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A general model of polyunsaturated fatty acid (PUFA) uptake, loss and transformation in freshwater fish

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ABSTRACT

Polyunsaturated fatty acids (PUFA) are crucial nutrients for fish and have been identified as globally-limited nutrients that are needed for ecosystem and human health. Greater understanding is needed into the processes influencing observed PUFA levels in fish because human demand for PUFA is increasing due to their nutritional benefits and because anthropogenic stresses threaten to reduce PUFA production. We present a general, process-based mass balance model for freshwater fish that estimates concentrations of n-3 (α-linolenic acid: ALA, eicosapentaenoic acid: EPA and docosahexaenoic acid: DHA) and n-6 (linoleic acid: LIN and arachidonic acid: ARA) PUFA from prey food items. Our model considers the processes of dietary uptake, absorption efficiency, egestion, transformation (elongation and/or desaturation) and β-oxidation. The model relies on rate constants derived from multiple regression analysis for egestion, transformation and β-oxidation based on ecological and physiological variables (i.e. body weight, diet, PUFA interactions). All regression equations had adjusted $R^2 \geq 0.47$ and p values <0.001. Application of the model to Yellow Perch (*Perca flavescens*) from the Upper Bay of Quinte, Canada provided estimates of ALA, EPA, DHA, LIN and ARA contents that were within a standard deviation of measured values without model calibration. The model showed that diet was the main source of ALA, EPA, LIN and ARA. Transformation of EPA to DHA was the dominant source of DHA. We hypothesize that within-fish transformation of precursor and the resultant product PUFA can compensate, to some extent, for dietary deficiencies in long chain PUFA in the diet of this freshwater fish.

1. Introduction

Polyunsaturated fatty acids (PUFA) play a role in mitigating cardiovascular disease (Lemaitre et al., 2003; Yokoyama et al., 2007), moderating tissue inflammation (Ruxton et al., 2004; Uauy and Valenzuela, 2000), and contributing to the development of nervous (Burdge, 1998), reproductive (Sidhu, 2003), and visual (Kim and Mendis, 2006) systems in humans and other vertebrates. The essentiality of some PUFA (i.e. α-linolenic acid and linoleic acid) stems from the inability of animals, including humans, to synthesize them de novo and/or, in the case of long-chain PUFA (LC PUFA = PUFA with 20 or more carbon atoms), at rates sufficient to maintain optimal health (Bell and Tocher, 2009).

In addition to a vital role in human health, lipids are critical components in fish nutrition as sources of energy, essential fatty acids (EFA) and sterols (Hansen et al., 2011). PUFA are crucial for fish because they affect metabolic activity, growth rates and reproduction. When available in adequate supply, EFA reduce the likelihood that fish will exhibit various pathologies (Watanabe, 1982). Additionally, PUFA are required for regulating hormonal processes (Arts and Kohler, 2009). In fish, the physiologically essential PUFA include the n-3 FA α-linolenic acid (ALA, 18:3n-3), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) and the n-6 FA linoleic acid (LIN, 18:2n-6) and arachidonic acid (ARA, 20:4n-6) (Parrish, 2009 inter alia). Parrish (2009) also proposed that docosapentaenoic acid (DPA, 22:5n-3) is an EFA but we do not consider it further because of a lack of information.

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The low melting points of PUFA (around −50 °C) are an important factor in the maintenance of cell membrane fluidity and consequently PUFA play an important role in biochemical adaptation to cold, aquatic environments (Arts and Kohler, 2009). As noted above, fish cannot synthesize ALA or LIN at all or transform these FA de novo into EPA, DHA and ARA, respectively, at rates sufficient to maintain themselves in an optimum physiological state (Parrish, 2009). As such, ALA and LIN must be obtained largely through the diet whereas longer chain PUFA can be supplied, to some extent, by transformation (Bézard et al., 1994; Hessen and Leu, 2006; Kainz et al., 2004). These requirements for specific FA contribute to the general finding that dietary FA composition is, at least in part, reflected in fish tissues (Karalazos et al., 2011; Richard et al., 2006; Teoh et al., 2011). Thus, PUFA have been used in conjunction with other FA as biomarkers to elucidate trophic relationships (Bérge and Barnathan 2005; Gomes et al., 2010; Van Biesen and Parrish, 2005).

The importance of PUFA to human and aquatic ecosystem health led Budge et al. (2014) and Arts et al. (2001) to propose that PUFA have a limited global supply and as such, should be managed in order to ensure adequate supplies. At the same time as global demand for PUFA is increasing, their production at current rates may be threatened by several anthropogenic stressors. For example, eutrophication of freshwater systems can cause lowered production due to a shift from high PUFA quality algal communities (i.e. diatoms) to low PUFA quality algae communities (i.e. cyanobacteria) that then limit the flow of mass/energy through the food web (Brett and Müller-Navarra, 1997). Similarly, increased water temperatures associated with climate change has the potential to reduce PUFA production at the base of aquatic food webs (Fuschino et al., 2011). These competing pressures of increasing PUFA demand by human consumers and potentially reduced production due to human stressors invite a better understanding of the associated processes and emphasize the need for more effective management of PUFA supply and demand. Here we present a model that allows for exploration of these competing pressures in the context of a single fish.

Several FA transport and accumulation models have been proposed for humans, rats and fish. For example, Pawlosky and coworkers (2001, 2003) examined the metabolism of ALA in humans using stable isotope tracers and developed a multicompartmental model to elucidate FA contributions to the maintenance of n-3 PUFA in plasma. Cunnane and Anderson (1997) first proposed a whole-body FA mass balance model for rats, emphasizing the importance of accumulation, transformation and β-oxidation pathways. Finally, Turchini and coworkers (2006, 2007) were the first to develop a compartmental FA mass balance method for fish, focusing on freshwater Murray Cod (*Maccullochella peelii*). Their method requires knowledge of the fish's initial and final body weight, initial and final quantitative fatty acid composition of the whole body, the total food intake, the quantitative fatty acid content of the diet, and the fatty acid digestibility of the quantitative fatty acid content of the total feces produced during the experiment. Using their mass balance method, it is possible to measure the fate of individual fatty acids with respect to transformation and β-oxidation. This method has been well received due to its simplicity and reliability. However, this method requires data that are made available through feeding trials of sufficient duration and thus cannot be applied to fish in natural environments.

We present a mechanistic, whole-body mass balance model for PUFA-specific uptake and elimination in a "generic" predatory freshwater fish. The model presented here can be used as a heuristic tool to understand the consequences of limiting individual PUFA on patterns of PUFA accumulation within a freshwater fish. Such circumstances can arise when anthropogenic stressors impact PUFA availability at the base of the food web as well as effects of changing the characteristics of the fish itself.

