User's Manual for Basic Version of MCnest –

Markov Chain Nest Productivity Model

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I. Introduction

A. *What is MCnest?*

The Markov chain nest productivity model, or MCnest, is a set of algorithms for integrating the results of avian toxicity tests with reproductive life-history data to project the relative magnitude of chemical effects on avian reproduction (Bennett et al. 2005, Bennett and Etterson 2007). MCnest was designed and optimized for MATLAB (www.mathworks.com), but most users will run a compiled version as a standalone program that does not require the user to have MATLAB software. The mathematical foundation of MCnest is in the analysis of Markov chains, which provides a flexible template for modeling the variation in avian breeding cycles among species.

B. *How to use this Manual*

This manual is organized into four major sections. Section I gives some basic background on MCnest and the justification for its development. Section II is a quick start guide to running simulations in MCnest that walks users through several examples. Section III describes the MCnest outputs and their interpretation. Section IV is a complete list of user inputs that can be modified in MCnest.

C. *What does MCnest do?*

The purpose of MCnest is to quantitatively estimate the relative change in the number of successfully fledged broods per female per year of avian species exposed to a specific pesticide application scenario. The relative change in the number of successful broods is estimated by comparing model results based on a defined pesticide application scenario with a no-pesticide scenario. To express the results in terms of annual reproductive success, the model estimate of the number of successfully fledged broods per female is multiplied by the expected number of fledglings per successful nest, which is taken from the literature.

Each model simulation follows the breeding activities of a population of females each day throughout a breeding season. The breeding activity is described by a series of phases through which the female transitions (i.e., pair formation, egg laying, incubation, nestling rearing, and waiting periods prior to starting a new nest attempt). The temporal pattern of breeding activity of each female varies due to differences in the initiation date of the first nest attempt and due to a specific probability each day that the nest attempt could fail from ecological causes such as predation or weather. When a nest attempt fails, each female can make a new attempt if there is time remaining in the breeding season, and for many species, females make a new attempt after completing a successful brood. If the simulation incorporates one or more pesticide applications, the pesticide exposure may represent an additional cause of nest failure depending on the types of pesticide effects observed in tests and the timing of the application relative to the phase of the nesting attempt for each female. When a nest attempt fails due to pesticide exposure, each female may make a new attempt if there is time remaining in the breeding season and pesticide residues decline to levels that would not affect parental well-being. As MCnest follows each female of the population through the breeding season, it tabulates the number of nest attempts and successful broods (i.e., broods surviving to fledging) per female in the population.

In this basic version of MCnest, our goal has been to limit the list of model inputs so that the model is applicable to as many species as possible. We have also focused on using model inputs that are readily available from published literature and from toxicity data submitted as part of the current pesticide

registration process. MCnest is designed to accommodate the structure of quantitative dose-response data from reproduction tests if it becomes available.

D. Why is MCnest needed?

The current pesticide registration process uses deterministic risk quotients (RQ) as the primary metric for assessing the potential risks of pesticide exposure to avian reproduction. While RQs can be used to classify the potential risk, they do not quantify the magnitude of risk or provide information on differences in risk among species or pesticide application scenarios. MCnest offers an approach for more quantitatively estimating the magnitude of avian reproductive effects when the ultimate goal is to probabilistically describe the risk of reproductive effects or to estimate the magnitude of effects to a population.

In population modeling, we need to know how a pesticide application scenario affects the annual reproductive success of a species compared to a scenario without pesticides. Annual reproductive success, also known as the fecundity rate, is here defined as the number of fledglings produced per female in the population over an entire breeding season. It represents the cumulative production of fledglings during a breeding season, including renesting attempts after nest failures and multiple successful nesting attempts. In MCnest, it can be estimated as the average number of successfully fledged broods per female per season times the average number of fledglings per successful brood.

E. What information is needed to use MCnest?

The basic version of MCnest requires data from avian toxicity tests, avian species life-history profiles, and a pesticide-use scenario that defines the timing and temporal pattern of exposures. The most common sources of toxicity data are the results from three standard avian toxicity tests: 1) the acute oral LD50 test, 2) the dietary LC50 test, and 3) the reproduction test. When available, additional toxicity data from alternative sources or from alternative interpretations of standard toxicity tests may be useful. The selection and use of toxicity data in the model is discussed in the MCnest Technical Manual.

In the basic version of MCnest, model simulations are run on avian species of interest (i.e., life-history profiles developed for specific species or generic species intended to represent certain life-history strategies). A series of life-history parameters is required for each species of interest. The user can select one of the species for which a suite of default life-history parameters has been developed, create a new species, or modify the profiles of existing candidate species by directly inputting the list of life-history parameters. The use of default species profiles and the creation of new or modified species profiles are discussed later in the manual.

Finally, MCnest requires that the user define a pesticide-use scenario. For the basic version of MCnest this requires, at a minimum, specifying the date(s) of application, the application rate(s), and the half-life of residue degradation on foods relevant to the species of interest. The user also has four options for how the application rate is converted to the ingested daily dose on the day of each application, which are explained later is Section IV.C.

II. Quick start guide

A. Installation of MCnest

Probably the most efficient way to understand MCnest is to begin using the model with default life-history data. To begin using the program, copy the MCnest files to an appropriate directory on your

