-}P} \cdot C_P \quad (25)$$

$$C_{PL} = K_{PL\text{-}P} \cdot C_P \quad (26)$$

$$C_w = K_{W\text{-}P} \cdot C_P \quad (27)$$

In order to determine the contribution of each chemical storage medium to the bioaccumulation of PFAAs (i.e., neutral lipids, phospholipids, protein, and water), the total mass of PFAAs in each tissue is expressed as the sum of the masses of the three storage media considered, in addition to water:

$$M_{TOTAL} = M_{NPL} + M_{PL} + M_{PR} + M_W \quad (28)$$

where M_{TOTAL} is the total mass of the chemical in an organism, M_{NPL} is the mass of non-polar lipids, M_{PL} is the mass of polar lipids, M_{PR} is the mass of protein, and M_W is the mass of water in the organism. Further, the total mass can be expressed as the sum of the concentration and volume of each tissue :

$$M_{TOTAL} = C_{NPL} \cdot V_{NPL} + C_{PL} \cdot V_{PL} + C_{PR} \cdot V_{PR} + C_W \cdot V_W \quad (29)$$

where C_X represents concentrations of each tissue in the organism, and V_X represents the volumes of each tissue in the organism. To determine the total concentration of a contaminant in each tissue, we can divide by the total volume:

$$C_{total} = C_{NPL} \cdot f_{NPL} + C_{PL} \cdot f_{PL} + C_{PR} \cdot f_{PR} + C_W \cdot f_W \quad (30)$$

where f represents the fraction of tissue in an organism.

Whereas previous models did not normalize measured concentrations, or normalized the concentrations based on only one tissue, such as lipids or protein, this methodology ignores the influence and sorption of the substance to other tissues, which may influence the overall food web magnification patterns. It is important to consider the relative sorption of non-polar lipids, polar lipids (i.e., phospholipids), and protein in order to increase the chemical behaviour of PFAAs and other contaminants predictions in aquatic food webs.

Measured concentrations were then normalized and expressed the same way as model calculations by protein normalizing and expressive relative to all tissues [77]:

$$C_{NORM} = C_B/(f_{PR} + (K_{NPL\text{-}PR} * f_{NPL}) + (K_{PL\text{-}PR} * f_{PL}) + (K_{W\text{-}PR} * f_W)) \quad (31)$$

where C_B is the geometric mean of the measured concentrations of a PFAA in an organism, and $K_{NPL\text{-}PR}$, $K_{PL=PR}$, and $K_{W\text{-}PR}$ are the partition coefficients of non-polar lipid, polar lipid, and water, respectively relative to protein.

3.3.1. PFOA and PFOS Concentration in Environmental Media

Water and bottom sediment were sampled in Charleston Harbor in 2004 and analyzed for PFAA concentrations (see Houde et al. (2006) for detailed methodology). The pore water concentration (i.e., concentration in water between grains of sediment) was calculated for PFAAs based on the fraction of organic carbon in sediment and sorption to protein:

$$C_P = C_{sed}/(OC_{sed} \cdot DOC_{sed})/0.35 \cdot K_{PW} \quad (32)$$

where C_P is the pore water concentration of PFAAs, C_{sed} is the concentration of PFAAs measured in sediment [77], DOC_{sed} is the density of organic carbon in sediment (0.9 kg/L), and K_{PW} is the protein-water partition coefficient, which indicates the partitioning from water to protein. Pore water concentrations are important when considering bioaccumulation potential for organisms such as benthic invertebrates and fish which are frequently in contact with bottom sediment, as chemicals are available for exchange with biota in concentrations above that of measured water concentrations [64,154]. This can impact bioaccumulation if the sediment-water column disequilibrium is large. Freely dissolved water concentrations represent the amount of chemical available for uptake in the water medium. For more hydrophobic compounds, this is not necessarily the same as the water concentration measured, as they have a high affinity for organic matter (i.e., DOC, POC), and are unavailable for uptake through diffusion [64,155-157]. However, because PFOA and PFOS do not have notably high K_{OW}s, the freely dissolved water concentrations of PFAAs were assumed to be the same as the total measured water concentrations in this version of the model.

It is assumed that the concentration of PFAAs in air is zero, given its high K_{OA} and lack of volatility of long-chain perfluorinated acids [73]. Neutral fractions of PFOA and PFOS have appreciable Henry's Law Constant values, but this does not play a large role in sorption because these compounds are virtually completely ionized at relevant pH.

3.3.2. Biota Body Weights and Composition

The mass of each aquatic organism in the food web was estimated based on the hypothetical food web described in [158], ranging from 10^{-7} kg to 1 kg, mass values were not provided in the Houde et al study (Table 3-2). Fish were categorized as 'small' (10^{-2} kg), 'medium' (10^{-1} kg), or 'large' (1.0 kg). Pore water ventilation values were selected according to fish types. To remain consistent with the observed bioaccumulation measurements, the bottlenose dolphin mass used was that of the deceased dolphin from Charleston Harbor (708.4 kg) reported in [77].

For fish and marine mammals, trophic level was assigned based on stable nitrogen isotope ($\delta^{15}N$) analysis (explained further in [113]):

$$TL = 2 + (\delta^{15}N_{consumer} - \delta^{15}N_{zooplankton})/3.8 \quad (33)$$

For invertebrate species not evaluated in the field study, trophic levels were assigned based on the dietary composition table presented in Alava et al. (2012) or described in SeaLifeBase and FishBase [111].

Table 3-2. Weights assigned to species within the Charleston Harbor bottlenose dolphin food web used to calculate BCFs, BMFs, and TMFs (from Houde et al., 2006 and Gobas et al., 2015).

Organism Type	Organism Name	Trophic Level	Wet Weight (kg)	Pore Water Ventilation (%)
Phytoplankton	n/a	1.0	n/a	n/a
Zooplankton	Copepoda	2.0	10^{-7}	0
Invertebrate	Oligochaete	2.1	10^{-4}	100
	Grass shrimp	2.2	10^{-3}	5
	Hard clam	2.2	10^{-2}	5
	Eastern oyster	2.3	10^{-2}	5
	Blue crab	2.8	10^{-2}	5
Fish	Striped mullet	3.4	10^{-1}	5
	Pinfish	3.9	10^{-1}	5
	Red drum	4.2	1.0	5
	Atlantic croaker	4.2	1.0	5
	Spotfish	4.3	1.0	0
	Spotted seatrout	4.3	1.0	0
Marine Mammal[a]	Bottlenose dolphin	4.4	$7.08 \cdot 10^{2}$	n/a

[a] Bottlenose dolphin weight obtained from deceased female dolphin in Charleston Harbor [77].

Fractions of non-polar (neutral) lipids, phospholipids, and protein in each organism are described in (Table 3-3) [69]. See Appendix C for an overall review of inputs for species specific biological and physiological parameters for the food web model).

Table 3-3. Fraction (%) of non-polar lipid, polar lipid, protein, and water within each species evaluated in the food web model (anthropods, invertebrates, fish, and mammals). Tissue fractions of organisms evaluated in marine food web model (from Hendriks et al., 2005).

Species Type	Non-Polar (Neutral) Lipid Fraction	Polar Lipid Fraction	Protein Fraction	Water Fraction
Arthropods	2%	1%	13%	75%
Other Invertebrates	2%[a]	1%	10%	69%
Fish	4%	1%	18%	77%
Mammals	9%	1%	21%	69%

[a] Neutral lipid fraction not identified for 'other invertebrates'; fraction of 2% identified for arthropods extended to other invertebrates.

3.3.3. Comparison to Empirical Food Web Data

Note that PFAA concentrations for phytoplankton, zooplankton, and marine invertebrates were not collected in the Charleston Harbor food web. Therefore, direct comparisons of measured concentration with model-calculated concentrations were limited to fish and marine mammal species. Concentrations of PFOA and PFOS for species from the bottlenose dolphin food web were calculated using the modified model, in one scenario considering only the species assessed in the field study (i.e., TLs 3.4 to 4.4) using the measured water and sediment concentrations as input values, and in a second scenario considering all species in the food web for which the model is capable of evaluating (i.e., TLs 1 to 4.4).

Whole body homogenates were measured for fish. PFAA concentrations were measured in the plasma of living dolphins in Charleston Harbor, as well in the individual organs of a deceased dolphin in the study area [77].

Measured concentrations for several fish (striped mullet and pinfish) and bottlenose dolphin were reported as <0.5 ng/g (below detection). For both fish and dolphin, multiple nondetect values were reported for PFOA and PFOS. To maintain consistency with the methodology used in the field study, random values (less than half of the minimum detection limit, or MDL) were used to replace nondetect values for the

calculation of means. These values are not expected to be the same as those calculated and used in the original research, though the same range (below half of the MDL) was utilized in the field study.

Where measured concentration values were below the MDL but above the instrument detection limit (IDL), the reported value was used, assuming that such concentrations were reasonable for the bottlenose dolphin food web.

Geometric means were calculated from individual concentrations of PFOA and PFOS measured in Charleston Harbor water, sediment, fish, and dolphin [119]. Because the range of concentrations varies between species in the food web, the geometric mean was used in order to obtain a suitable average of the ranges to ensure that no single concentration range rules the calculation of the mean.

3.4. Sensitivity Analysis

Sensitivity analyses were conducted to determine the variability within calculated concentrations. If the variability in calculated concentrations is smaller than the variability in observed concentrations from Charleston Harbor, it may indicate that there are factors in real life that are not being accounted for by the model [144].

Several input parameters involved in the calculation of TMF were evaluated in a sensitivity analysis. To test model uncertainty, @RISK was used to measure the sensitivity of model-calculated TMFs to the following parameters: water temperature, water pH, fraction of chemical ionized, log $K_{OW,}$ and log K_{PW}. These parameters were chosen based on at least one of the following criteria: the parameter had not previously appeared in the food web model, the parameter was associated with ionization and potential effects on bioaccumulation behaviours of IOCs, the parameter was location-specific and therefore subject to fluctuation or improper measurements (i.e., temperature and pH), or because there is inherent uncertainty in the values themselves (i.e., partition coefficients).

4. Results and Discussion

4.1. Partition Coefficients

Non-polar lipid-water, polar lipid-water, protein-water, and water distribution (or partition) coefficients were compared for PFOA, PFOS, and PCB 153 (a neutral, lipophilic compound) to determine which tissues are most important in the accumulation of lipophilic substances with a high affinity for protein, specifically albumin (Figure 4-1).

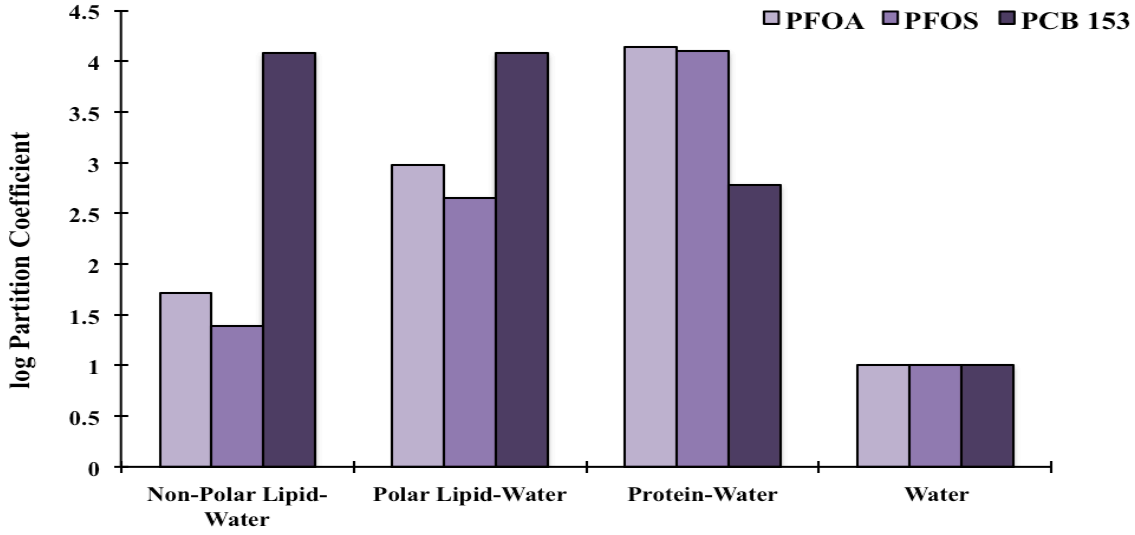


Figure 4-1. Tissue-water distribution or partition coefficients for non-polar lipid-water (neutral) lipid (log D_{OW}), polar lipid-water (log D_{MW}), protein-water (log K_{PW}), and water for PFOA and PFOS, as well as PCB 153 (a neutral, lipophilic compound). The non-polar lipid-water distribution coefficient is elevated for PCB 153 compared to PFOA and PFOS, whereas the protein-water partition coefficient is higher for PFAAs. Note that because PCB 153 is not an IOC, the membrane-water partition coefficient for this compound is assumed to be equivalent to log D_{OW} for PCB 153.

Values for both log D_{MW} and log K_{PW} for PFAAs are higher than log D_{OW} values for these compounds. This observation is consistent with reports suggesting that sorption of

IOCs (including PFAAs) to polar membranes and protein are more important for bioaccumulation compared to sorption to non-polar lipids [62,85]. These sorption patterns are in contrast to that of neutral, hydrophobic compounds, which sorb preferentially to non-polar lipid and bioaccumulate in tissues with high quantities of non-polar molecules in aquatic organisms [92].

There are notable differences between the distribution (or partition) coefficients for PFOA and PFOS compared to PCB 153. Affinity for non-polar lipid (log D_{OW}) is more than two-fold higher for PCB 153 than for PFOA and PFOS. This is due to the higher log K_{OW} value of PCB 153 (log K_{OW} of PCB 153 = 7.18). For neutral compounds such as PCBs, log K_{OW} ≈ log D_{OW}. This occurs because PCB 153 is not subject to ionization at environmental or physiological pH, and therefore the contribution of the ionized fraction (i.e., K_{MW}) to the distribution coefficient is negligible. These values are consistent with the lipophilic nature of PCBs [2]. Membrane-water partitioning is not as relevant for PCBs as for PFOA and PFOS, as this group of neutral compounds has little interaction with polar membranes [159]. Ionogenic compounds, on the other hand, such as PFOA and PFOS, interact with polar membranes, and have a higher affinity for phospholipids than neutral lipids, based on the concepts and calculations presented in Armitage et al. (2013). This theory states that charged species have a high affinity for phospholipids, and can lead to bioaccumulation of IOCs in biota. This is due to electrostatic interactions that occur between the charged species and the various components of the phospholipid membranes (i.e., electrostatic interactions with the zwitterionic head group and specific or nonspecific interactions elsewhere. [62].

Protein-water partition coefficients (K_{PW}) are higher for PFOA and PFOS compared to PCB 153. This is consistent with the protein-binding nature of PFAAs. Previous work has emphasized the need to account for protein sorption in bioaccumulation models to allow for adequate predictions of PFAA concentrations throughout food webs [85]. Such relationships are already established for neutral compounds ($K_{PW} = 0.05 \cdot K_{OW}$; see [79]); however, this rule is not necessarily applicable to IOCs. According to this method, log K_{PW} of PCB 153 is equal to 2.8, approximately 20 times lower than the log K_{PW} of PFOA and PFOS, signifying that protein partitioning is less relevant to overall bioaccumulation of neutral substances.

Body-water distribution coefficients (log D_{BW}) for PFOA and PFOS vary depending on the source of chemical properties used in the calculation (e.g., log K_{OW}). The D_{BW} values calculated for PFOA and PFOS in this model are lower than log K_{OW} values for these compounds. Nevertheless, log K_{OW} is used in many models to calculate bioaccumulation, even though this coefficient is most appropriate for estimating partitioning into non-polar lipids (Table 4-1). The lower body-water distribution coefficients applied in this approach represent the distribution and relative affinity of PFOA and PFOS within multiple biological media.

Table 4-1. Partition coefficient values for PFOA and PFOS used to calculate concentrations in an aquatic food web. This modified model is able to account for different partition coefficients of neutral and ionic chemical speciation, as well as non-polar and polar tissues.

	log K_{OW}[a] (Neutral, Non-Polar)	**log K_{OW}[b]** (Ionic, Non-Polar)	**log D_{OW}[b]** (Non-Polar)	**log K_{MW}[b]** (Neutral, Polar)	**log K_{MW}[b]** (Ionic, Polar)	**log D_{MW}[b]** (Polar)	**log K_{PW}[c]**	**log D_{BW}[b,d]**
PFOA	4.81	1.71	2.1	4.98	2.98	3.0	4.14	3.14 (P, Z, MI1,3,4) 3.26 (MI2,5) 3.40 (F) 3.46 (MM)
PFOS	4.49	1.39	2.6	4.65	2.65	2.7	4.10	3.10 (P, Z, MI1,3,4) 3.22 (MI2,5) 3.36 (F) 3.42 (MM)

[a] Calculated using KOWWIN v.1.68.
[b] Calculated at pH = 7.9 using methodology described in [62].
[c] Measured experimentally [82].
[d] P = phytoplankton; Z = zooplankton; MI*x* = marine invertebrate; F = fish; MM = marine mammal.

4.2. Ionization

The pK_a values used for PFOA and PFOS in the model (3.4 and 4.0, respectively) are among the highest reported values [160]. It is acknowledged that lower pK_a values are likely more accurate [93]. Ionization patterns remain largely uncertain for

PFAAs. For instance, under- or over-estimation of pK_a will underestimate the fraction of chemical in neutral and ionized form, respectively. Such errors may affect estimates of chemical sorption. If the proportion of neutral compound is underestimated, the model will assume less sorption capacity (and less bioaccumulation), and vice versa [62]. Regardless, substituting lower pK_a values into the model had a negligible impact on model results. Application of all practical pK_a values at pH = 7.9 resulted in >99% ionization for both PFOA and PFOS. It is important to recognize that ionization is a considerable source of uncertainty in the model.