Our model builds on the approach previously proposed by Cunnane and Anderson (1997), Pawlosky et al. (2001, 2003), and Turchini et al. (2006, 2007). Physiological and ecological data (i.e. body weight, diet matrices and temperature) are used to predict rate constants for physiological processes, which allow application to temperate freshwater predatory fish species. This approach differs from the model of Turchini et al. (2006, 2007) that relies on laboratory measurements of PUFA in fish to calculate transformation and β-oxidation rates. Our general mass balance model for freshwater fish simultaneously examines the uptake and elimination of n-3 (ALA, EPA and DHA) and n-6 (LIN and ARA) PUFA in an individual fish consuming multiple prey species. The mass balance model simplifies the complexity of PUFA metabolism by considering the processes of dietary uptake, egestion, transformation (elongation and/or desaturation), and β-oxidation. Expressions to estimate rate constants for each of the above processes were developed using literature data and multiple regression analysis. We provide justification for the mass balance model equation and discuss the specific rate constant regression equations. We then apply the mass balance model to a single fish species, Yellow Perch (*Perca flavescens*), to illustrate the model's utility and to demonstrate how the model can provide insights into what controls observed patterns of PUFA in fish.

2. Model development

2.1. Mass balance equation

Both marine and freshwater fish PUFA profiles are affected by and reflective of their dietary PUFA (Benedito-Palos et al., 2011; Richard et al., 2006; Turchini et al., 2006). Following ingestion, a PUFA is either digested or egested (Fig. 1). Upon digestion, a PUFA may be transformed by elongation and/or desaturation to longer chain FA or β-oxidized for energy production (Fig. 1).

Chemical similarities of PUFA can lead to competitive interactions in the biochemical and physiological reactions undergone by the parent compounds, precursors and products (Sargent et al., 1999). The mass balance model presented here considers uptake, loss and interconversion of five (5) PUFA (ALA, EPA, DHA, LIN and ARA). PUFA content is expressed on a dry weight basis (i.e. mass fractions).

We express the mass balance for PUFA metabolism in a predator using the generic equation as follows:

$$\frac{dm_{i,x}}{dt} = \sum_{i,j=1}^{n} \gamma_{Gi,x} m_{j,x} k_{Aji,x} - m_{i,x}(k_{Ei,x} + k_{Oi,x}) \pm m_{Ti,xy}(k_{Ti,xy}) \qquad (1)$$

where 'm' is the content (mg D.W.) of PUFA 'x' and transformation product PUFA 'y'. The form of this mass balance equation is generic – independent of any dataset or species. Five dependent mass balance equations are generated for ALA, EPA, DHA, LIN and ARA. The consumer (i.e. predator) and diet (i.e. prey) are 'i' and 'j', respectively. γ_G is a gut absorption coefficient (unitless). The 'k' values are uptake/elimination rate constants (h^{-1}) and subscripts A, E and O are diet (i.e. food), egestion and β-oxidation, respectively. Subscript 'T' is the net transformation of PUFA 'x' through elongating and/or desaturating to PUFA 'y'. If PUFA 'x' transforms to PUFA 'y', then the sign is negative as PUFA 'x' is 'lost'. The sign is positive when PUFA 'x' has an additional input from PUFA 'y' that has elongated and/or desaturated to PUFA 'x'. We assume that a transformed PUFA is subsequently available for additional elimination processes (i.e. egestion and β-oxidation).

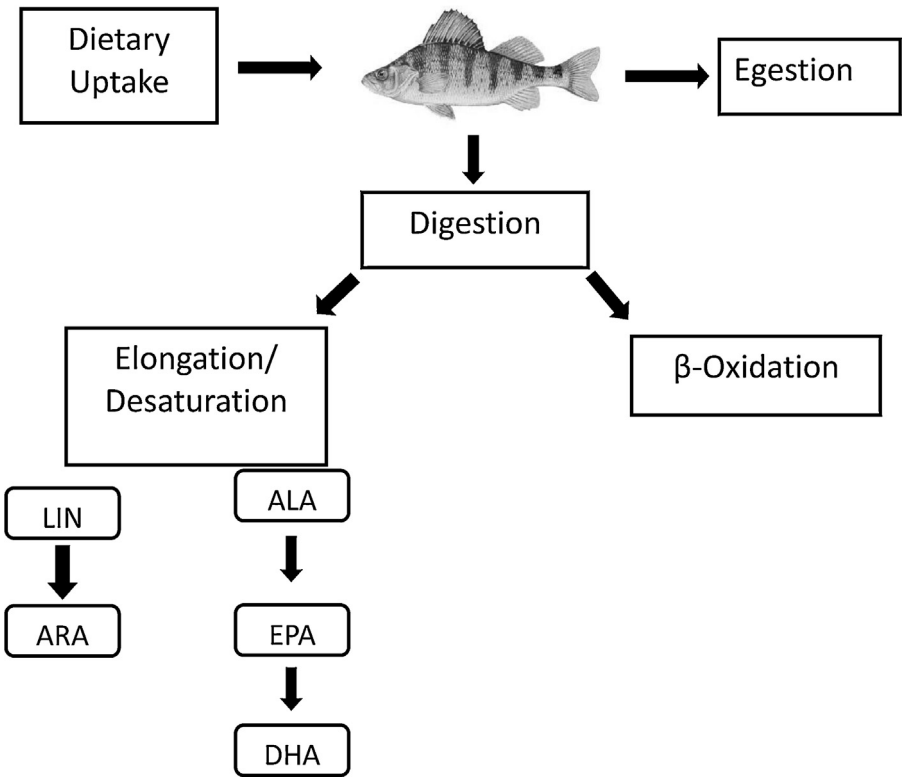


Fig. 1. Major PUFA metabolic pathways in a freshwater fish.