computer. The "MCnest.exe" file and the "SpeciesLibrary.xlsx" file <u>must</u> be in the same directory or MCnest will not open. If this is your first time using MCnest, you will need to install the MATLAB compiler runtime library (MCR). It can be downloaded from Mathworks at: http://www.mathworks.com/products/compiler/mcr/ by selecting the Windows 32-bit R2012a version. Installation of the MCR requires administrator privileges. To install, double-click "MCR_R2012a_win32_installer.exe" or select "Run…" and browse to the MCR file. Installation may take several minutes due to the very large file size. Once the MCR is installed, you may run "MCnest.exe" file without administrator privileges. Be patient; it can take up to 60 seconds to open!
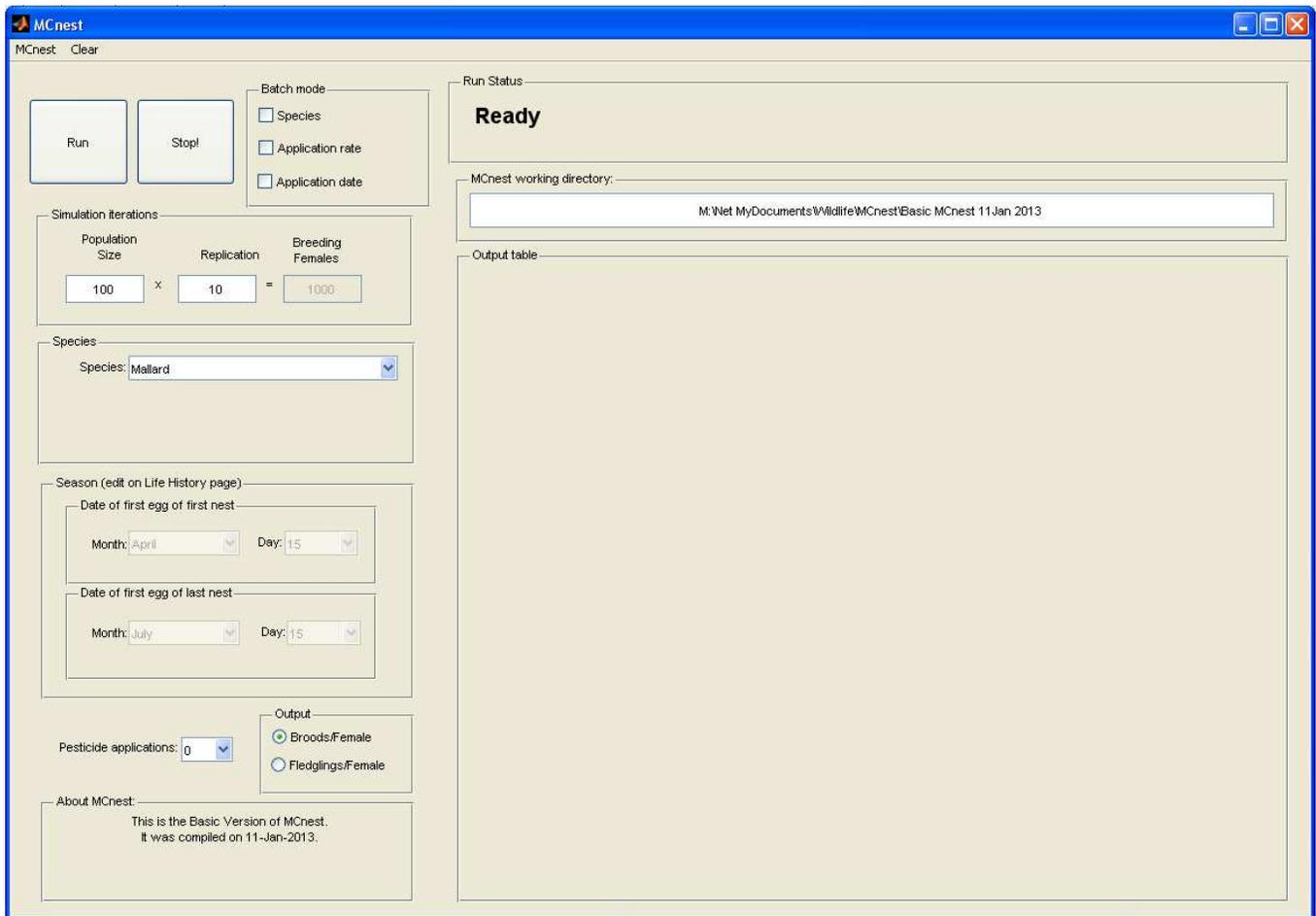
B. *MCnest startup window*

Once the MATLAB compiler runtime library is installed, MCnest is called by double-clicking the executable file (MCnest.exe) or selecting "Run…" from the Windows Start menu and then navigating to MCnest.exe. Doing so brings up the main MCnest screen (Figure 1). Of the several blocks of controls visible on the main MCnest screen, only two need be set to run simple simulations, the "Simulation iterations" block and the "Species" block. The "Batch mode" block is explained in greater detail in Section IV. The "Season (edit on Life History page)" block is not editable on the main MCnest page, but is provided for user information. It can be edited on the Life History page (explained in Section IV). Note, however, that the season changes as you select different species from the "Species" drop-down menu. The "Pesticide applications" control will be explained below and in further detail in Section IV.

Simulation iterations: The Simulation iterations block defines the simulation sample size (number of breeding females) as the product of a "Population size" parameter and a "Replication" parameter. The default is a population of 100 females, replicated 10 times for a total population size of 1,000 breeding females. In general these simulations will run fairly quickly; for getting to know the program we recommend these sample sizes. Note that the number of "Breeding Females" cannot be edited directly, except as the product of "Population size" and "Replication."

