FweB4 v1.0 - Technical Guide

R-based Model of MeHg Bioaccumulation in Aquatic Food Webs built off the Fish Bioenergetics 4 (FB4) Model

DRAFT

Prepared for:
Idaho Power and the English-Wabigoon River Remediation Panel

AZIMUTH
Azimuth Consulting Group Inc.
218-2902 West Broadway
Vancouver, B.C., V6K 2G8

and

Reed Harris Environmental Limited
180 Forestwood Drive
Oakville, ON, L6J 4E6

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1 BACKGROUND

Two projects are currently underway to investigate factors resulting in increased fish mercury levels in (1) Hells Canyon Complex, a series of three reservoirs on the Snake River, Idaho, and (2) the English-Wabigoon River system in Ontario, which has mercury contamination from a chlor-alkali facility that released mercury from approximately 1962-70. In both cases the projects include field studies and simulation modeling. The modeling in both studies involves the use of well-established high spatial resolution models for hydrodynamics, sediment transport and water quality:

- CE QUAL W2, originally developed for the US Army Corps, with current development and management by Portland State University (Wells, 2020), is being applied to the Hells Canyon Complex.
- Delft3D (Deltares, 2023) is being applied to the English-Wabigoon River system.

Mercury cycling in the water column and surface sediments has recently been added to both models. Inorganic Hg(II), MeHg and elemental mercury are each simulated. Inorganic Hg(II) and MeHg are simulated in the dissolved and particle phases, while elemental mercury is only dissolved.

Neither model includes the ability to simulate aquatic food webs. There is a need for a model that can use predicted MeHg concentrations in water, sediments, and potentially lower food web compartments (*e.g.* zooplankton) in CE QUAL W2 or Delft3D and subsequently predict MeHg bioaccumulation and concentrations in higher aquatic trophic levels, including sportfish.

A review of available contaminant food web models was carried out by Reed Harris Environmental Ltd., but did not identify a suitable model:

- There are standalone models that simulate MeHg in individual fish but not food webs with multiple biota and trophic levels, including the FB4 model (R Core Team, 2021) and US EPA FGETS model (US EPA, 2022).
- The D-MCM model (Harris *et al*., 2012, 2013) simulates MeHg in food webs but the food web module is integrated into the broader model that also simulates MeHg in water and sediments. D-MCM can't use predictions of MeHg in water from other models to predict bioaccumulation in food webs. D-MCM is also a proprietary model owned by EPRI and available only through licensing.
- The US EPA also has a food web bioaccumulation model (BASS) with the necessary features but it was not clear that BASS would be supported going forward in time. This

could be problematic for future applications. The model also runs on Windows XP and did not function during Windows 10 tests.

Overall, none of the above models was considered a suitable candidate for application in the Hells Canyon Complex or English-Wabigoon River system studies. It was however deemed possible and practical to modify and extend FB4, which is open source, to produce a model with the features needed. This document describes the extension of FB4 to produce a model called FweB4 that simulates MeHg bioaccumulation through simplified aquatic food webs. The document also provides basic instructions to use FweB4.

2 APPROACH

A numerical model has been developed to simulate MeHg dynamics and concentrations over time in aquatic food webs. The approach varies for different trophic levels as shown in **Figure 1**. MeHg concentrations in trophic levels below fish are either input by the model user or calculated by the model using a combination of MeHg concentrations in water and/or sediments in combination with simple instantaneous equilibrium partitioning specified by the user. Processes affecting MeHg in fish do not operate fast enough to assume simple instantaneous equilibrium with their surrounding conditions. Fish are instead simulated using an energy and mass balance (bioenergetics) approach based on FB4.

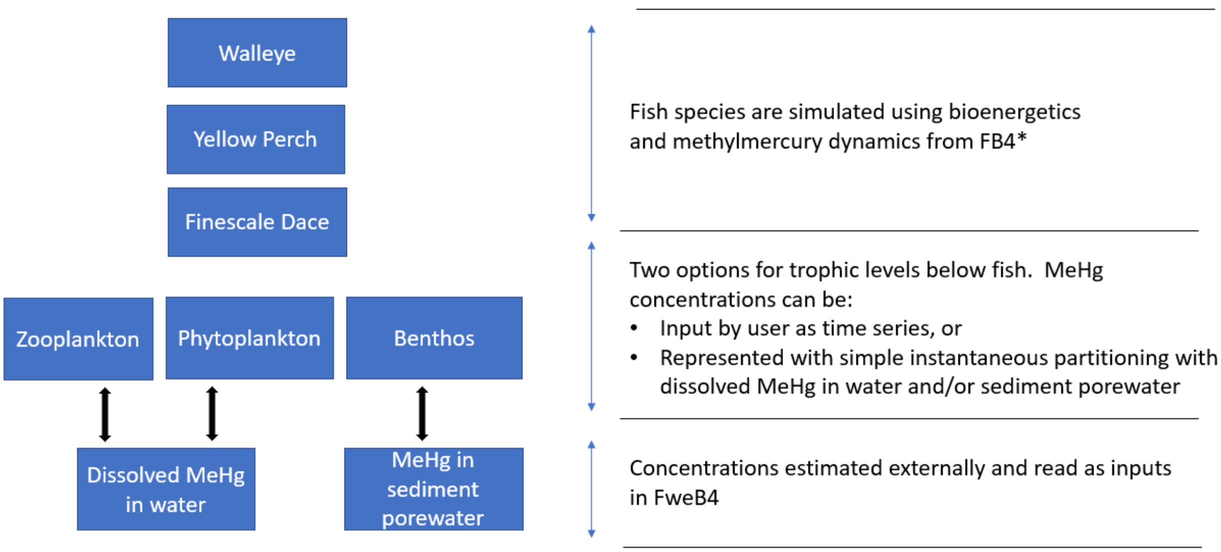

Figure 1. Example of how FweB4 simulates different food web compartments

<u>Bioenergetics and MeHg in Fish</u>

FB4 is written in R (R Core Team, 2021) and is open source. The model code to simulate bioenergetics and MeHg dynamics in an individual fish in FB4 was extended to simulate multiple fish species and multiple cohorts of each species simultaneously. This is shown in **Figure 2**. Cohorts for each fish species are represented by simulating a single representative fish using code from FB4, with minor modifications discussed below. The model does not currently include full fish population numbers or biomass. At the beginning of a simulation, fish are present for each age class for each fish species (**Figure 3**). As a simulation progresses through time, a new cohort hatches for each species each year and is simulated.

Fish diets are assigned by the model user using a table that specifies who eats what (fractions of the diet by weight). Fish diets can change with age. For piscivorous fish, the user assigns the fraction of the overall diet represented by each prey fish species. For example, adult walleye might consume 50% whitefish and 15% yellow perch along with other dietary items. It is also necessary to specify what size prey fish are preferred. Piscivorous fish eat prey fish based on the prey fish length as a fraction of the predatory length. For example, the model user may specify that predators seek prey fish ranging from 5-30% of their own length (**Figure 4**).

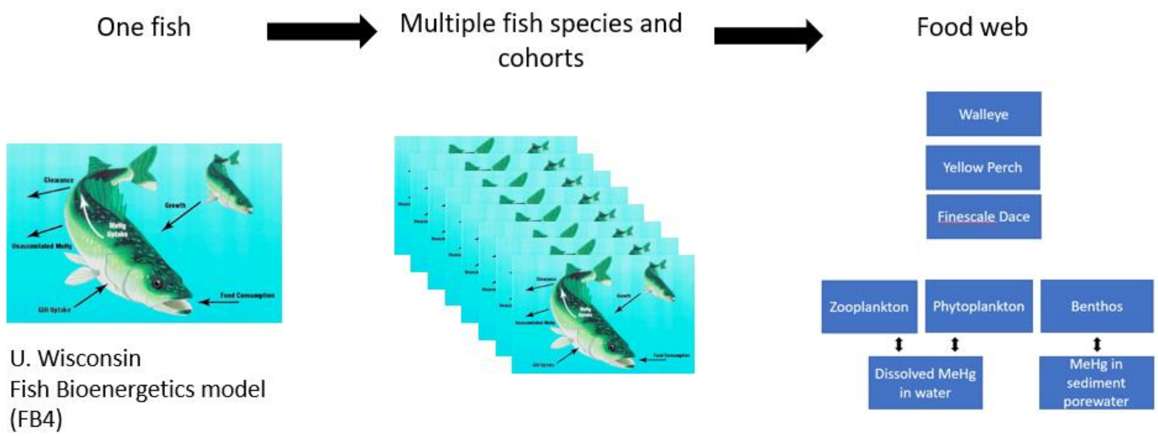

Figure 2. Conceptual sketch of extending FB4 to represent a food web

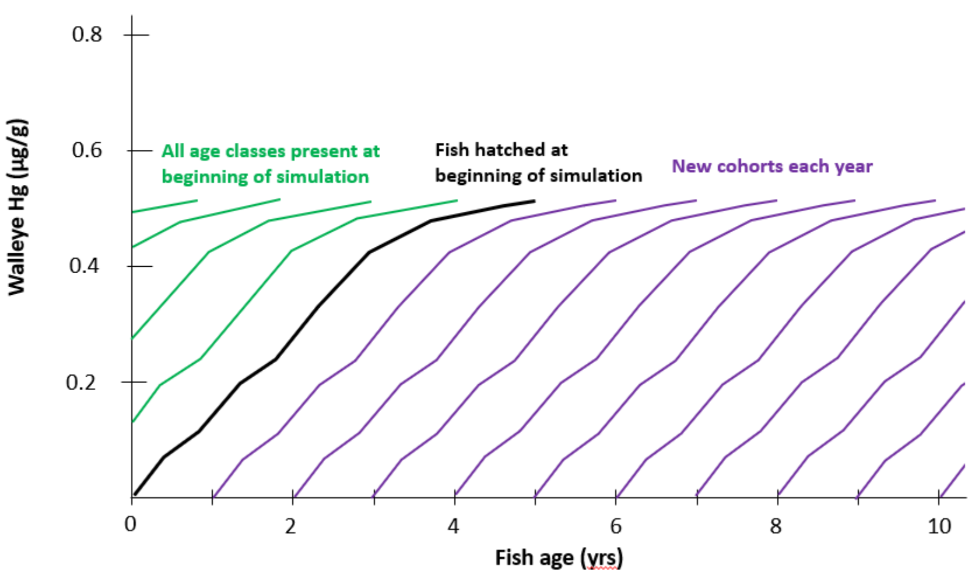

Figure 3. Conceptual sketch showing the representation of different cohorts of a fish species in a simulation. This is done for each fish species.

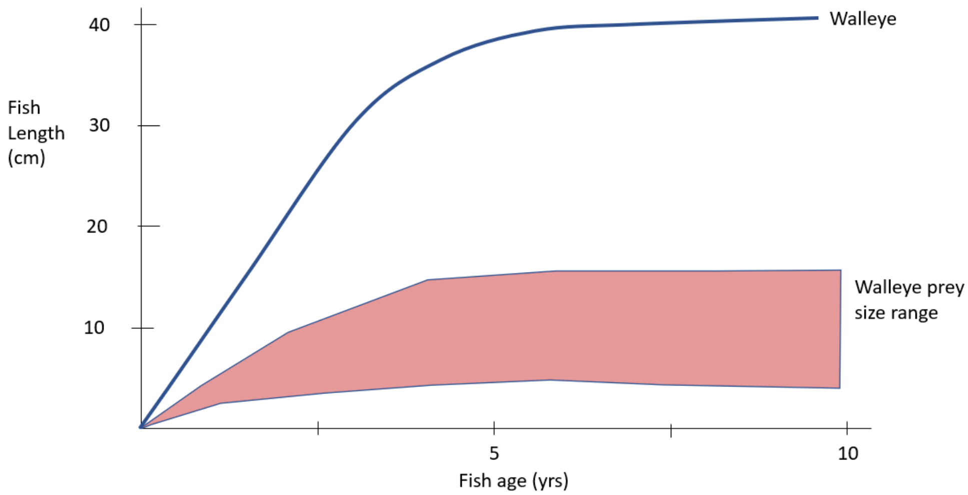

Figure 4. Conceptual sketch showing the preferred prey fish length range for a predatory fish. Preferred prey length ranges are assigned by the model user for each species.

Regarding fish growth, the user sets an initial weight and a target weight at a specified age (*e.g*. 1 kg at age 5 yrs). The model adjusts food consumption to achieve the target weight. Fish can gain and/or lose weight during a simulation.

The model also converts weight into length and includes provisions to estimate MeHg concentrations for a standard-length fish over time (**Figure 5**).

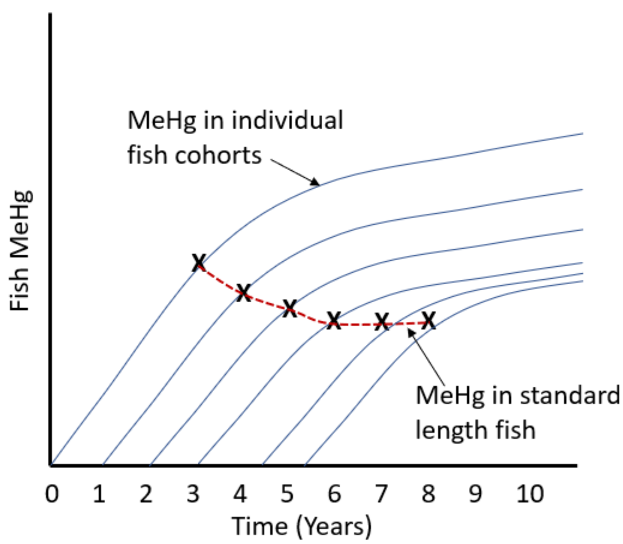

Figure 5. Sketch of MeHg concentrations in individual cohorts and standard size fish in a simulation.