Under steady-state conditions, Eq. (1) can be rewritten as (Eq. (2), Fig. 2):

$$m_{i,x} = \frac{\sum_{i,j=1}^{n} \gamma_{Gi,x} m_{j,x} k_{Aji,x} \pm m_{Ti,xy} k_{Ti,xy}}{k_{Ei,x} + k_{Oi,x}} \quad (2)$$

As this is a first generation model, a steady-state strategy was preferred over a dynamic solution because of the former's mathematical simplicity along with the paucity of temporally explicit data required for the latter approach. The mass of the 5 PUFA, as expressed in Eq. (2), can be solved for simultaneously using a matrix form modified from Gandhi et al. (2006):

$$Am = 0 \quad (3)$$

where matrix A includes the dietary matrix as well as all possible uptake and elimination pathways. For 5 PUFA the matrix is:

$$\begin{bmatrix} A^a & -T^{ba} & -T^{ca} & 0 & 0 \\ -T^{ab} & A^b & -T^{cb} & 0 & 0 \\ -T^{ac} & -T^{bc} & A^c & 0 & 0 \\ 0 & 0 & 0 & A^d & -T^{ed} \\ 0 & 0 & 0 & -T^{de} & A^e \end{bmatrix} \begin{bmatrix} m^a \\ m^b \\ m^c \\ m^d \\ m^e \end{bmatrix} = \begin{bmatrix} 0 \\ 0 \\ 0 \\ 0 \\ 0 \end{bmatrix} \quad (4)$$

where a, b, c, d and e are ALA, EPA, DHA, LIN and ARA, respectively.

2.2. Rate constant equations

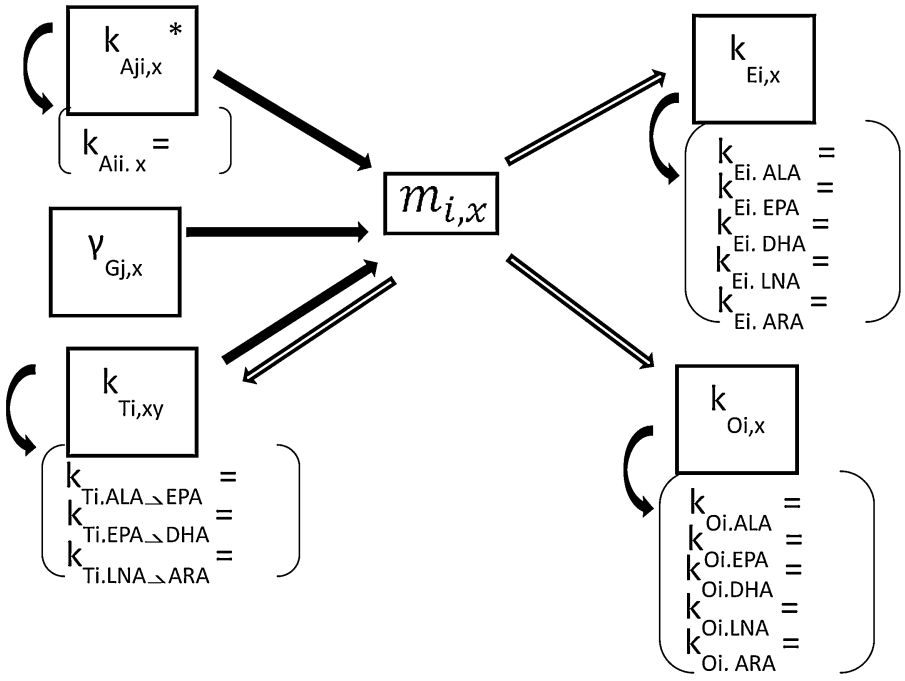
Empirical equations to predict egestion, transformation and β-oxidation rate constants were developed using multiple regression modeling (Statistica 7.0) (Eqs. (1) and (2)). These regression equations were developed to estimate rate constants for these processes for freshwater fish species in general, rather than using rate constants from the literature which were developed under specific conditions and therefore cannot be used in a general manner (i.e. Alhazzaa et al., 2011; Teoh et al., 2011). The regression equations were derived as best-fits to a data training set compiled from six studies by Alhazzaa et al. (2011), Francis et al. (2007, 2009), Teoh et al. (2011), Turchini and Francis (2009), and Turchini et al. (2006) (Table S1). The data training set included a total of 22 data points for the freshwater species Murray Cod (*Maccullochella peelii*) (13 data points), Rainbow Trout (*Oncorhynchus mykiss*) (2 data points), Barramundi (*Lates calcarifer*) (freshwater life-cycle phase; 3 data points) and Nile Tilapia (*Oreochromis niloticus*) (4 data points). Diet, temperature, fish weight and study duration varied within and between each study. All studies were conducted in the context of aquaculture, the significance of which is discussed below. Grubbs' Test (1969) was used to determine outliers ($\alpha < 0.05$) that were not included in the multiple regression analysis.

Various permutations were evaluated for their overall fit (adjusted-R^2), residuals and variance using multiple linear regression. The final regression equations for each rate constant (except for uptake) had the overall best fit and were consistent with the current understanding of physiological processes. The outcomes of these regressions in the form of $[PUFA]_x = mg\,[PUFA]_x$ in the prey per g of predator and expressed in units of mg PUFA $g^{-1}\,h^{-1}$, were mathematically manipulated to provide a rate constant (h^{-1}, Eqs. (5)–(18)). Table 1 summarizes the symbols and definitions of inputs for the multiple regression and mass balance model.

Below we present detailed discussions of the egestion, transformation and β-oxidation processes, followed by the PUFA-specific regression formulations.

2.2.1. Egestion

Species-specific egestion rates from the literature for individual species and dietary content of ALA and LIN were $\ln(x)$-transformed while content of EPA, DHA and ARA were $\ln(x+1)$-transformed to obtain a normal distribution of the data prior to conducting



k – uptake/elimination rate
γ_G – gut absorption coefficient
A – PUFA-based diet (i.e. food) of the consumer
T – PUFA transformation (i.e. elongation/desaturation)
E – PUFA egestion
O – PUFA β-oxidation
i – the consumer (i.e. predator)
j – the diet (i.e., prey)
x, y – 'generic' PUFA notation
m – mass of PUFA 'x'
* $k_{Aji,x}$ is not a PUFA-specific process. It represents the modified, rate constant version of Equation 5.

Uptake processes

Elimination processes

Sub-model equations

Fig. 2. PUFA model schematic diagram for a single freshwater fish.

Table 1
Summary of 1 fish PUFA model inputs.