Species: The Species block contains a listbox menu of species names for which life- history profiles have been pre-coded for use in MCnest. The default is the first species in the list, Mallard (*Anas platyrhynchos*). Specific details about the life-history of species in the library, and functions to edit life-history parameters to create new species are found on the Life History page, which can be accessed under the main MCnest menu. More details about editing and creating life-histories are given in Section III.
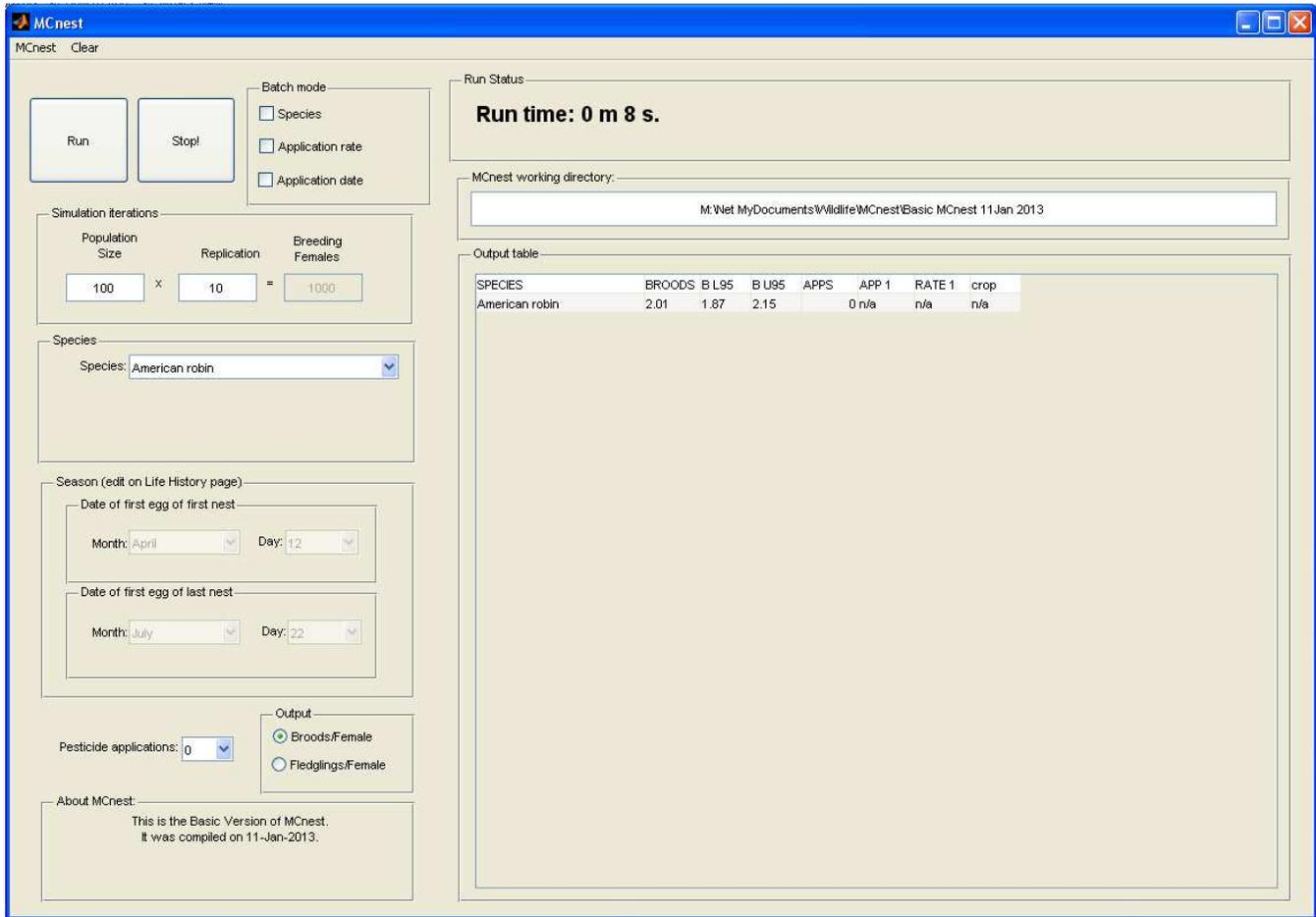
Figure 1. Main MCnest window.

C. *Running a simulation with no pesticide exposure for a selected species*

Once the program is loaded, verify that the "Population Size" edit box and the "Replication" edit box are set to 100 and 10, respectively. In the "Species" edit box, click the down arrow and select the American robin. Then click "Run" in the upper left corner. While the simulation is running the "Run Status" status box in the upper right of the main MCnest window will initially display the message "Simulation running, please wait…" while the Markov chain transition matrix is constructed. Once the simulation begins to run, the "Run Status" box will display the updating message "Iteration X of Y," where X is the recently completed iteration and Y is the total number of iterations (breeding females). When the simulation finishes, the window will revert to a run time message (Figure 2).

Figure 2. Sample output from a simulation with 10 replicates of 100 breeding American robins.

The default tabular output to a MCnest simulation gives the species name, the expected number of successful broods per female (BROODS), the upper and lower 95th percentile confidence intervals for BROODS (based on the ten replicates), and the number of pesticide applications simulated (APPS), which is 0 in this case. As more simulations are run, new output is appended to this table.
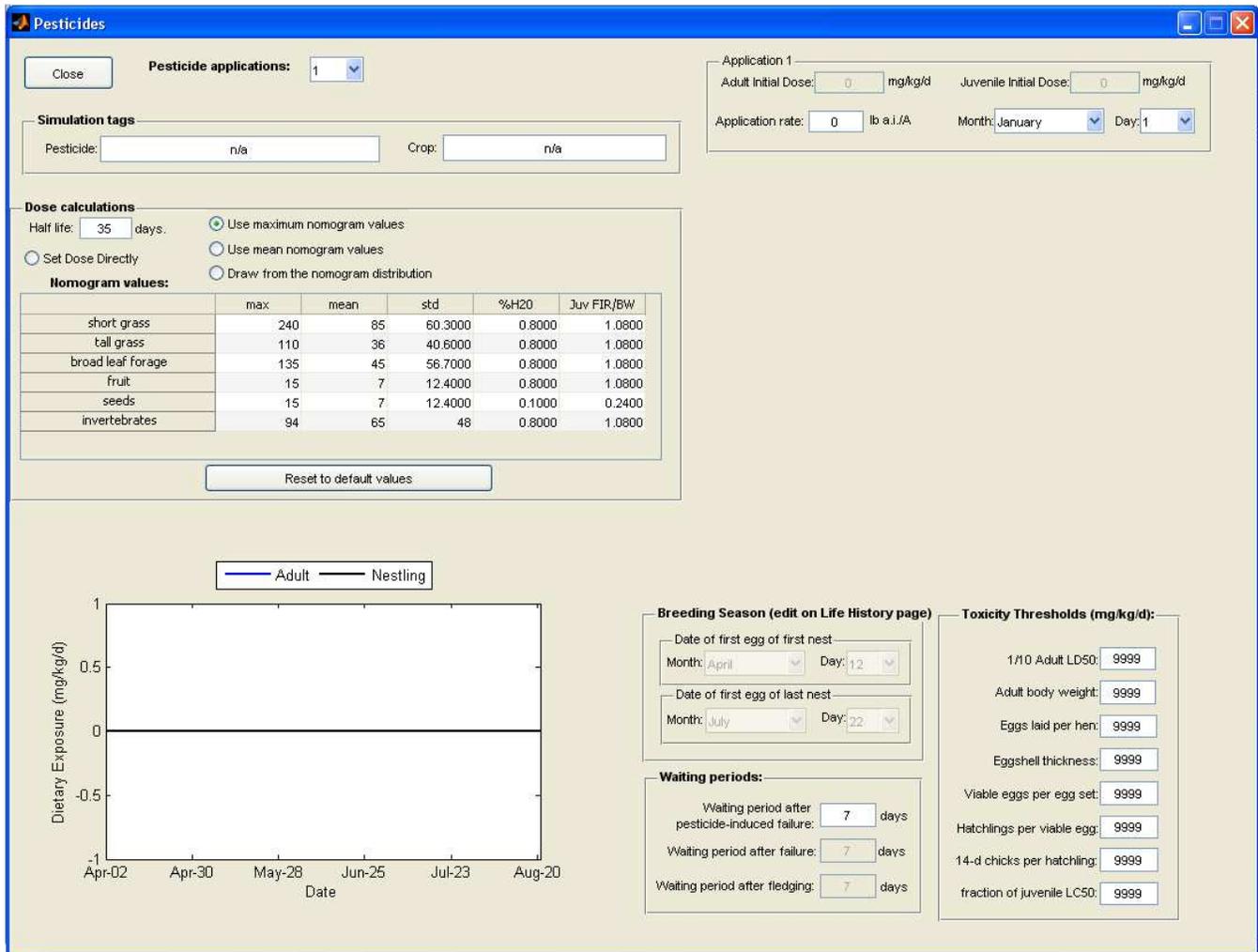
Note that the numerical results on your screen may differ slightly from Figure 2 because of variation in the actual sequences of random numbers utilized by the program. MCnest does give you the ability to set the random seed so that simulations may be replicated exactly. These functions are covered in detail below in Section IV.

