



# **AQUATOX**

## **MODELING ENVIRONMENTAL FATE AND ECOLOGICAL EFFECTS IN AQUATIC ECOSYSTEMS**

### **Guidance in AQUATOX Setup and Application**

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## Introduction

AQUATOX is a simulation model for aquatic ecosystems. AQUATOX predicts the fate of various pollutants, such as nutrients and organic chemicals, and their effects on the ecosystem, including fish, invertebrates, and aquatic plants.

This document is intended to be a “quick-start” guide to introduce major model features as well as being a type of “cookbook” to guide basic model setup, calibration and validation. It is designed to supplement the [AQUATOX Users’ Manual](#) and [Technical Documentation](#) which are frequently referenced within this document. This guidance document primarily pertains to Release 3.1 of the AQUATOX model, produced and supported by the [U.S. Environmental Protection Agency](#).

## Model Installation

AQUATOX is fairly easy to install on a Windows system (Windows XP or subsequent releases), however, administrator privileges are generally required for installation. To get started, download the [AQUATOX installer](#) from the EPA or other website.

For more information on model installation, please see the [AQUATOX Release 3.1 Installation guide](#).

## Very First Steps

Once the model has installed and is running, you may start to explore the features of the model. AQUATOX is a multiple-document interface in that you can load many different simulations and they will appear on the AQUATOX desktop under the main window.

To get started, we recommend working with the “Simple Tutorial” that is available by clicking on “Help, Tutorial” from the main menus (at the top of the AQUATOX screen after startup), or can also be found in the [AQUATOX Users’ Manual](#). This tutorial will guide you through adding and deleting a state variable, setting initial conditions, viewing parameters, running a simulation, and viewing output. There is a more advanced “stream tutorial” also available in these documents.

## Understanding Simulation Modeling

An AQUATOX model is primarily composed of the following components

- “state variables”—components of the modeling system, such as animal biomasses, in which masses or concentrations are modeled and tracked;
- “driving variables”—time series inputs, such as temperature, that are not changed by the model;

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- “parameters”—constant model inputs, such as “maximum photosynthetic rate,” that are used to calculate the modeled state variables;
- “boundary conditions”—information about state variables from outside the model domain, such as upstream loadings, or point-source loadings;
- “physical characteristics”—constant model parameters or time series such as “mean depth” that describe the site being modeled.

As a further introduction to the model, we recommend reading the section 1.1 “overview of the AQUATOX model” in the [Technical Documentation](#), along with section 1.8 regarding the intended application of the model.

AQUATOX can be run as a point model, a stratified model (with seasonally varying epilimnion and hypolimnion layers), and as a two- or three-dimensional model with linked segments. The spatial resolution depends on the modeling goal. However, given the trade-off between ecological realism and spatial resolution, the model domain is usually spatially aggregated, in contrast to hydrodynamic models.

When embarking on a modeling project, it is important to understand the assumptions regarding physical model setup and spatial and temporal resolution. Many of the basic points regarding the AQUATOX model construction can be found in Chapter 2 of the [Technical Documentation](#). That could be a good section to read before running AQUATOX.

Other resources include “What is AQUATOX,” lecture materials that start on page 3 of the AQUATOX one-day [web training materials](#) followed by the discussion of analytical capabilities.

### Common Problems with Simulation Setup

Many users decide to start a new simulation “from scratch” with all parameters zeroed out. This is probably seen as a “safe” way to start because this method ensures that all parameters will then reflect what is appropriate for their site, as opposed to some other location. However, when we have worked with studies created in this manner, we have found many parameters left as blank or “zero” as a default. This will not result in a successful ecological simulation. For this reason, we generally recommend starting with a surrogate simulation and modifying it to match your site’s characteristics. This also has the benefit of working with a calibrated parameter set as opposed to having to perform all model calibration yourself. For more information about choosing a surrogate site, see the section on *Working with Example Simulations*, which is later in this document.

The first consideration when setting up an AQUATOX model is defining the physical characteristics for your site properly. The majority of errant simulations that we have

seen have problems with water volume, water depth, or water velocity.

**Water Volume:** There are many options to model water volume, so the user must be careful in setting up this component of the model. The full set of water volume options can be found in the section on “Water Volume Data” in the [AQUATOX Users’ Manual](#). The step that users tend to neglect is graphing water volume after specifying their choices and running the model. This is an important check that the parameters that were imported into the model have the correct units and are being properly accounted for by the model. To examine the water volume output, you should click on the “output” button, then select “New” under Graph Library, then under “other” graphs, select “water volume.” You can then look at the water volume results for each segment as well as an accounting of inflow and outflow of water to that segment. Many early implementations of models have water volumes near zero or unreasonably large due to misspecification of inputs. This error is such a common problem that a technical note has been written on the subject and is available on the EPA website: [Modeling Water Flows with AQUATOX Release 3.1](#).

Specifying water volume in a linked-segment model is complicated and requires a full external accounting of the water balance for the entire linked system. (AQUATOX is not a hydrodynamic model so it does not account for the effect of river slope on water volume, for example. The large cell sizes of AQUATOX preclude hydrodynamics from being added to the model, but aggregation of data from smaller-cell-size hydrodynamic models is often utilized.) If a linked-segment model takes too long to run and causes problems debugging the water volume setup, a useful trick is to delete all biotic state variables and then to iron out the water volume setup with the faster linked model. The debugged water volume setup can then be imported into the slower full model once all segments have appropriate water volumes calculated.

**Water Depth:** There are three options for modeling mean-water depth at your site. This physical characteristic has an important effect on light climate, especially for periphyton that reside at the bottom of the water column. As a first option, in the site “underlying data” the “use bathymetry” option should be turned off, in which case mean depth is calculated in a time-varying manner as volume over surface area. In this case, the surface area does not change as volume increases, meaning this option has limited utility for some sites. If mean water depth does not change much, it can be entered in the underlying site data and kept as a constant. However, if mean depth is dynamic then it is useful to determine a time-series of mean-depth data using a hydrodynamic model or an external relationship of water volume to mean depths. This can then be imported into the model by clicking the “Site” button and then “Show Mean Depth / Evaporation” at the bottom of the window, and then importing a dynamic mean depth time series.

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As with water volume, whenever model input is changed, it is useful to look at a graph of the output to ensure that the expected model output has been produced. In this case, select :”new” graph, select then “custom” graph, and then add “ZMean (Dynamic (m))” to one of the axes.