Symbols	Units	Definition
i	–	The consumer (i.e. predator) (subscript notation)
j	–	The diet (i.e. prey) (subscript notation)
x	–	'Generic' PUFA notation (subscript notation)
y	–	'Generic' PUFA notation (subscript notation)
γ_G	–	Gut absorption coefficient
k	h^{-1}	Uptake/elimination rate constant
R	$kg\,d^{-1}$	Uptake/elimination rate
A	–	PUFA-based diet (i.e. food) of the consumer
E	–	PUFA egestion
O	–	PUFA β-oxidation
T	–	PUFA transformation (i.e. elongation/desaturation)
[ALA]	$mg\,ALA_j\,g_i^{-1}$	Mass fraction of α-linolenic acid (mg) in the diet per gram of predatory fish
[EPA]	$mg\,EPA_j\,g_i^{-1}$	Mass fraction of eicosapentaenoic acid (mg) in the diet per gram of predatory fish
[DHA]	$mg\,DHA_j\,g_i^{-1}$	Mass fraction of docosapentaenoic acid (mg) in the diet per gram of predatory fish
m	g	Mass of PUFA '*x*'
[LNA]	$mg\,LNA_j\,g_i^{-1}$	Mass fraction of linoleic acid (mg) in the diet per gram of predatory fish
[ARA]	$mg\,ARA_j\,g_i^{-1}$	Mass fraction of arachidonic acid (mg) in the diet per gram of predatory fish
BW	kg	Body weight
β_A	Dietary fraction	The fraction of species '*j*' consumed by species '*i*'
Temp	°C	Water temperature

the multiple regression analysis (Table S1). The regression equations for the egestion rate constants of each PUFA considered (Eqs. (5)–(9)) had adjusted R^2-values ≥0.69 and $p < 0.001$ (Table S2). No major problems of heteroscedasticity or influential outliers were observed (Fig. S1a). Egestion rate constants $k_{Ei,x}$ (h^{-1}) were therefore calculated as:

$$k_{Ei,\mathrm{ALA}} = \frac{(3.13 \times 10^{-6} * [\mathrm{ALA}]^{1.19} * ([\mathrm{EPA}]+1)^{0.47})}{[\mathrm{ALA}]} \quad (5)$$

$$k_{Ei,\mathrm{EPA}} = \frac{(1.45 \times 10^{-5} * ([\mathrm{EPA}]+1)^{0.79})}{[\mathrm{EPA}]} \quad (6)$$

$$k_{Ei,\mathrm{DHA}} = \frac{(1.49 \times 10^{-5} * ([\mathrm{EPA}]+1)^{-1.68} * ([\mathrm{DHA}]+1)^{2.66})}{[\mathrm{DHA}]} \quad (7)$$

$$k_{Ei,\mathrm{LIN}} = \frac{(1.24 \times 10^{-5} * [\mathrm{LIN}]^{0.43} * [\mathrm{ALA}]^{0.43} * ([\mathrm{EPA}]+1)^{0.36})}{[\mathrm{LIN}]} \quad (8)$$

$$k_{Ei,\mathrm{ARA}} = \frac{(1.64 \times 10^{-5} * ([\mathrm{ARA}]+1)^{2.25})}{[\mathrm{ARA}]} \quad (9)$$

where [PUFA] is the PUFA '*x*' content in prey "*j*" per gram body weight of predator "*i*" (mg FA g fish D.W.$^{-1}$).

2.2.2. Transformation

Freshwater fish can have high dietary PUFA demands that differ substantially from what their diet provides. The major difference between marine and freshwater fish is that the freshwater species studied to date appear to have a greater ability to desaturate and elongate dietary 18C n-6 and n-3 PUFA to LC-PUFA (Karahadian and Lindsay, 1989; Sargent et al., 1999; Simonetti et al., 2008). This is considered an evolutionary adaptation of freshwater fish to compensate for lower dietary n-3 LC-PUFA content, especially DHA availability. PUFA transformation can follow two pathways (Fig. S2). The n-3 pathway elongates and desaturates ALA to EPA and EPA is further elongated and desaturated to DHA. Alternatively, the n-6 pathway uses LIN for conversion to ARA. Details outlining the elongation and desaturation process can be found elsewhere (Bézard et al., 1994; Nakamura and Nara, 2004; Sargent et al., 2002).

Species-specific transformation rates and content of dietary and predator ALA and LIN from the literature were ln(*x*)-transformed, while dietary content of EPA, DHA and ARA were ln(*x*+1)-transformed prior to performing multiple regression analysis (Table S1). For the regression models, we considered precursor PUFA '*x*' transforming to end product PUFA '*y*' individually because n-3 and n-6 PUFA follow distinct transformation pathways (Eqs. (10)–(12)).

The final regression equations for ALA transformation to EPA and LIN transforming to ARA (Eqs. (10) and (12)) had adjusted R^2-values above 0.47 and $p < 0.001$ (Table S2) and no major problems of heteroscedasticity or influential outliers were observed (Fig. S1b). A statistically significant relationship was not found for EPA transforming to DHA. Therefore, until further data become available, we recommend modeling this transformation using the same regression equation for ALA transforming to EPA but replacing the ALA content with EPA (Eq. (10)). In Eqs. (10)–(12), $k_{Ti,xy}$ is the transformation rate constant of precursor PUFA '*x*' to end product PUFA '*y*' in h^{-1}.

$$k_{Ti,\mathrm{ALA}\rightarrow\mathrm{EPA}} = \frac{(8.19 \times 10^{-5} * [\mathrm{ALA}]^{0.73})}{[\mathrm{ALA}]} \quad (10)$$

$$k_{Ti,\mathrm{EPA}\rightarrow\mathrm{DHA}} = \frac{(8.19 \times 10^{-5} * ([\mathrm{EPA}]+1)^{0.73})}{[\mathrm{EPA}]} \quad (11)$$

$$k_{Ti,\mathrm{LIN}\rightarrow\mathrm{ARA}} = \frac{(6.08 \times 10^{-5} * ([\mathrm{EPA}]+1)^{-0.69} * [\mathrm{LIN}]^{0.98})}{[\mathrm{LIN}]} \quad (12)$$

2.2.3. β-Oxidization

β-Oxidation is the process by which FA are broken down to generate acetyl-CoA, the entry molecule for the Krebs cycle (Marýìn-Garcýìa and Goldenthal, 2002). Acyl-CoA is a temporary compound formed when coenzyme A (CoA) attaches to the end of a long chain FA. DHA and EPA, in their fatty acyl-CoA forms, are esterified to cellular lipid and undergo β-oxidation and other metabolic transformations including oxygenation reactions to eicosanoids (Arts et al., 2001).