3 MODEL DEVELOPMENT

As noted above, the starting point for the development of FweB4 was the FB4 model (version 1.1.2; Deslauriers et al. 2017), the latest version of a well-established bioenergetics model. Here, we briefly describe FB4 and then detail the additions and modifications made to develop FweB4. This includes minor changes to some equations used for individual fish, and additional features to extend the model to simulate food webs.

3.1 Core FB4 Functions

FB4 is a bioenergetics model that simulates fish consumption, metabolism, waste production, and growth, while maintaining an energy balance. (Deslauriers et al. 2017). FB4 is an open-source, freely available model that can be downloaded at http://fishbioenergetics.org/. FB4 is described in detail in a peer-reviewed journal publication and a user guide, both available at the above link. These resources describe the modelling approach used by FB4, the specific equations underlying the model, the bioenergetic parameters used in the equations, and provide guidance on running and troubleshooting the model. ***We assume that*** FweB4 ***users will have familiarized themselves with FB4 using the resources available.***

The basic equation of FB4 is an energy balance equation of the form:

$$C = R + A + SDA + F + U + G \quad (1)$$

Consumption (C) is balanced by metabolism (R), energy expenditure from activity (A), specific dynamic action (SDA), egestion (F), excretion (U), and growth (G). Each element of this energy balance equation is calculated by FB4. The equations and parameter values are applicable to individual species or groups of species. FB4 simulates a single fish but does not simulate multiple species or cohorts simultaneously.

FB4 includes an optional module to calculate contaminant accumulation using two possible equations. One equation considers only contaminant dietary uptake, while the other allows for contaminant clearance as well as dietary uptake. Gill uptake of a contaminant is not simulated in FB4.

3.2 New Functionality in FweB4

The primary features added during the development of FweB4 include:

1. The ability to simulate multiple cohorts and fish species simultaneously.
2. Predatory fish eat prey fish on the basis of user-defined species preferences and a preferred length range (fraction of the predator length).
3. MeHg uptake in fish via gills (although this is usually a small term compared to dietary uptake).
4. The option to predict lower food web MeHg concentrations on the basis of (1) the dissolved MeHg concentration in water or sediments and a bioaccumulation factor (BAF), or (2) user-defined time series.
5. Estimation of fish length as a function of weight, and the ability to report fish mercury concentrations as a function of length.
6. The ability to estimate the MeHg concentration in a standard-length fish over time.
7. The ability to estimate MeHg concentrations in muscle, in addition to concentrations on a whole-body basis

Further information is provided below.

3.2.1 Multiple Fish Species and Cohorts

FweB4 can simulate multiple fish species and multiple cohorts for each species simultaneously in a model run. The core functionality of FB4 to simulate individual fish has been retained without substantial modification, while enabling a full food web to be modelled. For backwards compatibility, FweB4 includes an option to run the model for only a single species and cohort.

Because FweB4 has multiple cohorts of different ages for each species it is important to follow not only the model simulation day (as FB4 does) but also the age of each fish. Furthermore, over the course of a model run FweB4 accounts for cohorts dying and being removed from the model as well as spawning resulting in new cohorts being added to the model.

The number of cohorts for each species is dictated by the spawning date (*e.g*. day 122 each year) and maximum age provided by the user for each species. On the first day of a simulation, there will be a cohort for each age class of each species present in the system. For example, if a species is defined by the user to live 10 years there will be 10 cohorts for that species on day 1 of the simulation. As a simulation proceeds, new cohorts are added when spawning occurs. Cohorts are removed from the model when they reach the specified maximum age.

3.2.2 Fish Bioenergetics, Growth, Length and Die-off

Fish Growth

In FweB4, the user provides a target fish weight at a given age for each fish species. The model adjusts food consumption to achieve the desired weight, using a scaling factor called the p value. The same p value is applied for all cohorts of a given species throughout a simulation. Similar to FB4, FweB4 adjusts the p-value iteratively to reach the target weight before running other components of the simulation.

Temperature and prey availability affect fish energy intake, expenditure, and growth. Because temperatures and prey availability can vary among years, fish growth rates may also vary among years. To fit fish growth to the target weight, the model assumes the user-specified diet dietary items and preferred prey fish lengths are available and uses the average daily temperatures provided for the simulation period when adjusting the p-value and scaling consumption. Thus simulated growth rates for a given year may deviate from the fitting procedure if the user assigns different temperatures to different years or if prey availability differs from the dietary matrix. It is also possible to manually assign a p-value for one or more species, in which case the fitting procedure is not conducted and the provided p-value is used.

The growth fitting procedure in FweB4 begins with a new cohort spawned on the day specified by the model user. This differs from FB4 which begins the growth fitting procedure on day 1 of the simulation, at which time a fish can be any desired age/weight.

FweB4 outputs a plot and summary csv file for each species, illustrating the growth trajectory of the 'average fish' and the selected p.value that achieves the desired weight on the specified day.

Initial fish weights

In FweB4, initial weights are needed for each cohort of each species present. The user must specify initial weights for a newly spawned fish, however the user does not specify initial weights for each cohort. Instead,the growth curve predicted by the model for a fish is used. Prior to simulating mercury, the model calibrates fish growth to reach a target weight at a given age. The model then uses the weight estimates at different ages to assign weights for each fish cohort at the beginning of the mercury component of the simulation. . For example, there might be 7 cohorts of smallmouth bass present at the beginning of a simulation. Each has a different age and weight. FweB4 will use the fitted growth curve to estimate the weight at each of these ages at the beginning of the mercury simulation.

New fish cohorts are added during a simulation whenever spawning occurs. The initial weight for a newly spawned fish is provided by the user on the 'Species_Info' tab of the input file.

Fish Length

Additional functionality was added to FweB4 for the model to predict the length of each fish. The starting point to estimate length is the well-established equation of Le Cren (1951).

$$W = aL^b \tag{2}$$

Where W is fish weight (g wet weight), L is fish length (cm), and a and b are species-specific parameters which control the length:weight relationship. The parameters a and b are required as user inputs for each species. FweB4 diverges from this relationship when the model predicts a fish loses weight (i.e., during spawning or periods where consumption is not meeting metabolic requirements). When weight is decreasing, length is held constant. In other words, it is assumed that although fish may get thinner, they do not get shorter. Once the fish starts to increase in weight again, its length is only increased once it has regained the weight lost. In practice, this means that length only increases when the predicted length from Equation 2 is greater than the length predicted by the model for the previous day.

Spawning
Spawning in FweB4 functions similarly to spawning in FB4. The user specifies the day on which spawning occurs, as well as the fraction of biomass lost during spawning. FweB4 requires the user to specify the minimum weight each species has to achieve before they spawn. There is also an option to turn spawning off.

Food availability
Dietary items are assumed to be available as specified in the dietary preferences table. Limited food availability is not explicitly considered, with the exception of prey size discussed below.

Prey Size Range
As described earlier, FweB4 considers a preferred prey length range for predators. Prey length range is expressed as a fraction of the predator length (e.g., a user might specify that at a given age a fish can consume prey ranging from 5% to 30% of its own length).

It is possible that on some days of a simulation no prey species will be available that satisfy the length requirements for a predator. These situations are resolved using the following logic:

1. If one or more prey items is not available in the correct length range, the predator compensates by consuming more of the other prey items in its diet. The predators diet proportion changes to accommodate prey availability. For example, if a diet matrix specifies a predator should eat 30% perch, 50% dace, and 10% each of benthos and zooplankton, but no perch are available in the correct size range, then the other dietary items are increased proportionally to sum to 100%. This results in a final diet composition of 71.4% dace and 14.3% each for benthos and zooplankton in this example.
2. If no prey fish (any species) are available in the desired length range, then the predator expands its prey size range to consume the closest size available fish to its preferred range for each prey item. The predators diet proportions are held constant, but its size range expands. Using the same example as above, if no perch or dace were available in

the correct size class then the predators size range would be expanded to include perch and dace cohorts closest to the preferred size range. Diet composition would stay the same (30% perch, 50% dace, and 10% each of benthos and zooplankton).

Energy Density
The model user must provide energy densities for all fish and dietary items (J/g ww). In FB4, these energy densities were allowed to vary over time. However in FweB4, energy densities are fixed for each model group and do not vary with age.

3.2.3 Changes to Simulating Contaminants

MeHg Uptake and Clearance
FB4 includes the option whether to include dietary uptake and clearance, or just dietary uptake. To simplify the extension of FB4 during the development of FweB4, this option was maintained, although it is recommended to include clearance. FweB4 also includes the option to include gill uptake of a contaminant. This option is selected when the parameter 'contam.eq' is set to three or four. Contaminant simulation equations three and four differ only in how clearance of MeHg is estimated.

Dietary uptake (U_D) (µg/day) is calculated as in FB4:

$$U_D = (C * W) * Prey_{Hg} * AE \quad (3)$$

Where C is the weight-specific consumption rate (g/g ww/d), W is the fish weight (g ww), $Prey_{Hg}$ is the diet-weighted mean concentration of mercury in prey (ug/g ww), and AE is the diet-weighted gross assimilation efficiency of Hg in prey (unitless).

Uptake from water across the fish's gills (U_{Aq}) is calculated as:

$$U_{Aq} = W * K * UF * MeHg_{D.Aq} \quad (4)$$

Where W is the weight of the fish (g ww), K is the water flow across the gills (L/g/day), UF is the contaminant uptake efficiency across the gills (unitless), and $MeHg_{D.Aq}$ is the dissolved concentration of MeHg in water (µg/L). Note that the user input file requires water MeHg concentrations to be specified in ng/L. The model then converts to µg/L.

Water flow across the gills ("K"; L/g/day) is calculated by the model based on the fish's bioenergetic oxygen demand.

$$K = \frac{Req.O_2}{Conc.O_2} \quad (5)$$

Where $Req.O_2$ is the oxygen required by the fish (mg O_2/g/day) and $Conc.O_2$ is the oxygen concentration in water (mg O_2/L). See the description of respiration parameters and equations in the FB4 documentation for additional information on calculating the oxygen required by the fish.

MeHg clearance ($Clear$) is calculated for 'contam.eq' two and three following the empirical relationship derived by Trudel and Rasmussen (1997) and also implemented in FB4.

$$Clear = MeHg_{Fish} * e^{(0.066\,T - 0.20(\ln(W)) - 6.56)} * M \quad (6)$$

Where $MeHg_{Fish}$ is the burden of MeHg in the fish (ug), T is temperature (Celsius), W is the weight of the fish (g ww), and M is a contaminant clearance multiplier (unitless). By default M should be set to 1, but is included to allow users to manually increase or decrease clearance rates if necessary to better fit field data or other empirical expectations.

An alternative formulation of MeHg clearance equation is included in FweB4 for 'contam.eq' four, which models clearance as a function of excretion. This equation has previously been implemented in another mercury cycling and bioaccumulation model (D-MCM, Harris *et al*., 2012, 2013).

$$Clear = \frac{Ex}{E} * MeHg_{Fish} * M \quad (7)$$

Where Ex is nitrogenous waste excretion (J /g bw / day), E is the energy density of the fish (J / g), $MeHg_{Fish}$ is the burden of MeHg in the fish (µg), and M is a contaminant clearance multiplier (unitless).

Units for Biota

FweB4 operates on a wet weight basis when performing calculations for biota. However, input parameters for lower trophic level model groups (e.g. zooplankton, benthos) are more often available on a dry weight basis. FweB4 has therefore been configured to expect dry weight input values (ng/g dw) for all lower trophic levels below fish, while initial fish species concentrations must be in wet weight (ug/g ww). Users are then required to provide dry to wet weight conversion factors for all non-fish model groups (on the 'Dry wet conversion' tab in the input spreadsheet).

Calculation of Lower Trophic Level MeHg Concentrations with Bioaccumulation Factors

The model user can assign lower trophic level MeHg concentrations using time series inputs, or by calculating concentrations using bioaccumulation factors. For example, if dissolved MeHg concentrations are available but not zooplankton MeHg, the option exists for the model to use a user specified bioaccumulation factor (BAF) to estimate MeHg concentrations in zooplankton, in lieu of assigning concentrations in a time series. The BAF is the ratio of MeHg concentration zooplankton (in this example) to the dissolved concentration in water. The units for BAFS are L/g if based on water concentrations or g/g if based on sediment. This functionality is provided in the user input excel file on the "Contam_Pre-Processing" tab.

We note that BAFS at a given site can change if water quality changes, particularly DOC. More DOC shifts MeHg from particles to the dissolved phase. More DOC may also reduce the fraction of dissolved MeHg available for uptake at the base of the food web. Changes in DOC at a site could therefore result in more dissolved MeHg, but a smaller fraction of that pool being available to the food web. We therefore recommend caution using the BAF approach in this model to estimate lower trophic level MeHg concentrations for scenarios where water quality may change, unless the above issues are considered.