**Water Velocity:** Water velocity may also be imported or calculated by the model. By clicking on the site button, a “Water Velocity” time series is visible along with the option to have AQUATOX calculate water velocity or to import water velocity for the site. Whichever option is selected, outputs for water velocity should also be graphed and inspected. Water velocity has important implications for the scouring of periphyton, breakage of macrophytes, oxygen reaeration, and scour of bottom sediments.

**Light:** Now that some critical variables in the physical setup of the site have been entered and double-checked, a proper accounting of light climate is required. Primary producers cannot function if light boundary conditions and water clarity are not within the bounds of reason. Light boundary conditions are available by clicking “light” in the AQUATOX state-variable list and are generally set using the annual mean and annual range (“Average Light” and “Annual Light Range”) parameters in the site’s “underlying data” record. Light penetration in the water column is a function of suspended inorganic matter (usually TSS), organic matter, and algal growth. To double-check the light climate in your model, graph the “secchi depth” output from your model. If this value is small then light cannot penetrate deeply enough to enable algal growth. The “secchi depth” time-series can also be plotted against observed data from your site, if available. See “Importing Observed Data” in the [AQUATOX Users’ Manual](#) for more information about plotting observed data against model results.

**Organic Matter:** Organic matter in the water column cannot be ignored in the model for many reasons. Some important examples are its role as a food source for invertebrates, its effect on water-column light extinction, and its effect on biochemical oxygen demand. To specify initial conditions and boundary conditions for organic matter, click on “Susp. and dissolved detritus” in the state-variables list associated with every simulation. Inputs in terms of “organic matter,” “organic carbon,” or “BOD” can also be specified as percentage breakdowns into faster-reacting labile and slower-reacting refractory components. More information on setting these parameters is available in section 5.1 (especially Table 10) of the [Technical Documentation](#).

**Food Web:** One more common mistake made when setting up the model is to forget to specify linkages in the model’s food web. A “food web” button is available on the main simulation’s screen along with a help button at the bottom to assist in setting up the trophic interactions (feeding preferences and egestion coefficients) appropriate for your ecosystem.

### Working with Example Simulations

AQUATOX 3.1 is delivered with 39 example studies (.aps or .als files) that include single-segment models, multi-segment models, and also include rivers, streams, ponds, lakes, reservoirs, and estuaries. A full list of these studies is provided in a color-coded table in the appendix at the end of this document.

As mentioned above, the best way to apply AQUATOX to your particular site is usually to select a “surrogate” site from the library of simulations that most closely matches your simulation and then modify the physical setup, biota, nutrients, and organic matter parameters to match your site.

A complete example of how to modify a surrogate site to match the characteristics of your own may be found in Day 1 (especially Labs 2 and 3) of the [web-training materials](#), which were developed for a three day course on AQUATOX. These exercises (study files and loadings files included) guide the user through modifications of a site’s physical setup, organic matter, inorganic matter, nutrient specifications in the water column, and selection of biota.

AQUATOX is also has a “wizard” interface that allows you to step through many of the most important parts of model setup. The parameters shown in the wizard are the same as those accessed through the state variables lists, loadings screens, and underlying data in AQUATOX’s primary interface, but the organization is different. There are 19 primary steps that the AQUATOX wizard uses sequentially or the user can select a step from the progress window (Figure 1). The wizard doesn’t include all of the parameters and flexibility of the primary AQUATOX interface, but it provides a user-friendly interface to work with the model, especially in adding and removing plant and animal state variables.

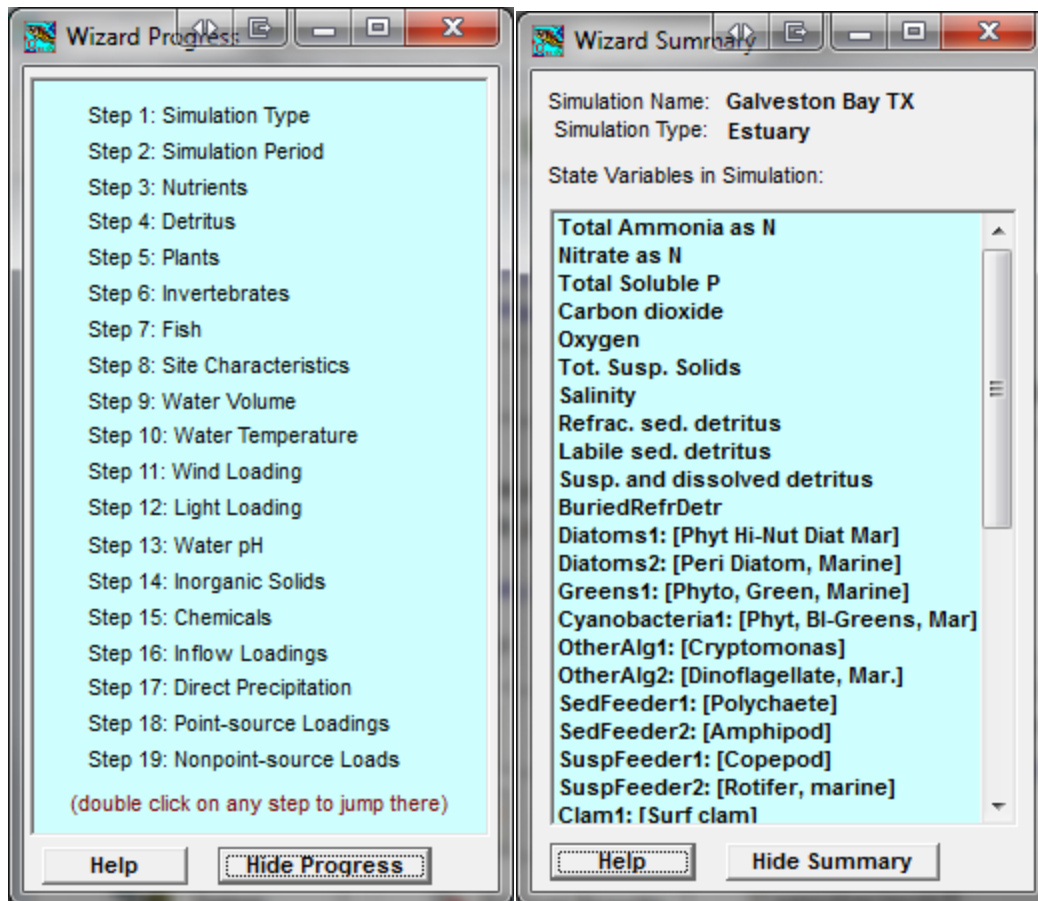


Figure 1. AQUATOX Wizard Progress and Wizard Summary screens

## Changing Site Characteristics to Match Your Site

When modifying a surrogate site to match your site, you should generally consider the following groups of variables:

- **Dates of simulation** in the “setup” window. The period of your simulation depends on what data sets are available for calibration and verification and what your needs are for model projections.
- **Site characteristics** in the site “underlying data” window (under the “Site” button). Initial focus should be on length, surface area, depths, temperature ranges, latitude, and average-light variables. Time-series of site characteristics such as water velocities and mean depths can also be found in the site-type window under the “Site” button.
- **Water volume setup** can be found by double-clicking on “water volume” towards the end of the state-variables list. See the discussion of modeling water volume in the section above on common problems above.