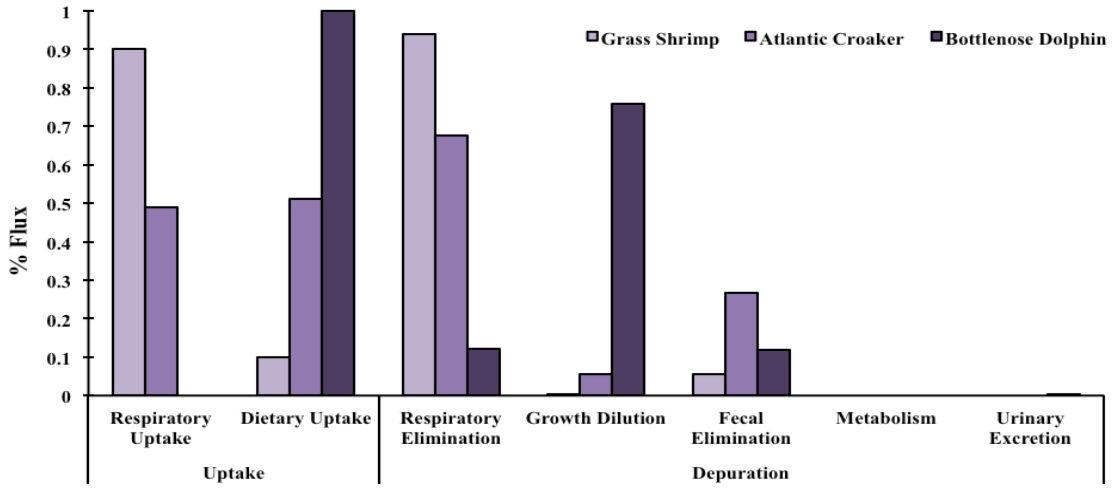
Assuming pK_a values of 3.4 and 4.0, respectively, PFOA and PFOS were virtually fully ionized (99.997% and 99.986%, respectively) at pH = 7.9. Because the pK_a values of these substances are below the pH of water, their bioaccumulative behaviour is only slightly affected by fluctuations in pH. For instance, the water immediately surrounding fish gills typically drops to pH ≈ 4.2 due to increased CO_2 levels from gill respiration, resulting in increased acidity [161]. Even at this relatively low pH, more than half of PFOA and PFOS remain ionized, according to model predictions (86% and 61% ionized, respectively). Similarly, in the stomach, pH ≈ 4, and a higher fraction of neutral compound is anticipated. In this scenario, where a low pH results in a higher fraction of neutral PFASs, there is a decrease in bioaccumulation of PFOA and PFOS with respect to all bioaccumulation metrics (BCF, BMF, and TMF) with decreasing pH. Although changes in speciation with varying pH is not important for PFOA and PFOS, it may substantially affect bioaccumulation behaviour of other IOCs (see [162]). These modifications to the model allow for the evaluation of chemical speciation as a function of pH, which was not evaluated in previous versions of this model.

4.3. Chemical Uptake and Elimination

Dynamics of uptake and elimination for PFOA and PFOS in a marine food web were calculated by the model based on chemical properties, environmental characteristics, diet, and species physiology. Relative contributions of various uptake and elimination routes for PFOA and PFOS are illustrated here using calculated concentration fluxes for three different species in the food web: marine invertebrate (grass shrimp), fish (Atlantic croaker), and marine mammal (bottlenose dolphin) (Figure

4-2; see Appendix D for rate constant values for all species). Influx and efflux patterns differ considerably between species, but mostly correspond to physiological characteristics.

(a) PFOA



(b) PFOS

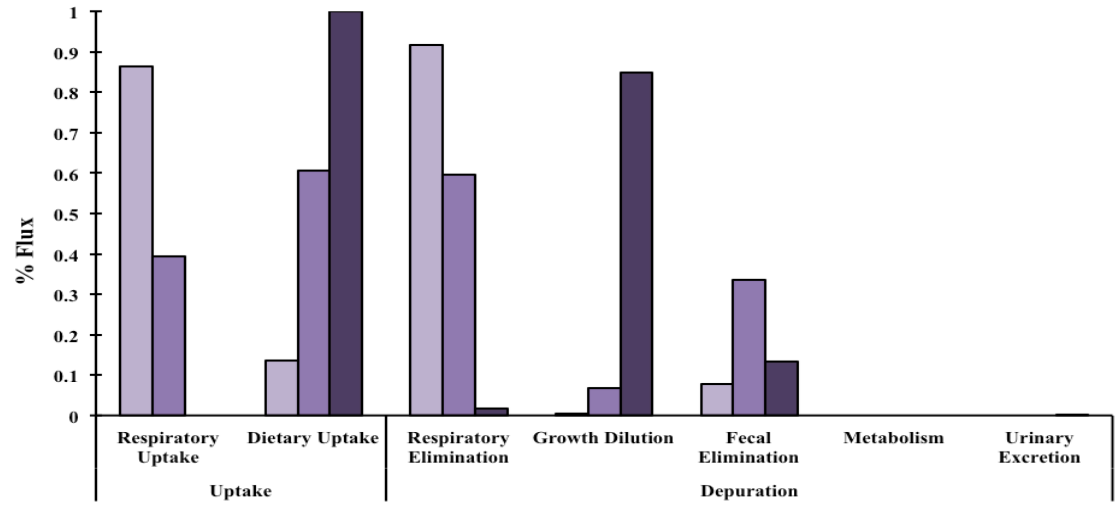


Figure 4-2. Relative fraction of chemical uptake and elimination fluxes for (a) PFOA and (b) PFOS, calculated for select species in a marine food web. Respiratory uptake via gill respiration is more important for lower trophic level aquatic species, whereas dietary uptake is more relevant for the air-breathing bottlenose dolphin. Elimination rate constants vary between species, but are mostly restricted to respiratory elimination (k_2), fecal elimination (k_E), and growth dilution (k_G). Note that biotransformation (k_M) is not applicable for PFOA and PFOS in this model.

Marine Invertebrate

Respiratory uptake and elimination are more important for lower level trophic species compared to other routes of exposure, driven by the water-respiring nature of invertebrates and fish. Large volumes of water pass through the gills to allow for exchange, resulting in a high respiratory uptake and elimination rates [162]. Although bioaccumulation of PFAAs has been documented in both aquatic and terrestrial organisms, these substances are more easily eliminated into surrounding water environments compared to air because of their relatively low K_{OW} compared to other legacy POPs [2]. Considering that the exposure of invertebrate species to PFOA and PFOS is dominated by water respiration, substantial bioaccumulation of PFAAs should not be a major concern in marine invertebrates, though this is not anticipated to hold true for higher trophic level organisms.

Fish

Respiratory and dietary uptake both contribute to chemical flux for Atlantic croaker (approximately 50% and 60% of uptake is via dietary uptake for PFOA and PFOS, respectively), highlighting the potential for both bioconcentration and biomagnification in this species. Respiratory elimination is important for fish (approximately 60% of total efflux), for similar reasons as described for invertebrates.

The Atlantic croaker experiences the highest proportion of fecal elimination flux (about 40% of total efflux) compared to the invertebrate and marine mammal. High fecal excretion rates are inconsistent with studies reporting negligible contributions of fecal elimination to total depuration for hydrophobic substances (e.g., PCBs) in fish [163]. This discrepancy may be attributable to the reduced hydrophobicity of PFAAs compared to other POPs.

Marine Mammal

Because PFAAs are non-volatile compounds, uptake from air is considered negligible for bioaccumulation [126]. Therefore, lung uptake was not considered for marine mammals in the model. This is likely because pulmonary respiration (utilized by

marine mammals but not invertebrates and fish) is a more efficient process than gill respiration, requiring smaller volumes of oxygen-containing media to achieve sufficient gas exchange [162]. Dietary uptake was the only relevant route of uptake for the dolphin. Likewise, low respiratory elimination rates in marine mammals is consistent with the understanding that higher-K_{OA} substances are not readily eliminated from air-breathing organisms via exhalation due to slow transport from biota to air [73].

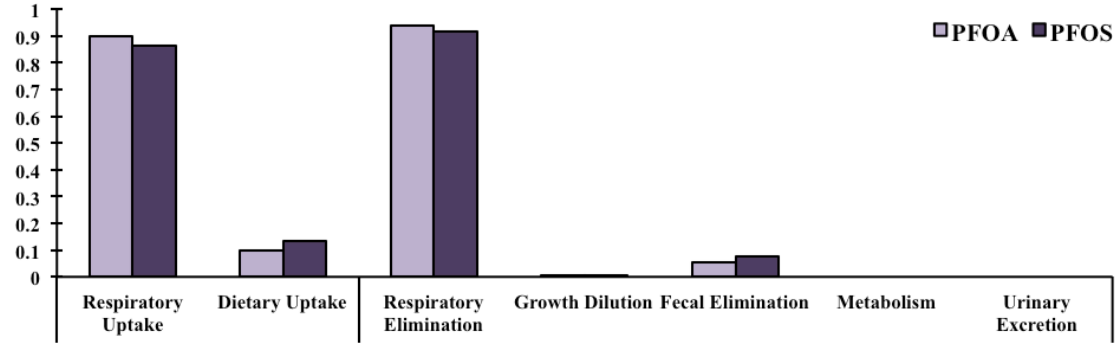
Relatively, growth dilution is highest for bottlenose dolphin, accounting for approximately 75% of apparent elimination for PFOA and 85% of apparent elimination for PFOS. Marine mammals have considerably larger masses than all other species in the food web. In this model, bottlenose dolphin mass is 708 kg, whereas the next largest species (i.e., spotted seatrout) has a mass of only 1 kg. Therefore, dilution resulting from mammal growth is a main source of apparent chemical elimination for marine mammals, suggesting that patterns of PFAA accumulation may vary as dolphins age and grow. This model suggests a general positive relationship between species mass and the contribution of growth dilution to overall elimination rate constants for PFOA and PFOS in marine mammals.

In this model, urinary excretion applies only to marine mammals. Given that marine mammals are large animals, urinary excretion is expected to occur at a higher rate than is observed in the model. Low urinary excretion rate constants are not consistent with high empirical concentrations of PFAAs measured in bottlenose dolphin urine. Although studies have highlighted the importance of urinary excretion for the overall elimination of PFAAs, such observations are not reproduced in this model [104]. The model does however estimate a urinary excretion rate (G_U) of 0.26 L/day, and a urinary excretion rate constant (k_U) of $1.1 \cdot 10^{-7}$ day^{-1} for PFOA and $1.2 \cdot 10^{-7}$ day^{-1} for PFOS, consistent with urinary excretion rates in other mammals [111]. The difference here is that the previous studies investigated absolute concentrations eliminated via urinary excretion, whereas this assessment evaluates urinary excretion compared to other elimination routes. The model estimates that 34.6 ng/L of PFOA and 48.2 ng/L of PFOS are excreted daily via urine. Though this is not a substantial quantity compared to other routes of elimination, this is likely comparable with reported concentrations measured in dolphin urine, though these concentrations were measured in ng/g and according to wet weight concentrations [104]. For instance, a previous study by Houde

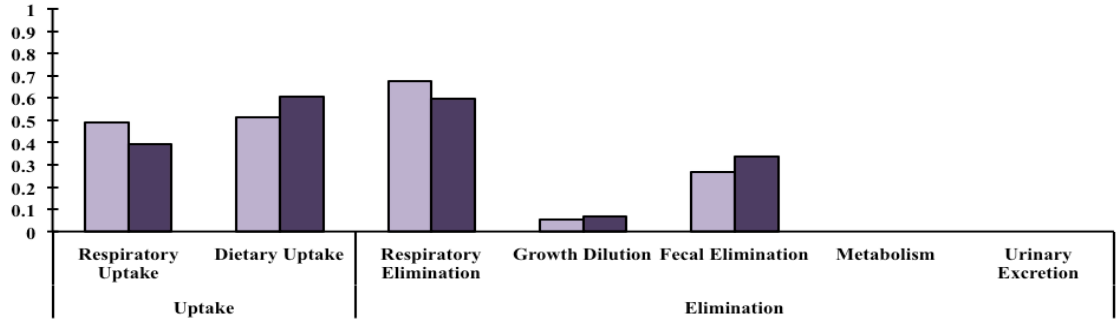
and colleagues reported 25.6 ± 78 ng/g ww of PFOS detected in urine [104]. No information was available for PFOA, as concentrations in urine were below the MDL. The authors also note that the excretion of PFAAs in urine decreased for compounds with a chain length between 8 to 11 carbons. This could explain the small quantity of PFOS eliminated in urine, as this compound has 8 fluorinated carbons. It appears as though the absolute quantity of PFOA and PFOS eliminated through urine was not negligible for the model, but rather urinary excretion is not considered substantial compared to other routes of elimination, such as growth dilution and fecal elimination. Additionally, there may be quantities of PFOA and PFOS subject to uptake by bottlenose dolphins not accounted for in this model, as PFAAs can be present as marine aerosols and in the boundary layer [164,165]. Therefore, the fraction of PFOA and PFOS expected to be present through respiratory uptake may not be negligible when the boundary layer and marine aerosols are included in the analysis; however, data is largely insufficient at this time to include such conditions in the model.

Intra-species evaluation of PFOA and PFOS fluxes were also conducted to compare chemical uptake and elimination of both compounds within the same species **(**Figure 4-3).

(a) Grass shrimp



(b) Atlantic croaker



(c) Bottlenose dolphin

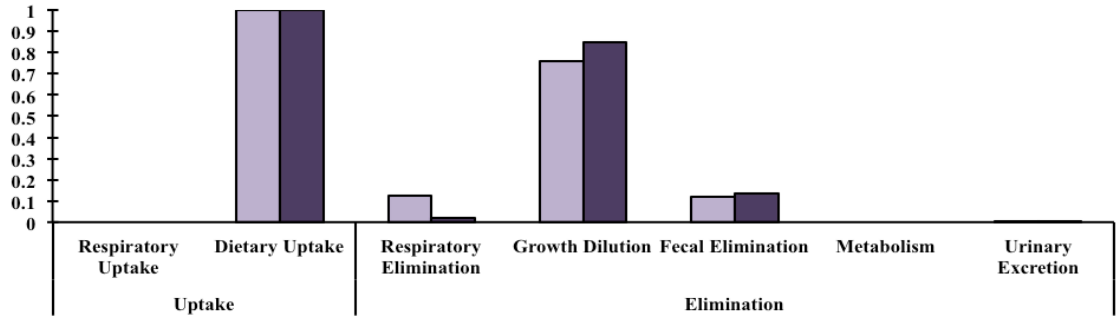


Figure 4-3. Relative chemical fluxes of PFOA and PFOS for various uptake and depuration routes expressed as the fraction of total uptake or depuration flux for (a) grass shrimp, (b) Atlantic croaker, and (c) bottlenose dolphin in a marine food web. Differences in fluxes are related to animal physiology and physicochemical properties of PFAAs.

Respiratory uptake and elimination are somewhat larger contributors to the overall PFOA flux than PFOS flux in aquatic species (about 0.5% higher in grass shrimp and 1% higher for Atlantic croaker), whereas dietary uptake and fecal elimination flux are higher for PFOS. For dolphin, chemical uptake of both compounds occurs exclusively via diet, but in terms of efflux, respiratory elimination is approximately 12-fold higher for PFOA than PFOS, where respiratory elimination of PFOS is effectively negligible. Meanwhile, growth dilution and fecal elimination contribute more than respiratory elimination to the depuration of PFOS in bottlenose dolphin. The large difference in respiratory elimination between PFOA and PFOS is likely related to the variation in K_{OA} values. The K_{OA} for PFOS is roughly four times higher for PFOS than for PFOA, indicating a greater inability for PFOS to move from biota to air compared to PFOA. Overall, however, respiratory elimination and fecal elimination of both PFOA and PFOS are low compared to growth dilution, which accounts for 76% and 85% of total depuration for PFOA and PFOS (respectively) in dolphin.

4.4. Estimated Concentrations of PFOA and PFOS in Biota

Protein-normalized PFOA and PFOS concentrations in biota were estimated for a full food web (TLs = 1 to 4.4), including phytoplankton, zooplankton, and marine invertebrates (hypothetical; derived according to species evaluated in Alava et al. (2012) and not measured in Charleston Harbor study), in addition to fish and marine mammals (measured in Charleston Harbor study; Figure 4-4; see Appendix E for full food web concentration values). Concentrations of PFOA and PFOS in biota increase throughout the modeled food web ($p < 0.05$; $r^2 = 0.32$ and 0.34, respectively), implying that these chemicals are subject to bioconcentration and biomagnification. Concentrations of PFOA and PFOS in dolphin are five and six times higher than in water (respectively) and five times higher than that in spotted seatrout.

High dietary uptake rate constants in the model are responsible for bioaccumulation in dolphins. Without the ability to effectively eliminate PFOA and PFOS via exhalation, the remaining elimination pathways (i.e., urinary and fecal excretion) become important for chemical removal. However, the rate constants for these remaining elimination routes are low compared to dietary uptake rate constant (dietary

uptake rate constants range from 10^1 higher than for fecal elimination to 10^4 higher for urinary excretion).