Contaminant Loss During Spawning

In FB4, contaminant burden is not affected by spawning, this produces an increase in concentration when spawning occurs, as bodyweight decreases but contaminant burden stays the same. In FweB4, the user can control the amount of contaminant burden lost during spawning. Using the "spawn.cont.ratio" parameter, the user can specify the ratio of the contaminant concentration (µg/g) in spawning material to the whole body concentration. A value of 1 will result in whole body concentration staying the same during spawning, while a value less than one (spawning material has lower concentration of MeHg) or greater than one (spawning material has higher concentration of MeHg) will increase and decrease, respectively, whole body concentrations when spawning occurs.

Additional Model outputs

FweB4 reports total dietary MeHg uptake (like FB4) and also dietary uptake from each prey group. For example, if a simulation includes smallmouth bass eating yellow perch, the model will report on the overall uptake of MeHg via yellow perch (but not how much from different age classes of yellow perch). This additional detail on dietary uptake provides insights into the relative importance of prey items for overall contaminant uptake and burden.

FweB4 estimates contaminant concentrations in fish muscle as well as whole-body. Muscle concentrations are often more relevant for comparisons with field measurements. The user supplies a conversion factor ('Muscle.body.ratio' for each species. which is a simple scaling factor to scale up from whole body to muscle concentration. This value applies for the entire life of a fish.

FweB4 also estimates contaminant concentrations in muscle for a standard-length fish. The user specifies the appropriate length for each species ('Standardized.length.cm'), and the model estimates the contaminant concentration for a fish of this size on each day of the model. This is achieved by linearly interpolating between concentrations simulated for each cohort and is possible as long as the specified size is within the range of sizes of the cohorts. If on a given day the specified length is outside the range of sizes for the cohorts in the model, then no standardized result is estimated. This feature is useful when comparing model results to monitoring data over multiple years, based on concentrations in standard length fish.

3.3 FB4 Functionality not Active in FweB4

Some functions in FB4 are not available in FweB4. These functions could be added in the future if warranted.

- **Nutrient Regeneration** – FB4 allows users to estimate the role of fish in nitrogen and phosphorus nutrient cycling, and specifically in regeneration of these nutrients through excretion. This functionality was not thought to be important for the purpose for which FweB4 was developed and so this functionality was not retained.

- **Population Effects** – FB4 provided a limited ability to estimate population level processes if provided with an initial population size and mortality rates over time. FweB4 follows a single fish for each species and cohort. Populations, and by extension population-level effects, are not currently represented. From a MeHg perspective, the current approach of representing individuals rather than populations assumes that fish populations do not affect MeHg concentrations in their surrounding environment (water and sediments).

4 USING THE MODEL

4.1 Hardware/software platform and requirements

The model runs on a Windows based PC in R, a statistical programming language (R Core Team, 2021). The model was developed using the software versions listed below:

- R - R version 4.1.2 (2021-11-01)
- RStudio - Build 382 2021.09.2
- Packages
    - **readxl** version 1.3.1
    - **ggplot2** version 3.4.0
    - **dplyr** version 1.0.10
    - **purr** version 0.3.4

FweB4 should be compatible with newer versions of R software and packages, however compatibility cannot be guaranteed. R and the packages built in this framework are all free and open source and have no warranty on functionality.

4.2 How to Install the Model

It is assumed that the user is sufficiently familiar with R to install the software and load an existing application. It is easiest to run the model and use R from an integrated development environment (IDE) such as RStudio. In order to run the model, it is therefore necessary to download and install both R and RStudio.

1. **Download and install R** (https://cran.rstudio.com/).
2. **Download and install RStudio** (https://posit.co/products/open-source/rstudio/).

   You may skip either of the first two steps if you already have R or RStudio installed, though if you run into issues with software versions it may be necessary to download newer versions of one or both. If you run into issues downloading either piece of software, various resources exist online to assist you downloading and installing both R and RStudio.
3. **Download FweB4 and extract zipped folder -** the model was likely provided to you as a zipped folder containing the necessary code and input files to develop a simulation and run the model. Extract the zipped folder to a location of your choosing on the hard drive. You may rename the folder if you wish.
4. **File folders** – An important consideration is where model outputs are stored when there are multiple simulations that a user wishes to keep track of. Users may wish to keep outputs for different projects in different file locations. They may also wish to keep outputs separate for simulations for a given project. To assist with this issue, the folder structure for model files is set up as shown in Error! Reference source not found.. It allows outputs to be saved in d ifferent folders based on the project, location and scenario. For example, a user might want to store results for simulations on the Mississippi River at different locations, with multiple runs at each location. A folder scheme could be set up using the following path:

/Fweb4/Mississippi River/Location1/Run1. The user would then edit the model code to point to the correct file path, as described in a later step. The model will also use this information when creating captions for output plots.

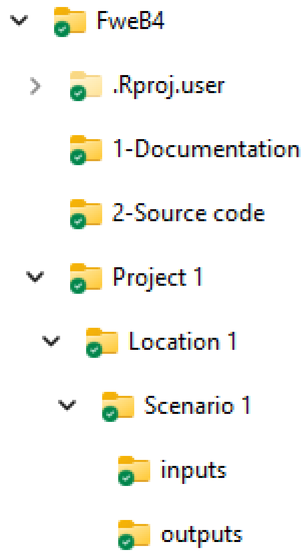

Figure 6. Folder structure for Fweb4

Note that the "Project" level folder and subfolders could be in any location on a computer. For example, a user may wish to place these files under a folder associated with a broader project set of files, above and beyond just model files. The user just needs to edit the code as described below to point to the proper file path.

At this point the model is ready to run (See **Section 4.3** below). Note that the first time you run the model, additional packages will be downloaded to RStudio. This will happen automatically when the model is run. This may take a few minutes but is only required the first time the model is run.

4.3 How to Run the Model

4.3.1 Model Inputs

FweB4 relies on two separate input files. The first file is called “Parameters_official.csv” and is located in the folder called “Inputs”. It contains bioenergetic parameters for 105 species of fish or invertebrates and is directly from FB4. In most instances the user will not need to edit these parameters. Refer to the FB4 documentation for a description of these parameters.

The second input file is an Excel file called “FweB4 input file.xlsx” with 46 input parameters listed across multiple tabs. It is also located in the “Inputs” folder and is the primary file used to assign study-specific inputs for simulations. **Appendix B** provides a detailed description of these parameters.

The following information guides the user through input editing in each tab in the input spreadsheet. Assigning values for many of these parameters will rely on a user’s knowledge of the study system and species

‘Scenario_Info’ tab

First, open “FweB4 input file.xlsx”. If you wish, save the file with a new name in the same “Inputs” folder as the original file. Click on the 1st tab, called “Scenario_Info” (**Figure 7**). Set values for the name of the project, the location for which the scenario applies (e.g., Lake 1), the scenario name, last day of the simulation, whether spawning is allowed (“calc.spawn”) and whether the model will simulate a single fish or a food web (“single.cohort”).

	A	B	C	D	E	F
1	Project name for model run	Location name for model run	Scenario name for model run	Last day of model. The first day is always 1, but can correspond to any day of the year.	Should spawning be allowed?	If TRUE then run model with single cohort to match original FB4
2	**Project_name**	**Location_name**	**Scenario_name**	**Last_day**	**calc.spawn**	**single.cohort**
3	LowerBrownlee	Loc1	Sc1	3650	TRUE	FALSE
4						

Figure 7. Scenario_Info tab of input file with example values.

Assign the Model Fish Species (‘Species_Info’ tab)

Choose the “Species_Info” tab. You can assign fish species based on the list in the ‘Parameters_Official.csv’ file. It is also possible to add new species as additional rows in the ‘Parameters_ Official .csv’ file, however this would require compiling the necessary bioenergetic parameters.

To select a species, enter a species name exactly as it appears in the ‘Parameters_Official.csv’ file in the corresponding columns of the input file. Copying and pasting the species name is recommended. In the example in **Figure 8**, the model has three species ‘Bluegill (juvenile)’, ‘White crappie (adult)’, and ‘Smallmouth Bass (adult)’.

To add a fish species, you would simply add a new row of values, while to remove a species simply delete a row.

Note, lower trophic level groups (e.g., invertebrates, phytoplankton, etc.) are not included here. Information for these lower trophic level groups are defined elsewhere in the input sheet.

For each fish species included in the model, various parameter values need to be specified. These parameters relate to:

- fish initial weights, growth, and length;
- age of fish death (and consequently number of cohorts);
- timing of spawning and loss of mass and contaminant burden; and
- contaminant uptake equation and efficiencies.

	A	B	C	D	E	F
1	sequential number for each species	Species name from parameters_official.csv It is critical that this matches the name in Parameters_official exactly.	p.value is the proportion of maximum consumption (unitless). If provided, the model uses this p.value rather than fitting to weight. If left blank then the p.value is estimated based on weight fitting procedure.	Age (days) at which the fish should hit the weight specified in column F.	Weight (g ww) that each species should weigh at the age specified in column E.	A standardized length (cm) for which to provide contaminant concentrations. Should be an integer.
2	**Nspecies**	**Species_txt**	**p.value**	**age.at.fit**	**fit.weight**	**Standardized.length.cm**
3	1	Bluegill sunfish (juvenile)		1460	80	10
4	2	White crappie (adult)		1825	125	20
5	3	Smallmouth bass (adult)		1825	450	25
6						
7						

Figure 8 Species Info tab with example values. Not all columns are shown.

Define Initial Fish Contaminant Concentrations ('Cohort_Contam_Info')

Next, it is necessary to define the initial contaminant concentrations for each cohort of each fish species. The number of cohorts present when a simulation starts is determined by the lifespan of the species and its spawn date. This is calculated in the Excel input spreadsheet (Column D, **Figure 9**). Initial fish contaminant concentrations should be in units of ug/g ww and be on a whole-body basis.

Initial MeHg concentrations should be entered for each cohort of each species, see the example below (**Figure 9**). Concentrations for other cohorts in the table can be left blank. In the example, bluegill require concentrations for five cohorts, while crappie and bass require concentrations for additional cohorts (all columns not shown in figure). If you need to add values for more cohorts than there are columns, simply copy the column header and create a new column ("Cohort 11 Initial contaminant concentration (ug/g)", "Cohort 12 Initial contaminant concentration (ug/g)", etc.). Ideally the initial fish MeHg concentrations would be informed by field data.

	A	B	C	D	E	F	G	H	I	J	K	L
1	Do not edit. These cells copied or calculated from info on Species_Info tab. Don't need to edit. Not read by R.						Enter initial contaminant concentrations for the number of cohorts in column D. Cohort 1 is youngest, then cohort 2, etc. Feel free to add additional columns as required. Values should be in **ug/g ww** and are assumed to be **whole body** concentrations.					
2	Species_txt	age.at.death	spawn.day	Lifespan in years = Number of Cohorts	Age of youngest cohort (days)	Age of oldest cohort (days).	Cohort 1 Initial contaminant concentration (ug/g)	Cohort 2 Initial contaminant concentration (ug/g)	Cohort 3 Initial contaminant concentration (ug/g)	Cohort 4 Initial contaminant concentration (ug/g)	Cohort 5 Initial contaminant concentration (ug/g)	Cohort 6 Initial contaminant concentration (ug/g)
3	Bluegill sunfish (juveni	1825	122	5	244	1704	0.05	0.07	0.1	0.13	0.16	
4	White crappie (adult)	2555	122	7	244	2434	0.05	0.07	0.1	0.13	0.16	0.18
5	Smallmouth bass (adul	3650	122	10	244	3529	0.05	0.07	0.1	0.13	0.16	0.18
6	0	0	0	0	0	0						
7	0	0	0	0	0	0						

Figure 9 Cohort Contam Info tab with example values.

Define Environmental Variables ('Environmental_vars')

This tab is used to provide water temperature inputs and dissolved oxygen concentrations if including contaminant uptake across the gills in a simulation (**Figure 10**). These values should span the full simulation period (day 1 to final day). Both inputs (water temperature and dissolved oxygen) use the same dates listed in column A, which can be provided at any time interval desired. Daily values are estimated in the simulation by interpolation.

If information on temperature and dissolved oxygen are only available at different times it is possible to leave empty cells in the time series and these will be computed by the model through linear interpolation. In the below example, temperature values are provided monthly (day 1, day 30, etc.). Dissolved oxygen is provided for the same time steps, but is held constant.

	A	B	C
1	Day for time series. Missing days are linearly interpolated. Must extend to the final day of the model.	Temperature (C)	Dissolved oxygen (mg/L). DO sat is only used if contaminant uptake across gills is consider (contaminant equation 3)
2	**day**	**temperature**	**DO**
3	1	5	9.5
4	30	4	9.5
5	90	9	9.5
6	120	14	9.5
7	150	19	9.5
8	180	23	9.5
9	210	25	9.5
10	240	23	9.5
11	270	19	9.5
12	300	14	9.5
13	330	9	9.5
14	360	5	9.5
15	365	4	9.5

Figure 10 Environmental vars tab with example values.

Define the Food Web Structure (‘Diet_matrix’)

Trophic levels below fish, and fish diets, are defined using a diet matrix (**Figure 11**). The user defines which lower trophic levels should be included as well as the diet proportions for the modelled fish species. There is no mathematical limit on the number of lower trophic levels and fish species, although more compartments can add to the complexity when interpreting results. Prey items are specified in columns, while predators are listed in rows. Diet proportions can vary by age, as defined by different rows for a given fish species. Dietary information should be given that spans the lifespan of each fish species (at minimum, there should be two rows for a predator species). Diet proportions at any given age (i.e., row) should sum to 1. Diet proportions are defined on a wet weight basis.