- **Water temperature setup** is found by double-clicking on “temperature” towards the end of the state-variables list. If you set the temperature ranges in the site characteristics screen properly you can select to “use annual mean and range”; otherwise you can use a constant or a time series to model water temperature.
- **Nutrient and inorganic matter loadings** and initial conditions may be found by double-clicking on each of these state variables at the top of the list of state and driving variables. Depending on your available data, inputs of phosphate and nitrate can be loaded as TN or TP using the “inflows are Tot. N” (or “Tot. P”) checkboxes located on these screens. Point source, non-point source and direct precipitation can also be separately specified. Total suspended solids may be input as a driving variable and this can have a significant effect on light climate.
- **Organic matter loadings** are available under “suspended and dissolved detritus.” See the discussion of modeling organic matter in the section on common problems above.

All of the above variables may be viewed in the simulation output window after running a preliminary simulation. Some examples are the “Nutrients,” “Detritus,” “Temperature,” and “Water Volume” graphs that can be selected after opting to create a “New” graph for the graph library. Custom graphs can also be produced for site mean depths and water velocities, for example. (For more information on creating and editing graphs, including a full list of the types in AQUATOX, see page 45 of the [AQUATOX Users' Manual](#).) Whenever a model input has been modified, it is important to double-check its effects on relevant model outputs before assuming that a variable has been properly set up. This can quickly solve potential problems with units or check-boxes not set correctly.

Animal and plant state variables are discussed in the next section of this document.

### Choosing Which State Variables Should Be Included

AQUATOX is distributed with an abundance of state variables to facilitate application to many different situations. The analyst should not feel compelled to use all the biotic groups, and in fact, that is probably a bad idea! Depending on the goal, many groups can be removed from a simulation, making it faster to run, simpler to calibrate, and easier to defend.

Adding and removing state variables is often easiest through the AQUATOX wizard interface which significantly facilitates the adding of size-class fish and selection of appropriate animals depending on plant, fish, or invertebrate type (see Figure 2).

Some examples of simple vs. more complex AQUATOX simulations may be found on pages 18-19 of the AQUATOX one-day [web training materials](#). Before selecting the food-web conceptual model for your site, it might also be worth reading the lectures on

modeling plants (p52), modeling animals (p80), and modeling chemicals (p115) of those same [web training materials](#).

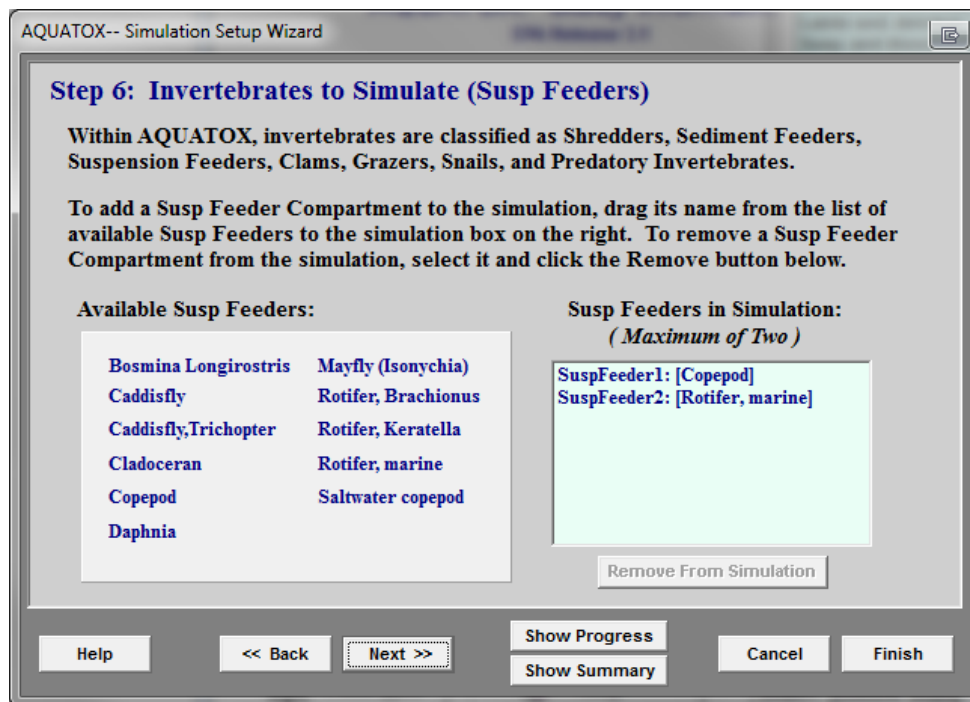


Figure 2. Selection of suspension feeders from AQUATOX database

Parameters are often available for individual species, but in food-web modeling, a species is usually representative of a larger group of species with similar life histories. Circumstances under which you would choose to model specific organisms with explicit attention to parameter values include:

- Commercially important species (salmon, oysters)
- Invasive species (*Cylindrospermopsis* cyanobacteria, *Hydrilla*, zebra mussels)
- Keystone species (Pacific salmon, gizzard shad)
- Species near the top of the food web (lake trout, largemouth bass).

Simpler food webs will be more subject to disruption from losses or gains in biomass of one component of that food web. For example, a simple food-chain model would be devastated by the loss of its single primary producer whereas a complex food web might continue to function fairly well if that same primary producer were lost as a result of prey switching and opportunistic feeding. Our [comprehensive sensitivity analysis](#) of AQUATOX found that the model is sensitive to food-web construction:

“Simpler food-web models are more sensitive to effects from food-web interactions. For example a food web with five zoobenthos categories is less sensitive to perturbations in a single zoobenthos parameter than a food web in which all zoobenthos are represented by a single category.”