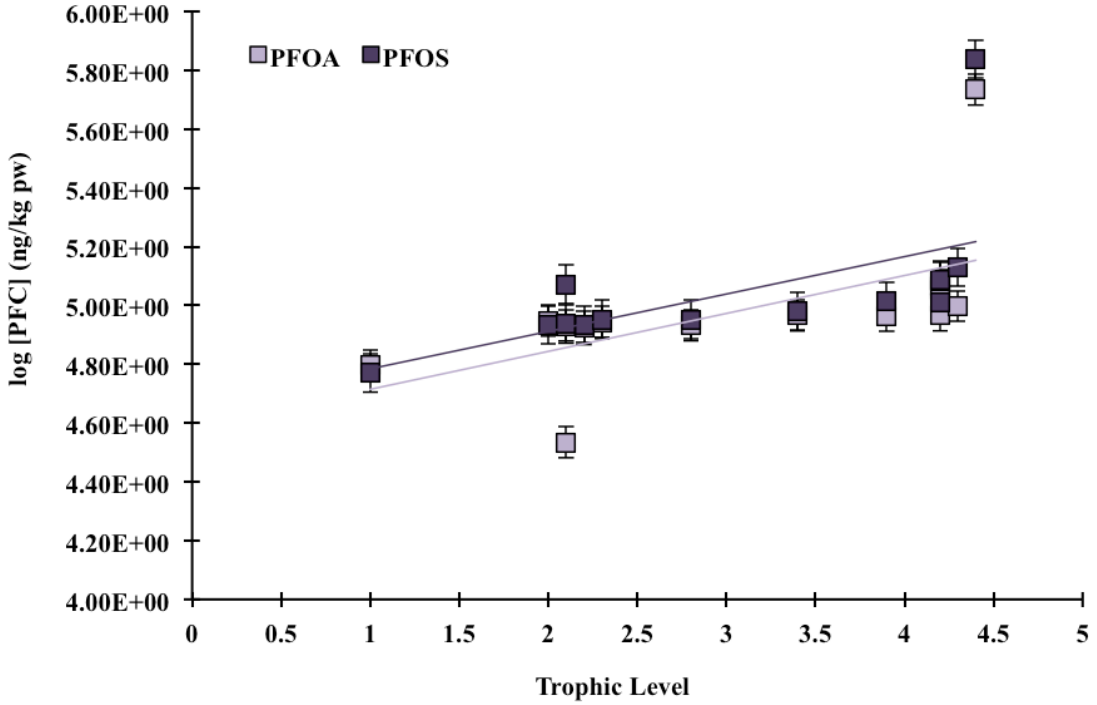


Figure 4-4. Model-estimated concentrations of PFOA and PFOS (log ng/kg) ±1 standard error in a marine food web (including phytoplankton, zooplankton, marine invertebrates, fish, and marine mammal). Increasing concentrations of PFOA and PFOS throughout the food web ($p < 0.05$) indicates that biomagnification occur in this food web. Input water (ng/L) and sediment (ng/kg) concentrations obtained from Charleston Harbor (Houde et al., 2006).

Estimated concentrations of PFOA and PFOS in biota are not significantly different from each other. Model estimated concentrations of PFOA are higher than concentrations of PFOS in biota for lower trophic level organisms, including phytoplankton (TL = 1) and zooplankton (TL = 2). This may be because the body-water distribution coefficient values (log D_{BW}s) are larger for PFOA than for PFOS (see Table 4-1), which is more important for bioconcentration in lower trophic level organisms due to substantial gill respiration. However, in higher trophic level organisms, where PFOS shows greater accumulation via dietary intake, the model suggests that PFOS concentrations may be higher than PFOA. Concentrations of PFOA and PFOS increase considerably in marine mammal (9- and 12-fold greater, respectively, for PFOA than

PFOS) compared to phytoplankton, revealing evidence for bioaccumulative properties of these compounds within the bottlenose dolphin food web, given water and sediment levels sampled in Charleston Harbor [77]. Additionally, concentrations of PFOS in sediment are higher than that of PFOA, which may contribute to higher PFOS concentrations in higher trophic level biota, as trophic magnification occurs throughout the food web.

The concentration of PFOA in the marine invertebrate oligochaete (TL = 2.1) should be further investigated due to its inconsistency with the general patterns of the chemical in the food web. The low concentrations of PFOA compared to PFOS are the result of an average concentration of PFOA in sediment that is almost 4 times lower than that of PFOS. This impacts oligochaete because 90% of its diet is from sediment, which is higher than that in other invertebrates. Linear regressions reveal that the TMF is not sensitive to this apparent outlier.

It is also noted that FOSA concentrations from the study area were <1% that of PFOS, suggesting that contributions to PFOS body burden from precursor can be considered negligible in this particular food web.

4.4.1. Tissue Distribution

Chemical concentrations in each organism – normalized to non-polar lipid, polar lipid, and protein – depends on the biochemical composition of its tissue. This is demonstrated here using the characteristics of fish species from the model as an example (Table 4-2). The product of the distribution or partition coefficients (i.e., D_{XW} or K_{XW}) and total fraction of each tissue (i.e., ϕ_X) expresses the relative mass of PFAA expected in each compartment. Total protein makes up the largest tissue fraction in fish (18%; [69]), and protein (serum albumin) makes up the highest partition coefficient (log K_{PW} = 4.14 for PFOA; 4.10 for PFOS). Therefore, the majority of PFOA and PFOS in biota are expected to sorb to serum albumin. Although polar lipids comprise a small percentage (1%) of fish, the affinity of PFOA and PFOS for this media is approximately 20 times higher than that for non-polar (neutral) lipids. This finding highlights the need to consider the role of polar tissues and protein in bioaccumulation modeling, as K_{OW} alone fails to capture the unique partitioning behaviour of PFAAs.

Table 4-2. Distribution of PFOA and PFOS among non-polar lipids, polar lipids, and protein within fish species calculated in the food web bioaccumulation model.

	PFOA			PFOS		
	Partition Coefficient	Fraction in Biota (%)	Relative Chemical Mass (%)	Partition Coefficient	Fraction in Biota (%)	Relative Chemical Mass (%)
Non-Polar Lipid	50	4.0	**0.080**	25	4.0	**0.044**
Polar Lipid	1000	1.0	**0.400**	500	1.0	**0.221**
Protein	14000	18.0	**>99**	13000	18.0	**>99**
Water	1.0	77.0	**0.031**	1.0	77.0	**0.034**

Chemical distribution patterns within fish tissues differ between neutral and ionized substances (Figure 4-5). For estimated concentrations of PFOA and PFOS in fish, virtually all of the total chemical concentration (> 99%) is expected to accumulate within protein. Sorption to neutral and polar lipids is less important for bioaccumulation f PFOA and PFOS (< 1% of total chemical mass). Conversely, the majority of PCB 153 (82%) is expected to be stored in non-polar lipids, consistent with the behaviour of neutral, lipophilic compounds [2]. Only 18% of PCB 153 is expected to accumulate in protein, assuming the protein-water partition coefficient is 5% of the K_{OW} [79]. Because the methodology used to calculate the log D_{MW} for PFAAs is relevant only for IOCs [62], it is assumed that $D_{MW} = D_{OW}$ for PCB 153. Because of this assumption, the fraction of PCB 153 in polar lipids (approximately 17%) exceeds the fraction of PFOA and PFOS in polar lipids (0.4% and 0.2%, respectively), despite the ionizable nature of PFAAs. Overall, this model demonstrates the unique distribution of PFOA and PFOS in biota compared to neutral, lipophilic compounds.

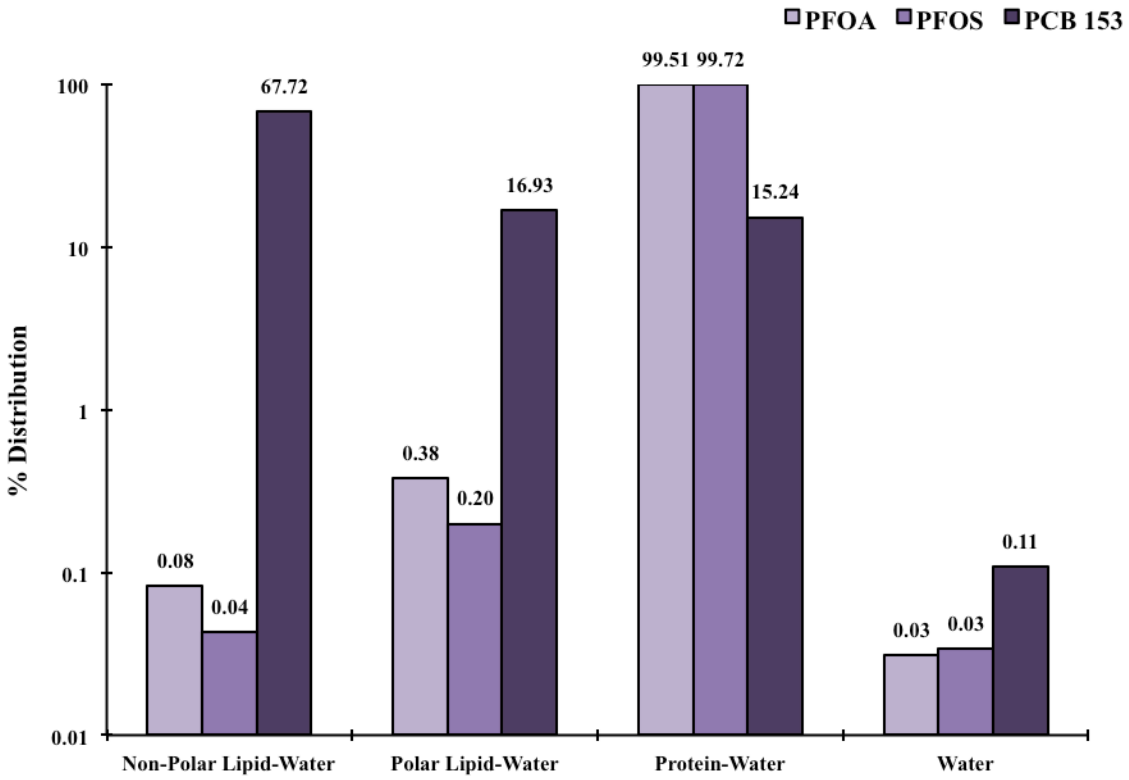


Figure 4-5. Fractions of PFOA, PFOS, and PCB 153 in non-polar lipid, polar lipid, protein, and water compartments of fish (log %). PFOA and PFOS are distributed almost exclusively within albumin (protein), due to the high K_{PW} of these ionogenic compounds. A very small fraction of PFOA and PFOS accumulate in polar lipid, as the total fraction of polar lipid is only 1%.

For neutral organic chemicals that are not metabolized, contaminant levels in biota often strongly correlate with lipophilicity of the compounds. Higher log K_{OW} values are associated with higher levels of bioaccumulation. However, the correlation between lipid content and contaminant levels is less pertinent for PFAAs. In model simulations, for example, when the fraction of non-polar lipid within spotted seatrout was increased from 1% to 50% of total body mass, the estimated BCF value of PFOA increased only by 4%. The model, therefore, is not sensitive to changes in non-polar lipid content, implying that the contribution of non-polar lipids to bioaccumulation of PFOA and PFOS is minimal. At the same time, however, it is recognized that protein partitioning alone is not sufficient to describe the bioaccumulation behaviour of PFASs (specifically, PFAAs), despite a high affinity of these compounds for protein [166]. For instance, the hydrophobicity of PFAAs varies with fluorinated carbon chain length. Longer carbon

chains are associated with higher degrees of bioaccumulation in neutral lipids, contributing to higher overall bioaccumulation in PFAAs with longer chain lengths [77,123,125,167,168].

4.5. Bioaccumulation Metrics

4.5.1. Bioconcentration

Estimates of bioconcentration factors (BCF) were calculated by the modified model (Table 4-3). Protein-normalized BCFs of PFOA and PFOS were < 5000 L/kg (the bioaccumulation threshold under CEPA) for all aquatic organisms (phytoplankton, zooplankton, marine invertebrates, and fish). However, BCFs for the marine mammal in the modified model were equal to 134,000 L/kg for PFOA and 150,000 L/kg for PFOS, exceeding the CEPA threshold of 5000 L/kg (Figure 4-6), though the regulations are designed explicitly for aquatic species.

Table 4-3. Model-calculated BCFs in a marine food web.

Organism Type	Organism Name	Trophic Level	BCF (L/kg)	
			PFOA	PFOS
Phytoplankton	*n/a*	1	1545	1405
Zooplankton	Copepoda	2	1352	1214
Marine Invertebrate	Oligochaete	2.1	1375	1244
	Grass shrimp	2.1	1699	1507
	Hard clam	2.2	1339	1197
	Eastern oyster	2.3	1312	1164
	Blue crab	2.8	1712	1523
Fish	Striped mullet	3.4	2025	1706
	Red drum	3.9	1691	1649
	Atlantic croaker	4.2	1695	1353
	Spotfish	4.2	2024	1357
	Pinfish	4.3	1974	1705
	Spotted seatrout	4.3	1669	1331
Marine Mammal	Bottlenose dolphin	4.4	134,000	150,000

Elevated bioconcentration factors of PFOA and PFOS in the dolphin compared to aquatic organisms suggests a lack of respiratory elimination in marine mammals. Respiratory elimination of PFAAs for dolphin in this model was very low and almost negligible due to the high K_{OA} of perfluorinated chemicals and slow transport from biota to air via exhalation in air-breathing organisms. For aquatic species, however, gill respiration allows for sufficient depuration to produce relatively low bioconcentration factors (i.e., < 5000).

Empirical BCFs for PFOA are typically lower than that of PFOS (e.g., [125]); however, the model calculates similar BCFs for the two compounds in this food web, a combination of the specific diet composition patterns and partition coefficients used within the model.

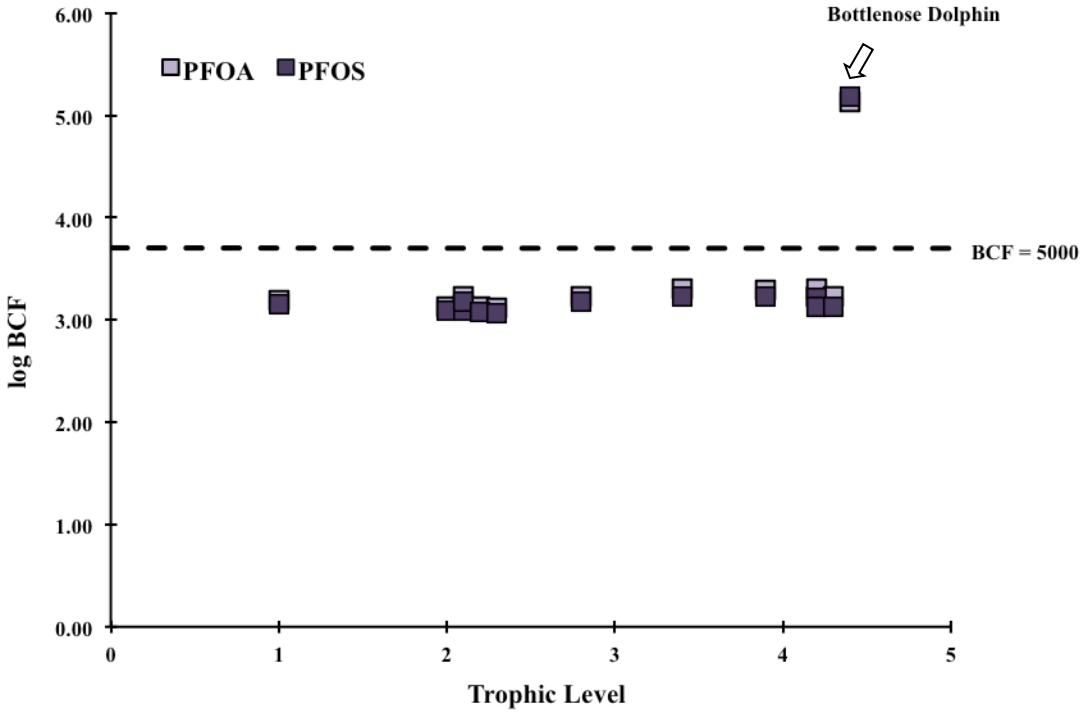


Figure 4-6. BCFs for PFOA and PFOS calculated from protein-normalized concentrations estimated by the modified bioaccumulation model. BCF values for all aquatic organisms are < 5000 L/kg, whereas the BCF for bottlenose dolphin is >5000 L/kg (exceeding the regulatory threshold for bioaccumulation under CEPA).

By definition, BCFs apply exclusively to water-respiring species (i.e., expressed as the ratio of the concentration in biota to the concentration in the surrounding water

environment) [3,4]. Therefore, the BCF cannot be relied upon to evaluate bioaccumulation behaviour in air-breathing organisms, including the bottlenose dolphin. It is important, then, to be cautious if attempting to extrapolate BCFs < 5000 in fish and other aquatic species to the entire food web, where elevated concentrations in marine mammals (as estimated by the model) are not explicitly accounted for in BCF analyses. Despite the fact that the BCF is not meant to be applied to non-aquatic organisms (due to a lack of respiration via water diffusion), this metric is useful for identifying the bioaccumulative properties of PFOA and PFOS in this particular food web. Model calculations estimate BCFs >> 5000 L/kg for PFOA and PFOS in dolphins, highlighting the potential for increased concentrations of PFOA and PFOS in marine mammals compared to air-breathing species.

4.5.2. Biomagnification

The model indicates no substantial biomagnification in the aquatic food chain for water-breathing organisms (BMFs range between 0.76 and 1.15 when calculated based on dietary uptake versus elimination; Table 4.4). In bottlenose dolphins, however, BMFs increase by a factor of six for PFOA (BMF = 7.4) and by a factor of seven for PFOS (BMF = 8.3) compared to spotted seatrout. These trends occur due to ionization of PFAAs at environmental and physiological pH. Ionization increases the solubility of the chemical in water, which increases depuration for water-breathing species (reducing tendencies for biomagnification; aligned with BMFs < 1), but reduces elimination via pulmonary respiration in mammals (elevating tendencies for biomagnification; aligned with BMFs > 1).

PFOA and PFOS, along with other PFAAs, are known to biomagnify in air-breathing mammals from both marine [11,71,169] and terrestrial [74] food webs, highlighting the influence of air-breathing organisms in the overall biomagnification of perfluorinated substances (summarized in [2]). For example, experiments with fish show a high degree of elimination to water through gill respiration; however, because protein to air exchange is slow, perfluorinated substances biomagnify in air-breathing animals [2,73].

Table 4-4. Model-calculated BMFs in a marine food web.