In the diet matrix, and subsequent tabs, it is critical that species names match exactly the names used in the ‘Species Info’ tab. For lower trophic levels (e.g., inverts) it is also critical that spelling is consistent across all tabs.

In the example below (**Figure 11**), two lower trophic groups are defined (inverts and benthos). To add an additional prey compartment simply insert a column into the diet matrix. Rows can be added or removed to change the time series information for diets for a given fish species.

Diet composition matrix, with consumer in column A, predator age (days) in column B, and prey items in subsequent columns. Proportions for missing ages are linearly interpolated.
Ages must span the lifespan of each species, as specified on the Species_info tab. This is true even if the length of the model is shorter than the fish lifespan.
Proportions are in proportion wet weight.
Predator and prey names must match Species_info tab exactly.

pred.prey	age	Zooplankton	Chironomids	Amphipods	Bluegill sunfish (juvenile)	White crappie (adult)	Smallmouth bass (adult)
Bluegill sunfish (juvenile)	1	0.7	0.2	0.1	0	0	0
Bluegill sunfish (juvenile)	1825	0.7	0.2	0.1	0	0	0
White crappie (adult)	1	1	0	0	0	0	0
White crappie (adult)	2555	1	0	0	0	0	0
Smallmouth bass (adult)	1	0.9	0	0	0.05	0.05	0
Smallmouth bass (adult)	700	0.9	0	0	0.05	0.05	0
Smallmouth bass (adult)	730	0.5	0.05	0.05	0.25	0.15	0
Smallmouth bass (adult)	3650	0.5	0.05	0.05	0.25	0.15	0

Figure 11 Diet matrix tab with example values.

Specify Size Limits for Prey ('Diet_size_matrix')

For many piscivorous fish species, prey length is an important consideration. This is specified in the model using the 'Diet size matrix' (**Figure 12**). The minimum and maximum prey length is defined as a proportion of the predator's length and can vary by age. The ages provided should span the lifespan of each species, and at minimum there should be two rows for each species.

In the below example, minimum prey size for all species and all ages is set at 5% of the predator's length, however maximum prey size varies by species and could increase as fish get older.

Diet size matrix, with predator in column A, predator age (days) in column B, and minimum and maximum size of prey items as a proportion of the predator size in other columns.
Proportions for missing ages are linearly interpolated.
Ages must span the lifespan of each species, as specified on the Species_info tab.
Predator names must match species names specified on the Species_info tab exactly.

pred.prey	age	min_size	max_size
Bluegill sunfish (juvenile)	1	0.05	0.2
Bluegill sunfish (juvenile)	1825	0.05	0.2
White crappie (adult)	1	0.05	0.2
White crappie (adult)	2555	0.05	0.2
Smallmouth bass (adult)	1	0.05	0.15
Smallmouth bass (adult)	3650	0.05	0.15

Figure 12 Diet Size Matrix with example values.

Specify Remaining Prey Information ('Prey_Info')

This tab defines various prey parameters that are used to model consumption and contaminant accumulation. For each parameter, values are required for every dietary group in the model. The first parameter is energy density (J/g ww), while the second is digestibility (%) for each model group. These values are described in detail in the FB4 documentation, including a discussion of how to calculate or estimate energy densities for different species if necessary. For some fish species (when PREDED EQ in column AK is 2 or 3 in Parameters_official.csv) then energy density is predicted as a function of bodyweight using the equations outlined in the FB4 user manual. For all other fish species and lower trophic levels the user-supplied energy densities are used.

The third and fourth parameters specify the dietary uptake efficiency of contaminants from different prey items. Depending on the contaminant equation selected (see **Section 3.2.3** and **Table 6**), one of transfer efficiency (Contaminant Equation 1) or assimilation efficiency (Contaminant Equation 2, 3, or 4) will be used. These two tabs therefore provide somewhat redundant information, and this redundancy is a carry over from FB4.

As with other parameters, it is critical that the names of model groups (lower trophic level groups or fish species) are specified consistently.

Provide Contaminant Concentrations ('Contam_conc' and 'Contam_Pre-Processing')

This tab is used to specify MeHg concentrations in water, sediment, and lower trophic level groups. Concentrations should be provided as a time series, with model day indicated in the first column. These time series inputs can be entered at regular or irregular time intervals. The model will interpolate daily values. Water concentrations are only used for considering gill uptake (contam_eq = 3), while sediment concentrations are only needed to calculate lower trophic level concentrations if specified in the 'Contam_Pre-Processing' tab.

Concentration values must be provided in the expected units. These are:

- Water = ng dissolved MeHg/L;
- Sediment = ng/g dry;
- Lower trophic model groups = ng/g dry; and
- Fish species = µg/g wet.

For lower trophic level MeHg concentrations, it is alternatively possible to use bioaccumulation factors (BAF's) to calculate concentrations based on MeHg concentrations in water (dissolved phase) or sediment solids. This is done in the 'Contam_Pre-Processing' tab. To use a BAF instead of a user-input time series, the user includes a row in the "Contam_Pre_Processing" tab column called "Active" and sets the value to TRUE. Also required are values for the BAF numerator and denominator ("End.compartment" and "Start.compartment" respectively. In the example below (**Figure 13**), all lower trophic level MeHg concentrations are user-specified, and

BAFs were not used (“Active” = FALSE). If the BAF is based on the MeHg concentration on sediment solids, the BAF is dimensionless (i.e. based on concentrations in ng/g dry for biota and sediment solids). Conversions in ‘Contam_Pre-Processing’ are only applied when ‘Active’ is set to TRUE. Note that if the Contam_Pre-Processing tab is used to estimate values for a given lower trophic compartment, these estimated values are given priority over any values provided in ‘Contam_conc’.

This tab allows you to calculate contaminant concentrations in model compartments if they are not available directly (e.g., calculate benthic invertebrate concentration from sediment; zooplankton from water). The contaminant concentrations provided in the 'Contam_conc' tab for the group in the 'start.compartment' is multiplied by the 'conversion.factor' to calculate the contaminant concentration in the 'end.compartment'. Group names must match those used elsewhere exactly.

Units are as described in 'Contam_conc' - ng / L for water and ng / g dry for sediment and non-fish model groups.

If 'Active?' is set as TRUE, then this conversion will be used for the 'end.compartment' group, if it is FALSE then the model will look for and use values provided on the 'Contam_conc' input file.

Active	Start.compartment	End.compartment	Conversion.factor	conversion note
FALSE	water	Zoo	1.5	calculating zooplankton conc from water
FALSE	sediment	Benthos	2	calculating benthos conc from sediment

Figure 13. Contaminant Pre-Processing tab with example values.

Dry Weight to Wet Weight Conversion (‘Dry_wet_conversion’)

While MeHg concentrations for lower trophic level groups are required to be provided on a dry weight basis, FweB4 converts these values to wet weight for bioenergetic calculations. Fweb 4 also converts lower trophic level MeHg concentrations back to dry weight when reporting outputs. It is therefore necessary to provide dry to wet weight conversion factors for all lower trophic level model groups. **Figure 14** shows an example for a model with three lower trophic groups (phytoplankton, zooplankton, and benthos). A value of 0.2 as a dry to wet weight conversion factor corresponds to a moisture content of 80%.

Dry to wet weight ratio for non-fish model groups. These values are used to convert the dry weight inputs to the wet weight equivalents used in the model.

g dry / g wet	g dry / g wet	g dry / g wet
Zooplankton	**Chironomids**	**Amphipods**
0.12	0.15	0.2

Figure 14. Dry to Wet weight conversion tab with example values.

4.3.2 Running FweB4 in R

To load and run the model:

1. **Open the Model Project** (‘FweB4.Rproj’) using RStudio, located in the root folder where the model was unzipped to. Note that your most recent version of R is used by RStudio in the background, so it is not necessary to open R directly.
2. **Open the Model R Script** in R Studio. The script is called “FweB4_model_v4.5.3.R”, located in the ‘2-Source code’ folder, and can be accessed in the “Files” tab. This should open in the R Project that you opened in step 1.
3. **Edit references to input and output files –** Edit the code (Lines 17, 20, and 23) to reference the correct input files and output location (**Figure 15**). There are two input files and one output location required; note that all three paths start at the “Project” level (i.e., the paths are relative to the root folder of the FweB4 model). If copying the directory path from file explorer, note that the slashes may need to be replaced (R expects forward slashes ‘/’ while Windows often uses back slashes ‘\’).
    - **‘parms_file’ –** This is the file containing the bioenergetic parameters for each species. Unless you have edited this file, this should always reference the ‘Parameters_official.csv’ file.
    - **‘main_inputs’** – This is the Excel file containing the parameters for your specific model run. This is the file described in **Section 4.3.1**.
    - **‘out_loc’ –** The folder where you would like output files to be saved. This name should always end with a forward slash.

```
14 # Set file paths for input and output files ========================================
15
16 # Bioenergetics parameters, by species
17 parms_file <- "Project 1/Location 1/Scenario 1/inputs/Parameters_official.csv"
18
19 # model parameter file
20 main_inputs <- "Project 1/Location 1/Scenario 1/inputs/FweB4 input file.xlsx"
21
22 # Output directory
23 out_loc <- "Project 1/Location 1/Scenario 1/outputs/"
24
```

Figure 15. Specify input files and output location. In this example, the model Your values may be different to those shown here.

4. **Run the model** – Highlight all the text in the model R script (e.g.‘FweB4_model_v4.5.3.R’) and then click the ‘Run’ button at the top of the page (**Figure 16**). Warnings, errors, or other messages will be displayed in the ‘Console’ at the bottom of the screen.

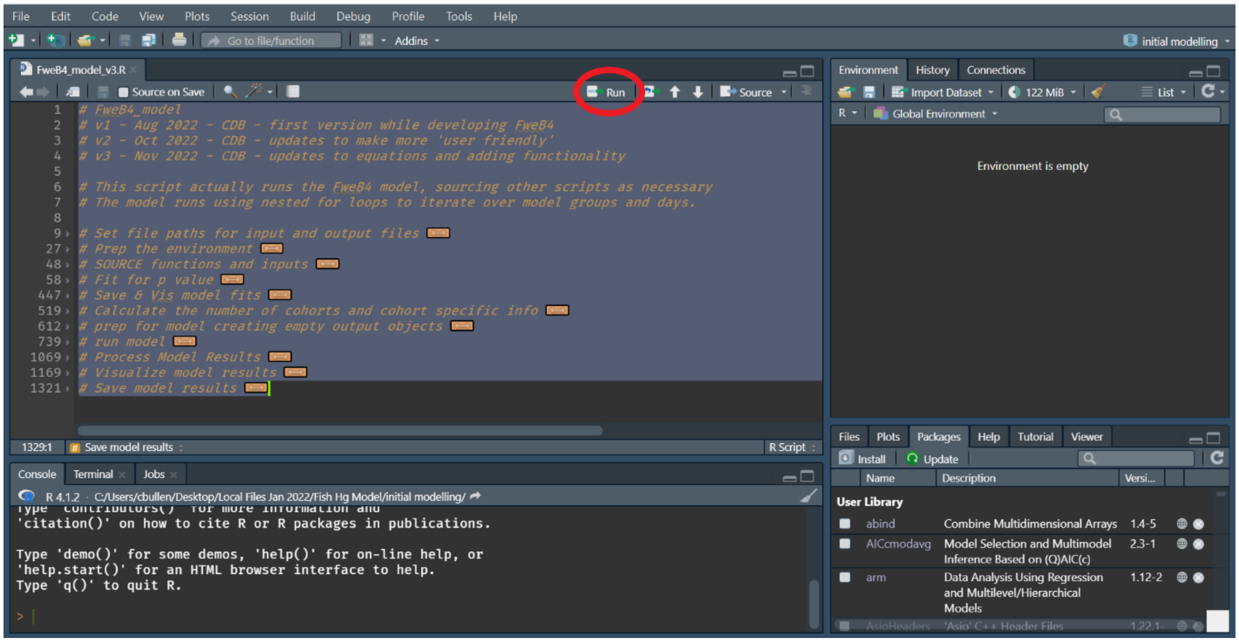

Figure 16. Running FweB4**. Highlight all code lines and click the '*Run*' button (circled in red).**

4.3.3 Model Outputs

The model saves several output files to the location specified when running the model (see **Section 4.3.2**). These outputs are related to both the fish growth-fitting procedure and the overall model simulation.

Model Output Data

The model generates four types of output csv files:

1. **A summary of input parameters** used in the simulation. The values reported in this file are daily estimates. If inputs are entered at other time intervals, the model interpolates to daily values, which is the information reported in this file. Information is provided for water temperature, dissolved oxygen, dissolved water MeHg concentrations, sediment MeHg concentrations, and lower food web MeHg concentrations. This file has the text "input_timeseries" as part of the file name.

2. **Daily results for MeHg in standard-sized fish.** This file has the text "Standard_Size" as part of the file name.

3. **Daily results for all fish species and cohorts**. Information is provided for a wide range of parameters related to MeHg concentrations and fluxes, growth and bioenergetics fluxes. Columns are different parameters; rows are outputs for a given cohort on a given day. This is often a large file, depending on the number of species, the number of cohorts, and the length of the model run. This file has the text "ALL" as part of the file name.