D. Running a simulation with a pesticide exposure

MCnest is designed to simulate the effects of pesticide applications on the reproductive output of breeding birds. There are many options in MCnest for how this is done and only the simplest is explained in this portion of the user's guide.
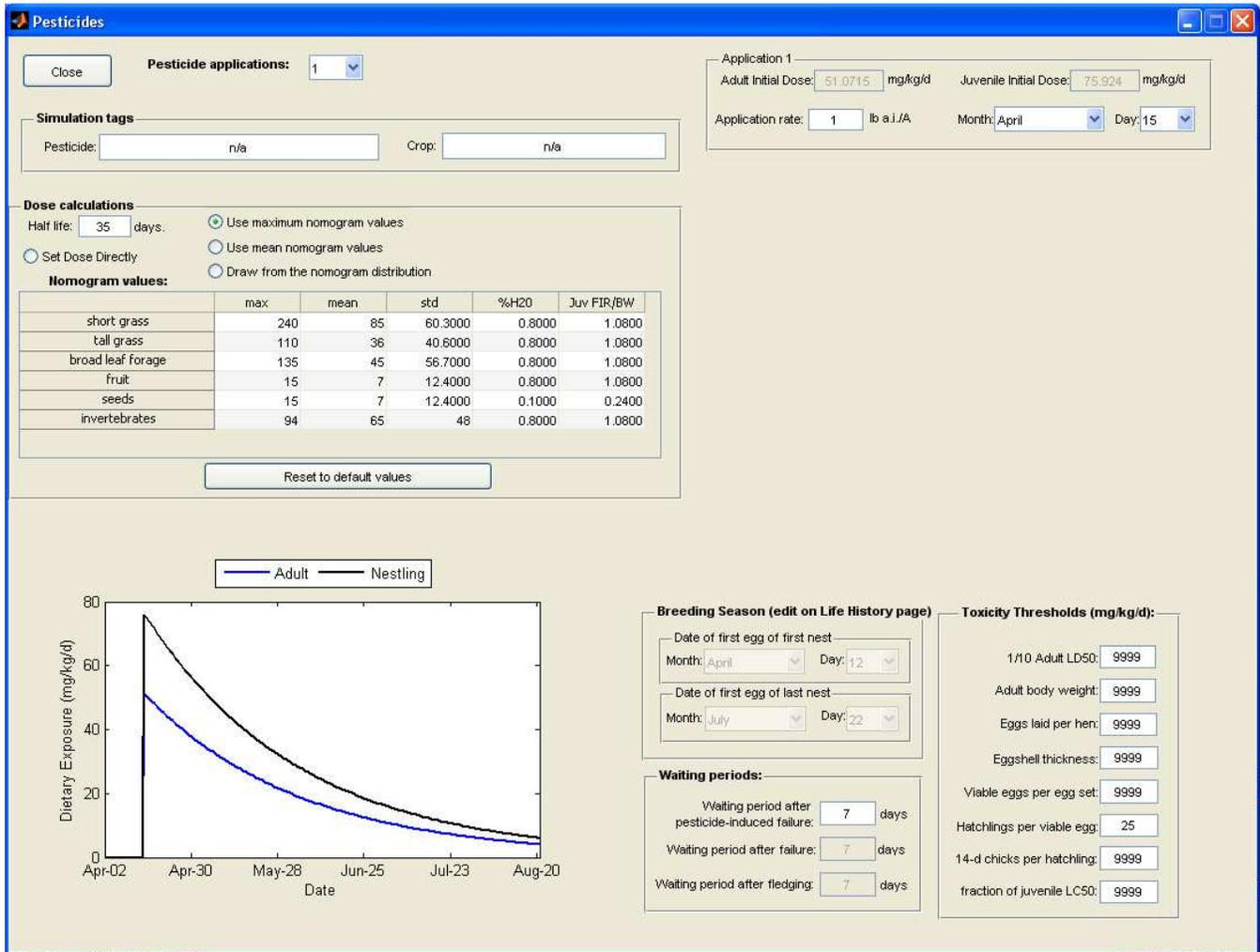
With the MCnest main screen still displaying the American robin simulation, find the "Pesticide applications" control at the lower left of the main MCnest screen and choose "1." After a short delay, the Pesticides window should appear (Figure 3)
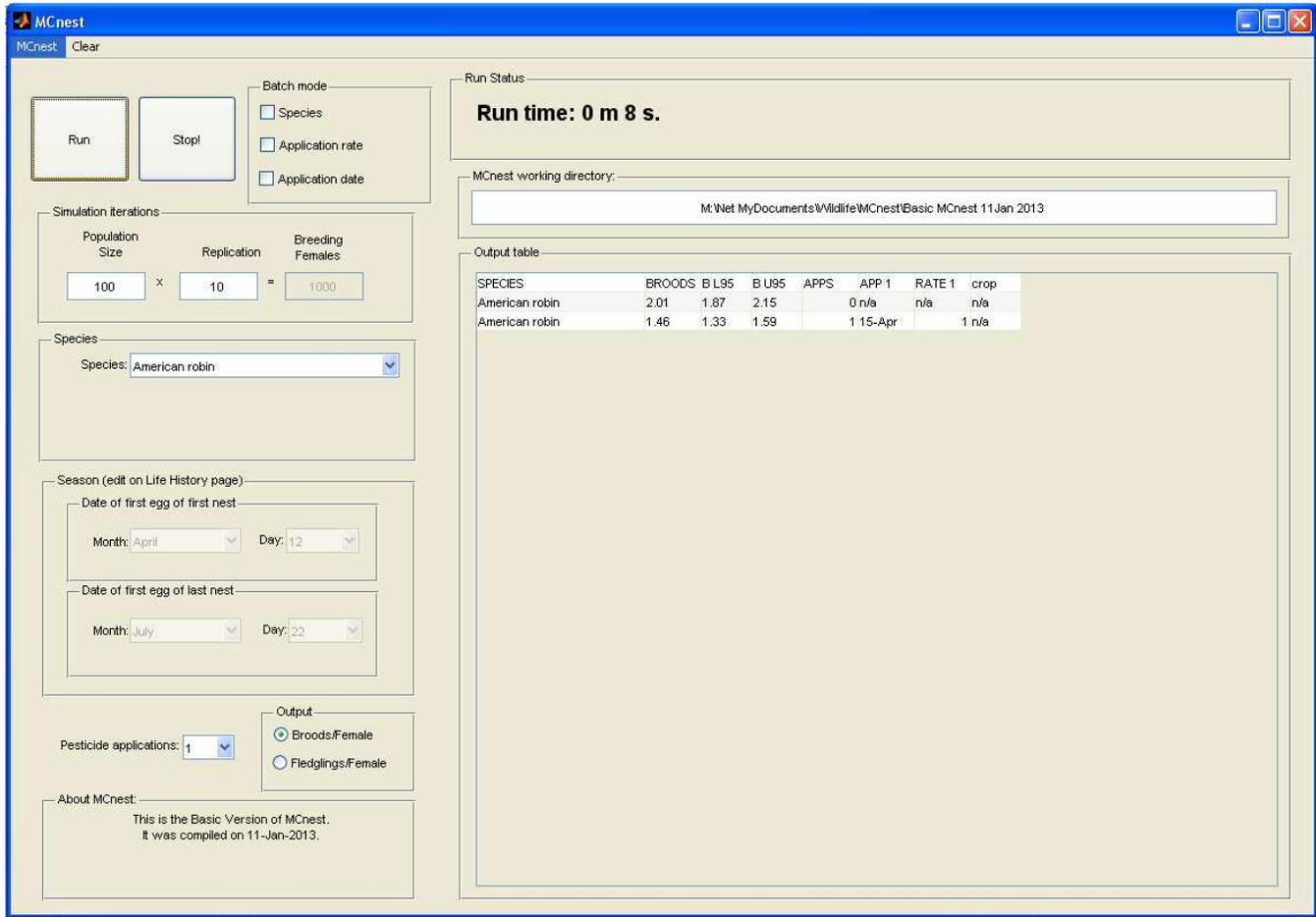
Figure 3. The Pesticides window in MCnest.