To obtain equations for calculating general β-oxidation rate constants, EPA, DHA and ARA in the species-specific diet were ln(*x*+1)-transformed prior to multiple regression analysis (Table S1). The species-specific ARA β-oxidation rates were ln(*x*+1)-transformed. The mass fractions of ALA and LIN in the diet and prey were ln(*x*)-transformed. All resultant regression equations had adjusted R^2-values >0.80 and $p < 0.001$ (Table S2). The resultant dataset was homoscedastic and had no influential outliers (Fig. S1c). The following equations for calculating the β-oxidation rate constants (Eqs. (13)–(17)) were arrived at after analysis of various options:

$$k_{Oi,\mathrm{ALA}} = \frac{(2.84 \times 10^{-4} * [\mathrm{ALA}]^{0.94} * ([\mathrm{EPA}]+1)^{-0.18})}{[\mathrm{ALA}]} \quad (13)$$

$$k_{Oi,\mathrm{EPA}} = \frac{(3.25 \times 10^{-5} * ([\mathrm{DHA}]+1)^{2.39} * ([\mathrm{ARA}]+1)^{-5.00} * [\mathrm{LIN}]^{2.39})}{[\mathrm{EPA}]} \quad (14)$$

$$k_{Oi,\mathrm{DHA}} = \frac{(8.57 \times 10^{-5} * ([\mathrm{DHA}]+1)^{2.92} * ([\mathrm{EPA}]+1)^{-2.39})}{[\mathrm{DHA}]} \quad (15)$$

$$k_{Oi,\mathrm{LIN}} = \frac{(1.46 \times 10^{-4} * [\mathrm{LIN}]^{1.07} * ([\mathrm{ARA}]+1)^{-0.62})}{[\mathrm{LIN}]} \quad (16)$$

$$k_{Oi,\mathrm{ARA}} = \frac{(([\mathrm{ARA}]+1)^{1.68 \times 10^{-4}}) - 1)}{[\mathrm{ARA}]} \quad (17)$$

2.2.4. Other elimination processes

Other removal processes in addition to egestion, transformation and β-oxidation occur, however their contribution to PUFA elimination is limited. For example, some conversion of ARA and EPA to eicosanoid products occurs, but at low rates (Turchini et al., 2007). In rats, the rate of conversion was found to be less than 1 μg d^{-1} rat^{-1} (Hansen and Jensen, 1983). Therefore, we did not explicitly model this elimination process. Additionally, Turchini and co-workers (2006, 2007, 2009) considered PUFA conversion to dead-end products (i.e. ALA elongation to eicosatrienoic acid (ETE), LIN elongation to eicosadienoic acid (EDA)) in their mass-balance equations. However, Turchini et al. (2007) reported less than 2% conversion of ALA to its dead-end products relative to its total net intake. Thus, we did not include these processes in our model.

2.2.5. Dietary uptake and gut absorption coefficient

The regression model for the dietary uptake rate R_{Ai} (kg d^{-1}) was previously defined by Arnot and Gobas (2004) as:

$$R_{Ai,x} = 0.022\mathrm{BW}_i^{0.85} e^{0.06*\mathrm{Temp}} \quad (18)$$

where Temp is water temperature (°C) and BW_i is the mass (kg W.W.) of the predator. This equation is specific for species and temperature, but not PUFA. The equation was modified for expression as a dietary uptake rate constant $k_{Ai,x}$ (h^{-1}).

Uptake of FAs into enterocytes, including PUFA, was originally thought to occur by passive diffusion. However, it is now widely believed that, whereas medium-chain FA are primarily taken up by passive diffusion, the uptake of long-chain FA involves various

transport proteins and thus, uptake in the gut is highly efficient (Andre et al., 2000; Concha et al., 2002; Denstadli et al., 2004). Sire and Vernier (1981) suggested that transport proteins in fish have similar characteristics to FA binding proteins found in mammals. The gut absorption coefficient ($\gamma_{G,i}$), ranging from 0–1 (unitless), is the fraction of PUFA that the predator absorbs from its diet. In a situation where PUFA content is limited, the predator would absorb all available PUFA (i.e. $\gamma_{G,i} = 1$). Where PUFA content is abundant, the predator may not physiologically require the entirety of the PUFA available in its diet and so $\gamma_{G,i} < 1$.

3. Model application

We applied the steady-state mass balance model (Eq. (2) to and derived rate constants Eqs. (5)–(18)) for Yellow Perch (*Perca flavescens*), a small, predatory fish in the upper Bay of Quinte, Ontario, Canada. This was the only system for which we had ecological and PUFA data. The data for Yellow Perch were not used to develop the rate constant equations. During the period for which we had ecological and PUFA data the system's water temperature was 20 °C and the Yellow Perch maintained a diet of bivalves (15%), benthic invertebrates (70%), planktivores i.e. Alewife *Alosa pseudoharengus* (5%), and benthivores i.e. Brown Bullhead *Ameiurus nebulosus* (10%). Dietary fractions were modified from Blukacz-Richards and Koops (2012) and Koops et al. (2006). We estimated that the PUFA diet of these Yellow Perch was 0.28, 1.4, and 2.3 mg/d of ALA, EPA and DHA and 0.38 and 0.90 mg/d of LIN and ARA, respectively. The estimates were based on the diet fractions mentioned above and unpublished PUFA data on the prey (T. Johnson, Ministry of Natural Resources, Picton, Ontario, Canada, personal communication).

A sensitivity analysis was performed by changing each parameter by increments of ±10% in order to identify sensitive parameters. We used Monte Carlo analysis to propagate the error associated with the regression equations through our PUFA model equations. The standard error of the estimate (SEE; Table S2) for each PUFA's regression equations was used to estimate each parameter's normal distribution. The residual variability of the uptake, egestion, transformation, and β-oxidation rate constants was propagated independently through our model because of the lack of knowledge about their covariance.

It should be noted that the only system-specific data used in the model application were dietary intake, the PUFA content in diet, fish body weight and system temperature. The mass balance model rate constants were obtained from the regression equations which used a training set assembled from the literature that did not include the Yellow Perch data (Section 2.2).

4. Results and discussion

4.1. Rate constants

Using multiple regression analysis, dietary PUFA content were used to estimate egestion, transformation and β-oxidation rate constants in fish. Rate constant equations were positively correlated with the corresponding PUFA's dietary content except for EPA's β-oxidation rate constant equation (Eq. (14)). In 8 of 13 rate constant equations, competition existed between PUFA. For example, in Eq. (14) DHA, LIN and ARA content in the diet all contribute to the β-oxidation rate constant equation for EPA. The interpretation of this equation was that the rate of β-oxidation of EPA increased as a function of the content of DHA, LIN and ARA in the fish's diet in the data training set used to develop the equations (which makes sense intuitively). In contrast, in Eq. (10) only ALA content in the diet is considered when calculating the ALA to EPA transformation rate constant, i.e. the more ALA available, the greater its rate of conversion to EPA. Competition did not exist for rate constant equations predicting ARA values.