### Working with Boundary Conditions

“Boundary conditions” are state variables from outside of the spatial domain of a modeled system, and the loss of state variables to a region beyond the spatial domain. These are especially important to properly characterize in rivers or other systems with low retention times. For example, concentrations of nutrients in a system with a water retention time of less than one hour will largely reflect the loadings without modification. There simply is not enough time for the nutrients to react within the system to noticeably change their concentration in the water column. For this reason, the “retention time” of the modeled system is another output variable that should be plotted and considered.

In standing-water systems with longer retention times, initial conditions become much more important and boundary conditions have a smaller, more subtle effect on overall model predictions.

Because phytoplankton and zooplankton wash out of stand-alone stream segments rapidly, a special assumption has been put into place to handle plankton retention times. In this case, AQUATOX takes into account the “Total Length” of the river being simulated, as opposed to the length of the river segment or reach, so that phytoplankton and zooplankton production upstream can be estimated. The “Total Length” parameter can be directly entered at the bottom of the “Site Data” screen or estimated from the watershed area in that same location. This parameter essentially slows down the residence time for phytoplankton and zooplankton to account for up-stream production and allows more reasonable in-stream predictions to be produced. For more information about this feature, see the “Phytoplankton and Zooplankton Residence Time” section in Chapter 4 of the [Technical Documentation](#).

In a multi-segment system such as a river with multiple reaches or a complex reservoir, tributaries are important sources of water, nutrients, organic matter, and toxicants. There could be point-source inputs, such as effluent from wastewater treatment plants, and nonpoint-source inputs, like direct runoff and groundwater input. All of these sources may be separately characterized using a structure called “tributary input segments.” See [Tributary Input Segments](#) in the [Users’ Manual](#) file for more information on how to set up and utilize these model structures.

## Model Calibration and Validation

### Order of Model Calibration

In general one should work with the portions of the model domain least affected by other categories you will be calibrating in the future. Ordinarily the sequence would be:

- Boundary conditions including nutrient loadings;
- Plants;
- Animals; and,
- Toxicants.

### Which parameters are likely to be most important?

The [Sensitivity Analysis report](#) provides many insights into the importance of various parameters. As presented in the report's summary:

- Biotic state variables are sensitive to temperature parameters.
- Consumption and respiration parameters are sensitive, especially when allometric formulations are used for fish.
- Algae are sensitive to their maximum photosynthesis rate ( $P_{max}$ ).
- Simpler food-web models are more sensitive to effects from food-web interactions due to lack of alternative prey sources.
- Periphyton biomass is quite sensitive to sloughing parameters such as “percent lost in slough event.”
- Log octanol-water partition coefficient ( $K_{ow}$ ) is a highly sensitive parameter for toxicant fate and effect.

### Generality

Models cannot be realistic, general, and precise at the same time. However, for many applications, models *should* be both realistic and general, and precision is not necessary or even attainable (Park and Collins 1982). Generality is especially important because models are usually used to predict responses under changing conditions. Mechanistic constructs can extend the applicability beyond the immediate domain—unlike empirical models that are constrained by the limits of observed data (DeAngelis and Mooij 2003). AQUATOX can be set up so that multiple sites, linked only by a common parameter set, can be calibrated simultaneously. An example is the Minnesota Rivers study in which rivers with low-, moderate-, and enriched-nutrients and turbidity were calibrated together. In another study, the model was calibrated across diverse reaches of the 60-mile long Lower Boise River in Idaho. Because of this generality the periphyton parameter set can be easily applied to represent periphyton in other wadeable rivers with little additional calibration.

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However, there are limits to generality. If the expectation of goodness of fit is too demanding (see next section) then site-specific calibration may be required, keeping in mind the tradeoff between precision and applicability to changing conditions. Perhaps more important is the fact that not all processes are represented by mechanistic constructs. For instance, calibration may be required to capture the responses of phytoplankton to the fine-scale hydrodynamics that are not represented by AQUATOX.

### Acceptance Criteria

A weight-of-evidence approach is usually recommended in terms of deciding whether simulated results are acceptable and defensible. From simple to more complex lines of reasoning, some of the following lines of evidence may be used:

- Reasonable behavior based on general experience;
- Visual inspection of data points and model plots;
- Do model curves fall within error bands of data?
- Do point observations fall within model bounds obtained through uncertainty analysis?
- Regression of paired data and model results—is there concordance or bias?
- Comparison of mean data and mean model results;
- Comparison of frequency distributions;
  - Relative bias;
  - F test; and,
  - Kolmogorov-Smirnov test.

For more information on measures of model performance please see section 2.6 of the [Technical Documentation](#)

### Possible Model Applications

AQUATOX is a comprehensive but flexible ecosystem model, and has an almost unlimited variety of potential applications. In this section we highlight some of the types of model applications that we have worked with or are aware of. A good starting point for investigating AQUATOX applications would be the [AQUATOX bibliography](#) and the [Annotated Bibliography for AQUATOX](#) on the EPA website. An additional source of information regarding model applications may be found in the “potential applications” lecture starting on page 6 in the AQUATOX one-day [web-training material](#). In the discussion below, relevant example studies included with AQUATOX 3.1 are highlighted in grey; more information about these studies can be found in the color-coded appendix at the end of this document.

### Screening-level Model

Perhaps the simplest application of AQUATOX is as a screening-level model, applied without calibration where relative, not absolute, differences are of interest. For example, AQUATOX was highlighted in a recent Water Environment Research Foundation report on using models to develop site-specific nutrient goals (Bowers and Bell 2013). In this case study, AQUATOX was applied without calibration to four streams in Virginia. Two streams had greater periphytic biomass and were dominated by green algae, and two had lower biomass and were dominated by diatoms.

This application of AQUATOX was:

- Capable of distinguishing between “higher biomass” and “lower biomass” sites.
- Capable of predicting when filamentous greens would predominate

The default parameters were taken from (Park et al. 2009). Changing the critical force ( $FCrit$ ) values for scouring periphyton probably would have provided the biggest adjustment.

### Total Maximum Daily Load

At press time, AQUATOX was in the draft stages of being utilized as part of a TMDL project on the Lower Boise River (LBR), Idaho. The model had previously been applied to the LBR, so that study file was used as a starting point (see Lower Boise R. ID Seg\_1-3.als). The goal of the model application was to estimate the necessary reductions in nutrient loads to the river in order to meet state water quality standards. (The TMDL, when completed, is subject to review and approval by EPA.) The Idaho water quality standards include the following applicable requirement:

- **“Excess Nutrients.** Surface waters of the state shall be free from excess nutrients that can cause visible slime growths or other nuisance aquatic growths impairing designated beneficial uses.”