A full description of the Pesticide controls is given in Section IV. For now, leave the "Pesticide applications" listbox set to "1." Under "Toxicity Thresholds," all of which are expressed as mg/kg body weight/day, set the "Hatchlings per viable egg" edit box to "25." In the "Dose calculations" frame, leave the default radio-button "Use maximum nomogram values" checked and leave the default half-life set to 35 days. Finally, in the "Application" 1 frame set the "Application rate," expressed as pounds active ingredient (AI) per acre, to "1" and the "Month" to "April" and the "Day" to "15" (Figure 4). Press "Enter" to register the changed parameter values into program memory.

Figure 4. Pesticides window with application date set to "April 15," Application rate set to "1," and the Toxicity Threshold for Hatchlings per viable eggs set to "25."

MCnest uses algorithms from OPP's Terrestrial Residue EXposure model or T-REX (http://www.epa.gov/oppefed1/models/terrestrial/trex/t_rex_user_guide.htm) to translate the application rate (expressed as pounds AI/acre) into doses (expressed as mg/kg body wt./day) for both adult and juveniles taking into account the species' typical diet. This produces the values for "Adult Initial Dose" (51.1) and "Juvenile Initial Dose" (75.9) that appear in the two associated boxes. These initial doses will be realized on the application date (April 15) and will decay geometrically with a residue half-life of 35 days. Click "Close" to close this window and return to the main MCnest window. Once back at the main window, click "Run" to run the pesticide simulation. A second row of output should appear on the main MCnest window (Figure 5).

Figure 5. Main MCnest window displaying the results of two simulations.

A quick glance at the Output table suggests that the model predicts an approximately 30% reduction in the expected number of broods per female due to pesticide exposure compared to the expected number in the absence of pesticide effects. However, such simple comparisons may be insufficient for understanding potential risks of a pesticide application. For this, MCnest offers many kinds of output that are stored and accessed in several ways. These are covered in detail in the next Section III (MCnest outputs).

This is the end of the Quick Start Guide. Congratulations! You now know how to run rudimentary simulations in MCnest. However, we recommend you continue through the next section to see how to extract more information from MCnest simulations. The above two simulations will be used as examples throughout Section III to demonstrate the diagnostic and output features of MCnest.
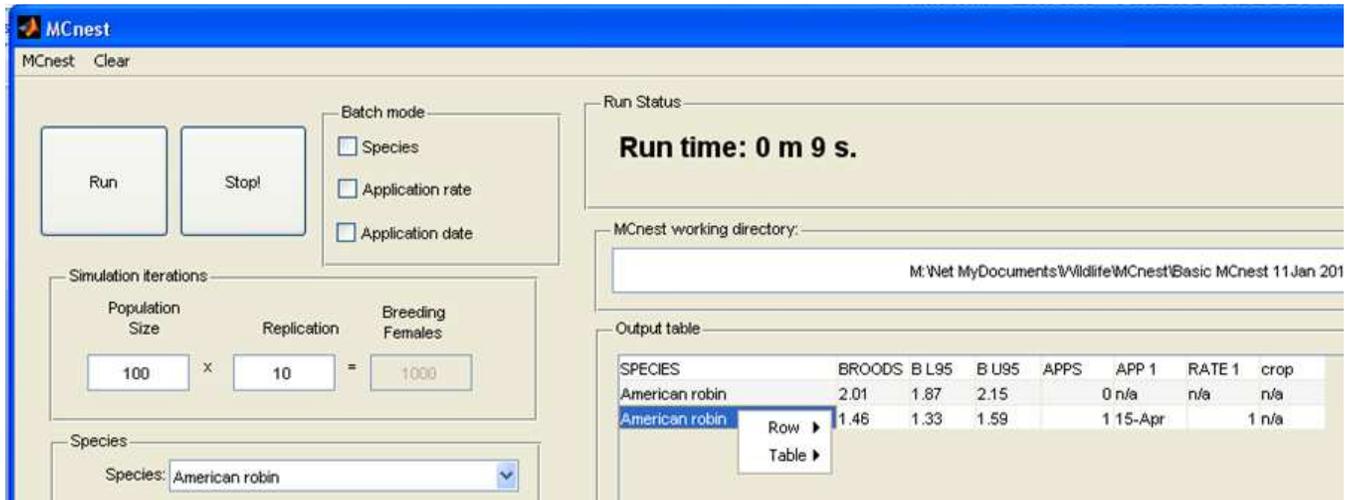
III. MCnest outputs

A. *Using the table*

The Output table on the main MCnest screen provides a snapshot of the overall results for each simulation as well as functional access to more detailed information about MCnest results. Because each MCnest simulation is separate, the basic version of MCnest does not automatically calculate the

percent reduction in seasonal productivity due to a pesticide exposure relative to a no-pesticide scenario. However, the model user can copy the contents of the Output table into a spreadsheet, such as Excel, to calculate comparisons among simulations. Use the cursor to highlight the contents of the Output table and copy the contents by simultaneously hitting the Control key and letter "C." In Excel, place the cursor in a destination cell and simultaneously hit the Control key and letter "V" to paste the table.