4. **Results from fish growth fit procedure.** As discussed in **Section 3.2.2** the model fits fish growth rates to reach a target weight at a target age. This is done prior to the full MeHg simulation using average daily temperatures during a simulation. Because temperatures can vary in a simulation on a given day of the year from one year to the next, the actual growth rate simulated may vary from the results of the growth-fitting exercise. Fitted growth results are available for each species, identified in the output folder by the "SpcX_model_fit" in the file names, where X is a number associated with each fish species in the model. The model saves a plot (.png file) illustrating the growth curve and best-fitting p.value for the species, as well as a table (.csv file) with the daily growth data (including temperature, age, starting weight (g), ending weight (g), predator and prey energy densities (J/g), and consumption rate (g/g/d). Output file names also include information on the Project, Location and Scenario as well as the date and time of the model run. It is important to note that the *fitted growth curves are not the same as the growth rates actually simulated*, which are available in other output files as described in bullet #3.

Model Output Plots

A plotting module is included to visualize a wide range of model results. Recognizing that users may wish to create other plots, underlying data for all plots can be exported and users can produce their own plots using software of their choosing. Before starting, you should create a folder called "plots" in the "outputs" folder (i.e., the 'out_loc' specified in the model run). Then add a folder called "plotdata" to the "plots" folder. It should look as shown in **Figure 17**.

Note that the plotting code is more interactive than the model code. Rather than simply select all the code at once, you will run a number of code chunks as explained in the instructions below.

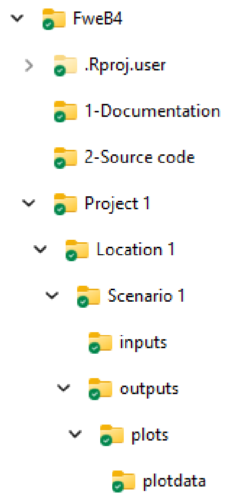

Figure 17. Directory structure showing "plots" and "plotdata" folders

Instructions to run the plotting module are as follows (code lines shown in parentheses are approximate):

1. **Open the Model Project** ('FweB4.Rproj') using RStudio, located in the root folder where the model.
2. **Open the Plotting Module R file -** Open the 'FweB4_plotting_v2.R' file from your FweB4/2-Source Code/ folder.
3. **Paths to input files** (Line 21 – 26) **and output files** (Lines 29 – 30) – set the path and file name for each of the four input files (summary of input parameters, all model results, results for standard sized fish, and the original model input file; see section **Model Output Data** for more information) and the paths for the two output folders (the 'plots' folder will contain all the plots and the 'plotdata' will contain plot-specific data files if that option is selected).
4. **Run Lines 1 through 81** – The code will automatically install packages that are not already in your package library. You only need to install a package once. There will likely be some warnings related to versioning and functions; these are unlikely to be important.
5. **Data Upload & Organization** (Lines 82 - 157) – this section of code reads in the data, adds a few new variables for plotting and cleans up the data. It is run in two steps:
    a. Run Lines 82 through 115 to import the data and get "pretty" names (i.e., each name part is capitalized, so "Rainbow trout" would become "Rainbow Trout"). The code prints the species names from your model run so that you can update the fish names and their order in Lines 132 and 155. These lines are initially setup for the example, so show *c("Bluegill Sunfish","White Crappie","Smallmouth Bass");*

this is the part of the code you would change if your model contains different species.

b. After your species names and their order have been set (see above), you can run Lines 116 to 157.

6. **Species/cohorts list** (Lines 161 - 171) – Run those lines of code. R will generate a new csv file with rows for each species/cohort combination. That file ("Species_Cohorts_to_plot.csv") gets saved to the 'outputs/plots' folder. It contains a column called 'Plot' that is set to 'NA'. Open the csv file (one option is to open it in Excel) and change the Plot variable for any cohort to 'yes' to include in the detailed plots; save the file.
7. **Add Species/Cohort Info and Set global plotting options** (Lines 173 through 178)– run these lines to add the species/cohort numbers back to the original data and to set the ggplot2 theme and plot title alignment. Note that ggplot2 has a number of themes to choose from; details can be found here (https://ggplot2.tidyverse.org/reference/ggtheme.html).
8. **Export data for plotting** (Line 181) – Toggle 'writeDat' to "TRUE" if you want detailed data outputs for all plots to be able to produce custom plots. The data files are exported to the 'outputs/plots/plotdata' folder. These files can be large, so keep this as "FALSE" unless you really need the data, particularly if you have many cohorts selected for plotting. Run this line.
9. **Diet size processing** (Lines 184-211) - Run these lines which will process the information on diet sizes necessary for some of the later plots.
10. **Input Parameter Plots** (Lines 214-244) – nothing to modify here; run all the code to produce a plot with a panel for each input parameter.
11. **Species Hg Plots** (Lines 248 - 302) – run lines 248 through 302. This code produces a panel for each fish species that shows MeHg concentrations in muscle tissue for each cohort and for a standard sized fish across the simulation timeline; it also exports plot data if you selected that option in Step 8.
12. **Detailed Cohort Plots** (Line 336 to end) – run this code to produce the detailed output plots for specific species/cohort combinations; this assumes that you modified the "Species_Cohorts_to_plot.csv" file by changing at least one "NA" to "yes" in the 'Plot' column (i.e., selecting at least one species/cohort combination; see Step 6 above). If you run the code but have not selected at least one cohort you will get an error. So, first run lines 336 through 347; if you don't get a warning message about species/cohort combinations, then put the cursor at the beginning of Line 351 and select 'run'. That will produce the following files for each species/cohort selected (note that the plots are saved to the plot output directory [specified in line 27] but are not actually shown on screen like the previous plots were):

a. **Series 1** (shown by "S1" in file name) – plot panels of tissue MeHg, MeHg burden, MeHg uptake and clearance, and daily MeHg clearance are shown relative to fish age and simulation year.
b. **Series 2** (shown by "S2" in file name) – plot panels showing MeHg uptake in food vs water and uptake across all sources (amount per day and proportion of daily uptake) relative to fish age and simulation year.
c. **Series 3** (shown by "S3 in file name) – plot panels showing age-weight, age-length, length-weight, and age-growth efficiency relationships. The latter is net production/consumption.
d. **Series 4** (shown by "S4 in file name) - plot showing fish length over time (model day and fish age) and the preferred diet size range for the selected cohorts.

5 REFERENCES

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APPENDICES

APPENDIX A:
MODEL TEST SCENARIOS

Various tests were carried out to ensure FweB4 functions as intended, including:

- Comparisons of results simulating MeHg with FweB4 and FB4.
- Tests of the effects of new features in FweB4:
  - MeHg uptake across the gills
  - Using BAFs to estimate MeHg in lower trophic levels
  - Estimating MeHg in standard length fish at two different lengths
  - Changing the preferred prey length range

Additional information is provided below.

FweB4 vs FB4

It was important to confirm that FweB4 was able to replicate the results of FB4 if simulating the same scenario. Because FweB4 includes some features not available in FB4, comparison tests could only be conducted at the level of complexity available in FB4 (i.e., one species, one cohort). Here we present the results of two such comparison tests, to illustrate that FweB4 and FB4 function identically for the tests carried out. The specific test scenarios are fictional; the purpose of these tests was to compare the models to one another rather than to assess the realism of either model.

<u>Comparison Test 1 – Lake Whitefish</u>

For the first test, FB4 and FweB4 estimated growth and MeHg concentrations in Lake Whitefish over 800 days. Fish were assumed to spawn at the beginning of the simulations in each model. FweB4 was run in a single cohort configuration.

The consumption fitting procedure was conducted in both models, with a target final weight of 400g on day 800 (while FweB4 can fit to weight on any day, FB4 requires that it is the final day of the model). Both FB4 and FweB4 predicted that the p.value best fitting this weight was 0.1345, indicating that the models are functioning identically in this regard.

Lake Whitefish was assigned a diet of invertebrates and fish, with the proportion of fish in the diet increasing over time. Spawning was set to occur on the final day of each year (day 365), and contaminant accumulation was modelled using the second of the two equations available in FB4, which allows for accumulation and clearance. The weights and contaminant concentrations predicted by FB4 and FweB4 were identical (**Figure 18)**.

While the test confirmed that FB4 and FweB4 produced the same results for growth and MeHg concentrations, the growth pattern in both models was slow at first, then faster. This may have resulted in the quick initial increase in MeHg concentrations after hatching. Further exploration of this trend, which also occurs for some other fish species (e.g. northern pike) is warranted.

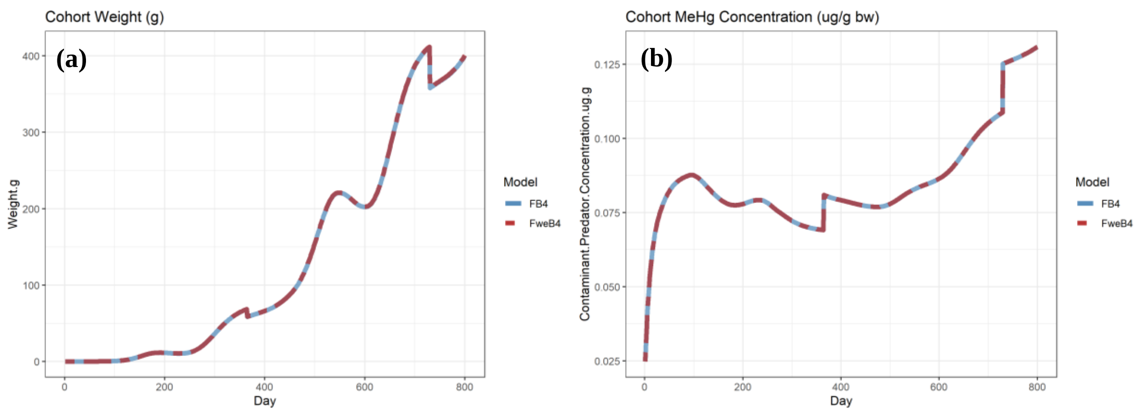

Figure 18. Comparison of FB4 and FweB4 estimates of weight (a) and contaminant concentration (b) for the Lake Whitefish test.

<u>Comparison Test 2 – Simple Food Web</u>

The second comparison involved a 10 year simulation of a simplified food web consisting of invertebrates, benthos, dace, yellow perch, and walleye. This test was included to assess the simultaneous multi-species aspects of FweB4. To keep the scenario relatively simple and align FweB4 with FB4, the following model settings were applied in FweB4:

- only one cohort of each species was used (as in FB4);
- all cohorts were presumed to survive for the entirety of the simulation;
- predators were allowed to consume prey of any size;
- cannibalism was not allowed;
- spawning was allowed at any weight (as in FB4);
- contaminant burden was assumed to be retained during spawning (as in FB4); and
- contaminant uptake and clearance were calculated using 'Equation 2' from FB4 (which does not consider uptake from water).
- invertebrate and benthos MeHg concentrations were set to increase slowly over time (initially 0.02 and 0.04 ng/g dw for invertebrates and benthos, respectively), peak at 5 years (0.04 and 0.06 ng/g dw), and then decrease back to initial values at 10 years.

As FB4 is unable to simulate multiple species at the same time, only the highest trophic compartment (Walleye) was simulated in FB4. MeHg concentrations in Walleye dietary items in FB4 were based on the corresponding concentrations from FweB4 for dace and yellow perch. Both models were given a target final Walleye weight of 1500g on day 3650. FB4 and FweB4 both calculated p.values of 0.393, and the resulting growth and methylmercury concentrations were well aligned and practically identical. (**Figure 19**). Simulated MeHg in finescale dace increased then decreased, due to the same pattern assigned to intertebrates and benthos concentrations in their diet.