Organism Type	Organism Name	Trophic Level	BMF	
			PFOA	PFOS
Phytoplankton	*n/a*	1		
Zooplankton	Copepoda	2	0.11	0.16
Marine Invertebrate	Oligochaete	2.1	0.09	0.13
	Grass shrimp	2.1	0.21	0.29
	Hard clam	2.2	0.21	0.26
	Eastern oyster	2.3	0.18	0.25
	Blue crab	2.8	0.23	0.33
Fish	Striped mullet	3.4	0.78	1.03
	Red drum	3.9	1.16	1.00
	Atlantic croaker	4.2	1.17	1.45
	Spotfish	4.2	1.39	1.46
	Pinfish	4.3	0.76	1.83
	Spotted seatrout	4.3	1.15	1.43
Marine Mammal	Bottlenose dolphin	4.4	7.39	8.27

Patterns of biomagnification in aquatic organisms (i.e., where BMFs ≈ 1) illustrate the influence of diet on concentrations of PFOA and PFOS in higher trophic levels. Increased BMFs for marine mammals are expected based on the higher relative body mass, high dietary uptake rates, low respiratory elimination, and negligible biotransformation of PFAAs. Although BMFs calculated for aquatic organisms are not always good indicators of biomagnification in mammals due to differences in respiratory elimination, they appear useful for PFOA and PFOS in this particular marine food web.

BMFs for PFOS are typically higher than that for PFOA (primarily because of lower gill elimination values for PFOS), with the exception of red drum (TL = 3.9) and Atlantic croaker (TL = 4.2). This is likely a reflection of diet composition, and could change with any modifications to the quantities and species of prey considered in the model.

4.5.3. Trophic Magnification

To evaluate the influence of air-breathing species on the trophic magnification of PFOA and PFOS, TMFs were estimated from the model with and without the dolphin (Figure 4-7). When the bottlenose dolphin was excluded from TMF calculations, the TMF for PFOA was equal to 1.2 ± 0.029 SE ($p < 0.05$, $r^2 = 0.34$) and the TMF for PFOS was equal to 1.2 ± 0.015 SE ($p < 0.05$, $r^2 = 0.6$). Alternatively, when the full food web (i.e., with dolphin) was included in estimates of trophic magnification, TMFs were equal to 1.3 ± 0.052 ($p < 0.05$, $r^2 = 0.32$) for PFOA and 1.3 ± 0.050 ($p < 0.05$, $r^2 = 0.33$) for PFOS. TMFs for PFOA and PFOS were not statistically different from each other in both scenarios (t-test;$p > 0.05$).

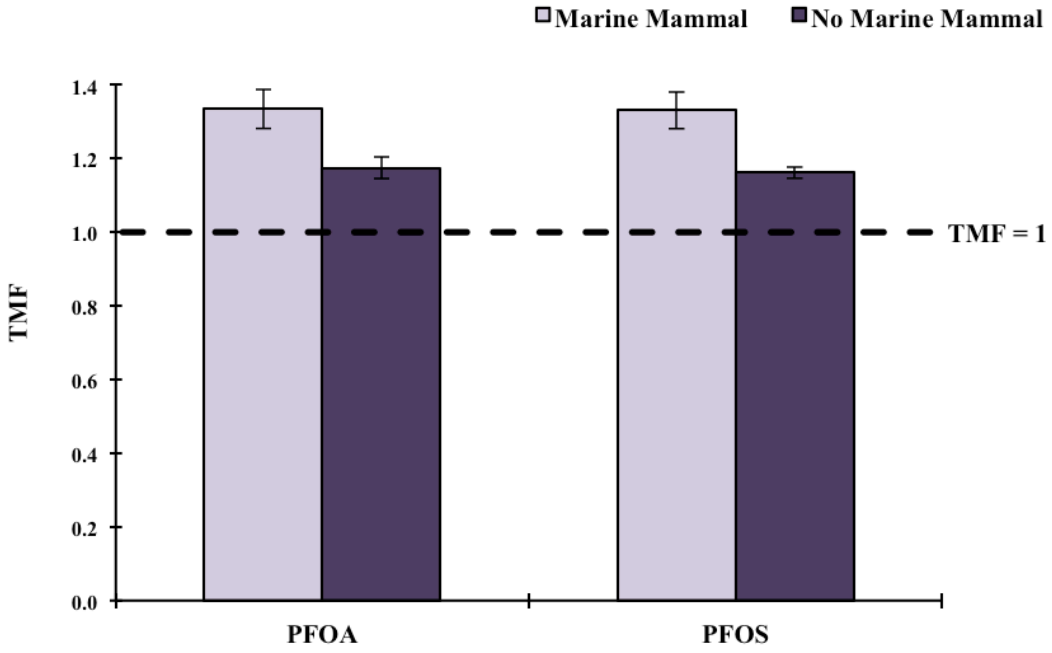


Figure 4-7. TMF estimates derived from model calculations for PFOA and PFOS in a marine food web (±1 standard error) under two scenarios: with marine mammal species (plankton + invertebrates + fish + marine mammal; TMFs = 1.3), and without marine mammal species (plankton + invertebrates + fish; TMFs = 1.2). Trophic magnification occurs in both scenarios ($p < 0.05$). Although calculated TMF values are lower when marine mammals are excluded from analysis (likely a result of higher bioaccumulation of perfluorinated compounds in air-breathing organisms), the difference in TMFs is not statistically significant ($p = 0.48$ for PFOA and $p = 0.40$ for PFOS) between TMFs with and without the marine mammal considered.

TMFs close to 1.0 depict scenarios where chemical exchange is occurring predominantly between the organism and the water, and the substance is absorbed from water and is not rapidly metabolized. Chemicals with high biota-water exchange rates and a lack of biotransformation in aquatic organisms are expected to exhibit TMFs ≈ 1, as calculated by the model (TMF = 1.2). This is in contrast to trophic dilution, where TMF < 1. Based on the model calculations, neither trophic dilution nor trophic magnification was expected for PFOA and PFOS in marine invertebrates and fish.

Observed TMFs of PFOA and PFOS from Charleston Harbor are consistently higher than calculated TMFs. Higher measured TMFs from Charleston Harbor may be due to multiple possible factors, including spatial and/or temporal concentration gradients of the compounds in water and sediment, as well as inclusion of different trophic level ranges. This is discussed further in Section 4.6.2.

TMFs are calculated from a linear regression of log-normalized concentrations within individual species in a food web. The slope, used to determine the TMF (i.e., TMF = 10^b, where b = slope), is dependent on the number of data points included in the regression, as well as the range of trophic levels considered. Therefore, calculating the TMF for a food web with few species or a large proportion of high trophic levels, for example, is bound to have a different TMF compared to different variations of the same food web (i.e., with more individual species or an even distribution of low and high trophic levels). Though a more substantial difference in TMFs were anticipated between the two versions of the food web, the lack of difference likely occurs because the inclusion or exclusion of one marine mammal in a food web of 13 other aquatic species does not greatly influence the linear regressions used to calculate TMF. Therefore, its influence on the overall TMF (i.e., slope of concentrations versus trophic level) was not significant. Caution should be taken when classifying PFOA and PFOS as biomagnifying substances within this particular food web, as the model input values (i.e., water and sediment concentration data) were calculated from a single sampling study, and similar environmental concentrations might not be replicated in future experiments or in other study locations.

Compounds with log K_{OW} < 5 and BCFs < 5000 are readily eliminated via gill respiration and rarely biomagnify in aquatic organisms [2,51]. Both PFOA and PFOS

have log K_{OW}s < 5 and BCFs < 5000, yet TMF > 1 for both substances, regardless of whether or not marine mammals are included in the model. This observation fails to highlight the differences in uptake and elimination between water- and air-breathing species. There are several possible explanations for this. Firstly, if most of the chemical accumulates in protein (Figure 4-5), then K_{OW} does not serve as an appropriate indicator of bioaccumulation. Secondly, available K_{OW} values for PFOA and PFOS are potentially unreliable, which is related to the feasibility of using conventional methodologies for determining physicochemical properties of PFAAs. Whereas the shake-flask method is commonly used to experimentally determine partition coefficients between octanol and water, this is not practical for perfluorinated compounds because the surfactant nature of these substances causes them to aggregate at the interface of a liquid-liquid system, creating 3 separate layers [125]. Therefore, computational approaches are often used to estimate the K_{OW} (and other properties, such as K_{OA} and pK_a) based on chemical and molecular structure relationships. Estimates of K_{OW} from software programs such as EPI Suite and SPARC depend on generalized computation algorithms providing reasonable, yet potentially erroneous values for modeling input parameters. Actual K_{OW} values for PFOA and PFOS may indeed be > 10^5 and therefore considered lipophilic enough to be considered bioaccumulative in aquatic organisms. For that reason, modeling approaches (such as this one) utilizing K_{OW} values estimated via computational programs (in this case, SPARC) may be applying unreliable estimates for physicochemical properties. The specific consequence here is the assumption that because log K_{OW} < 5, PFOA and PFOS should not demonstrate trophic magnification in the water-breathing component of a marine food web. Meanwhile, this assumption could be incorrect if K_{OW} and other physicochemical property data estimates are erroneous.

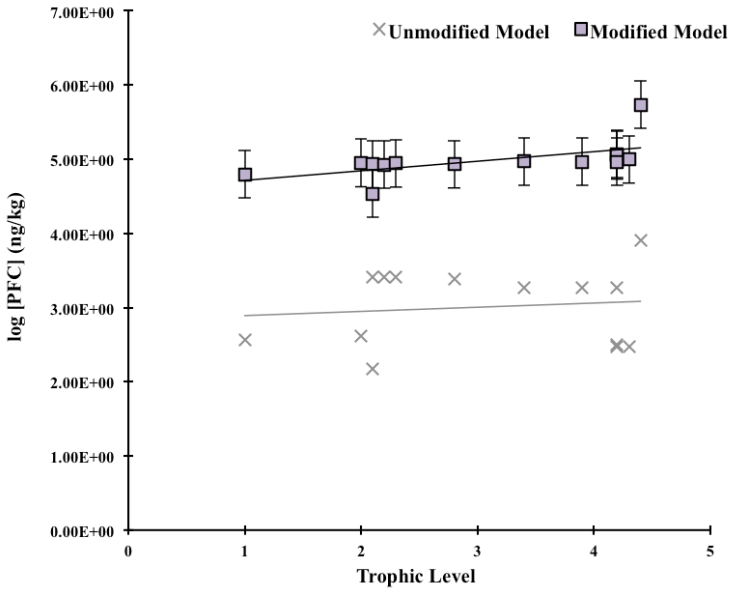
4.6. Model Analysis

4.6.1. Model Performance

To test the model performance of the modified model against the unmodified food web model, concentrations of PFOA and PFOS in biota were also estimated for this food web using the original, unmodified aquatic model developed by Arnot and Gobas [64]. PFOA and PFOS concentrations estimated using the modified model were

significantly higher ($p < 0.05$) than output concentrations from the original model (Figure 4-8).

(a) PFOA



(b) PFOS

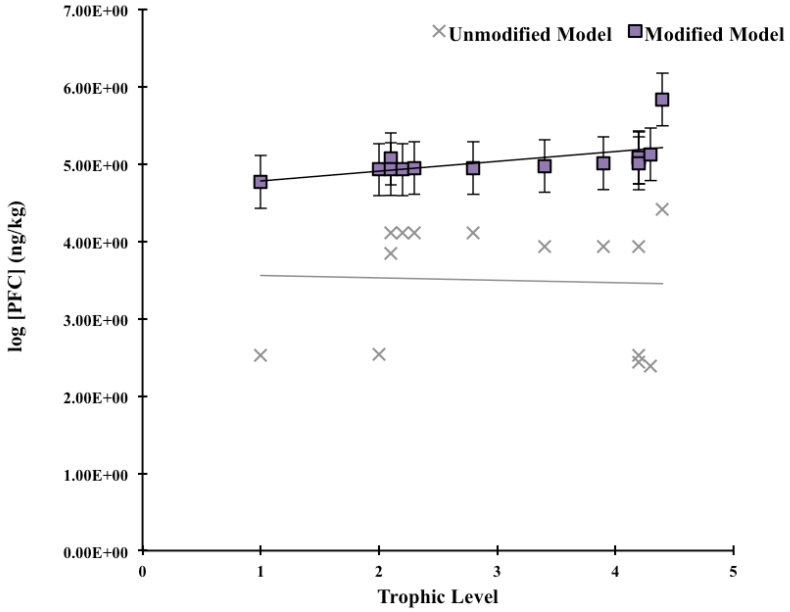
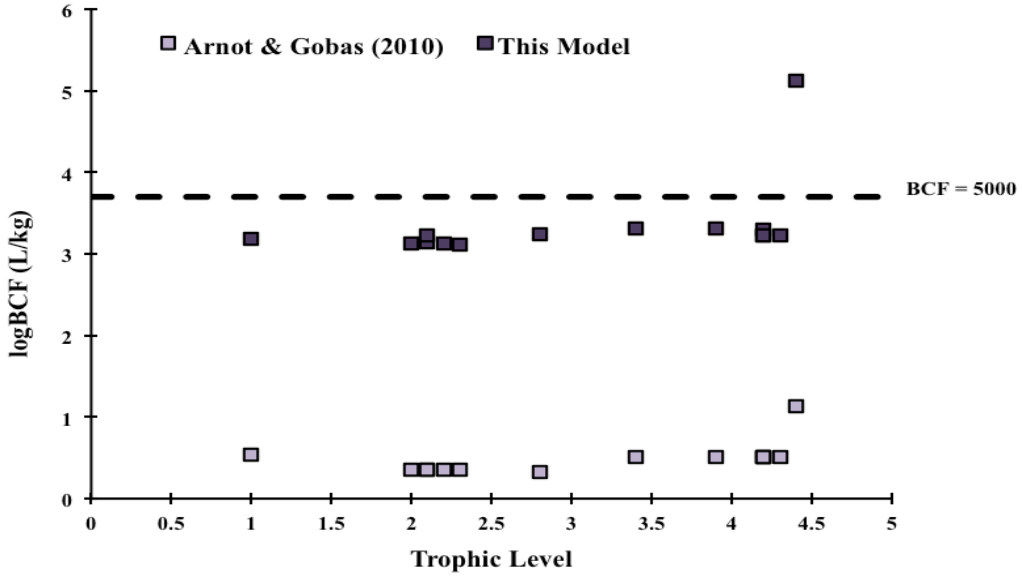


Figure 4-8. Concentrations of (a) PFOA and (b) PFOS in a marine food web calculated using the original food web bioaccumulation model developed by Arnot and Gobas (2004) and the modified model developed in this study.

BCFs were calculated for both versions of the model (Figure 4-9). BCFs calculated for PFOA and PFOS in the original model were higher ($p < 0.05$) than BCFs calculated from the modified model. This is due to differences in chemical partitioning algorithms of the two models. In the original model, only partitioning to non-polar lipid is considered. Because partitioning into tissues with a higher affinity for PFAAs (i.e., polar lipid, protein) were not considered in the original model, BCFs for PFOA and PFOS were far below the CEPA bioaccumulative threshold of 5000 L/kg. Partitioning into other tissues, particularly protein-rich tissues, contributed to higher BCF estimates for PFOA and PFOS.

(a) PFOA



(b) PFOS

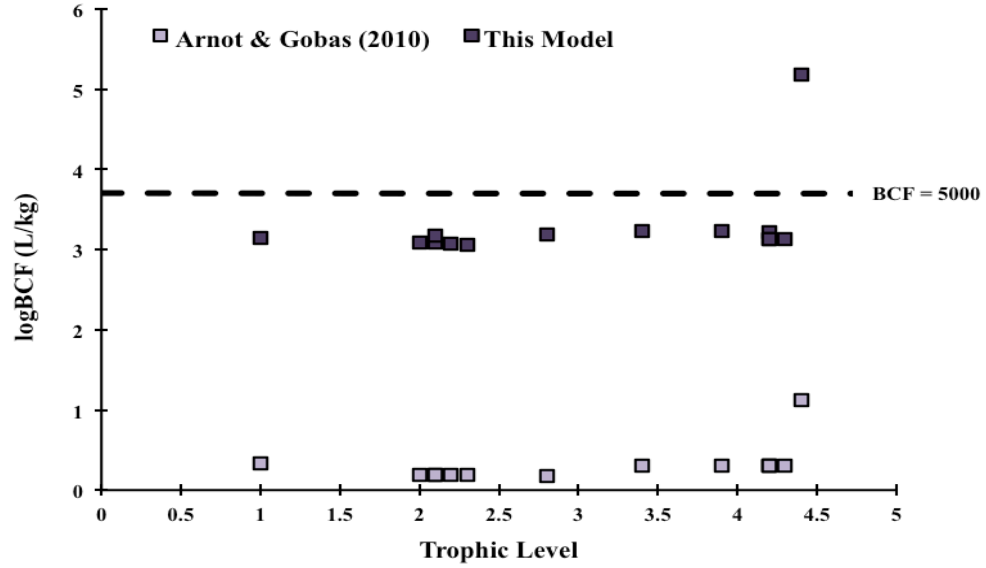


Figure 4-9. BCF estimates for (a) PFOA and (b) PFOS from the unmodified and modified food web model. The adjusted model provides higher ($p < 0.05$) BCF values for air-breathing marine mammal species (i.e., bottlenose dolphin) exceeds a BCF of 5000 only in the modified model.

BCFs in PFOA and PFOS in fish did not exceed 5000 L/kg. Aquatic organisms are able to readily eliminate low K_{OW} substances via gill respiration [2]. The BCF of PFOA and PFOS for the dolphin in the modified model exceeds the regulatory threshold of 5000 L/kg (134,415 L/kg for PFOA and 150,379 L/kg for PFOS), due to the slow elimination of PFAAs in air-breathing organisms [2]. Bioaccumulative concerns for PFOA and PFOS in air-breathing animals from a marine food web are only flagged as a concern, according to relevant Canadian regulations, when the modified model is used, since BCF > 5000. The difference in estimated BCF values demonstrates the importance of using appropriate partition coefficients to measure bioconcentration in food webs with both aquatic and mammalian species.

TMF values calculated by the old model for PFOA and PFOS for fish and mammals were equal to 0.8 for both compounds (r^2 = 0.026 and 0.022, respectively). This suggests a lack of trophic magnification, and in fact suggests that trophic dilution could occur (since TMF < 1) across trophic levels.