Because of the emphasis on nuisance algal endpoints, but not higher organisms, while also trying to minimize overparameterization of the model relative to the available data, the modeling team felt that the food web included in the model could be simplified. All invertebrate and fish state variables were removed, and the only algal groups used were those necessary to obtain an acceptable fit to the chlorophyll *a* observations.

### Site-specific Water Quality Criteria

Using a process-based model such as AQUATOX can help to provide a mechanistic link between nutrients and algal responses. This can be used in conjunction with other efforts and approaches to develop nutrient targets. For example, AQUATOX could be used to evaluate which factor or factors are controlling algae levels or to evaluate the

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effects of agricultural practices or land-use changes on chlorophyll a concentrations. Some examples of recent use follow:

- Short course Day 2 demonstration: “Modeling Nutrients for Criteria Support in Tenkiller Lake, OK” (Tenkiller Ferry Lake OK.als). For more information, see page 41 of the second-day lectures in the AQUATOX three-day [web-training materials](#). Please note, when accessing these lecture materials you can look at the slides only or you can also view slides with detailed notes.
- AQUATOX is being used at the present time to develop nutrient standards for Nevada (Smith and Fritsen 2011, Smith et al., In press). This model application remains under development and is subject to review and approval by all applicable agencies.

### Thermal Pollution and Climate Change

AQUATOX has realistic temperature responses that include limited adaptation. Therefore, boundary conditions for temperature can be varied to represent a variety of climate scenarios. In particular, the impacts of climate change on Lake Onondaga, New York, were forecast using AQUATOX (Taner et al. 2011). The AQUATOX [sensitivity analysis report](#) suggests that AQUATOX biotic state variables are sensitive to temperature parameters both due to direct effects (on metabolism rates, for example) and indirect effects (food-web interactions).

### Invasive Species

AQUATOX can be used to evaluate potential responses to invasive species and to evaluate various mitigation measures. For example, direct and indirect impacts on native species may be simulated. As a teaching example of this type of model application, control of *Hydrilla* in Clear Lake, California, was simulated. For more information, please see Lab 6 in the second-day of the three-day [web-training materials](#).

To model invasive species, an AQUATOX user needs a good idea of why an invasive species is expected to be more successful than native species (faster growth rates or better feeding efficiency, for example) and include those characteristics within the parameter set for that species.

### Risk Assessment of New or Existing Chemical

AQUATOX can be used in risk assessment of bioaccumulation and ecotoxicity of pesticides and industrial chemicals. Examples include:

- Microcosms
  - Replication of aquarium with HCB and macrophytes (Gobas et al. 1991) (HCB Tank.aps)
- Mesocosms

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- Probably one of the best examples is an application in France (Sourisseau et al. 2008) in which the model was successfully calibrated and validated to biomass dynamics of various biological compartments in artificial streams designed for measuring pollutant effects on aquatic communities.
- Estuarine environment
  - Galveston Bay, Texas in which the bioaccumulation of PFOS throughout the estuarine food web was simulated. For more information, please see “Modeling Estuarine Conditions” at the start of the third-day lectures within the three-day [web-training materials](#).

### Impacts of Contaminated Sites on Aquatic Ecosystems

The model has been used to analyze the potential bioaccumulation of, ecotoxicity of, and recovery from chemicals in a variety of ecosystems, including:

- A small stream in Denmark polluted with TCE from a leaking tank (Funder 2009, McKnight et al. 2010a, McKnight et al. 2010b) ([Skensved Denmark TCE.aps](#)). The model predicted limited ecological changes in the aquatic life in the stream as a result of the TCE contamination.
- A small creek in Oregon with chlorpyrifos and legacy dieldrin, ([Zollner Creek OR w chlorpyr dieldrin-pulse.aps](#)).

### Combined Sewer and Stormwater Discharge

“Lake Onondaga is arguably the most polluted lake in the United States.” This excerpt comes from the preface of a Effler’s book (1996) which served as the source of data for this study. The lake has significant nutrient inputs from wastewater treatment plant (“Metro”) and combined sewers, resulting in successive algal blooms, hypoxic hypolimnion, and build-up of organic sediments ([Onondaga Lake NY Sed Diagenesis.aps](#)). An earlier version of AQUATOX was verified using data from Lake Onondaga (see page 1-1 of the [Release-1 Model Validation Reports](#)). The non-calibrated model successfully predicted oxygen and chlorophyll *a* dynamics in this vertically stratified system—further calibration subsequently improved model fit.

### Environmental Impact of Construction

Nearby construction can increase the flux of nutrients and sediment into receiving waters. In a hypothetical example, total suspended sediment (TSS) loadings to the Cahaba River were doubled, increasing the embeddedness and affecting both periphyton and zoobenthos ([Cahaba R AL X2 TSS.aps](#)).



## Common Error Messages

For the most part, AQUATOX error messages are comprehensive explanations that should help you to resolve whatever problem that you are having, but several common error messages have the potential to cause confusion.

- ***AQUATOX is not set up in a correct directory structure! AQUATOX will not function.*** AQUATOX must be set up in a directory structure with the PROGRAM, OUTPUT, DATABASE, and STUDIES directories in their original locations after the installer has been executed. If a new “exe” file is to be used, it must be copied into the PROGRAM directory of an existing installation.
- ***Because LipidFrac differs from that in the Chem Tox records, AQUATOX will update the LipidFrac fields in the chemical toxicity records for all toxicants in the study.*** To allow chemical toxicity records to be edited as a unique database (when not associated with a simulation) chemical toxicity records have their own set of “lipid fraction” data. However, this option can cause a discontinuity in a simulation if an animal’s “underlying data” has a lipid fraction different from that in the chemical toxicity record. This dialog informs the user that it will modify the lipid fraction in the chemical toxicity record to remove this discontinuity. Press cancel to leave all data unchanged.
- ***Because LipidFrac was changed, AQUATOX will update the LipidFrac fields for all toxicants in the study.*** AQUATOX is attempting to reconcile the lipid data in the chemical toxicity record with the lipid in the Animal’s “underlying data,” in this case because the underlying data has changed. Press cancel to leave all data unchanged.
- ***Because Lipid Fraction data may have been changed in the Toxicity Screen, each Lipid Fraction from this chemical's toxicity record will be copied over to the other toxicants in this study. Lipid Frac will change in each relevant (linked) organism's underlying data.*** Again, AQUATOX attempts to equalize the lipid data in the chemical toxicity record with the lipid in the Animal’s “underlying data,” in this case because the chemical toxicity record data has changed. Press cancel to leave all data unchanged.
- ***File version unreadable: File predates Version 1.03, or other error.*** This error is usually shown when a user tries to load a non-AQUATOX file (non .aps or .als file) into the model or drags and drops a non-AQUATOX file onto the AQUATOX desktop.
- ***Key violation. When importing a time-series.*** This error message is produced by the AQUATOX database manager when an imported time series has the same date repeated twice, which is not allowed in the AQUATOX data structures.
- ***No "Partial" runs are permitted when exporting or viewing Linked Results.*** Linked-result output and export relies on all segments being complete and having the same number of data points. When a linked-mode run is stopped by the user part of the way through a simulation, these functions are unavailable. A user can still view partial output for an individual segment by first clicking on that individual segment and then clicking on the “output” button associated with that segment.
- ***Warning, in the control run, variable {variable name} becomes zero or so tiny as to result in infinite differences being calculated. AQUATOX will plot these differences as zero. When plotting “difference” data in the output window.*** The difference graph