The additional output functions are accessed by first left-clicking on the species name for the output row of interest and then right-clicking the mouse within the outlined results block. They are divided into two basic categories, functions that give more detailed information about an individual simulation (under the **Row** submenu) and functions that operate on multiple simulations, e.g., for comparing among species, pesticide applications, etc. (under **Table** submenu). The examples below make use of the two simulations run above in Section II (Quick Start Guide). Throughout this section on model outputs we assume that you have two similar results available. For example, left-clicking on American robin in the first row of the SPECIES column and then right-clicking should bring up the main Output table menu (Figure 6).

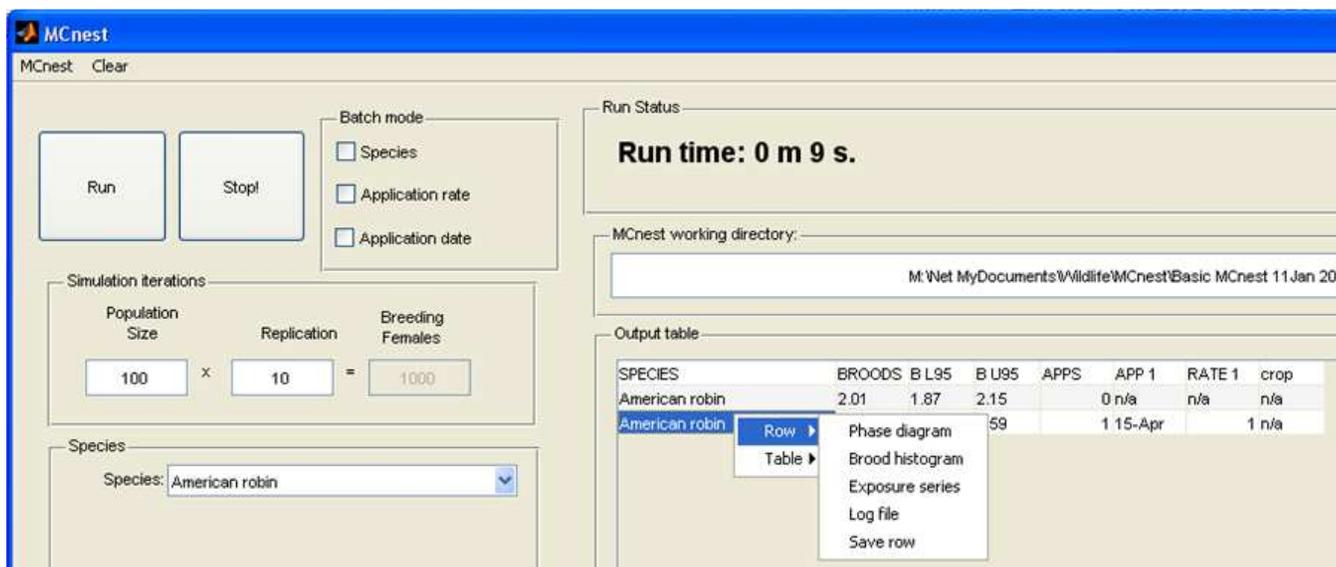Figure 6. The first dichotomy of choices in the context menu.

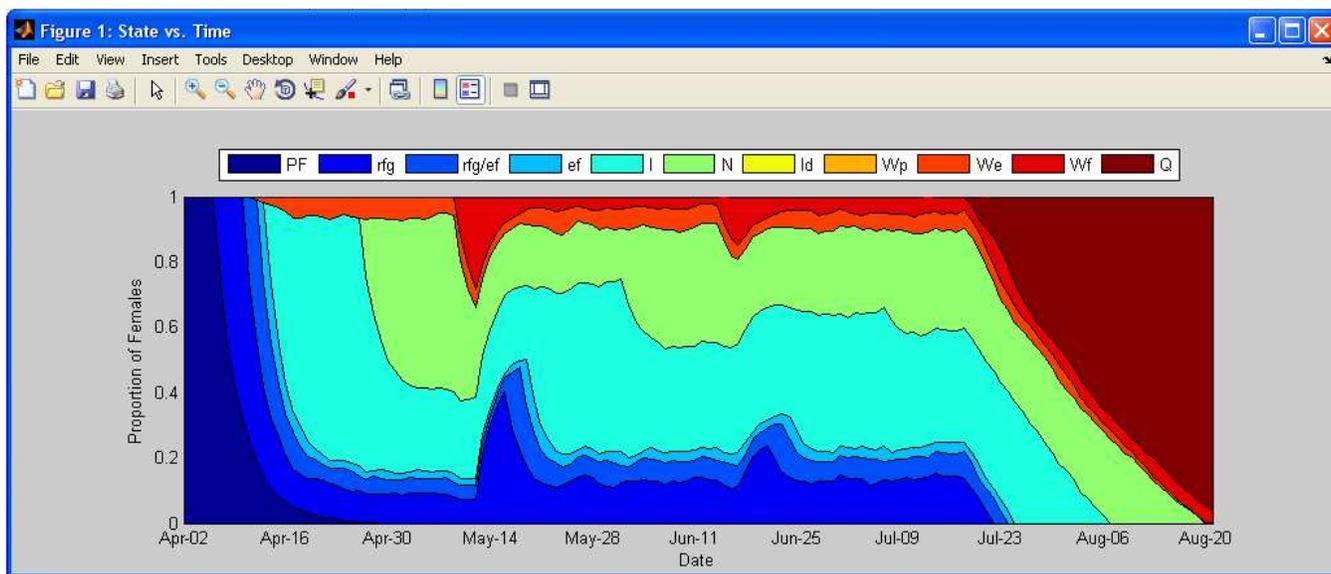

B. *The* **Row** *menu*

After left-clicking on the specific row of interest, place the cursor on "Row" in the results menu to get access to a sub-menu with five choices (Figure 7).