Although concentrations of PFOA and PFOS from the modified model project significantly higher contaminant levels in biota, the degree of food web magnification expected to occur does not significantly vary between the two versions of the model. Despite the fact that the inclusion of protein-water partitioning to the model effectively increased the fish-water partition coefficient, thereby reducing depuration rates, the TMF is not significantly different between the original and modified model. Since the adjusted model calculates concentrations in biota given a very high (> 99%) fraction of PFOA and PFOS in protein, higher K_{PW} values (compared to lower D_{OW} values used within the original model) are likely to at least partially account for elevated concentrations estimated by the adjusted model.

Although the original model, developed for neutral contaminants [64] was not explicitly designed to estimate bioaccumulative behaviour of IOCs, the overall trends of food web magnification are similar to those from a modified version of the model accounting for chemical ionization and partitioning into important tissues besides non-polar lipids. Accounting for the air-breathing nature of marine mammals in this modified bioaccumulation model does not result in significantly higher trophic magnification than

expected using models created for neutral, hydrophobic chemicals in an aquatic environment [62].

4.6.2. Modified Model vs. Empirical Measurements

Incorporating field data with model estimates of bioaccumulation allows for a comparison of predicted chemical behaviour with patterns observed in the real world [53]. Estimated concentrations, BMFs, and TMFs for fish and marine mammal were compared to measurements obtained from the Charleston Harbor bottlenose dolphin food web conducted by Houde et al. [77]. BCFs were not compared between the model and empirical data because this metric was not examined in the field study, and comparison to metrics reported in the original study are not available. Note that because PFAS concentrations measured by Houde et al. were only available for fish and bottlenose dolphin (TLs = 3.4 to 4.4), comparisons between the modified model and observed concentrations were limited to this portion of the food web (i.e., phytoplankton, zooplankton, and marine invertebrates were excluded from this analysis).

PFOA and PFOS Concentrations

Model estimates almost consistently over-predict concentrations of PFOA and PFOS in fish and bottlenose dolphin measured in Charleston Harbor, though agreement is generally better for PFOS than PFOA (Figure 4-10). There are several possible explanations for these trends. Firstly, because the majority of chemical is in protein, log K_{PW} values are an important driver for calculating estimated concentrations in the model. Consequently, the laboratory-based measurements of log K_{PW} values used in the model may not reflect the actual partitioning behaviour of perfluorinated substances in these species. Another possible reason for the apparent over-prediction of PFOA relates to the decreased affinity of PFCAs longer than six fluorinated carbons for BSA (i.e., protein) [82]. Additionally, the possibility of concentration gradients in the study area should be considered, as this may capture higher than average concentrations of PFOA and PFOS in water and sediment, which would be reflected in estimated concentrations of these compounds in biota [115]. Lastly, and perhaps most important, is that all protein content of the organisms is assumed to have the same K_{PW} as serum albumin, when in fact,

albumin makes up a fraction of total protein. This may lead to overestimation of PFOA concentrations in biota.

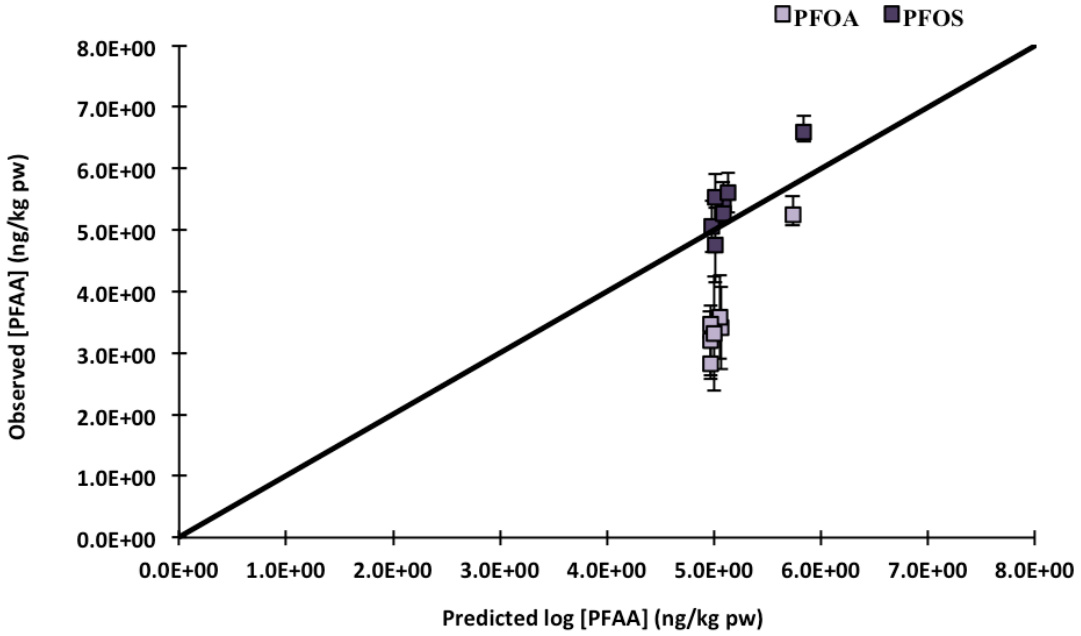


Figure 4-10. Protein-normalized model calculated concentration of PFOA and PFOS for fish and bottlenose dolphin (ng/kg pw) in the Charleston Harbor marine food web versus protein-normalized observed geometric mean concentrations (±1 standard error).

TMFs

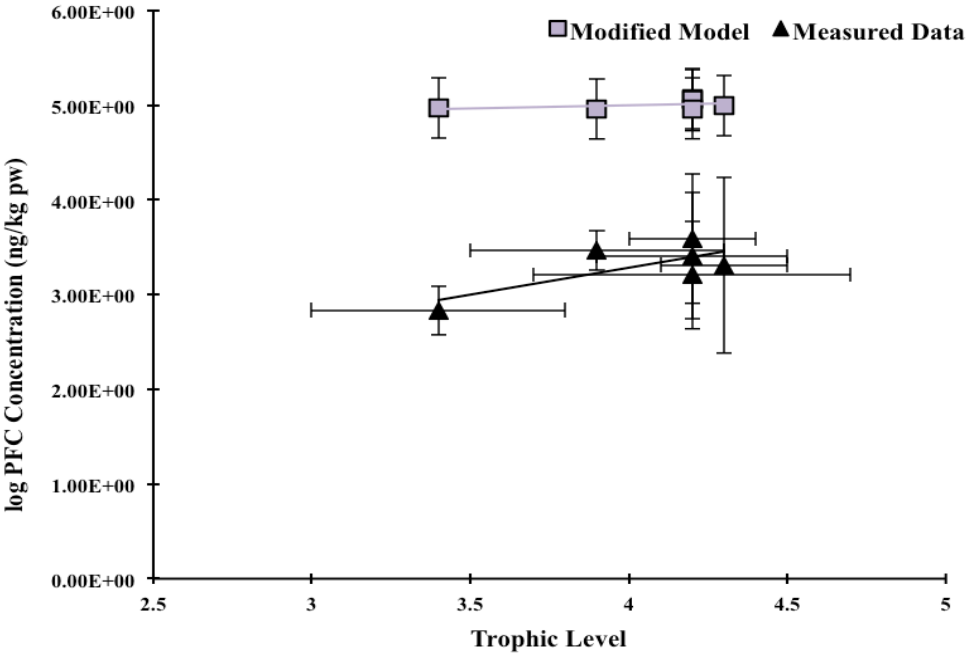
Lastly, TMF values were compared between the modified model and observed data. Once again, TMFs were compared with and without marine mammals in order to determine the influence of air-breathing organisms on bioaccumulation behaviours of PFOA and PFOS. Since only fish and dolphin are included in this comparison, excluding marine mammals means that bioaccumulation is evaluated in fish species only. Also note that observed TMFs used in this analysis are not the values reported in the Charleston Harbor study, but rather the protein-normalized values calculated here.

First, measured concentrations of PFOA and PFOS in aquatic organisms only (i.e., just fish) from Charleston Harbor were compared to calculated concentrations for the same fish species from the new model (Figure 4-11a; Figure 4-11c). The model-estimated TMF for PFOA was equal to 1.2 ± 0.060 but was not significantly different

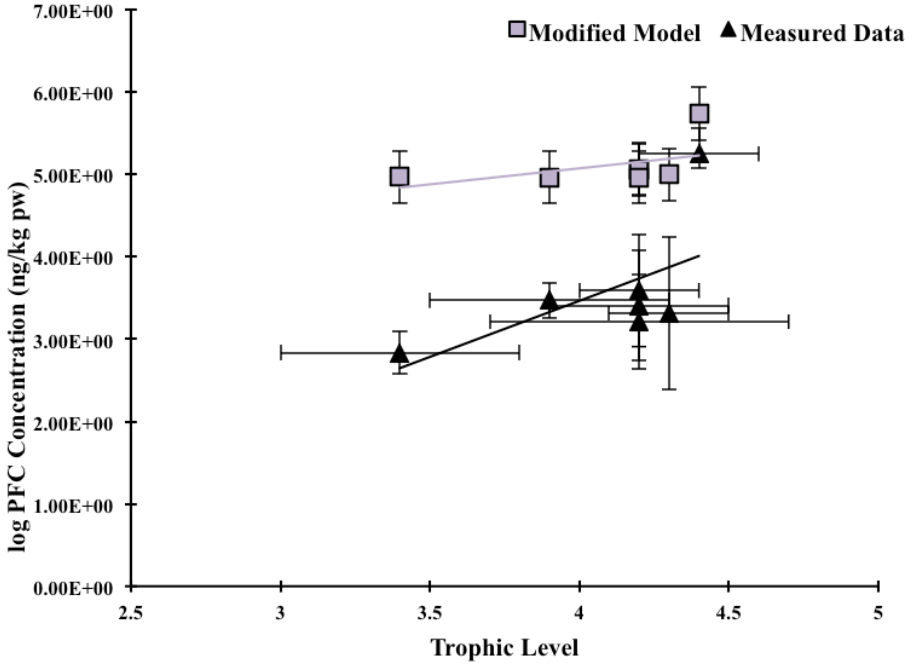
from the observed TMF ($p = 0.32$; $r^2 = 0.24$), and for PFOS, the TMF estimated by the model was equal to 1.4 ± 0.055 and was also not significantly different from the observed TMF ($p = 0.073$; $r^2 = 0.59$). This is compared to protein-normalized TMFs from the Houde et al. study, which were equal to 3.7 ± 0.267 ($p = 0.1$; $r^2 = 0.53$, testing whether slope is different from zero) for PFOA and 4.3 ± 0.82 ($p = 0.15$; $r^2 = 0.44$) for PFOS, and are also not significant. The modeled and measured TMFs without marine mammals (i.e., with fish species only) are significantly different from each other for PFOA ($p < 0.001$) but not for PFOS ($p = 0.084$).

To determine the influence of air-breathing organisms on trophic magnification, the second scenario evaluated concentrations of PFOA and PFOS in bottlenose dolphin as well as fish from the model and the measured Charleston Harbor data (Figure 4-11b; Figure 4-11d). The model-estimated TMF for PFOA was equal to 2.5 ± 0.33 ($p < 0.05$; $r^2 = 0.36$), and for PFOS, the TMF estimated by the model was equal to 3.0 ± 0.34 ($p < 0.05$; $r^2 = 0.44$), demonstrating trophic magnification. This is compared to protein-normalized TMFs from the Houde et al. study, which were equal to 23.0 ± 0.82 for PFOA and 13.4 ± 0.57 for PFOS. The modeled and measured TMFs including marine mammals are statistically different (slopes are not the same) for PFOA ($p > 0.001$), but not for PFOS ($p = 0.17$).

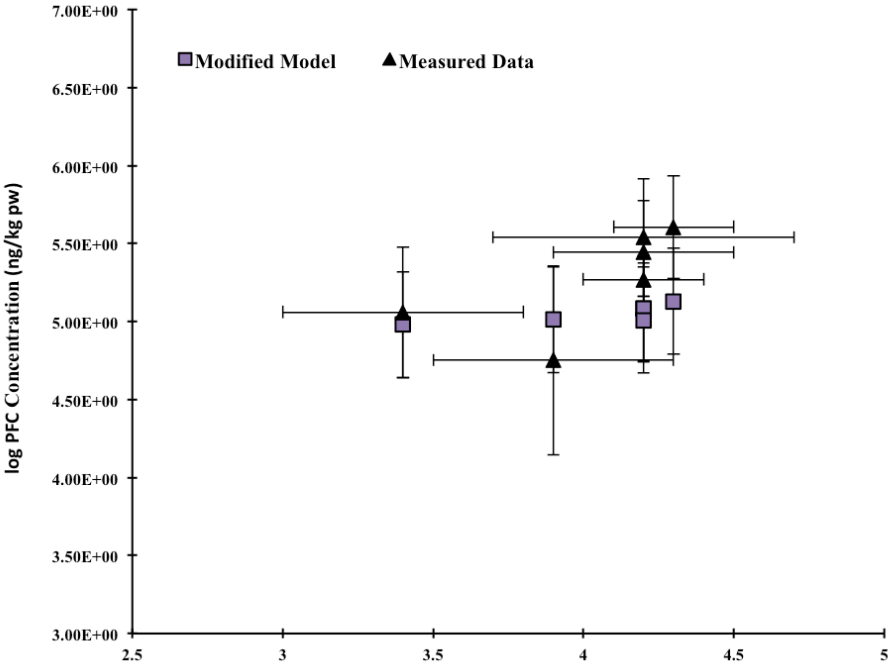
(a) PFOA (excluding marine mammal; water-respiring species only)



(b) PFOA (including marine mammal; water- and air-breathers)



(c) PFOS (excluding marine mammal; water-respiring species only)



(d) PFOS (including marine mammal; water- and air-breathers)

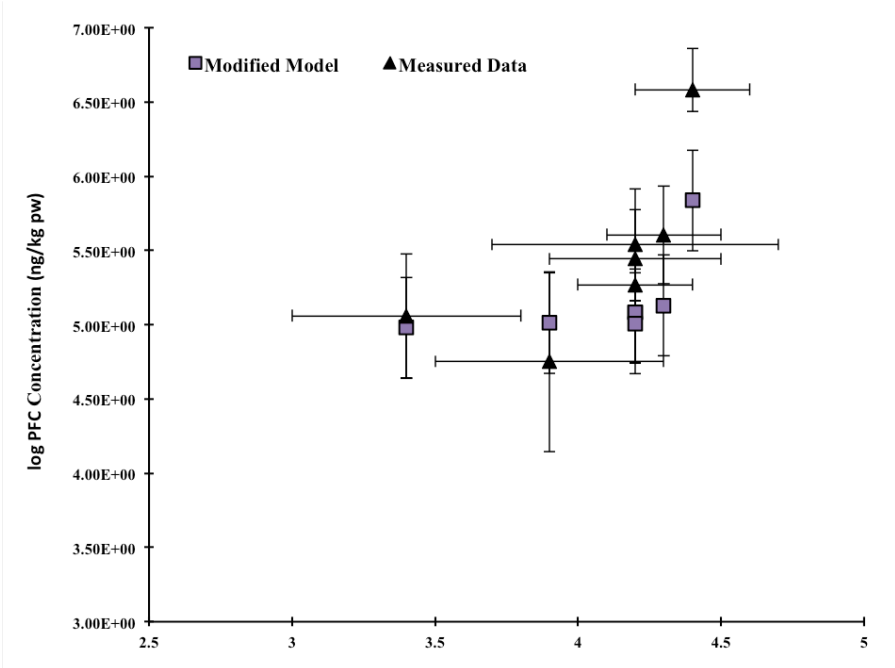
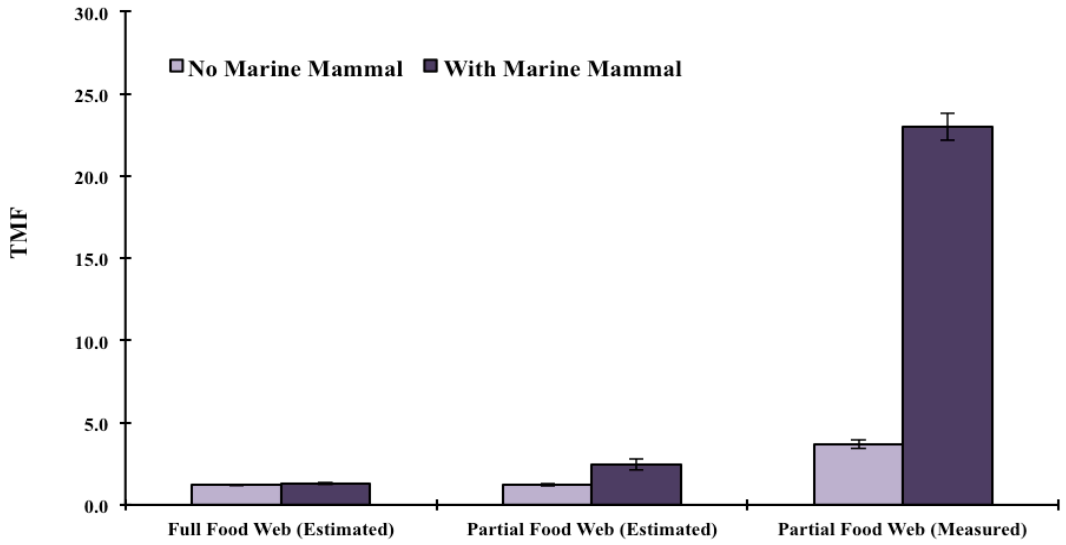


Figure 4-11. Comparison of modeled and measured PFOA (a,b) and PFOS (c,d) concentrations for food webs with and without marine mammals (±1 SE).