shows percent differences in perturbed data in comparison to the control run. If a variable in the control run has a value of zero, this quantity is not calculable (due to division by zero). This dialog warns the user that the differences are sometimes being plotted as “zero” because of this problem.

- **Warning: Periphyton {plant name} is not linked to a phytoplankton compartment. Chlorophyll may be undercounted in a scour event.** Observed chlorophyll *a* data at the simulated river could include the effects of scoured periphyton biomass. To allow for better comparison between observed and simulated time series, periphyton may be linked to a phytoplankton compartment so that simulated chlorophyll *a* will include the effects of periphyton sloughing. See the section on “periphyton-phytoplankton link” in chapter 4 of the [Technical Documentation](#).
- **Warning: Your study has inputs of organic matter in BOD units. AQUATOX 3.1 uses a different method than Release 3.0 for converting CBOD to organic matter, based on percent refractory rather than BOD5\_CBODu. (Please see equation 148c in the latest Tech. Doc.). The quantity of OM loaded into your system may be different than in previous model results. Note, the default BOD5\_CBODu ratio was 2.47 which corresponds to a 60% refractory loading.** This wordy dialog was designed to inform users of older versions of AQUATOX that our assumptions regarding the conversion of BOD to organic matter have been refined in the latest version of the model. The error message alerts the user to this fact as well as the relevant equation (148c) in the [Technical Documentation](#).
- **You have entered a value outside of the recommended range for Relative Error: (0.0005 to 0.01).** The “relative error” defines how much error is allowed by the AQUATOX Runge-Kutta differential equations solver before it moves on to the next step. Setting this to be too small could cause the program to execute too slowly; setting it too large could create large errors in model accuracy. See section 2.1 of the [Technical Documentation](#) and especially Figures 4 and 5 for more information.

## Appendix: Guide to AQUATOX 3.1 Simulations

AQUATOX is distributed with a variety of self-contained studies (Table 1) that can be used as tutorial examples, templates, or starting points for developing new applications. They are color-coded here to give the user a rough idea of their applicability. There are four general classes of studies:

- **Nutrient studies** that are designed to examine the effects of organic matter, nitrogen, and phosphorus levels on primary productivity and the consequent effects on the food web.
- **Microcosm and mesocosm studies** in which the model is applied to experimental facilities or sites that are in themselves physical models with controlled boundary conditions; these range from simple aquaria to experimental streams to pond enclosures.
- **Chemical fate and effects studies** that examine bioaccumulation and the direct and indirect effects of organic chemicals on the food web as well as the persistence of those chemicals.
- **Studies intended for teaching purposes** that are not closely based on observed data, but that are included to illustrate particular AQUATOX features or site types.

The table below is organized by study type in the following order: nutrient studies, micro- and mesocosm studies, chemical fate and effects studies, and teaching studies. Well-calibrated studies<sup>1</sup> for each type are presented first.

|   |  |
|---|--|
| Well-calibrated nutrient study              | Roughly-calibrated nutrient study              |
| Well-calibrated micro- or mesocosm study    | Roughly-calibrated mesocosm study              |
| Well-calibrated chemical fate/effects study | Roughly-calibrated chemical fate/effects study |
|   | Study intended for teaching purposes           |

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<sup>1</sup> In this case, the term “well calibrated” is a function of the available data to calibrate against and the goals of the study. The term does not necessarily mean that all state variables in the study have been calibrated against an extensive data set.

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**Table 1. Description of Example Study Files for AQUATOX 3.1**

| <b>Study Name</b>   | <b>Site Type</b> | <b>Location</b>     | <b>Run time</b><br>(h:mm;<br>2.66 GHz<br>Quad CPU) | <b>Notes</b>  |
|---|------------------|---------------------|--|---|
| <p><i>Blue Earth R.MN.aps</i></p> <p><i>(well-calibrated nutrient)</i></p> <p><i>Blue Earth R.MN BMP Criteria.aps</i></p> | River            | Southern MN         | <p>0:14 for 2 yr</p> <p>0:14 for 2 yr</p>          | <p>The Blue Earth River drains a watershed in the Western Corn Belt Plains ecoregion that is 95% agricultural, planted in corn and soybeans. Suspended sediments are important most of the time; otherwise, algal blooms predominate.</p> <p>Study set up to evaluate nutrient reduction due to best management practices (BMPs).</p>                               |
| <p><i>Cahaba R AL.aps</i></p> <p><i>(well-calibrated nutrient)</i></p> <p><i>Cahaba R AL X2 TSS.aps</i></p>               | River            | Near Birmingham AL  | <p>0:47 for 2 yr</p> <p>0:29 for 2 yr</p>          | <p>A shallow stream incised in the southern Appalachians, located in a rapidly urbanizing area and receiving effluent from wastewater treatment plants. Good calibration data on periphyton, invertebrates, and fish.</p> <p>TSS is doubled to demonstrate embeddedness and impact on zoobenthos; it also decreases periphyton growth and speeds up simulation.</p> |
| <p><i>Crow Wing R. MN.aps</i></p> <p><i>(well-calibrated nutrient)</i></p>  | River            | North central MN    | 0:13 for 2 yr                                      | Shallow, relatively low-nutrient river that drains a predominantly forested watershed in the Northern Lakes and Forests ecoregion. Mile 72 is in the headwaters and drains numerous small lakes.  |
| <p><i>DeGray Res AR.aps</i></p> <p><i>(well-calibrated nutrient)</i></p>  | Reservoir        | Near Hot Springs AR | 0:16 for 2 yr                                      | A mesotrophic-eutrophic impoundment of the Caddo River in the Ouachita Mountains ecoregion. Most of the watershed is forested. Study shows transient response to drowned forest shortly after dam construction. Uses sediment diagenesis model.   |
| <p><i>Lake George NY.aps</i></p> <p><i>(Well-calibrated nutrient)</i></p> <p><i>Lake George NY smelt.aps</i></p>          | Lake             | Upstate NY          | <p>0:01 for 3 yr</p> <p>0:03 for 13 yr</p>         | <p>Mesotrophic end of large, deep lake in Adirondacks.</p> <p>Introduction of smelt changes food web and favors diatom blooms.</p>  |