Figure 7. The Row submenu of the context menu.

Phase diagram: Clicking "Phase diagram" under the row menu brings up an area plot of the proportion of females (vertical axis) in different developmental stages (colors) as the breeding season progresses (horizontal axis) (Figure 8).

Figure 8. Phase diagram for American robin with no pesticide exposure.

The phase diagram is the primary diagnostic tool for understanding how the simulated chemical exposure was translated by the model into projected reproductive effects. Because there is no chemical exposure in the example simulation, there are no corresponding diagnostic effects in the phase diagram. Nevertheless the diagram is useful for tracing the general progress of females unexposed to pesticides

through various breeding phases over the course of the simulated season. Table 1 provides a more detailed explanation of the phases in the phase diagram. Each of the phases is defined in greater detail in the technical support document.

Notice that two phases in Table 1 do not occur in the phase diagram pertaining to the first simulation above (Figure 8). These are the doomed incubation period (Id: yellow) and the waiting period after pesticide-induced failure (Wp: orange). These two states can only be entered into after exposure to a pesticide exceeding the appropriate threshold. However, highlighting the second row in the Output table and opening the phase diagram will produce a figure (Figure 9) that shows a portion of females in the doomed incubation period (yellow) for a short time before transition to the waiting period after pesticide induced failure (orange).
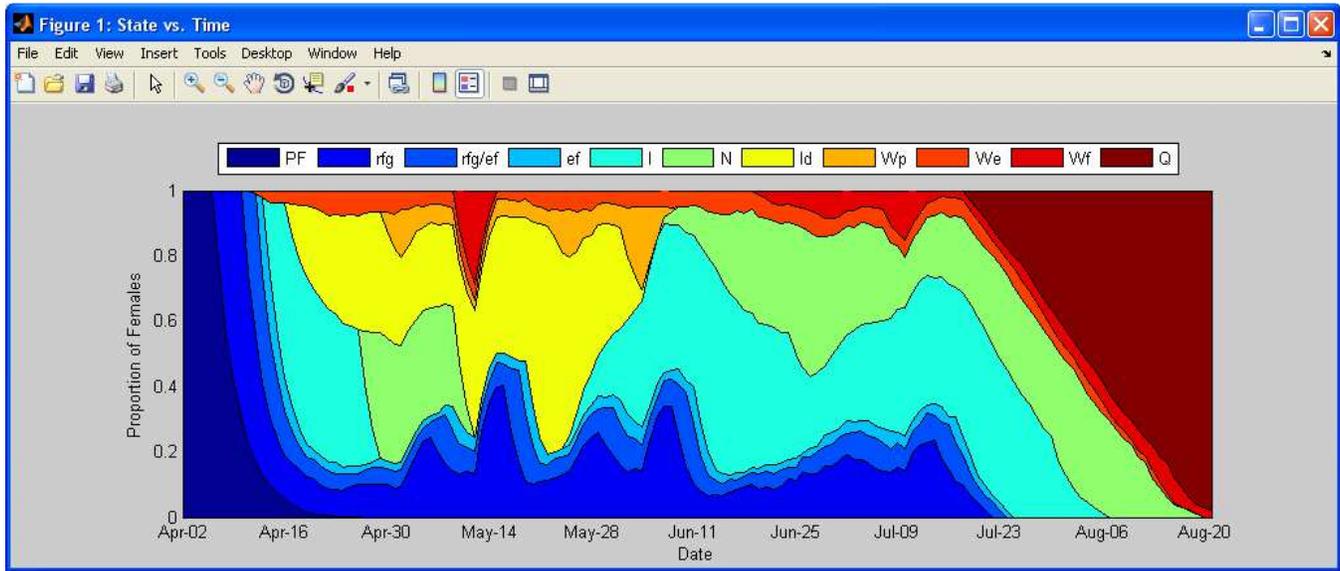
Table 1. Simulated breeding phases as represented in the phase diagram of MCnest output.

Phase	Legend abbreviation	Legend color (approx.)
Pair Formation	PF	
Rapid Follicle Growth	rfg	
Overlap of Rapid Follicle Growth and Egg Formation	rfg/ef	
Egg Formation	ef	
Incubation	I	
Nestling	N	
Doomed Incubation	Id	
Waiting after pesticide-induced failure	Wp	
Waiting after ecological failure	We	
Waiting after successful fledging	Wf	
Quit	Q	

Hint: you do not need to close the phase diagram for a previous simulation before generating one for the next. Thus for example, the two phase diagrams (Figures 8 & 9) could be open concurrently for direct comparison.
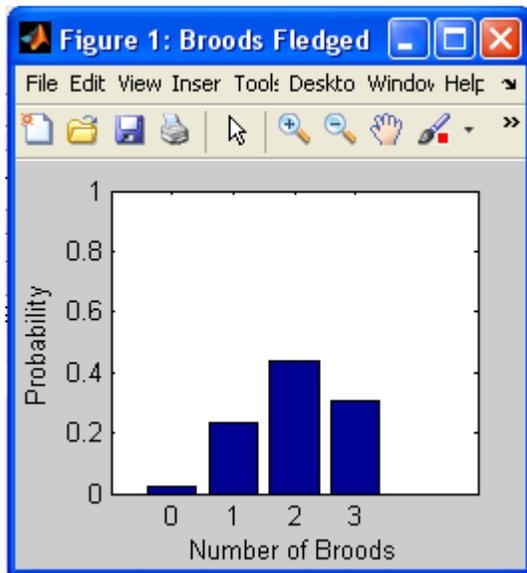
Further guidance on the interpretation of phase diagrams can be found in Appendix A.

Figure 9. Phase diagram for a simulation with a short period of effects on egg hatchability.