To evaluate the role of food web composition on trophic magnification, modeled and measured TMFs for the species included in the Houde et al. study (i.e., fish, either with or without marine mammals) were compared to calculated TMFs for all the species included in the food web (i.e., TLs 1 through 4.4; Figure 4-12). Estimated TMFs were higher for partial food webs compared to a full food web. The highest overall TMFs were from observed TMFs measured in Charleston Harbor. Excluding trophic levels from TMF calculations can either over- or under-estimate overall trophic magnification, depending on the accumulation behaviour occurring within the omitted trophic position(s). The model determined TMFs of 2.5 and 3.0 for PFOA and PFOS in the food web that included only fish and dolphin using the water and sediment concentration data provided by [77]. However, using these same environmental concentrations as model input parameters to estimate concentrations for all trophic levels results in lower TMFs = 1.3 for PFOA and PFOS. TMFs based on measured concentrations considering only fish and dolphin appears to capture a high degree of magnification, perhaps over representative of actual contaminant behaviour throughout the full food web. Comparing calculated TMF values from the modified model and observed TMF levels in scenarios with and without inclusion of the marine mammal does not support the hypothesis that TMFs calculated from food webs containing the bottlenose dolphin will have higher degrees of trophic magnification than TMFs calculated for food webs without marine mammals.

Although information regarding lower trophic level organisms were not reported in the Houde et al. study, it is expected based on results from the modeled food web that including PFOA and PFOS concentrations from marine organisms such as phytoplankton, zooplankton, and invertebrates would lower measured TMFs. This is because the TMF is a trophic level averaged biomagnification study and the model generally predicts less biomagnification in lower trophic levels (see Table 4-4).

(a) PFOA



(b) PFOS

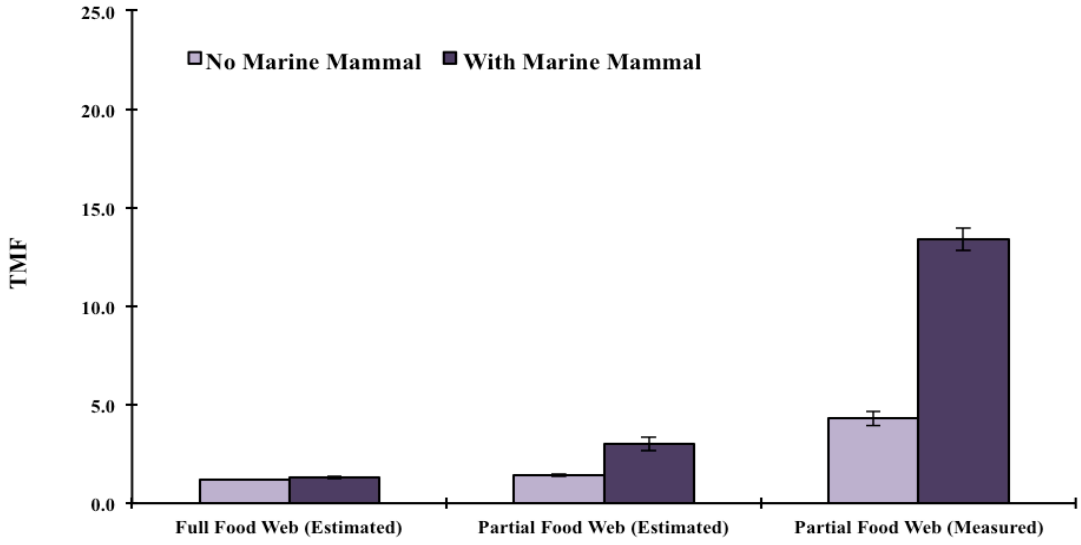


Figure 4-12. TMFs of (a) PFOA and (b) PFOS for calculated and measured concentrations in Charleston Harbor (±1 standard error). Modeled TMFs for PFOA and PFOS in the full food web are not statistically different with and without marine mammals. Empirical TMFs for the partial food web (fish and marine mammals) are higher than measured concentrations for PFOA ($p < 0.05$), but not for PFOS.

This analysis reveals that TMF values can change depending on the number and types of species included in the study. Variations in TMF values demonstrate the capacity of trophic magnification patterns to change throughout trophic levels within the same food web. Caution should be taken when applying TMFs determined for segments of a food web to a full food web, as TMF values are subject to change depending on species included in the analysis, as observed in this study. Calculating TMFs for different ranges of a food web brings to attention potential complications arising from the omission of not only air-breathing species, but also lower trophic level species.

Inconsistencies between modeled and empirical TMFs may not necessarily reflect errors with model development and execution, but rather inherent complications with field sampling research, including spatial variability and area-specific characteristics of the environmental or biota [115]. Explanations for lack of agreement in TMF estimates between modeled and calculated include the influence of spatial variability [115] and inaccurate diet composition (i.e., incorrect predator-prey interactions). Measurements of biomagnification and trophic magnification from field research is generally less reliable because of environmental variability and error, and should be taken into account when evaluating the agreement between modeled and measured trophic magnification [53,170].

4.6.3. Comparison to other ecosystems

TMFs derived from model calculations were also compared to TMFs of PFOA and PFOS determined for other empirical studies of trophic magnification in food webs containing marine mammals (Figure 4-14). With the exception of the TMF of PFOA in [30], all TMFs of PFOA and PFOS were found to be greater than 1, suggesting trophic magnification of both PFAAs in various ecosystems, including Lake Ontario [30] and the Canadian Arctic [73]. TMF calculations from [77] estimated higher TMFs for PFOA than for PFOS, which varies from model calculations and the other studies evaluating trophic magnification of these compounds showing TMFs of PFOA and PFOS to be approximately equal. High TMFs for PFOA in measured biota may be connected to elevated concentrations of PFOA in water and/or sediment. Also, the types and relative

number of species included in the trophic analysis can also influence absolute and relative TMFs (see Section 4.5.3).

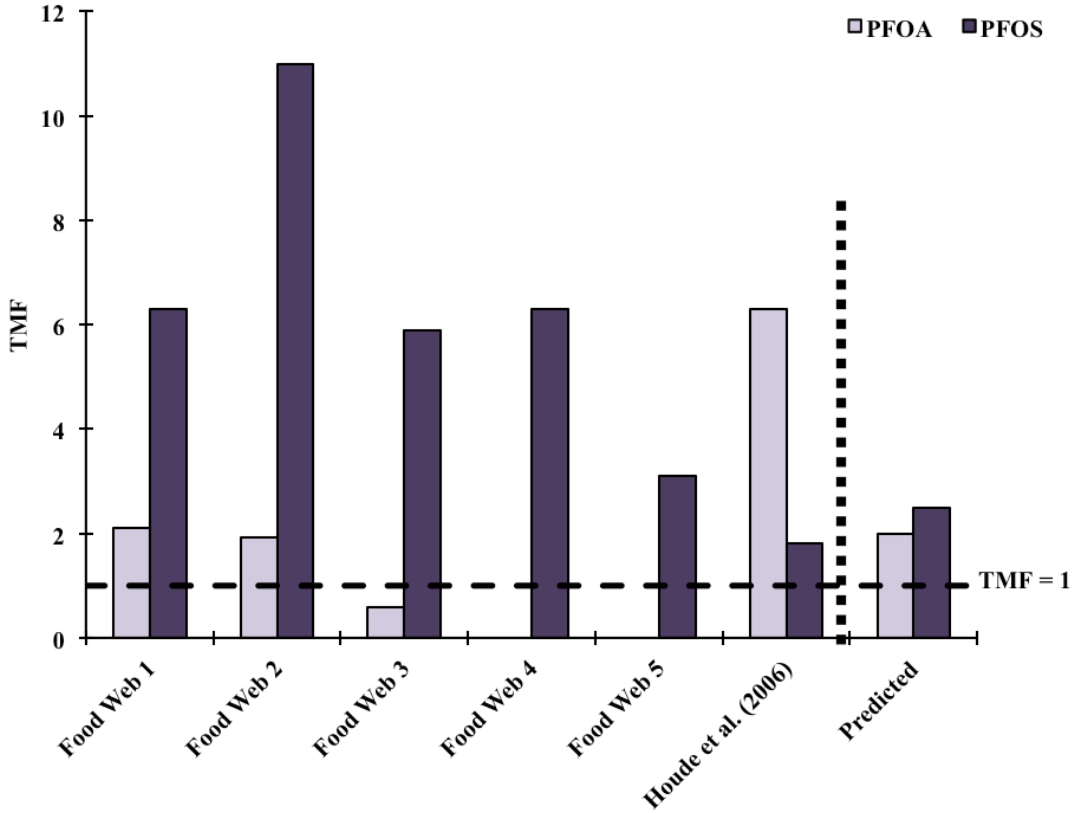


Figure 4-13. Measured TMFs of PFOA and PFOS (error not reported) from various marine food webs containing marine mammals compared to TMFs calculated by the model developed in this study, as well as Charleston Harbor bottlenose dolphin food web reported (not re-calcualted with normalized concentrations) in Houde et al. (2006). TMF values for PFOS in Food Webs 1 through 5, as well as calculated TMFs are higher than TMFs for PFOA; however, concentrations of PFOA are higher than PFOS for data from Houde et al. (2006). Most values exceed TMF = 1 (exception: PFOA concentrations in Food Web 3). TMFs for PFOA not reported in Food Webs 4 and 5.

PFAA concentrations detected in dolphin plasma from Charleston Harbor were some of the highest concentrations measured in marine mammals [42,77,169]. More recent studies have also revealed that PFAA levels in Charleston Harbor sediment can be up to an order of magnitude higher compared to other U.S. urban areas [98]. High levels of contamination likely come from point source pollution, resulting in concentrations of PFOA and PFOS in sediment that are much greater than the average

concentrations to which animals are exposed. Patterns of food web accumulation modeled in Charleston Harbor were similar to those from other ecosystems [42,73,173-175], implying that the bioaccumulative behaviour of PFAAs reported here may be independent of location. Elevated PFAA concentrations have also been measured in marine mammals, particularly top predators, such as bottlenose dolphins [41,77,96,97], as well as harbor seals [176], polar bears [177], and other air-breathing organisms in marine food webs, including the river otter, pygmy sperm whale, short-snouted spinner dolphin, striped dolphin, rough-toothed dolphin, California sea lion, and northern elephant seal [177].

Given the unusually high concentrations in these marine mammals, it is possible that renal re-uptake proteins are becoming saturated, resulting in more extensive elimination of PFOA in these organisms, hence the low TMFs for this compound compared to PFOS, which is not influenced by such processes [84].

Inconsistencies have been identified between reports on temporal changes of PFAA concentrations in marine mammals (e.g., [178]), despite substance phase-outs [115]. Production of several long-chain PFASs has ended or been or largely reduced, most notably, the decision by 3M Co. to cease manufacturing of PFOS in the early 2000s [45]. High concentrations of PFAAs in high trophic level marine mammals are expected to remain an issue in coming years, emphasizing the need to adequately determine the degree to which PFAAs bioconcentrate and biomagnify in marine mammals [179]. Long-range atmospheric and oceanic transport of PFAAs, for instance, can work on decadal scales, and have contributed to increased levels of PFASs in more remote and pristine areas of the world in recent years, such as the Arctic and Antarctic [180].

4.7. Sensitivity Analysis

Sensitivity analyses illustrated changes in TMF for each selected parameter. The pH of water had a relatively small impact on TMF, due to the low pK_a values of PFOA and PFOS, signifying that small shifts in pH are unlikely to change the ionized fraction (and thus bioaccumulative behaviour) of the substances. Water temperature also did not

have a large influence on TMF values, implying that relatively small shifts temperature (due to spatial variability or measurement inaccuracies) would not have a large impact on TMF calculations.

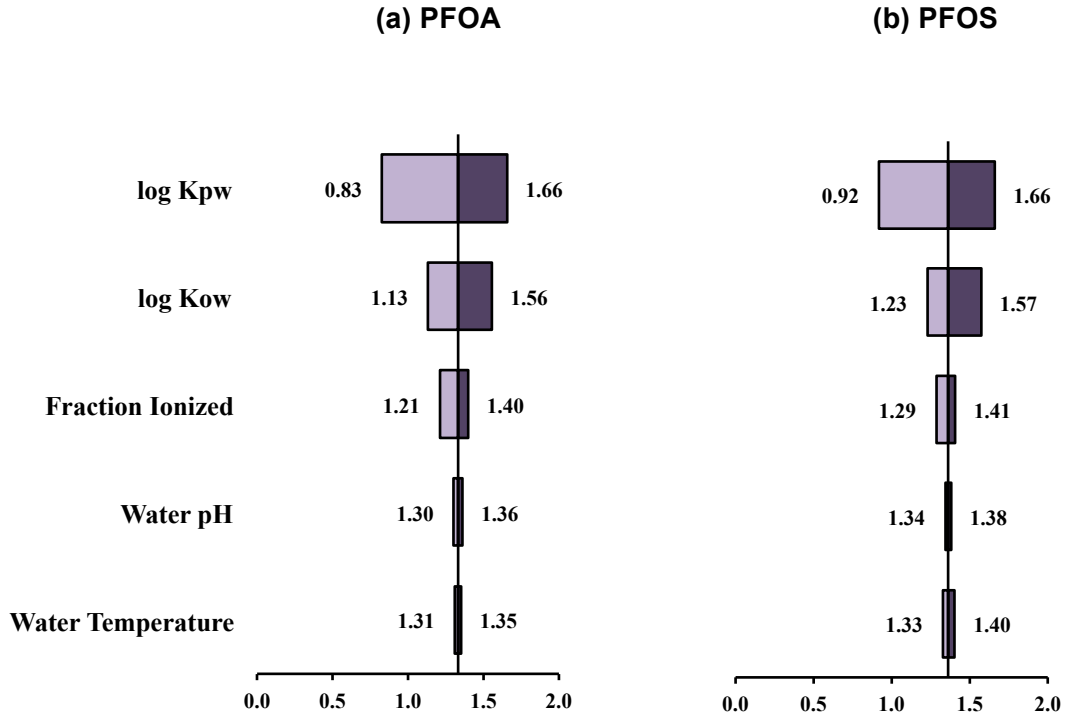


Figure 4-14. Sensitivity of TMF estimates for PFOA and PFOS to multiple input parameters (water temperature, water pH, fraction of compound ionized, log K_{OW}, and log K_{PW}). Bars illustrate the possible range of TMF values as the input parameters vary over their range.

The fraction of ionized PFOA and PFOS has a larger effect on mean TMF than temperature and pH. The effect of total ionized fraction was strong enough that a slightly higher proportion of neutral chemical can make the difference between a TMF < 1 and a TMF > 1. This finding calls for more accurate methods of calculating pK_a values of ionogenic substances, a method over which there has been much controversy (see [120]).

Lastly, log K_{PW} and log K_{OW} values had the largest overall impact on TMF. Numerous values of log K_{PW} (measured experimentally) and log K_{OW} (typically calculated using software such as SPARC or EpiSuite) have been reported for PFOA and PFOS. If the most accurate values are actually larger than the ones used within this model, this will contribute to a higher TMF. For instance, log K_{PW} values of 2.5 and 3 for serum albumin have been calculated for PFOA and PFOS, respectively, which are lower than measured values used in this study [73,166]. Lower log K_{PW} values might under-estimate TMF values for PFOA and PFOS.

4.8. Evaluation of Bioaccumulation Metrics

According to the BCF, PFOA and PFOS are not expected to bioaccumulate within water-respiring species from a marine food web, but BMFs and TMFs show that these compounds do have a tendency to biomagnify in water-respiring species. Conversely, exposure to high levels of PFOA and PFOS through diet, along with inefficient mechanisms for elimination, contributes to elevated concentrations of PFOA and PFOS in the bottlenose dolphin. Note that the goal of this study is not to identify one superior metric of bioaccumulation for PFOA and PFOS in general, but to determine whether all metrics can adequately describe the bioaccumulation behaviour expected to occur in the food web evaluated here.

High concentrations of PFOA and PFOS were estimated for marine mammals, creating a functional BMF between water-breathing species (i.e., all collective prey) and the marine mammal (i.e., top predator). This differs from a food web where concentrations increase with increasing trophic level (e.g., as observed for PFOS across a full marine food web examined in [172]).

This study demonstrates that, within a modeling context, the TMF is a fairly reliable tool for analyzing patterns of bioaccumulation and biomagnification of PFOA and PFOS within marine ecosystems. However, despite the benefits of using the TMF as an indicator of bioaccumulation, there are several limitations associated with the TMF. It is advised to exercise caution when describing food web magnification using TMFs [53]. Although the BCF has been identified as an inadequate metric for evaluating

bioaccumulation in air-breathing animals, the widespread application of this tool has promoted a rigorous approach to measuring bioconcentration. Experiments designed to measure BCFs in Canada, for example, must implement methods that follow the OECD guidelines [181]. BCF values are only considered satisfactory if the laboratory tests were conducted under precise conditions as outlined in the guidelines. Similar meticulous guidelines currently do not apply towards methodologies and techniques used to calculate TMFs from field research. Inconsistencies in the measurement and calculation of TMFs are further amplified by potentially high levels of uncertainty and variability within field research, depending on environmental conditions and sampling methods [3,53,112,170]. Levels of uncertainty and error that are considered reasonable for field research would be considered unacceptable for most laboratory-based tests. The same issues are generally also applicable to BMFs [3].

Up to half a trophic level of uncertainty from stable isotope ($\delta^{15}N$) analysis was reported in the Houde et al. bottlenose dolphin study, resulting in considerable overlap in trophic positions for species within the food web [77]. TMF values, then, may not have been calculated based on the true trophic positions of the species, perhaps leading to inaccurate estimates of trophic magnification occurring in the ecosystem. Potential complications arising from the use of stable isotope analysis in environmental toxicology are further discussed elsewhere [112,182].

It is important to note that applying TMFs as a metric of bioaccumulation remains a relatively novel concept at this time [3,53,112]. Uncertainties in temporal (and spatial) variability has contributed to the classification of information acquired from field studies (i.e., TMFs), as unsuitable for use within regulatory contexts, despite the recognized benefits of analyses focusing on food web magnification. The usefulness of TMFs within bioaccumulation assessments should not be discounted, as this metric is able to provide insight into the behaviour of environmental contaminants throughout food webs. This is particularly true for PFAAs, as the bioaccumulation behaviour of these substances is still not fully understood. The unconventional bioaccumulation patterns of PFAAs compared to many other environmental contaminants emphasizes the need to apply models, such as the one developed in this study, in order to capture bioaccumulation based on comprehensive food web dynamics.