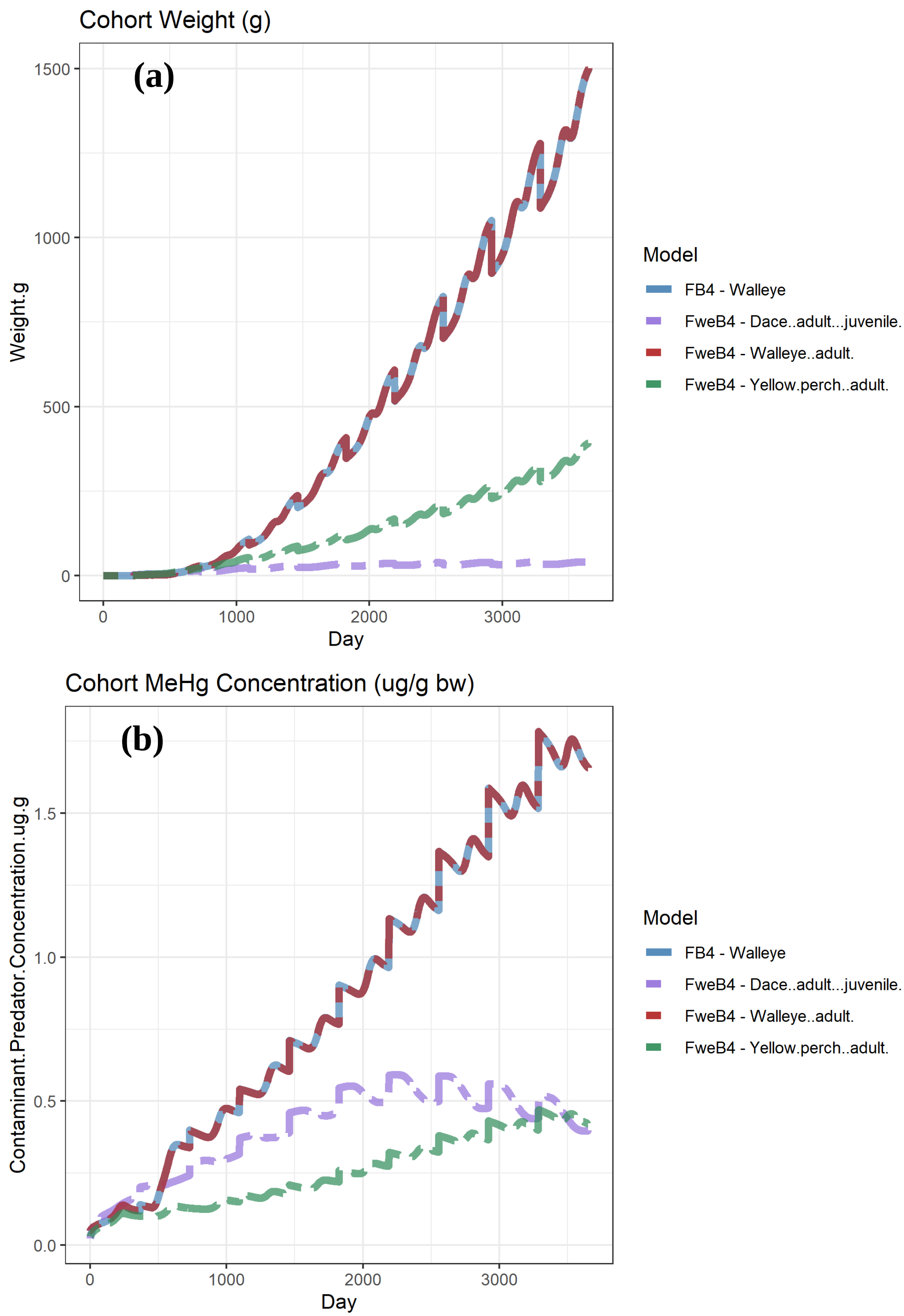

Figure 19. Comparison of FB4 and FweB4 estimates of weight (a) and contaminant concentration (b) for a simple food web. FB4 only modelled Walleye (see text for details).

Simulations with and without gill uptake of MeHg

Using the FweB4 scenario from Comparison Test 2, simulations were conducted excluding gill uptake of MeHg (as in Test 2) and allowing for uptake of MeHg using the third contaminant equation (contam.eq = 3). For the simulation with gill uptake, the concentration of dissolved MeHg in water was set at 0.1 ng/L while gill uptake efficiency was set at 25% ('Aq_MeHg_uptake' parameter).

Generally, simulated MeHg concentrations are very similar with or without gill MeHg uptake (**Figure 20)** with gill uptake resulting in marginally higher concentrations in all model groups. On the final day of the model, concentrations in the simulation allowing for gill uptake are 1.2% (walleye) to 1.4% (perch) higher than the simulation without gill uptake. This difference would be greater with increased aqueous concentrations or increased gill uptake efficiency.

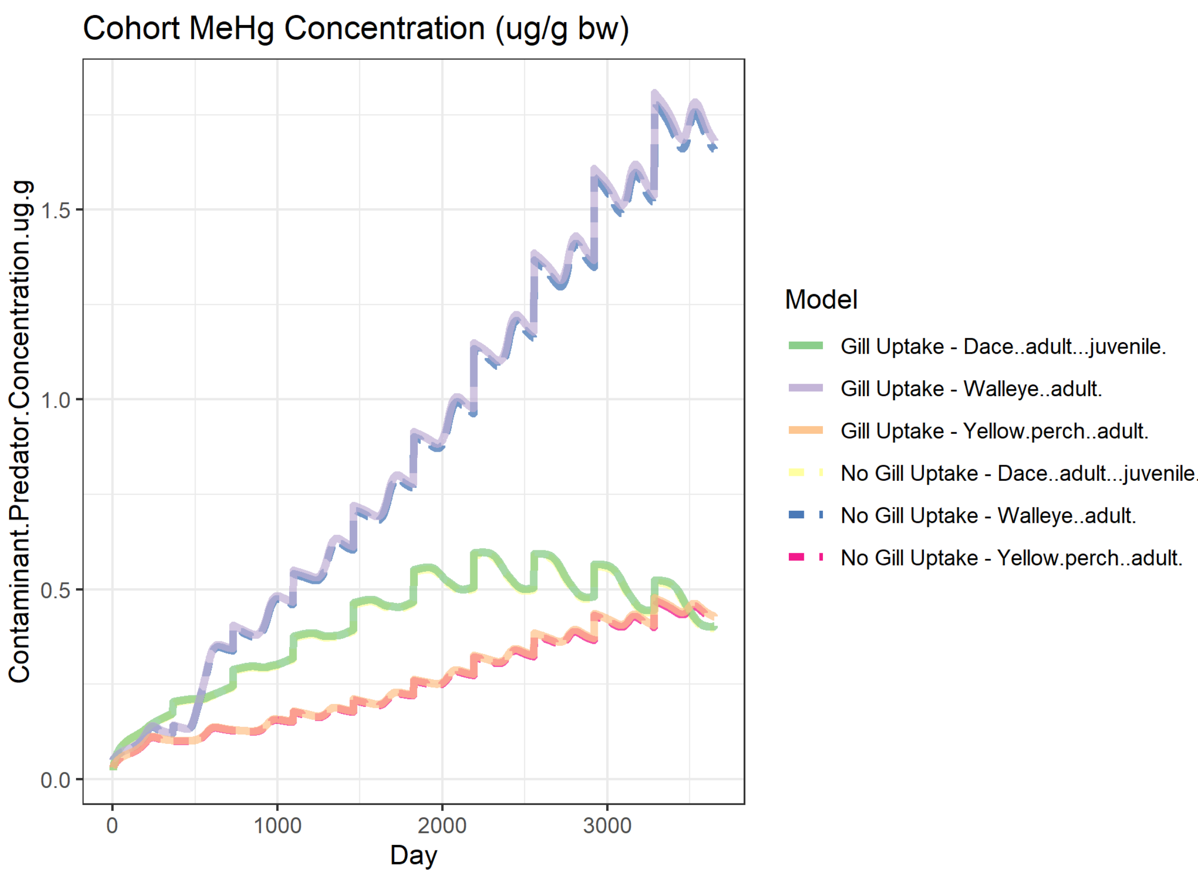

Figure 20. Comparison of FweB4 simulations with and without gill uptake of MeHg.

Simulation with zooplankton MeHg predicted by the model.

A simulation was carried out to test the model feature that predicts lower foodweb MeHg concentrations using a user-specified bioaccumulation factor (BAF). Two model scenarios similar to Comparison Test 2 were simulatied, including a simple food web consisting of invertebrates, benthos, dace, yellow perch, and walleye. In one scenario zooplankton concentrations were estimated by the model on the basis of a user-input concentrations of 0.1 ng/L for dissolved methylmercvury in water and a zooplankton BAF of 1000 L/g dw (1.0E+06 L/Kg dw). This resulted in a zooplankton MeHg concentration of 100 ng/g dw. In the other scenario, the zooplankton MeHg timeseries was input as 100 ng/g dw, with no use of the BAF. As expected, the two scenarios gave identical results. Note the BAF is based on a dry weight MeHg concentration in zooplankton.

Different standard length

Simulations were conducted to examine the influence of the selected standard fish size on the estimated MeHg concentration. Two simulations were run, parameterised as for the previous tests, with varying standard sizes selected for each species. In one simulation standard sizes for dace, perch, and walleye were set at 3 cm, 10 cm, and 30 cm, respectively. In the second simulation, all sizes were increased to 5 cm, 15 cm, and 40 cm for dace, perch, and walleye respectively. As expected, fish of a larger standard size have higher MeHg concentrations (**Figure 21**).

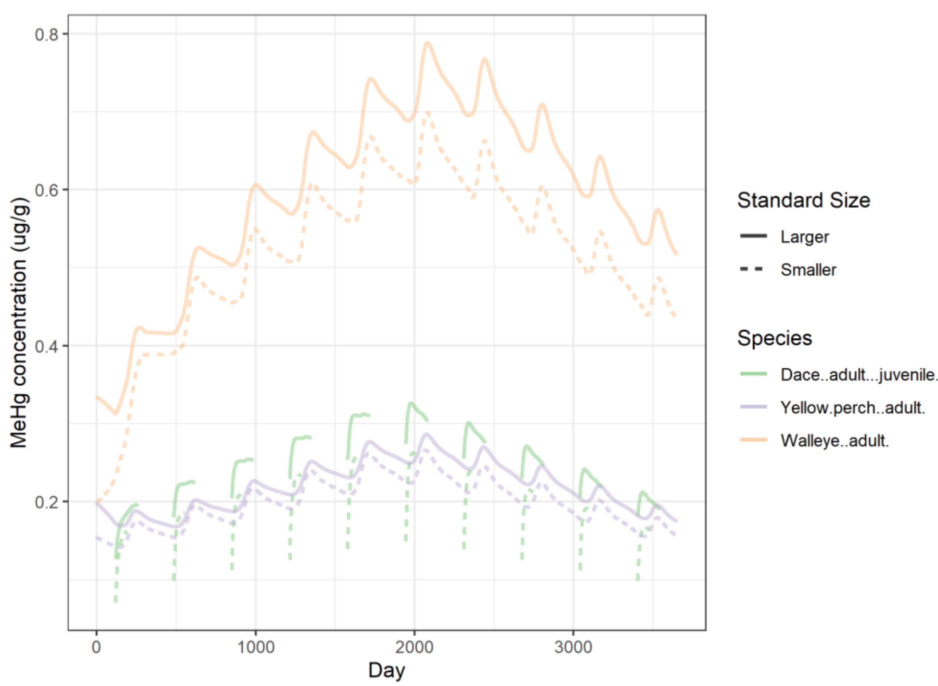

Figure 21. FweB4 estimated MeHg concentrations in walleye at two standard lengths (30 and 40 cm).

Different preferred length for prey fish

This test compared the influence of the preferred length of prey fish on estimates of fish weights and MeHg concentrations. The model used the same model parameters as previous tests, and was re-run with varying limits on the maximum prey size relative to predator body length. One simulation set the maximum prey size at 20% of the predator length, while the second simulation had a maximum prey size of 40%. For simplicity, the same values were used for all species and held constant over time, however users can vary this parameter as required.

Estimated weights and MeHg concentrations for the two simulations were then compared. Only Walleye results showed differences between the two simulations, suggesting that in this hypothetical food web dace and perch growth were not limited by prey size. Walleye, on the other hand have noticeably increased MeHg concentrations in standard size fish (**Figure 22**) when they are allowed to consumer larger prey. These results are consistent with expectations and suggest that with more restricted prey size Walleye consume younger cohorts with lower MeHg concentrations. Walleye cohort weights were identical between the two simulations however differences in weights may occur if more-restrictive size ranges result in predators sometimes being unable to find prey of a suitable size and compensating by consuming other model groups (see **Section 3.2.2** for an explanation of what happens when prey of the correct size are not available)

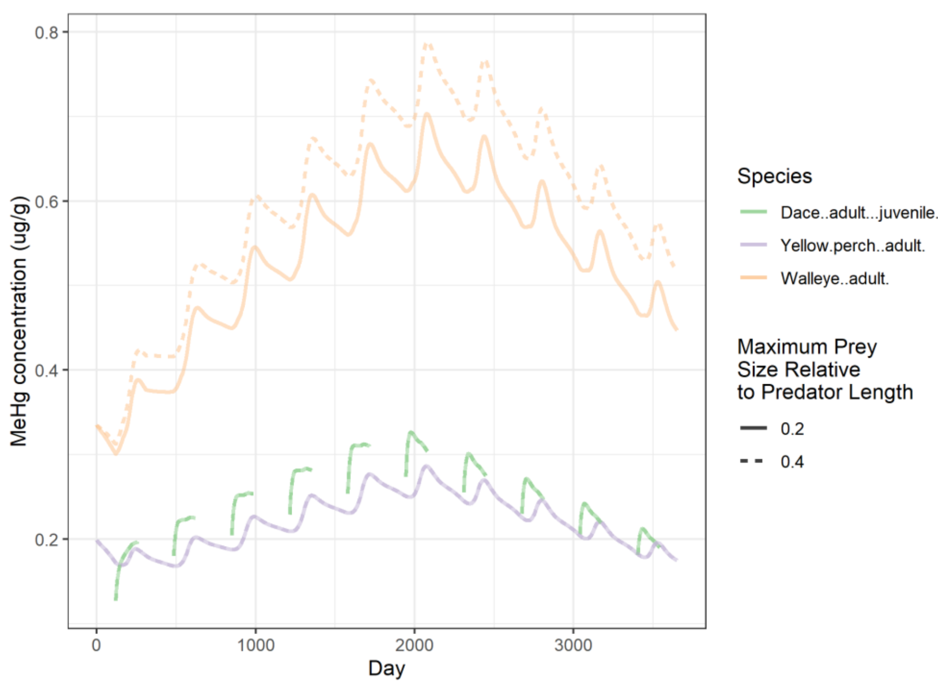

Figure 22. FweB4 estimated MeHg concentrations in standard size fish, with different maximum prey sizes. Standard sizes can be set by the user and here is 5cm for dace, 15cm for perch, and 40cm for walleye.