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| <b>Study Name</b>   | <b>Site Type</b>          | <b>Location</b>                 | <b>Run time</b><br>(h:mm;<br>2.66 GHz<br>Quad CPU) | <b>Notes</b>  |
|---|---------------------------|---------------------------------|--|---|
| <p><i>Lower Boise R. ID Seg_1-3.als</i></p> <p><i>(Well-calibrated nutrient)</i></p> <p><i>Lower Boise R. ID Seg_1-3 Diel.als</i></p> | <p>River</p> <p>River</p> | <p>Boise ID</p> <p>Boise ID</p> | <p>2:49 for 3 yr</p> <p>2:35 for 1 yr</p>          | <p>Three upstream linked segments of the lower Boise River, a shallow river with abundant periphyton. Flow is controlled by upstream releases and irrigation diversions. Two segments are low-nutrient and the third receives WTP effluent. Also has hourly simulation to predict diel oxygen, which is dominated by throughflow except during low flow.</p>  |
| <p><i>MN Rivers.als</i></p> <p><i>(Well-calibrated nutrient)</i></p>  | Rivers                    | North, central, and southern MN | 0:40 for 2 yr                                      | Crow Wing, Rum, and Blue Earth Rivers as linked segments sharing the same parameter set (Park et al. 2005).   |
| <p><i>Onondaga Lake NY Sed Diagenesis.aps</i></p> <p><i>(Well-calibrated nutrient)</i></p>  | Lake                      | North of Syracuse NY            | 0:01 for 2 yr<br>(steady-state aerobic layer)      | “Lake Onondaga is arguably the most polluted lake in the United States” from the preface of a book (Effler 1996), which served as the source of data for this study. The lake has significant nutrient inputs from wastewater treatment plant (“Metro”) and combined sewers, successive algal blooms, hypoxia in hypolimnion, build-up of organic sediments in bottom, and high mercury levels and high salinity (the latter two are not modeled at present). Run with sediment diagenesis submodel (Di Toro 2001), with steady-state aerobic layers. |
| <p><i>Rum R MN.aps</i></p> <p><i>(Well-calibrated nutrient)</i></p>   | River                     | north of St. Paul MN            | 0:13 for 2 yr                                      | Rum River is a shallow river, with moderate nutrients and low suspended solids that drains forests and dairy farms in the North Central Hardwoods Forest ecoregion.   |
| <p><i>Tenkiller Ferry Lake OK.als</i></p> <p><i>(Well-calibrated nutrient)</i></p>  | Reser-voir                | Eastern OK                      | 0:51 for 2 yr                                      | Linked segments representing a eutrophic reservoir impaired by nutrients and organics, especially from upstream poultry and swine farms; there are excessive algae, and the hypolimnion is anoxic during the summer. However, it is one of the most important recreational lakes in the state. The sediment diagenesis submodel is necessary to simulate the anoxic hypolimnion.  |

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| <b>Study Name</b>   | <b>Site Type</b> | <b>Location</b>  | <b>Run time</b><br>(h:mm;<br>2.66 GHz<br>Quad CPU) | <b>Notes</b>   |
|---|------------------|------------------|--|--|
| <i>Cheney Res KS.aps</i><br>(roughly-calibrated nutrient)                                 | Reservoir        | Near Wichita KS  | 0:01 for 15 mn                                     | City of Wichita acquires about 70 percent of its daily water supply from Cheney Reservoir. It is believed that objectionable tastes and odors in Cheney Reservoir result from cyanobacteria (blue-green algae), and there is concern with proliferation of algal growth. Both nutrients and suspended solids affect algal growth and could be a concern for taste-and-odor issues (USGS 2008). |
| <i>Lake Jesup FL.aps</i><br>(roughly-calibrated nutrient)                                 | Lake             | North of Orlando | 0:01 for 7 yr                                      | Lake Jesup is a large, shallow lake. Urban storm water and agricultural runoff impact the lake, as well as historic wastewater discharge. Blooms of the invasive cyanobacteria <i>Cylindrospermopsis</i> have been increasing.   |
| <i>Lake Pyhäjärvi Finland.aps</i><br>(roughly-calibrated nutrient)                        | Lake             | SW Finland       | 0:04 for 10 yr                                     | Mesotrophic boreal lake simulated by Anne Mäkynen, Jyväskylä University. The difference between observed and simulated phosphorus concentration corresponds perfectly with the mass removed by fishing.  |
| <i>Farm Pond MO.aps</i><br><i>Farm Pond MO Esfenval.aps</i><br>(Well-calibrated mesocosm) | Pond             | Central MO       | 0:01 for 1 yr<br><br>0:01 for 1 yr                 | Generic pond built to USDA specifications. Esfenvalerate loadings are the worst-case scenario using runoff from an adjacent corn field predicted by the PRZM model.  |
| <i>HCB Tank.aps</i><br>(Well-calibrated microcosm)  | Aquarium         | Experimental lab | 0:00:01 for 2 mn                                   | Represents an experiment in which an aquarium tank containing macrophytes was dosed with hexachlorobenzene (Gobas et al. 1991).  |
| <i>Ponds MN Chlorpyrifos.als</i><br>(Well-calibrated mesocosm)                            | Enclosures       | Duluth MN        | 0:00:15 (perturbed & control) for 3 mn             | Pond enclosures dosed with 0.5, 6, and 32 ug/L chlorpyrifos at an EPA lab.   |
| <i>Expr Stream Esfenval.aps</i><br>(Roughly-calibrated mesocosm)                          | Stream           | Idaho            | 0:15 for 10 mn (perturbed)                         | Based on Lower Boise River, this is a reach with a volume of 400 m <sup>3</sup> and a retention time of 0.1 day. Set up for constant dosing for a period of time. Study uses fixed time step so it can be used for detecting lowest effect levels.   |