4.9. Policy Implications

According to existing CEPA regulations, BCF estimates indicate that PFOA and PFOS do not pose a bioaccumulative concern for the aquatic species in this food web [5]. Empirically-derived TMFs, however, reveal that these PFAAs are indeed expected to magnify in the bottlenose dolphin food web.

Protein normalization is a key factor in assessing the difference in expected bioaccumulation patterns between lipophilic and protein-binding compounds. Protein-normalized BCFs reveal that the appropriate assessments based on the relevant type of binding (in this case, protein) raise concern about the degree of bioconcentration expected to occur in both aquatic and mammalian biota.

Adhering to the current CEPA classification system (i.e., BCF ≥ 5000 L/kg) to identify the bioaccumulation tendencies of PFOA and PFOS will suffice for the water-respiring species within this food web; however, application to marine mammals reveals that BCF is not necessarily universally applicable to all species. This discrepancy exists because bioconcentration is not the mechanism responsible for PFAA accumulation in air-breathing organisms. Rather, biomagnification, or exposure through diet, is the primary force driving bioaccumulation. It is necessary to create regulations according to the most vulnerable species, which in this case, refers to air-breathing organisms. Previous modeling studies have calculated BCF values for PFOA and PFOS in individual aquatic organisms (in contrast to full food webs), such as fish (see [62]). If BCF values < 5000 L/kg are calculated for individual aquatic species, as is true for PFOA and PFOS within this study, further regulatory attention may not be flagged for the overall food web, even if there are marine mammal species subject to elevated concentrations of PFOA and PFOS. However, despite concerns regarding the application of BCFs as indicators of bioaccumulation for ionizable substances such as PFAAs [5], BCFs calculated by this model in fact estimate BCFs ≥ 5000 L/kg for dolphins. Overall, shifting to a more comprehensive regulatory framework of bioaccumulation analysis is required to account for full food web biomagnification.

4.10. General limitations of study

Diet compositions remain largely unknown for the food web considered in this study and likely other food webs of interest as well. It is not expected that food web interactions are accounted for in their entirety, as quantitative diet analysis for fish species was partially estimated from a qualitative generalization of dietary intake patterns from other studies (e.g., [111]) or large databases (i.e., SeaLifeBase and FishBase). Inaccurate dietary consumption data can further result in inaccurate estimates of bioaccumulation. For instance, the model assumes that zooplankton (TL = 2) makes up 60% of the spotfish diet. If, in reality, spotfish only consumed 20% zooplankton, and the remaining 40% of that dietary intake was actually eastern oyster (TL = 2.3), the model may underestimate bioaccumulation of PFOA and PFOS in spotfish since a large portion of the assumed diet is from a lower trophic level.

Additionally, the partition coefficients used in this model were determined using different methodologies. Octanol-water (K_{OW}) and octanol-air (K_{OA}) partition coefficients were calculated using computational software (i.e., EPI Suite, SPARC), whereas K_{PW} was determined experimentally using BSA as a model protein [82]. Specific computational programs and experimental approaches can often yield variable values for physicochemical properties such as partition coefficients. Implementation of inconsistent calculations and measurements likely had an impact on model results. Furthermore, PFOA and PFOS are sometimes referred to as 'high-K_{OA}' substances in the literature (e.g., [2,73]), defined as compounds with $K_{OA} > 10^6$. According to SPARC calculations, however, K_{OA} values for both PFOA and PFOS are $< 10^6$, below the threshold of as a high-K_{OA} chemical by these standards. Using higher K_{OA} values (such as those reported in [73]) are expected to predict higher concentrations in dolphin, since elimination via respiration for air-breathing species is less efficient with increasing K_{OA} [2]. Variability in partition coefficients also largely impacts calculated TMFs (as determined by the sensitivity analysis described in Section 4.7); therefore, further exploration of partition coefficients will be important in future analyses.

This model does not account for sex-specific or life stage characteristics of bottlenose dolphins. For example, this study did not consider the influence of lactation or birth on PFAA concentrations, which often reduce maternal POP concentrations and

decreasing TMFs [104]. A female dolphin was examined in Charleston Harbor, but characteristics aside from sex and weight are not reported [77]. In other food web models, maternal factors such as fetus-mother chemical partitioning are considered, and are able to account for the higher proportions of blubber in female dolphins compared to male dolphins [144]. This model also does not examine concentrations in young dolphins. In contrast, the killer whale bioaccumulation model [111] predicts bioaccumulation in adult males, adult females, and juvenile killer whales.

Spatial concentration gradients may bias TMF values calculated with field measurements of concentration data [115]. Substantial differences may exist between TMFs calculated from individual studies if spatial concentrations are not consistent across the study area, even with random sampling measures, affecting the general applicability of TMF estimates [115]. There is no knowledge of spatial differences in sediment and water concentrations of PFOA and PFOS in Charleston Harbor, and therefore it is not possible to determine whether spatial gradients in environmental concentrations resulted in inaccurate estimates of bioaccumulation for the biotic components of the food web. The probability of observing a TMF ≥ 1 from field data decreases when spatial gradients are incorporated into analyses [115]. Measured concentrations of PFOA and PFOS in biota may not be an accurate reflection of the water and sediment concentrations measured from Charleston Harbor. Consequently, the observed values may fail to reflect environmental concentrations used as model input. Comparisons between model calculations and empirical measurements should be conducted with caution, as chemical concentrations measured in the Houde et al. (2006) study may not be representative of concentrations within Charleston Harbor. Spatial concentration gradients, in particular, can lead to measured or estimated contaminant levels unrepresentative of average chemical concentrations in environment and biota [115]. Empirical TMFs may be inaccurate due to spatial heterogeneity and temporal variability of PFAS concentrations. Spatial differences in concentrations may exist even on small scales, especially if PFAS pollution originates from a point source (such as discharged water), or enters Charleston Harbor via runoff in particular locations, creating a contaminant plume with a defined pollution gradient. Therefore, spatial concentration gradients can exist even within resident dolphin habitat areas. Such phenomena can occur even within carefully planned studies designed to reduce confounding factors.

Given that water and sediment concentrations serve as model inputs, any spatial concentration gradients present during sampling could influence model-calculated concentrations within biota. This may help to explain the lower estimated TMF values compared to the field-derived TMFs.

Specific binding to may affect bioaccumulation of these PFAAs, but were not thoroughly investigated in this model. Some research suggests that specific protein interactions are important for bioaccumulation of PFAAs in fish and mammals because of various pharmacokinetics associated with different types of protein [84]. For instance, organic anion transporter (OAT) proteins are associated with renal reabsorption of organic anions from urine to blood [183]. Therefore, concentrations of PFOA and PFOS normally expected to be excreted via urine are reabsorbed within biota, leading to higher concentrations in organisms than calculated from a model applying non-specific binding to protein [84]. Furthermore, the sorption of compounds to serum albumin is influenced by competing proteins, whereas this is not the case for muscle protein [153]. This introduces another potential consequence of assuming partitioning to serum albumin only. Integrating specific binding into this mechanistic model could improve overall estimates of bioaccumulation by capturing the unique physiochemical properties and pharmacokinetic interactions of PFOA and PFOS. Simultaneously, however, there is also value in maintaining more generalized models that apply non-specific binding, given that overall trends in bioaccumulation are established. As such, pharmacokinetic assessments are not included in this model, though it is recognized that interspecies and gender variability for clearance and circulation of PFAAs may influence bioaccumulation of these anionic compounds [84,184-186]. Similarly, the presence of branched versus linear isomers was not explicitly integrated into the model. Branched PFCA and PFOS molecules are eliminated from biota more efficiently than are their linear counterparts, but the model was designed only for linear isomers [187]. Branched isomers for PFOA, in particular, are rarely observed in biota and account for <1% of total PFOA concentrations in biota [187-189]. The model performed well for PFOS without considering branched isomers, suggesting that the parameters within the model adequately captured factors influencing PFOS bioaccumulation.

4.11. Future Directions

The model developed in this study can be applied to quantify the maximum allowable concentrations in environmental media (e.g., water, sediment) not to be exceeded in order to prevent concentrations associated with specific toxicological endpoints in higher trophic level organisms. Maximum concentrations of PFOA and PFOS assumed to maintain safe concentrations for species in all trophic levels may be set too high to protect top predators (e.g., bottlenose dolphins) if trophic magnification occurs. Evaluating suitable maximum concentrations involves determining whether, when the TMF > 1, trophic magnification occurs despite setting maximum environmental concentration limits. For example, a maximum permissible concentration for PFOS of 0.65 ng/L has been calculated for fresh water in parts of Europe [190], but in Canada, draft federal environmental quality guidelines for PFOS recommend a much higher limit of 6000 ng/L in water [191]. Mean water concentrations of PFOA and PFOS measured in Charleston Harbor were 4.8 ng/L and 4.2 ng/L, respectively, below the recommended concentration by Environment Canada. However, concentrations above the regulatory thresholds were observed in Charleston Harbor dolphins [77]. Threshold concentrations can be revised to protect higher trophic level species based on expected bioaccumulation trends.

Increasing concerns regarding the toxic and bioaccumulative effects of longer chain PFAAs has encouraged manufacturers (predominantly in North America and Europe) to eliminate these compounds (e.g., PFOA and PFOS) from commerce, producing shorter chain substitutes instead [45]. For example, perfluorobutane sulfonate (PFBS), a common chemical substitute for PFOS, is a degradation product of perfluorobutane sulfonyl fluoride (PBSF)-based compounds [192]. This chemical substitute is manufactured because a compound with four fluorinated carbons should pose fewer health and environmental risks compared to their longer-chain counterparts. (For instance, the EC_{10} of PFBS in chicken serum is 95x lower than that of PFOS and also has a much shorter half-life than PFOS) [132,193]. The question remains as to whether PFBS and other shorter-chain or structurally distinct alternatives are actually suitable substitutes in terms of bioaccumulation potential. Research on the toxicity and persistence of PFBS generally suggests fewer adverse effects on organisms compared

with PFOS (e.g., [193-196]). It follows that PFBS is expected to pose fewer overall health risks to humans and wildlife, yet its persistence is on par with that of long-chain PFAAs [197]. The shorter chain of PFBS also makes the compound more mobile in the environment. In some cases, bioaccumulation is observed to increase with decreasing chain length [197]. It remains necessary to adequately investigate the bioaccumulative potential of PFAA substitutes. If, through the application of an adapted bioaccumulation model, PFBS is projected to biomagnify in food webs, the toxic effects of this compound may be amplified, as the compound would reach higher concentrations than expected throughout the food web. For instance, there are concerns regarding the general lack of experimental data describing the physicochemical properties of fluorinated alternatives, stating in particular that qualitative analyses suggest no difference in terms of associated health risks between long-chain PFAAs and their shorter chain alternatives [121]. Similarly, studies have shown similar sorption of shorter chain PFAAs (i.e., PFBA and PFPeA) to BSA as for longer chain PFAAs, signifying that chain lengths may not be indicative of accumulation within certain types of protein [82]. The model developed in this study can be used to evaluate the suitability of chemical substitutes based on specific physicochemical properties. Future research should utilize models like the one developed in this study, as they can easily be applied to shorter-chain PFAAs (given knowledge of basic physicochemical properties) to determine their expected bioaccumulation behaviour.

5. Conclusion

To my knowledge, this is the first study to develop and apply a food web bioaccumulation model designed explicitly for PFAAs in food webs containing both water- and air-breathing species in order to capture the bioaccumulative tendencies of ionizable compounds, including PFOA and PFOS.

The food web model developed for PFOA and PFOS has made several modifications over previous versions of the model. First, the new model accounts for compounds that are completely or almost completely ionized at environmentally and physiologically relevant pH, rather than assuming neutral speciation. Second, the model includes marine mammals and is not limited to water-respiring species. Third, this study evaluates several metrics of bioaccumulation, allowing for a more comprehensive analysis of contaminant behaviour within individual species (BCF), predator-prey relationships (BMF), and the full food web (TMF).

This study adapted an existing food web bioaccumulation model from its original design (i.e., intended for neutral, lipophilic contaminants) for PFOA and PFOS, two contaminants of environmental concern for ecosystems, wildlife, and human health. The modified model presented here addresses several outstanding issues encountered when modeling food web accumulation of perfluorinated substances, effectively taking into account the necessary elements required to adequately estimate the behaviour of PFOA and PFOS in an aquatic marine food web.

This study supports the theory that PFAAs behave differently from neutral, lipophilic substances within aquatic food webs containing both water- and air-respiring species. According to the model, PFAAs are not expected to bioaccumulate in aquatic, water-respiring organisms. The modified model predicts BCFs < 5000 for aquatic species in this food web, indicating that PFOA and PFOS are not bioaccumulative under Canadian regulations. Without considering any other metrics of bioaccumulation, PFOA

and PFOS would not be considered bioaccumulative concern. However, investigation of BMFs (multiple predator-prey relationships >1) and TMFs (>1) expose the bioaccumulative tendencies of both compounds throughout the food web.

This study challenges the capacity of existing chemical regulations to protect top marine predators, especially air-breathing mammalian species from bioaccumulation of PFOA and PFOS. BCFs are the main metric of bioaccumulation measured under Canadian environmental regulations, where chemicals with BCF values <5000 L/kg are considered 'non-bioaccumulative'. However, even when BCFs remain below this threshold in aquatic organisms, application of the modified model reveals that this condition does not guarantee the protection of air-breathing organisms (i.e., bottlenose dolphin) at higher trophic levels, where BCF ≥ 5000. Without examining the actual bioaccumulation behaviour of PFAAs in air-breathing organisms, the elevated concentrations identified by this model and field observations would go unacknowledged. PFAAs could reach harmful concentrations in the bottlenose dolphin and other marine mammals, amplifying the toxic effects of these compounds within the ecosystem.

This study reiterates that all metrics of bioaccumulation evaluated here – the BCF, BMF, and TMF – have certain advantages, but also have inherent biases and potential disadvantages. This concept becomes increasingly important as an increasing number of chemicals are identified as environmental contaminants. Estimates of food web magnification determined by this model are fairly consistent with observed trophic magnification of PFOA and PFOS, due to modifications intended to sufficiently reflect the chemical properties and trophic behaviour of perfluorinated substances. This study highlights the usefulness of the TMF to provide a more holistic approach to food web bioaccumulation modeling. Future consideration of the TMF as a holistic bioaccumulation tool is encouraged in modeling and empirical research. In this study, the TMF best described the observed bioaccumulative behaviour of PFOA and PFOS in a Charleston Harbor marine food web. Though several concerns regarding the application of the TMF have been addressed [53,115,198], the TMF was a useful means to estimate and evaluate food web accumulation of PFOA and PFOS, and provided the best estimate of chemical behaviour in a food web compared to the BCF and BMF. This

framework can also be adapted and applied to other industrial and commercial ionogenic organic compounds aside from PFAAs.

Using environmental concentration data from Charleston Harbor, this study suggests that trophic magnification of PFOA and PFOS occurs in this food web with (TMF = 1.3) or without (TMF = 1.2) the presence of an air-breathing animal (i.e. dolphin), which is unexpected considering PFAAs are not expected to bioaccumulate in water-respiring organisms. This result suggests that PFOA and PFOS may bioaccumulate and biomagnify in aquatic ecosystems previously not considered as problematic.

This study also brings attention to the importance of food web composition on TMFs. Empirical concentration data was available for fish and dolphin in the Charleston Harbor food web (TLs 3.4-4.4). When just these species were considered in model analysis, TMFs were equal to 2.5 for PFOA and 3.0 for PFOS. However, in order to estimate a TMF more representative of a full food web, species from lower trophic levels included in similar studies (i.e., TLs 1 through 2.8) were integrated into the model. Under these conditions, TMFs decreased to 1.3 for both PFOA and PFOS. Trophic positions and the number of species included in the regression analyses to calculate TMF influence the resulting trophic magnification. It is important to acknowledge and account for food web composition when evaluating TMFs and trophic magnification in order to recognize or mitigate the influence of species distribution throughout food webs.

Continued development of food web specific bioaccumulation models is essential for reaching an improved understanding of the biological and physiological impacts of these problematic compounds within ecosystems. Ionogenic substances such as PFOA and PFOS remain a serious concern to ecosystems, and require a proactive response within science and policy. Expectantly, continued work in this area will further demonstrate the need for modified regulatory criteria in Canada and elsewhere in the world.

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Appendix A.

Ionogenic Concentration Model Equations

The bioconcentration model describes uptake and elimination via the respiratory route.

It views uptake as a result of gill ventilation, transport through aqueous boundary layers, and parallel transport through the membrane bilayers and pore transport.

The overall resistance encountered by bioconcentrating chemicals can be expressed as:

$R_{total} = R_{ventilation} + (1/R_{mem} + 1/R_{pore})^{-1} + R_{internal}$

Where:

R_{total} : Total resistance for uptake or elimination

$R_{ventilation}$: Resistance due to gill ventilation

$R_{internal}$: Resistance due to water phase diffusive transport

R_{mem} : Resistance due to lipid phase diffusive transport

R_{pore} : Resistance due to pore/joint gap transport

The resistance R is the reciprocal of the conductivity D, where D is the transport parameter in units of $mol.d^{-1}.Pa^{-1}$.