Comparison with D-MCM model

A comparison was made between FweB4 and an existing mercury cycling and bioaccumulation model known as the Dynamic Mercury Cycling Model (D-MCM) (Harris *et al*., 2021, 2020, 2015, 2013, 2012; RHE et al., 2020, Dastoor et al., 2016). D-MCM simulates three forms of mercury (methylmercury (MeHg), inorganic Hg(II) and Hg(0) in water, sediments and simplified food webs. D-MCM has been applied to numerous sites and the FweB4 comparison was done using D-MCM results for a location on the South River, VA.

DuPont used mercury in the production of acetate rayon fibers from approximately 1930-1950 near the small town of Waynesboro, Va. Elemental and inorganic Hg(II) were both inadvertently released and transported into surface water, sediments, floodplain soils, and biota of the South River and part of the South Fork Shenandoah River. Fish mercury concentrations remain elevated decades after the original contamination occurred. Smallmouth Bass mercury concentrations at some locations are still in the range of 2-4 µg/g in adult fish (Stahl et al., 2014; Harris et al., 2020).

Food web MeHg was simulated with D-MCM at several locations along the South River from 2006-2014. Here we applied FweB4 at a location 11.5 miles downstream of the original point of release. D-MCM-predicted MeHg concentrations in water and lower trophic level biota were used as well as water temperatures and fish dietary preferences used in D-MCM, to examine how closely the two models simulated MeHg in fish. This was therefore a test of how closely FweB4 matched results from another model rather than how well it fit field data.

MeHg concentrations in water and the lower food web items predicted by D-MCM and used as inputs to FweB4, are shown in **Figure 23** and **Figure 24** respectively.

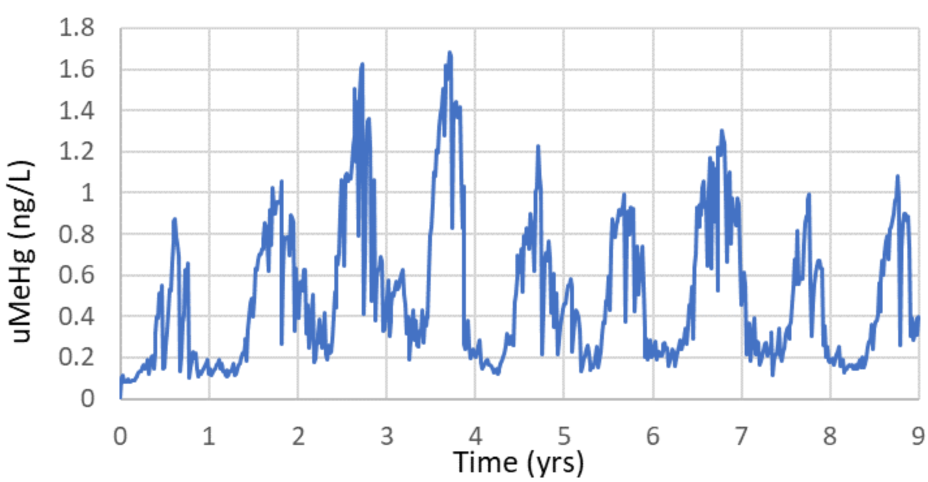

Figure 23. MeHg concentrations in water used in FweB4 simulation. Concentrations were generated by D-MCM model.

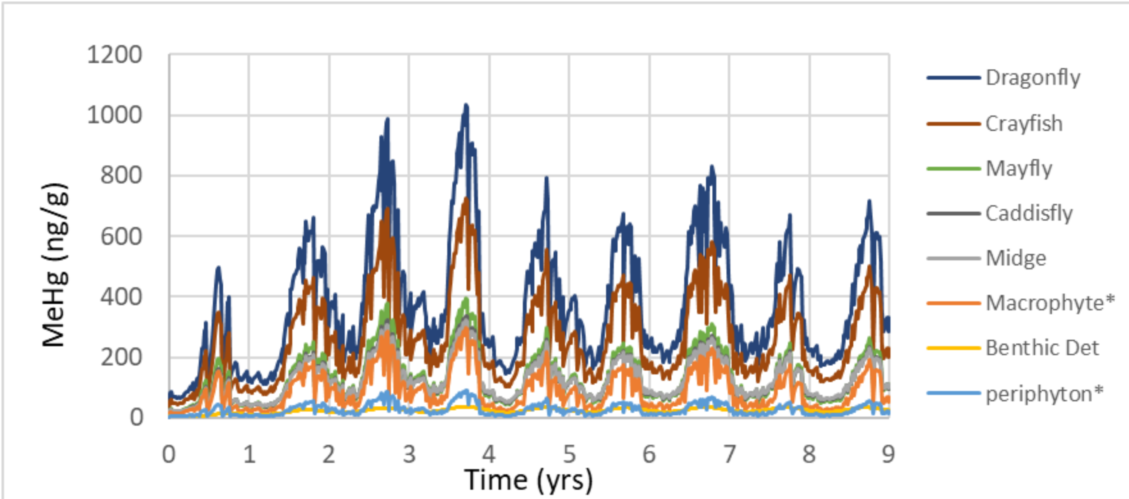

Figure 24. MeHg concentrations in lower trophic level items used in FweB4 simulation. Concentrations were generated by D-MCM model.

Fish growth is temperature dependent in both D-MCM and FweB4, although the effect is different. Water temperatures used by D-MCM and FweB4 are shown in **Figure 25**.

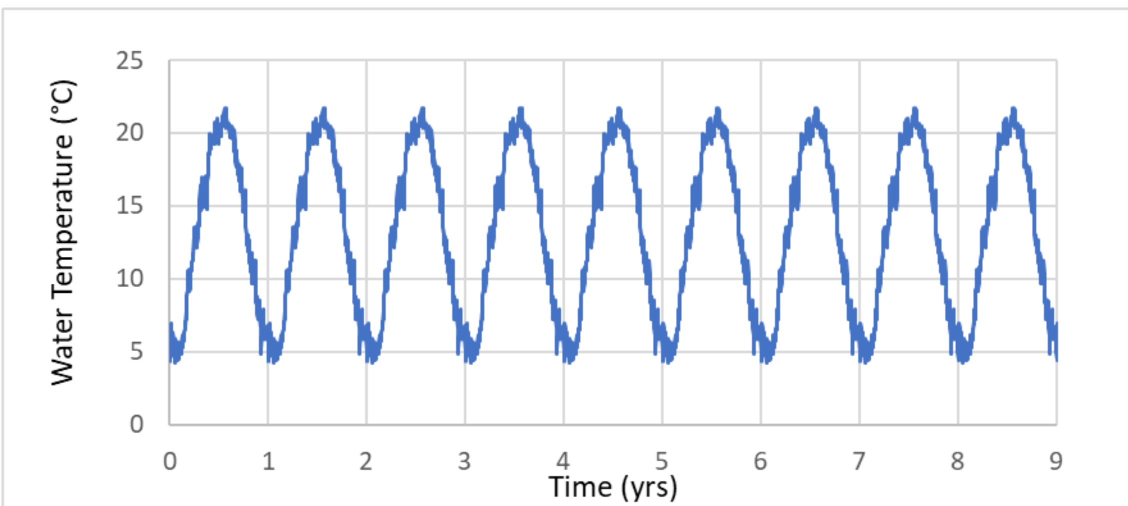

Figure 25. Water temperatures used in FweB4 and D-MCM simulation comparison for food web in South River, VA

Fish MeHg concentrations are strongly affected by what fish eat. The dietary preferences used by FweB4 was set up to match that of D-MCM (**Table 1** and **Table 2**).

Predator fish species	Age	Macrophyte	Midge	Benthic Detr.	Periphyton	Mayfly	Dragonfly	Crayfish	Caddisfly	Shiner	RB Sunfish	Smallmouth Bass
Shiner	0	0.16	0.17	0.27	0.19	0.06	0.03	0	0.12	0	0	0
Shiner	1945	0.16	0.17	0.27	0.19	0.06	0.03	0	0.12	0	0	0
RB Sunfish	0	0.11	0.28	0	0	0.21	0.1	0.03	0.27	0	0	0
RB Sunfish	2675	0.11	0.28	0	0	0.21	0.1	0.03	0.27	0	0	0
Smallmouth Bass	0	0.05	0.11	0	0	0.26	0.14	0.06	0.03	0	0.35	0
Smallmouth Bass	365	0.05	0	0	0	0.03	0.02	0.34	0.05	0	0.51	0
Smallmouth Bass	3650	0.05	0	0	0	0.03	0.02	0.34	0.05	0	0.51	0

Table 1. Dietary preferences used in FweB4 and D-MCM simulation comparison for food web in South River, VA. Values are fractions of the overall diet by weight. Diets can change with age for a given species.

Fish species	Age (days)	Min Fraction	Max Fraction
Shiner	1	0.15	0.4
Shiner	1945	0.15	0.4
RB Sunfish	1	0.15	0.4
RB Sunfish	2675	0.15	0.4
Smallmouth Bass	1	0.15	0.4
Smallmouth Bass	3650	0.15	0.4

Table 2. Prey size range preferences relative to predator length used in FweB4 and D-MCM simulation comparison for food web in South River, VA

Values used for selected model inputs related to MeHg fluxes in fish are given in **Table 3**.

Parameter	Shiner	RB Sunfish	Smallmouth Bass
Uptake efficiency food (fraction)	0.75	0.75	0.75
Uptake efficiency water (fraction)	0.36	0.36	0.36
Clearance multiplier (dimensionless)	1.9	0.08	1.8
Concentration of MeHg in spawning material relative to spawning adults (whole body)	0.01	0.01	0.01

Table 3. Selected parameter values related to MeHg dynamics in fish, used in FweB4 and D-MCM comparison for food web in South River, VA

Two scenario variations were tested with FweB4. The first scenario set the target weight for each fish species to match D-MCM at a given fish age (**Table 4**) and the default FweB4 bioenergetic parameters were applied.

Species	Age (days)	Target Weight (g)
Shiner	1824	34.8
RB Sunfish	2554	73
Smallmouth Bass	2188	620

Table 4. Target ages and weights used for calculating FweB4 P-values to match D-MCM results

While the simulated fish weights matched on one date, weights on other dates did not necessarily match (red vs blue lines in growth plots in Figure 26). This was not surprising given that the two models use different functions for growth. For example, fish can lose weight in FweB4 but in D-MCM fish growth is always positive. For Common Shiner, simulated growth and MeHg concentrations were similar overall in FweB4 and D-MCM (Figure 26). For Redbreast Sunfish, FweB4 growth was slower at first, then faster (to reach the target weight), while simulated MeHg concentrations were comparable. For Smallmouth Bass, growth was slightly faster in FweB4 and MeHg concentrations were similar but slightly greater than in D-MCM.

For the 2nd scenario, two FweB4 parameters (CB and RB) were adjusted for Redbreast Sunfish (Table 5). By modifying these two parameters, which control how specific consumption (CB) and respiration (RB) rates depend on fish weight, the growth trajectory for the Sunfish simulated in D-MCM could be more closely matched.

Scenario	Model Parameter	
	CB	RB
FweB4 default	-0.274	-0.2
Modified	-0.36	-0.21

Table 5. Modifications to FweB4 parameters to match D-MCM growth for Redbreast Sunfish

For Smallmouth Bass the final weight assigned to the p -fitting algorithm was decreased from 620 g to 603 g to better match the simulated D-MCM growth for Smallmouth Bass. No adjustments to the bio-energetic parameters were required.

The effects on MeHg concentrations are shown in **Figure 26**. Redbreast Sunfish MeHg concentrations were similar for the two models until approximately age 4 years, after which FweB4 concentrations were higher than in D-MCM. This may have been related to slower growth in later years for Sunfish in Scenario 2 than Scenario 1. Smallmouth Bass MeHg concentrations in FweB4 were remained quite similar to Scenario 1 and D-MCM.

Overall these results show similar results for FweB4 and D-MCM if fish are exposed to similar levels of MeHg in water and food. These results also showed a tendency noted in earlier tests for growth rates in some fish species to appear slow at first, then fast to catch up to the desired target weight for an adult. Further attention is needed on this issue.