Whereas some dependencies were mechanistically plausible, as noted above, others were not. In six of the eight rate constant equations that exhibited competition, EPA was most often (75% of the time) the competitive PUFA that appeared in the rate constant equation, suggesting that dietary EPA content plays an important role in rate determination. The multiple regression analysis picked up some competitive interactions of transformation (i.e. EPA content in the diet impacted transformation of LIN to ARA, Eq. (12)) where competing elongase and desaturase processes exist between n-3 and n-6 PUFA.

Interestingly, temperature was not a significant predictor variable in the rate constant equations other than dietary uptake (Eq. (18)), although it was evaluated for inclusion as an independent variable in all rate constant expressions. We expected that it might be included based on previous studies (i.e. Jiang and Gao, 2004) indicating that increased temperature over a range of 15 °C between 10 and 25 °C decreases organism PUFA content. Temperature may not have been a significant predictor variable due to a lack of temperature variation in the data used in the multiple regression analysis, as 22 of the 24 data points (90%) had study temperatures >24 °C.

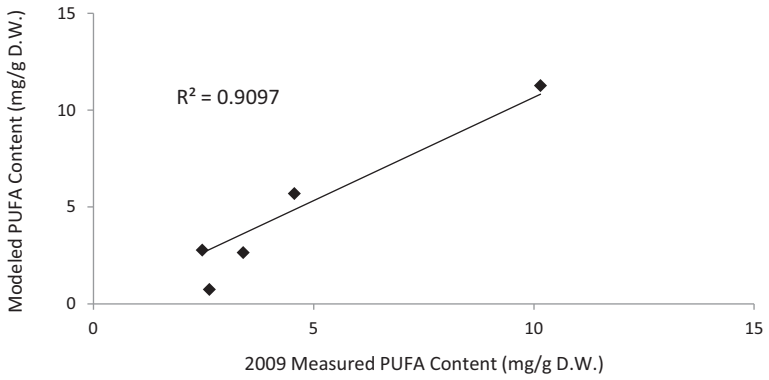
Clearly, the regression equations and resultant rate constants are a product of the data training set used to generate the regression equations. We note that the data in the training set were obtained experimentally in the context of aquaculture studies which likely resulted in some distortions, as discussed below.

4.2. Estimated PUFA content

The modeled values for Yellow Perch fit well with the 2009 measured upper Bay of Quinte values ($r^2 = 0.94$; Fig. 3a). This agreement was achieved without model calibration. All modeled values were within the standard deviation of the measured values, noting that the measured values were quite variable, for example measured DHA content was 10.1 mg/g ± 7.4 (Fig. 3b). The model was able to capture the general trend of the n-3 PUFA where ALA < EPA < DHA. EPA and DHA were within 20% and 10% of the 2009 Yellow Perch measured values, respectively. Although in line with the general n-3 trend, the model underestimated the ALA content by a factor of 3-fold. For both n-6 PUFA, the modeled values were within 25% of the measured values. However, the model had difficulty capturing the LIN < ARA trend, producing similar values (~2.7 mg/g) for LIN and ARA. Compared to the measured values, the model overestimated LIN by 10% while underestimating ARA by 25%.

From a physiological perspective, the comparison of measured and modeled data suggests that the model considers the dominant uptake and elimination pathways for PUFA in a fish. Those processes, ingestion, gut absorption, egestion, transformation and β-oxidation, are major pathways that others (i.e. Turchini et al., 2006) have considered and highlighted as important. Although there are additional pathways that could have been considered, we suggest that they would not have significantly altered the model's outcome. For example, we only considered PUFA elongation and desaturation but not retro-conversion (i.e. DHA transformation to EPA through chain shortening and increased saturation) based on reports that the latter is of minimal quantitative importance (Buzzi et al., 1997). A possible exception could be for DPA which retro-converts to 20:5n-3 (EPA). However, this primarily occurs when DPA is abundant and EPA is scarce (G. Turchini, Deakin University, Australia, personal communication, 2011), which is an unlikely scenario in freshwater environments.

a) Model fit. Each point represents an average of three measured concentrations plotted against one modeled concentration.



b) Error bars represent standard deviation for measured data (from 3 measurements) and modeled data (from ….).

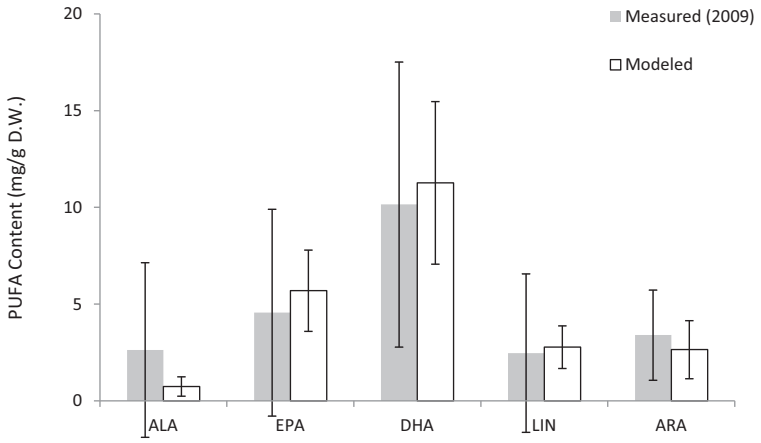


Fig. 3. Comparison of measured PUFA concentrations in Yellow Perch sampled in 2009 from the upper Bay of Quinte, Canada (T. Johnson, Ministry of Natural Resources, Picton Ontario, Canada, personal communication) and modeled PUFA concentrations. (a) Model fit. Each point represents an average of three measured concentrations plotted against one modeled concentration. (b) Error bars represent standard deviation for measured data (from 3 measurements) and modeled data were generated from a Monte Carlo analysis that varied all input variables simultaneously based on the standard deviation of each variable.

4.3. PUFA uptake and loss processes

The relative importance of uptake and loss processes varied among the PUFA considered. For ALA, the dominant loss mechanism was β-oxidation (66%), followed by transformation (33%) with minimal egestion (1%) (Fig. 4a). LIN had a similar distribution of losses in that β-oxidation was the dominant loss pathway (60%; Fig. 4b). This is consistent with evidence that these PUFA are good substrates for β-oxidation, especially when available through the diet at high levels (Karalazos et al., 2011; Richard et al., 2006; Teoh et al., 2011).