$1/D_{total} = 1/D_{ventilation} + 1/D_{internal} + (D_{mem} + D_{pore})^{-1}$

Where:

$D_{total} = k_1 \cdot V_F \cdot Z_W = k_2 \cdot V_F \cdot Z_F$

$D_{ventilation} = Q_V \cdot Z_W$

$D_{internal} = Q_{internal} \cdot Z_W = (1/Q_W - 1/G_V)^{-1}.Z_W$

$D_{mem} = Q_{mem} \cdot Z_L$

$D_{pore} = Q_P \cdot Z_W$

After substitution:

$1/(k_1.V_F.Z_W) = 1/(Q_{ventilation} \cdot Z_W) + 1/(Q_{internal} \cdot Z_W) + 1/(Q_{mem} \cdot Z_L + Q_{pore}.Z_W)$

Multiply both sides with Z_W:

$1/(k_1 \cdot V_F) = 1/(Q_{ventilation}) + 1/(Q_{internal}) + 1/(Q_{mem} \cdot K_{LW} + Q_{pore})$

Multiply by mass of fish (V_F):

$1/k_1 = V_F \cdot (1/Q_{ventilation} + 1/Q_{internal} + 1/(Q_{mem} \cdot K_{LW} + Q_{pore}))$

Where:

$Q_W = 88.3 \cdot V_F^{0.6}$, where Q_W is in L/d and V_F is in kg

$Q_{internal} = (1/Q_W - 1/G_V)^{-1}$

$Q_{mem} = 0.011 \cdot Q_W$

$Q_{ventilation} = A \cdot V_F^{0.8} / (E_{ox} \cdot C_{ox})$, where for rainbow trout: A = 0.14 mL $O_2/(g^{0.8} \cdot d)$

E_{ox} is oxygen uptake efficiency

C_{ox} is oxygen concentration

Also:

$K_{FW} = Z_F/Z_W = k_1/k_2$

Thus:

$k_2 = k_1 \cdot Z_W/Z_F$

Appendix B.

Charleston Harbor Diet Composition

Table A-1. Possible diet composition of the Charleston Harbor bottlenose dolphin food web used to calculate BCFs, BMFs, and TMFs (modified from Alava et al. 2012).

			Prey (% Diet)[1]													
		TL	Sed	Phy	Zoo	Marine Invertebrate					Fish					
			Sed	Phy	Zoo	Oli	GSh	HCm	EOy	BCr	SMu	RDr	ACr	Spt	Pin	SSt
Phytoplankton	n/a	1														
Zooplankton	Copepoda	2	0	100												
Marine Invertebrate	Oligochaete	2.1	0.9	0.05	0.05											
	Grass shrimp	2.1	0.3	0.35	0.35	0										
	Hard clam	2.2	0.3	0.35	0.35	0	0									
	Eastern oyster	2.3	0.15	0.6	0.25	0	0	0								
	Blue crab	2.8	0.45	0.05	0.2	0.1	0.05	0.05	0.1							
Fish	Striped mullet	3.4	0.15	0.55	0.3	0	0	0	0	0						
	Red drum	3.9	0	0.5	0.3	0.2	0	0	0	0	0					
	Atlantic croaker	4.2	0	0	0.75	0	0	0.1	0.1	0	0.05	0				

	Spotfish	4.2	0	0	0.8	0.1	0.1	0	0	0	0	0	0			
	Pinfish	4.3	0.6	0	0.3	0.1	0	0	0	0	0	0	0	0		
	Spotted seatrout	4.3	0	0	0	0.5	0.2	0.1	0.1	0.1	0	0	0	0	0	
Marine Mammal	Bottlenose dolphin	4.4	0	0	0	0	0.1	0	0	0.2	0.2	0.2	0	0.2	0	0.2

Legend: Sed = sediment; Phy = phytoplankton; Zoo = zooplankton; Oli = Oligochaete; GSh = Grass Shrimp; HCm = Hard Clam; EOy = Eastern Oyster; BCr = Blue Crab; SMu = Striped Mullet; RDr = Red Drum; ACr = Atlantic Croaker; Spt = Spotfish; Pin = Pinfish; SSt = Spotted Seatrout.

Appendix C.

Biological and Physiological Parameters for Food Web Model

Table A-2. Overall review of inputs for species specific biological and physiological parameters of the food web model.

Parameter	Value/Input	Reference
Species	***Phytoplankton***	
Trophic Level	1	Gobas et al. (2015)
Weight (kg)	N/A	Gobas et al. (2015)
Non-Polar Lipid Content (%)	2.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	10.0%	Hendriks et al. (2005)
Growth Rate Constant (1/day)	8.00E-02	Alpine and Cloern (1992)
Aqueous phase resistance constant (Ap) (1/day)	6.00E-05	Arnot and Gobas (2004)
Organic phase resistance constant (Bp) (1/day)	5.50E+00	Arnot and Gobas (2004)
Species	***Zooplankton***	
Species Name	*Copepoda sp.*	
Trophic Level	2	Gobas et al. (2015)
Weight (kg)	1.00E-07	Gobas et al. (2015)
Non-Polar Lipid Content (%)	2.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	10.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	0.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E+00	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	85.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	85.0%	Estimated based on Arnot and Gobas (2004)

Protein Digestion Efficiency (ε_{PR})	75.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_{W})	55.0%	Arnot and Gobas (2004)

Species	***Invertebrate 1***	
Species Name	*Oligochaete*	
Trophic Level	2.1	Based on Alava et al. (2012)
Weight (kg)	1.00E-04	Gobas et al. (2015)
Non-Polar Lipid Content (%)	2.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	10.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	100.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E+00	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	75.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	75.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_{W})	55.0%	Arnot and Gobas (2004)

Species	***Invertebrate 2***	
Species Name	Grass Shrimp (*Palaemonetes pugio*)	
Trophic Level	2.1	Based on Alava et al. (2012)
Weight (kg)	1.00E-03	Gobas et al. (2015)
Non-Polar Lipid Content (%)	2.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	13.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	5.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	75.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	75.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	50.0%	Arnot and Gobas (2004)

Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Invertebrate 3***	
Species Name	Hard Clam (*Mercenaria mercenaria*)	
Trophic Level	2.2	Based on Alava et al. (2012)
Weight (kg)	1.00E-02	Gobas et al. (2015)
Non-Polar Lipid Content (%)	2.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	10.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	5.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	75.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	75.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Invertebrate 4***	
Species Name	Eastern Oyster (*Crassostrea virginica*)	
Trophic Level	2.3	Based on Alava et al. (2012)
Weight (kg)	1.00E-02	Gobas et al. (2015)
Non-Polar Lipid Content (%)	2.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	10.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	5.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	75.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	75.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	50.0%	Arnot and Gobas (2004)

Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Invertebrate 5***	
Species Name	Blue Crab (*Callinectes sapidus*)	
Trophic Level	2.8	Based on Alava et al. (2012)
Weight (kg)	1.00E-02	Gobas et al. (2015)
Non-Polar Lipid Content (%)	2.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	13.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	5.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	75.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	75.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	50.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Fish 1***	
Species Name	Striped Mullet (*Mugil cephalus*)	
Trophic Level	3.4	Houde et al. (2006)
Weight (kg)	1.00E-01	Gobas et al. (2015)
Non-Polar Lipid Content (%)	4.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	18.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	5.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	92.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	90.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Fish 2***	
Species Name	Red Drum (Sciaenops ocellatus)	
Trophic Level	3.9	Houde et al. (2006)
Weight (kg)	1.00E-01	Gobas et al. (2015)
Non-Polar Lipid Content (%)	4.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	18.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	5.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	92.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	90.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	**Fish 3**	
Species Name	*Atlantic Croaker (Micropogonias undulatus)*	
Trophic Level	4.2	Houde et al. (2006)
Weight (kg)	1.00E+00	Gobas et al. (2015)
Non-Polar Lipid Content (%)	4.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	18.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	0.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	92.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	90.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Fish 4***	

Species Name	Spotfish (*Leiostomus xanthurus*)	
Trophic Level	4.2	Houde et al. (2006)
Weight (kg)	1.00E+00	Gobas et al. (2015)
Non-Polar Lipid Content (%)	4.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	18.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	0.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	92.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	90.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Fish 5***	
Species Name	Pinfish (*Lagodon rhomboids*)	
Trophic Level	4.2	Houde et al. (2006)
Weight (kg)	1.00E+00	Gobas et al. (2015)
Non-Polar Lipid Content (%)	4.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	18.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	0.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	92.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	90.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Fish 6***
Species Name	Spotted seatrout (*Cynoscion nebulosus*)

Trophic Level	4.3	Houde et al. (2006)
Weight (kg)	1.00E+00	Gobas et al. (2015)
Non-Polar Lipid Content (%)	4.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	18.0%	Hendriks et al. (2005)
Fraction of Respired Pore Water (%)	0.0%	Gobas et al. (2015)
E_D – Constant A	8.50E-08	Arnot and Gobas (2004)
E_D – Constant B	2.00E-08	Arnot and Gobas (2004)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	92.0%	Arnot and Gobas (2004)
Polar Lipid Digestion Efficiency (ε_{PL})	90.0%	Estimated based on Arnot and Gobas (2004)
Protein Digestion Efficiency (ε_{PR})	60.0%	Arnot and Gobas (2004)
Water Digestion Efficiency (ε_W)	55.0%	Arnot and Gobas (2004)

Species	***Marine Mammal***	
Species Name	Bottlenose Dolphin (*Tursiops truncatus*)	
Trophic Level	4.4	Houde et al. (2006)
Weight (kg)	7.08E+02	Houde et al. (2006)
Non-Polar Lipid Content (%)	9.0%	Hendriks et al. (2005)
Polar Lipid Content (%)	1.0%	Hendriks et al. (2005)
Protein Content (%)	21.0%	Hendriks et al. (2005)
E_D – Constant A	1.00E-09	Moser and McLachlan (2001)
E_D – Constant B	1.03E+00	Moser and McLachlan (2002)
Lung Respiration Rate (G_V) (L/day)	1.65E+05	Estimated in Model
Food Ingestion Rate (G_D) (kg food/day)	6.50E+00	Hickie et al. (2013)
Urinary Excretion Rate Constant (G_U) (L/day)	2.61E-01	Based on Hickie et al. (2013)
Non-Polar Lipid Digestion Efficiency (ε_{NPL})	100.0%	Kelly and Gobas (2003)
Polar Lipid Digestion Efficiency (ε_{PL})	95.0%	Estimated based on Kelly and Gobas (2003)
Protein Digestion Efficiency (ε_{PR})	98.0%	Kelly and Gobas (2003)
Water Digestion Efficiency (ε_W)	85.0%	Kelly and Gobas (2003)

Appendix D.

Output Parameters From Food Web Model

Table A-3. Model output parameters from the updated food web model. Final concentrations are protein-normalized.

(a) PFOA

Species Type	Species Name	Gill/Lung Uptake Efficiency E_W	Gill/Lung Uptake Rate Constant k_1 (L/kg·d)	Gut Uptake Efficiency E_D	Dietary Uptake Rate Constant k_D (kg/kg·d)	Gill/Lung Elimination Rate Constant k_2 (1/d)	Fecal Excretion Rate Constant k_E (1/d)	Growth Rate Constant k_G (1/d)	Diet Concentration C_D	[PFOA] (ng/kg pw)
Phytoplankton	n/a	n/a	2.49E+02	n/a	n/a	1.61E-01	n/a	8.00E-02	n/a	6.27E+04
Zooplankton	Copepoda	1.36E-03	2.72E+04	5.00E-01	8.72E+02	1.95E+01	2.19E+02	8.79E-03	6.30E+03	2.36E+05
Marine Invertebrate	Oligochaete	3.75E-01	1.71E+03	5.00E-01	1.18E-01	1.23E+00	5.93E-03	2.21E-03	1.68E+03	3.49E+04
	Grass shrimp	3.34E-01	6.83E+02	5.00E-01	8.34E-02	3.78E-01	2.25E-02	1.39E-03	1.06E+04	9.36E+04
	Hard clam	2.98E-01	6.08E+02	5.00E-01	8.34E-02	4.37E-01	1.59E-02	8.79E-04	8.37E+03	9.39E+04
	Eastern oyster	2.98E-01	6.08E+02	5.00E-01	8.34E-02	4.37E-01	2.51E-02	8.78E-04	9.75E+03	9.45E+04
	Blue crab	2.98E-01	6.08E+02	5.00E-01	8.34E-02	3.37E-01	1.67E-02	8.79E-04	7.55E+03	9.12E+04
Fish	Striped mullet	2.66E-01	1.08E+02	5.00E-01	4.18E-02	4.33E-02	7.88E-03	2.22E-03	1.06E+04	1.12E+05

	Red drum	2.66E-01	1.08E+02	5.00E-01	4.18E-02	4.33E-02	9.27E-03	2.22E-03	1.10E+04	1.11E+05
	Atlantic croaker	2.37E-01	4.31E+01	5.00E-01	2.96E-02	1.72E+02	6.83E-03	1.40E-03	2.07E+04	1.90E+05
	Spotfish	2.37E-01	4.31E+01	5.00E-01	2.96E-02	1.72E-02	6.76E-03	1.40E-03	2.06E+04	1.90E+05
	Pinfish	2.37E-01	4.31E+01	5.00E-01	2.96E-02	1.72E-02	2.63E-03	1.40E-03	7.60E+03	1.27E+05
	Spotted seatrout	2.37E-01	4.31E+01	5.00E-01	2.96E-02	1.72E-02	7.15E-03	1.40E-03	7.30E+03	1.03E+05
Marine Mammal	Bottlenose dolphin**	7.00E-01	1.63E+02	9.76E-01	8.95E-03	1.49E-04	1.45E-04*	9.18E-04	1.99E+04	6.96E+05

(b) PFOS

Species Type	Species Name	Gill Uptake Efficiency E_W	Gill Uptake Rate Constant k_1 (L/kg·d)	Gut Uptake Efficiency E_D	Dietary Uptake Rate Constant k_D (kg/kg·d)	Gill Elimination Rate Constant k_2 (1/d)	Fecal Excretion Rate Constant k_E (1/d)	Growth Rate Constant k_G (1/d)	Diet Concentration C_D	[PFOS] (ng/kg pw)
Phytoplankton	n/a	n/a	2.27E+02	n/a	n/a	1.61E-01	n/a	8.00E-02	n/a	5.89E+04
Zooplankton	Copepoda	8.69E+04	1.74E+04	5.00E-01	8.72E+02	1.37E+01	2.19E+02	8.79E-03	5.94E+03	2.26E+05
Marine Invertebrate	Oligochaete	2.40E-01	1.10E+03	5.00E-01	1.18E-01	8.67E-01	5.93E-03	2.21E-03	2.05E+03	1.19E+05
	Grass shrimp	2.14E-01	4.36E+02	5.00E-01	8.34E-02	2.66E-01	2.25E-02	1.39E-03	1.02E+04	9.74E+04
	Hard clam	1.91E-01	3.89E+02	5.00E-01	8.34E-02	3.07E-01	1.59E-02	8.79E-04	8.17E+03	9.82E+04
	Eastern oyster	1.91E-01	3.89E+02	5.00E-01	8.34E-02	3.07E-01	2.51E-02	8.78E-04	9.36E+03	9.85E+04
	Blue crab	1.91E-01	3.89E+02	5.00E-01	8.34E-02	2.37E-01	1.67E-02	8.79E-04	8.47E+03	9.72E+04
Fish	Striped mullet	1.70E-01	6.92E+01	5.00E-01	4.18E-02	3.04E-02	7.89E-03	2.22E-03	1.02E+04	1.19E+05
	Red drum	1.70E-01	6.92E+01	5.00E-01	4.18E-02	3.04E-02	9.28E-03	2.22E-03	1.22E+04	1.26E+05
	Atlantic croaker	1.51E-01	2.75E+01	5.00E-01	2.96E-02	1.21E-02	6.83E-03	1.40E-03	2.01E+04	2.10E+05
	Spotfish	1.51E-01	2.75E+01	5.00E-01	2.96E-02	1.21E-02	6.77E-03	1.40E-03	2.07E+04	2.15E+05
	Pinfish	1.51E-01	2.75E+01	5.00E-01	2.96E-02	1.21E-02	2.63E-03	1.40E-03	8.43E+03	1.45E+05

	Spotted seatrout	1.51E-01	2.75E+01	5.00E-01	2.96E-02	1.21E-02	7.16E-03*	1.40E-03	1.18E+04	1.40E+05
Marine Mammal	Bottlenose dolphin**	7.00E-01	1.63E+02	9.76E-01	8.95E-03	1.98E-05	1.45E-04	9.18E-04	2.30E+04	9.01E+05

*Urinary excretion rate constant for bottlenose dolphin (k_U) = 1.11E-07 day^{-1} (PFOA); 1.22E-07 day^{-1} (PFOS)
**For mammals, values describe parameters for pulmonary respiration in place of gill respiration.

Appendix E.

Estimated Concentrations of PFOA and PFOS in Biota

Table A-4. Calculated concentrations for PFOA and PFOS in biota from the Charleston Harbor marine food web using the modified model. Note: water and sediment concentrations were obtained from Houde et al. (2006) for use as input concentration values.

		TL[a]	PFOA (ng/kg pw)	PFOS (ng/kg pw)
Water[b]	*n/a*	*n/a*	*6.10E+00*	*6.33E+00*
Sediment	*n/a*	*n/a*	*1.95E+02*	*6.80E+02*
Phytoplankton	n/a	1	6.27E+04	5.89E+04
Zooplankton	Copepoda	2	8.88E+04	8.57E+04
Marine Invertebrate	Oligochaete	2.1	3.42E+04	1.18E+05
	Grass shrimp	2.1	8.54E+04	8.65E+04
	Hard clam	2.2	8.45E+04	8.55E+04
	Eastern oyster	2.3	8.79E+04	8.97E+04
	Blue crab	2.8	8.56E+04	8.95E+04
Fish	Striped mullet	3.4±0.4	9.30E+04	9.52E+04
	Red drum	3.9±0.3	1.15E+05	1.22E+05
	Atlantic croaker	4.2±0.2	1.13E+05	1.22E+05
	Spotfish	4.2±0.5	9.25E+04	1.02E+05
	Pinfish	4.3±0.4	9.21E+04	1.03E+05

	Spotted seatrout	4.3±0.2	9.96E+04	1.35E+05
Marine Mammal	Bottlenose dolphin	4.4±0.2	5.42E+05	6.89E+05

[a]TLs for phytoplankton, zooplankton, and invertebrates based on[111]; TLs for fish and marine mammal obtained from [77].
[b]Water concentrations in ng/L (pw).