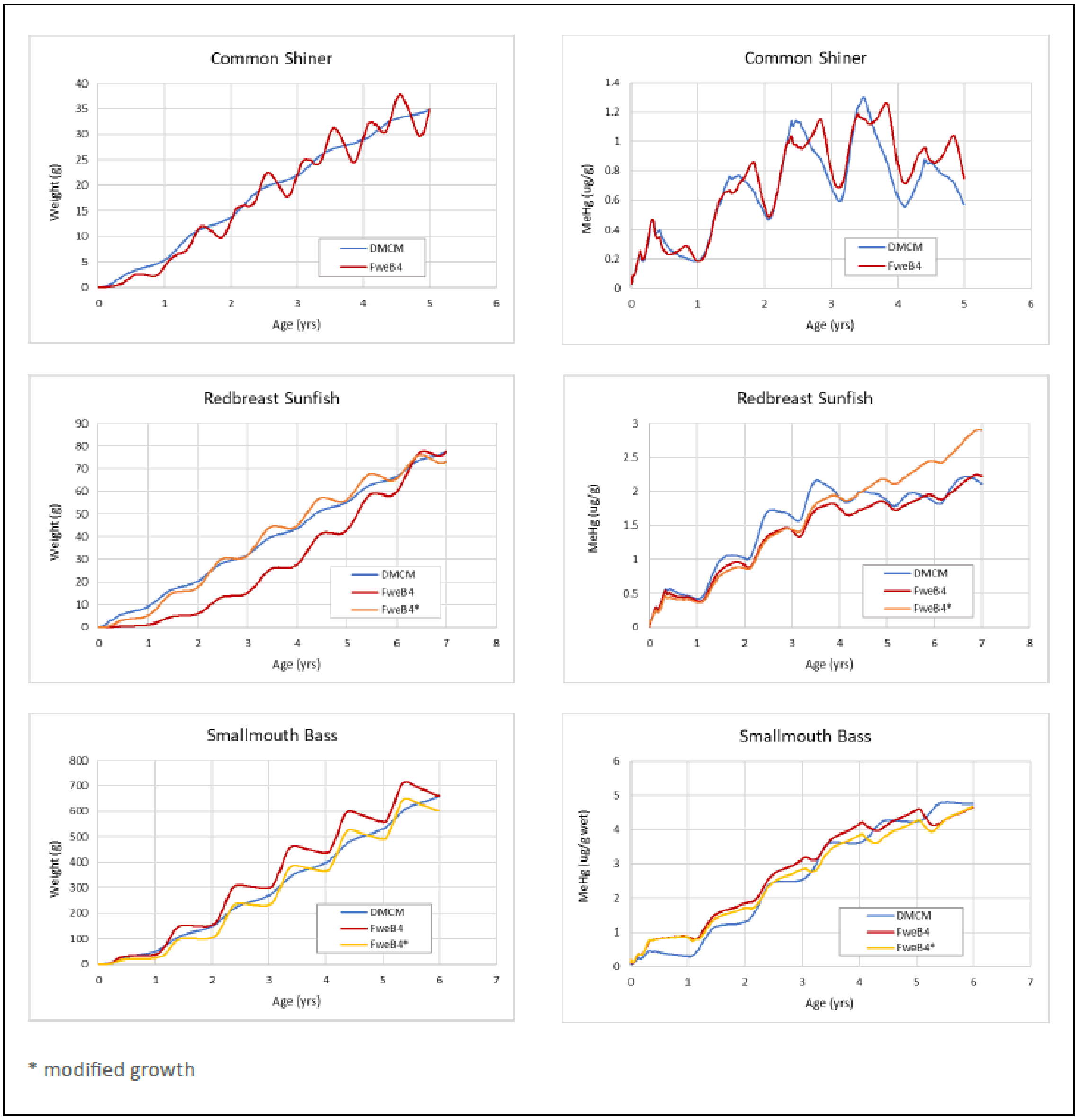

Figure 26. Comparison of simulated growth and MeHg concentrations in Common Shiner, Redbreast Sunfish and Smallmouth Bass in the South River, VA (11.5 miles downstream from original point of mercury release)

APPENDIX B:
MODEL PARAMETER DESCRIPTIONS

Table 6. FweB4 Model Parameter Descriptions

Location Sheet	Parameter	Category	Origin	Description
Scenario_Info	Project_name	Generic	New for FweB4	Name of the project. This name is used to label the output files.
	Location_name	Generic	New for FweB4	Name of the location for the scenario. This is used to label the output files.
	Scenario_name	Generic	FB4	Name of the scenario being run. This is used to label the output files.
	Last_day	Generic	FB4	Final day of the model. Can be any number.
	calc.spawn	Generic	FB4	TRUE or FALSE, should spawning be allowed? If FALSE, then cohorts are not allowed to spawn and no new cohorts start.
	single.cohort	Generic	New for FweB4	TRUE or FALSE, should the model be run with just a single cohort for each species? This is most useful for comparing FweB4 with FB4.
Species_Info	Nspecies	Generic	FB4	Number of species in the model, sequential from 1,2,3, … etc.
	Species_txt	Generic	FB4	The name of each species/model group from 'Parameters_official.csv'. It is critical that this matches the name in Parameters_official exactly.
	p.value	Bioenergetics	FB4	The p value is the proportion of the species-specific maximum consumption that each species is allowed to consume. If a p value is provided then the model will use this rather than fitting the model to weight data. Generally it will be preferable to fit the model to weight data and leave the p.value blank.
	age.at.fit	Bioenergetics	New for FweB4	The age (in days) at which each species should obtain the weight specified in 'fit.weight'. In FB4, this age must always be the final day of the model but in FweB4 it is possible to use any age. If a p.value is provided for a species, then this value is ignored.

Location Sheet	Parameter	Category	Origin	Description
	fit.weight	Bioenergetics	FB4	Weight (in g ww) that each species should obtain at the age specified by 'age.at.fit'. If a p.value is provided for a species, then this value is ignored.
	Standardized.length.cm	Bioenergetics	New for FweB4	The length (in cm) for which results should be reported for a 'standard' fish. This parameter is optional, but if provided allows the model to report results for a fish the specified size for each day of the model. If provided this value should be an integer.
Species_Info (Cont')	Oxycal	Bioenergetics	FB4	Oxycalorific Coeficient (J / g O2) is used to convert respiration (g 02 / g fish / day) into units of energy (J / g fish / day). 13560 is the default, but may vary by fish diet.
	Init_pred_conc	Contaminant	FB4	This is the initial contaminant concentration (ug/g ww). In FB4 this was a single value, but in FweB4 must be provided for each species and initial cohort. See 'Cohort_Contam_Info' for specifying these values in FweB4.
	contam_eq	Contaminant	FB4	The contaminant accumulation equation to be used. Options include 1 (FB4), 2 (FB4), or 3 (new in FweB4).
	Muscle.body.ratio	Contaminant	New for FweB4	The ratio of contaminant in fish muscle relative to the whole body average (unitless). The model uses whole body concentrations throughout, so this values is used to calculate contaminant concentration results for muscle tissue at the end of the model run.
	Aq_MeHg_uptake	Contaminant	New for FweB4	The fraction of MeHg in water that is taken up across the gills (unitless). This value is only used if 'contam_eq' 3 is selected.
	Contam.clearance.mult	Contaminant	New for FweB4	Multiplier of contaminant clearance rate (unitless). This value is only used if 'contam_eq' 3 is selected. A value of 1 has no effect, while smaller values decrease and larger values increase the rate of contaminant clearance.
	age.at.death	Bioenergetics	New for FweB4	Age (in days) at which fish cohorts should be removed from model.

Location Sheet	Parameter	Category	Origin	Description
	start.weight	Bioenergetics	FB4	Weight (in g ww) for a newly spawned fish of each species. In FB4, this was a single value indicating the starting weight on day 1 of the model. In FweB4, this is now the weight for a fish of age 1 day, which could occur on any day of the model.
	spawn.day	Bioenergetics	FB4	Day of the year (Julian calendar, 1-365) at which spawning occurs for each species. Day 1 is the first day of the model run.
	min.spawn.weight	Bioenergetics	New for FweB4	Minimum weight (g ww) required for spawning to occur for each species. Fish below this weight will not spawn.
	spawn.amount	Bioenergetics	FB4	Proportion of mass lost during spawning (unitless) for each species.
Species_Info (Cont')	spawn.cont.ratio	Contaminant	New for FweB4	Ratio of contaminant burden (ug) in spawning material to the whole body concentration. A value of 1 will result in whole body concentration staying the same during spawning, while a value <1 will increase whole body concentration and a value >1 will decrease whole body concentration.
	length.alpha	Bioenergetics	New for FweB4	Alpha coefficient for calculating fish length based on weight. The length:weight relationship is defined using the following equation W = aL^b
	length.beta	Bioenergetics	New for FweB4	Beta coefficient for calculating fish length based on weight. The length:weight relationship is defined using the following equation W = aL^b
Cohort_Contam_Info	Cohort 1 Initial contaminant concentration (ug/g)	Contaminant	New for FweB4	The contaminant concentration (ug/g ww) for Cohort 1 of each species on the first day of the model.
	Cohort 2 Initial contaminant concentration (ug/g)	Contaminant	New for FweB4	The contaminant concentration (ug/g ww) for Cohort 2 of each species on the first day of the model.

Location Sheet	Parameter	Category	Origin	Description
	Cohort N ….	Contaminant	New for FweB4	The number of starting cohorts for each species depends on the specified 'age.at.death' and the 'spawn.day'. The number of cohorts is calculated and provided in the input sheet. An initial starting concentration needs to be provided for all initial cohorts. If the units for the data do not match the units expected by the model (ug/g ww whole body) then it is possible to do unit conversions using the 'Contam Pre-Processing' tab.
Environmental_vars	day	Bioenergetics	FB4	Day for which environmental variables are provided. This series should span from 'First_day' to 'Last_day' of the model. Values can be given for each day, or if there are gaps (e.g., 1,30,60,90,…) values are interpolated by the model.
	temperature	Bioenergetics	FB4	Temperature (in Celsius) for the corresponding 'day'. Values can be given for each day, or if there are gaps (e.g., 1,30,60,90,…) values are interpolated by the model.
	DO	Contaminant	New for FweB4	Dissolved Oxygen (in mg/L) for the corresponding 'day'. These values are only used if 'contam.eq' is set to 3 and uptake across the gills is considered. Values can be given for each day, or if there are gaps (e.g., 1,30,60,90,…) values are interpolated by the model.
Diet_matrix	Diet Matrix (by model group)	Bioenergetics	FB4	Diet composition matrix specifies the diet proportions (by wet weight) for each predator over time. Rows should sum to 1. Predators are specified in the rows, while prey items are columns. Diet composition is allowed to vary by age for each predator. The age range entered should span the lifespan of each species (1-'age.at.death'), and can additionally change at user specified ages. Values can be given for each age, or if there are gaps (e.g., 1,365,1560,1825) values are interpolated by the model.

Location Sheet	Parameter	Category	Origin	Description
Diet_size_matrix	Diet Size Matrix (by model group)	Bioenergetics	New for FweB4	The 'Diet_size_matrix' specifies the minimum and maximum sized fish a predator species is able to consume. The minimum and maximum sizes are given as a proportion of the predator size (i.e., a minimum value of 0.05 indicates that fish must be at least 5% of the predators length to be consumed), and are given in 'min_size' and 'max_size', respectively. As with the 'Diet_matrix', the size range can vary by predator age as specified in the 'age' column. Values can be given for each age, or if there are gaps (e.g., 1,365,1560,1825) values are interpolated by the model.
Prey_Info	Energy_Density (by model group)	Bioenergetics	FB4	Energy density (J/g ww) for each model group. For some fish species, underlying bioenergetic parameters dictate energy density independent to the values specified here, in which case these values will be ignored. In FB4 this value was allowed to vary over time, but here the value is kept constant for each model group.
	Indigestible_Fraction (by model group)	Bioenergetics	FB4	Proportion of prey items that are indigestible. These values are only used for species where the egestion equation is 3 (this is set internally by the model based on bioenergetic parameters).
	Contam_Transfer (by model group)	Contaminant	FB4	Contaminant transfer efficiencies (unitless) for each prey item. These values are only used if 'contam.eq' is set to 1.
	Contam_Assimilation (by model group)	Contaminant	FB4	Contaminant assimilation efficiencies (unitless) for each prey item. These values are only used if 'contam.eq' is set to 2 or 3.
Contam_Pre-Processing	Active	Contaminant	New for FweB4	This sheet allows the user to calculate contaminant concentrations in model compartments if they are not available directly (e.g., calculate benthic invertebrate concentration from sediment; zooplankton from water). This parameter can be TRUE or FALSE, and indicates whether this conversion should be used (TRUE) or not (FALSE).

Location Sheet	Parameter	Category	Origin	Description
	Start.compartment	Contaminant	New for FweB4	Indicates the model compartment (often water or sediment) that should be used to calculate concentrations in the end group. Units are as described in 'Contam_conc' - ng / L for water and ng / g dry for sediment.
	End.compartment	Contaminant	New for FweB4	The model compartment for which concentrations should be calculated, and should be one of the non-fish model groups (e.g., benthos, zooplankton). Units are as described in 'Contam_conc' - ng / g dry for non-fish model groups.
	Conversion.factor	Contaminant	New for FweB4	The conversion factor used to calculate the concentration in the 'End.compartment' from the 'Start.compartment' as: End.compartment = Start.compartment * Conversion.factor Units for this value depend on the model compartments in question, but are likely L/g if start.compartment is water or g/g if start compartment is sediment.
Contam_conc	Contaminant Concentration (by model group)	Contaminant	FB4	This specifies the contaminant concentration over time for each model compartment and group. The 'Day' series should span from 'First_day' to 'Last_day' of the model. Values can be given for each day, or if there are gaps (e.g., 1,30,60,90,…) values are interpolated by the model. Water concentration (ng/L) is only needed for 'contam.eq' 3 or if required by 'Contam_Pre-Processing', while sediment concentration (ng/g dry) is only needed if required by 'Contam_Pre-Processing'. For lower trophic level model groups (e.g., benthos) concentrations must be in ng/g dry. For fish species (ug/g wet), concentrations are not needed as they will be calculated by the model.

Location Sheet	Parameter	Category	Origin	Description
Dry_wet_conversion	Dry weight to wet weight conversion (by model group)	Contaminant	New for FweB4	Dry to wet weight ratio for non-fish model groups. These values are used to convert the dry weight inputs to the wet weight equivalents used in the model. Values are required for all non-fish model groups and should be in units of g dry / g wet.