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| <b>Study Name</b>   | <b>Site Type</b> | <b>Location</b>   | <b>Run time</b><br>(h:mm;<br>2.66 GHz<br>Quad CPU)                                 | <b>Notes</b>  |
|---|------------------|-------------------|--|---|
| <p><i>Ohio stream Chlorpyrifos constant.aps</i></p> <p><i>(Roughly-calibrated mesocosm)</i></p> <p><i>Ohio stream Chlorpyrifos pulsed.aps</i></p> | Stream           | North central OH  | 0:07 for 2 yr  | A small creek draining agricultural area, used as a generic study for various pesticides. One study has constant exposure and other has pesticide runoff during summer storms.  |
| <p><i>Coralville Res IA Dieldrin.aps</i></p> <p><i>(Well-calibrated chemical fate/effects)</i></p> <p><i>Coral Res IA Sens.aps</i></p>            | Reservoir        | Near Iowa City IA | <p>0:13 (perturbed)</p> <p>0:10 (control)</p> <p>for 9 yr</p> <p>2:41 for 1 yr</p> | <p>Coralville Reservoir is a large, shallow, eutrophic reservoir. The drainage area is over 90% agricultural, especially corn. Runoff carries large amounts of fertilizer, animal wastes, silt, and pesticides into the reservoir. By the early 1970's, the population of largemouth bass and fish other than buffalofish began to decline and residues of the pesticides aldrin and dieldrin greatly increased in tissue samples (Mauriello and Park 2002).</p> <p>Study set up for sensitivity analyses, 54 parameters.</p> |
| <p><i>Evers Res FL.aps</i></p> <p><i>(Well-calibrated chemical fate/effects)</i></p>  | Reservoir        | Bradenton FL      | 0:05 for 5 yr (perturbed)  | A reservoir with increasing algal blooms, treated with copper sulfate and hydrogen peroxide. Simulated by Dr. Don Blancher, Sustainable Ecosystem Restoration, LLC  |

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| <b>Study Name</b>  | <b>Site Type</b> | <b>Location</b> | <b>Run time</b><br>(h:mm;<br>2.66 GHz<br>Quad CPU) | <b>Notes</b>  |
|--|------------------|-----------------|--|---|
| <i>Lake Ontario PCBs.aps</i><br><br>(well-calibrated chemical fate/effects)  | Lake             | US-Canada       | 1:55 for 4 yr                                      | Demonstration of bioaccumulation simulation for numerous PCB congeners compared to data of (Oliver and Niimi 1988)see also (Burkhard 1998); this implementation uses Barber (2003) k2 estimation.   |
| <i>Skensved Denmark TCE.aps</i><br><br>(well-calibrated chemical fate/effects)<br><br><i>Skensved Denmark Atrazine.aps</i> | Stream           | Denmark         | 0:15 for 1 yr<br><br>(perturbed)                   | Groundwater with trichloroethene from a leaking tank is polluting a small stream. Simon Funder and Dr. Ursula McKnight of the Technical Univ. of Denmark, used AQUATOX to show the <b>impacts are probably negligible</b> . The same setup with atrazine does show some direct and indirect ecotoxicological effects. Concentrations are near the no effects level so the option for a fixed time step was chosen.  |
| <i>Clear Lake CA Fluridone.aps</i><br><br>(Roughly-calibrated chemical fate/effects)                                       | Lake             | Central CA      | 0:14 (both perturbed & control)<br><br>for 3 yr    | Roughly based on Clear Lake CA, a large, shallow, eutrophic lake with cyanobacteria blooms. Sonar (fluridone) has been used successfully in Clear Lake to eradicate <i>Hydrilla</i> . Although <i>Hydrilla</i> did not appear until 1994, the study is set up with 1970-1971 ecosystem data. Note that the fluridone loadings are for 1971 but without bracketing the simulation period with 0 loadings. The fluridone loadings are repeated in each of the three years. Also note that the entire lake was modeled for convenience; in reality, <i>Hydrilla</i> spread slowly, so only selected areas needed to be treated. Our simulation is therefore a worst-case scenario. |
| <i>East Fork Poplar Creek TN PCBs.aps</i><br><br>(Roughly-calibrated chemical fate/effects)                                | Stream           | Oak Ridge TN    | 1:09 for 8 yr                                      | A small stream that drains the Y-12 plant at Oak Ridge National Lab with PCB contamination. The simulation runs for eight years to illustrate gradual recovery.   |
| <i>Galveston Bay TX.aps</i><br><br>(Roughly-calibrated estuary)  | Estuary          | Near Houston TX | 0:11 for 3 yr                                      | A shallow, productive bay that receives runoff from Central TX, including the Houston Ship Channel.   |



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| <b>Study Name</b>   | <b>Site Type</b> | <b>Location</b>                  | <b>Run time</b><br>(h:mm;<br>2.66 GHz<br>Quad CPU) | <b>Notes</b>   |
|---|------------------|----------------------------------|--|--|
| <i>Zollner Creek OR w<br/>chlorpyr dieldrin-<br/>pulse.aps</i><br><br><i>(roughly-calibrated<br/>chemical<br/>fate/effects)</i> | Stream           | Willamette<br>Valley<br>OR       |  | The watershed is >90% agricultural, with row crops, orchards and vineyards, grain and grass fields, and large poultry farms. It is a USGS National Water Quality Assessment Program (NAWQA) site, and also a principal TMDL site. State criteria for chlorpyrifos and legacy dieldrin were exceeded (Williams and Bloom 2008). |
| <i>Impact of<br/>anadromous fish.aps</i><br><br><i>(Study intended for<br/>teaching purposes)</i>                               | Lake             | Based on<br>Lake<br>George<br>NY | 0:01 for<br>3 yr                                   | Mesotrophic lake based on Lake George NY, with Chinook salmon representing anadromous fish. Nutrients are imported into lake.  |
| <i>Nockamixon Res<br/>PA.aps</i><br><br><i>(Study intended for<br/>teaching purposes)</i>                                       | Reserv-<br>oir   | eastern<br>PA                    | 0:00:30<br>for 2 yr                                | Heavily impacted reservoir downstream of the Quakertown wastewater treatment plant outlet.   |

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