EPA's predominant uptake pathway was through ingestion (94%) while only 6% of the uptake was via transformation from ALA (Fig. 4c). This suggests that, at the levels we described, dietary EPA was sufficient in quantity to meet most of the requirements of Yellow Perch and that only minimal amounts of ALA needed to be transformed to EPA to make up any shortfall. For EPA, the dominant loss mechanism was transformation to DHA (84%), followed by egestion (15%) with minimal β-oxidation (1%). In contrast to EPA, the dominant DHA uptake pathway was transformation from EPA (87%) suggesting that the DHA dietary content was insufficient and hence transformation of EPA to DHA was required to meet physiological demand of DHA (Fig. 4d). Thus, in this case, the model shows that transformation was critical for supplying the fish with adequate DHA whereas sufficient ALA and EPA could be acquired through diet. As such, we hypothesize that within-fish transformation of precursor and the resultant product PUFA can compensate, to some extent, for dietary deficiencies in long chain PUFA (LC-PUFA). This is consistent with the evolutionary adaptation of freshwater fish that allows for greater ability to transform PUFA than marine species (Karahadian and Lindsay, 1989; Sargent et al., 1999; Simonetti et al., 2008).

As noted above, the model did not replicate the LIN < ARA n-6 trend, instead LIN ≈ ARA. The underestimated ARA accumulation could be attributed to a slower than optimal LIN to ARA transformation rate (0.42 μmol fish^{-1} d^{-1}) which would have supplemented the dietary uptake. The LIN to ARA transformation only accounted for 14% of the uptake, compared to DHA, where the EPA to DHA transformation accounted for 87% of the uptake. The primary loss mechanism of ARA was through egestion, which accounted for 60% of the loss, while β-oxidation accounted for 40% (Fig. 4e).

The delta-6 desaturase used in the ALA to EPA transformation process is more active in some fish than that used in the LIN to ARA transformation process (Sargent et al., 2002; Stubhaug et al., 2007; Francis et al., 2009). However, our model calculated similar ALA and LIN transformation rates of 0.38 compared to 0.42 μmol fish^{-1} d^{-1},

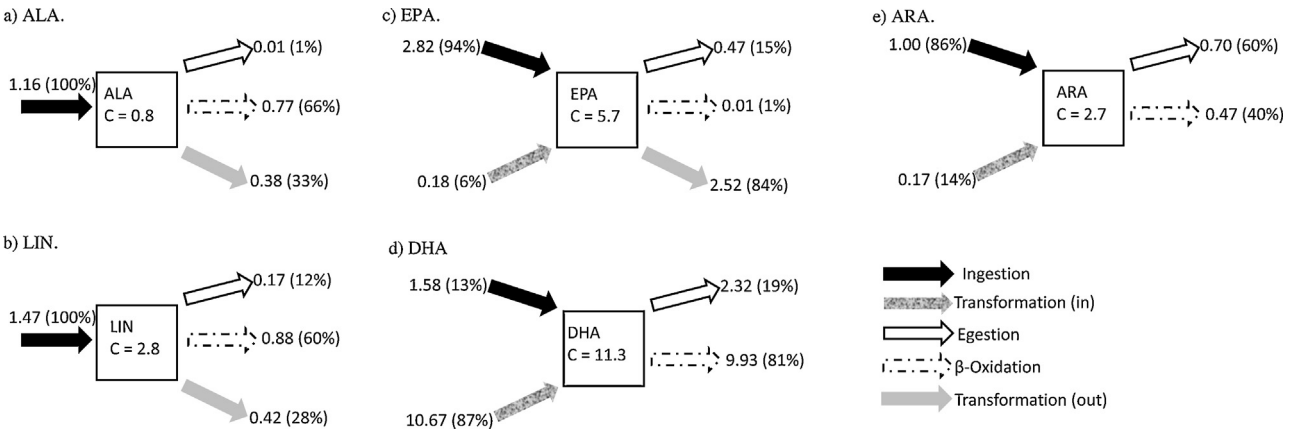


Fig. 4. Summary of content (C; mg/g D.W., numbers contained in boxes) and fluxes of inputs and losses (arrows; $\mu mol\, d^{-1}\, fish^{-1}$ and percent contribution to total input and losses) for each PUFA estimated by the model for the application of the model to a Yellow Perch in the upper Bay of Quinte.

respectively. Thus the relative contribution of transformation of ALA to EPA was only 5% greater than that of LIN to ARA. LIN had a greater proportion lost to egestion (12%) relative to ALA (1%). While the transformation of ALA to EPA and DHA was able to supplement any n-3 deficiency in the diet, the LIN to ARA transformation rate was unable to compensate for a lack of dietary ARA and therefore unable to capture the n-6 LIN < ARA trend, in spite of appreciable accumulation compared to measured values (Fig. 3b).

The model results suggest that β-oxidation was the main loss pathway of ALA, LIN and DHA which, especially for DHA, is puzzling given current knowledge of PUFA physiology in wild fish. For example, Morais et al. (2005) noted that DHA is not considered to be a good substrate for β-oxidation due to its essential role in membrane structure. Kainz et al. (2008) commented that DHA is generally the most highly retained PUFA in many freshwater fish. As noted above, PUFA can be good substrates for β-oxidation when dietary supply is abundant (Karalazos et al., 2011; Richard et al., 2006; Teoh et al., 2011). The potential overestimation of β-oxidation could be an artifact of the data training set that was used to calculate the rate constant regression equations. These data come from aquaculture studies that examined how changing contents of ALA and LIN influence EPA, DHA and ARA accumulation. In these studies, high ALA and/or LIN content was incorporated into the fish diet, while dietary EPA, DHA and/or ARA were kept low or completely excluded. These conditions, with minimal EPA, DHA and/or ARA in the diet, may only reflect a limited set of natural freshwater conditions such as a cyanobacteria bloom where phytoplankton ALA content is high while EPA and DHA content is very low (Müller-Navarra et al., 2004). This was not the case in the Bay of Quinte. Under such conditions, excess ALA and LIN may be lost to β-oxidation whereas under natural conditions these PUFA are either conserved or undergo transformation to EPA, DHA and ARA.

In particular, although ALA is a good substrate for β-oxidation, an overly aggressive rate constant reflective of the training set conditions could account for the model under-predicting ALA content; its β-oxidation rate constant ($0.77\ \mu mol\, d^{-1}\, fish^{-1}$) was 50% and 100% faster than that for transformation and egestion. Although the model overestimated LIN content by 10%, it too had a fast β-oxidation rate relative to the other elimination rates. The LIN β-oxidation rate ($0.88\ \mu mol\, d^{-1}\, fish^{-1}$) was 50% and 80% faster than the transformation and egestion rates. Both ALA and LIN were most sensitive to changing β-oxidation rates.

The high loss of DHA via β-oxidation (81%) may have another explanation – the assumption of steady-state conditions may force an unrealistically high loss rate. Temperate freshwater fish undergo seasonal variation in their PUFA content as a function of PUFA dietary availability and fish life stage (i.e., Ågren et al., 1987). Rather than DHA being lost to β-oxidation, it may be more likely that DHA is accumulated by Yellow Perch, especially in late summer/fall when it is needed for maintaining membrane fluidity over winter.

4.4. Model sensitivity

Results of the sensitivity analysis indicate that PUFA display unique sensitivities to the model parameters (Fig. S3). ALA and LIN content were most sensitive to the dietary uptake and β-oxidation rate constants (a 30% β-oxidation decrease produced a 22% increase in ALA and LIN). The dietary uptake rate constant equation used for this model (Eq. (18)) is well established, and as such, we have high confidence in this parameter's estimation. Conversely, the β-oxidation rate constants are poorly studied and hence we are less confident of these regressions (Eqs. (13)–(17)). Moreover, the importance of β-oxidation could be an artifact of the regression training set used to develop the rate constants, as discussed above and below.

Not surprisingly, EPA content was highly sensitive to its transformation rate; a 30% increase in this rate constant resulted in a 20% decrease in EPA content (Fig. S3b). This may be because the regression equation for EPA transformation rate constant (Eq. (11)) was the only rate constant that did not produce a statistically significant relationship to any of the predictor variables.

DHA content showed the greatest sensitivity to model parameters of all PUFA considered. DHA content was most sensitive to the egestion rate constant followed by β-oxidation, dietary uptake and transformation (Fig. S3c).

In future, examining the likelihood of covariance among the rate constants and effectively controlling the error propagation through our model should be considered, i.e., to reduce the width of the uncertainty bands shown in Fig. 3. By including these considerations one would avoid some extreme model outputs likely resulting from unrealistic combinations of the rate constants; namely, a high egestion rate for a particular PUFA cannot be combined with an excessively high transformation rate of the same PUFA. Along the same line of reasoning, an important augmentation of the model would be to include an internal logic that is conceptually on par with the adaptive fish physiology that disallows unrealistic values of metabolic rates to actually occur in the real world (see problem with the β-oxidation rate) (Perhar et al., 2013).

5. Implications

PUFA are crucial for fish because they affect metabolic activity, growth rates and reproduction. They are also crucial because their supply is limited and anthropogenic stressors are impinging on their production and/or availability. However, we lack quantitative tools to predict the effect of stressors on their production and availability. Here, we have presented a mechanistic mass balance model that quantifies the uptake and elimination of ALA, EPA, DHA, LIN and ARA in a "generic" freshwater fish. Our model simplifies the physiological dynamics of PUFA metabolism by focusing on the most significant pathways of PUFA accumulation: ingestion, egestion, transformation and β-oxidation. The regression equations for rate constants reflect the dietary composition in the prey species, highlighting the individual nature of each PUFA.

The application of the model to estimate PUFA uptake and loss in a Yellow Perch using data from the Bay of Quinte (the only system for which we had sufficient data for model application and evaluation) produced ALA, EPA, DHA, LIN and ARA content that was consistent with measured values. The agreement between measured and modeled PUFA content provides some confidence that the model is sound. This agreement was achieved without model calibration, but relied only on system-specific data of dietary composition, the PUFA content in diet, fish body weight and system temperature. The model results confirm the importance of diet as the main source of most PUFA. In addition, the results lead us to hypothesize that transformation, and not just diet, plays an integral role in DHA accumulation, which has not been previously shown. Indeed, estimating transformation rates is a strength of the model as such rates could not be obtained from field studies. The importance of the transformation pathways lies in it being a compensatory mechanism for dietary PUFA deficiency.

As a heuristic, rather than a predictive tool, the model can be applied to natural freshwater environments, facilitating a greater understanding of PUFA transfer and accumulation in fish. As a result of using regression analysis to formulate rate constant equations as opposed to using 'fixed' input values, the model is applicable to a range of scenarios and is not limited by the parameterization used in the aforementioned Yellow Perch model scenario. Thus, the model can be used to answer questions related to PUFA accumulation under conditions of low dietary availability, as can occur under eutrophic conditions or climate warming.

One weakness of the model application (but not, we submit, of the mass balance model itself) was the finding of potentially unrealistically high rates of PUFA loss via β-oxidation. We attributed this to the use of a data training set for estimating model rate constants, where the data training set came from the aquaculture literature (i.e. Alhazzaa et al., 2011; Francis et al., 2007, 2009; Teoh et al., 2011; Turchini et al., 2006; Turchini and Francis, 2009). Due to logistical impracticalities, egestion, transformation and β-oxidation rates have not been measured in natural freshwater environments. The current data training set is satisfactory for parameterizing a first generation model. However, one conclusion that can be drawn from the modeling exercise presented here is the need for measured rates of PUFA uptake and loss rates in wild fish, which, admittedly, are very challenging to measure. In addition, data from other systems are needed to further evaluate the model. Conversely, the model as parameterized here, can be applied to aquaculture scenarios to evaluate the effect of various PUFA dietary scenarios.

In terms of advancing our understanding of PUFA dynamics, our approach of using regression analysis to derive rate constants rather than using static numerical inputs, introduces an extra layer of causality into the model by connecting the rate constants with meaningful predictor variables, namely diet and body weight. Using this method, we are able to better define their uncertainty and the general model parametric error is now replaced by the SEE of the regression models (used to perform Monte Carlo analysis). In a broader context, this practice can be an excellent solution to the identifiability problem of complex over-parameterized models and may offer a more reliable solution to the use of numerical models for management purposes (Arhonditsis et al., 2007). For example, this method may render credibility to our PUFA model when used to guide environmental management under a wide variety of conditions such as changing diet due to invasive species and/or climate change, as the rate constants are not simply an input vector, stemming from a model fitting exercise, but rather evolving entities reflecting the specific conditions induced by the scenario examined. Another important implication of our approach is that it offers a different perspective toward the optimization of future data collection efforts. In particular, the model application was not a calibration exercise. Rather, we argue that the effective parameterization of a model requires more elegant experimentation focused on the development (and further refinement) of the causal characterization of model parameters, i.e., our regression equations used to estimate uptake, loss and transformation processes. In addition, depending on the nature of the dataset, the proposed method allows potential users to easily delineate the application domain and to determine to what extent a particular model has local or universal use.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ecolmodel.2015.12.004.

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