Brood histogram: This sub-menu item generates a histogram of the number of successful broods produced per female in the simulation (Figure 10). It is useful for gaining an intuitive sense for the expected variance in female reproductive success associated with a simulation scenario.
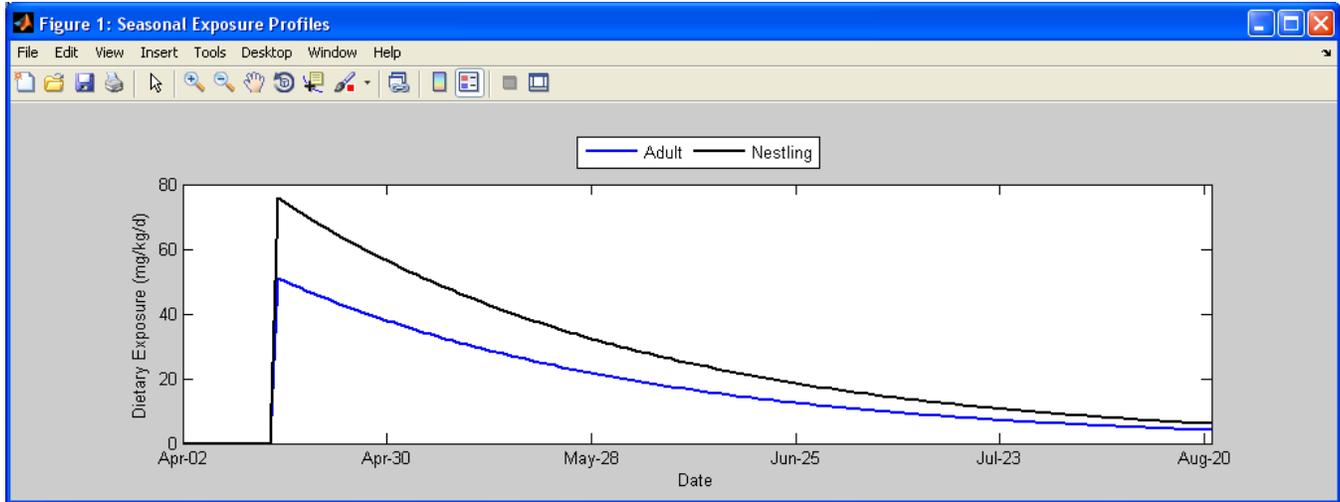
Figure 10. Sample brood histogram for default American robins with no pesticides.

Exposure series: Figure 11 shows the simulated Seasonal Exposure Profile for the second American robin simulation, in which the threshold (i.e., 25 mg/kg/d) for egg hatchability was exceeded for a short duration. Note the separate exposure series for adults versus juveniles. Although this choice is available

for each simulation, when no pesticide applications are simulated, there is simply a horizontal line at 0 mg/kg/d.

Figure 11. Example Seasonal Exposure Profile for American robins with pesticide parameters set as described in the pesticide example of Section II (Quick Start Guide).

Log file: This sub-menu choice opens a text file (using the computer's default text editor) and prints the main results and all of the input parameters used in the simulation. This file is virtual (it can be regenerated at any point, but it does not exist until generated). Therefore if it is needed for documentation, it should be explicitly generated and saved to disk.

Figure 12. Sample log file for American robin simulation with a single pesticide application.

Save row: This sub-menu choice saves the selected row to an output file that can later be opened in MCnest. The file is assigned a name by the program corresponding to the species name and the date and time that the simulation was run. A confirmation pop-up window is displayed (Figure 13). Files are saved into the directory specified in the "MCnest working directory" list above the "Output table."
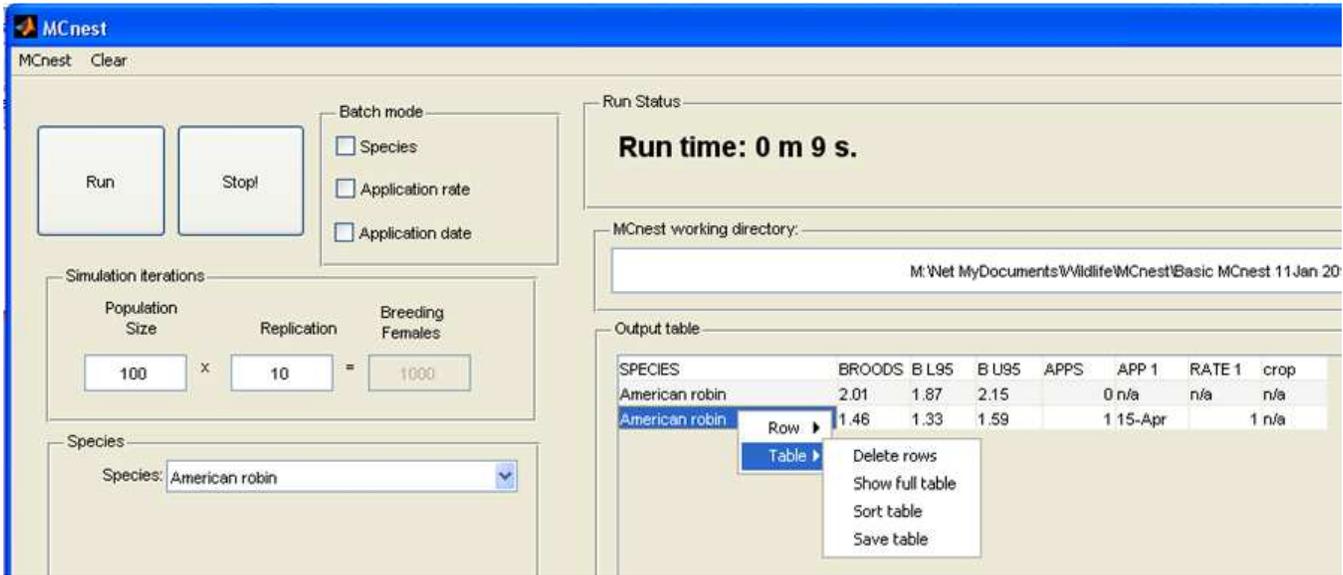
Figure 13. Example filename and popup window when a single row is saved to an output file.

C. The **Table** *menu*

After right-clicking anywhere in the "Output table" box, place the cursor on "Table" in the results menu gives access to a sub-menu with four choices (Figure 14).

Figure 14. The Table sub-menu of the context menu.

Delete rows: This sub-menu choice deletes the selected row(s) from the table. Multiple rows can be selected and all selected rows will be deleted. They cannot be recovered.

Show full table: This submenu choice opens a larger table window that presents more information about each simulation (Figure 15), including the expected nest success rates (S, with confidence intervals) and nest attempt rates per female (N, again with confidence intervals). It also displays information concerning the pesticide exposure scenarios. A definition of each code used as column heading in the full table is located in Table 2. In the default simulations above these should all be set to n/a because no pesticide was simulated. Right-clicking on this table gives access to a context menu that is identical to that of the default table on the main MCnest screen, except that the "Show full table" option is omitted.

Just as with the main Output table, the model user can copy the contents of the full Output table into a spreadsheet. Use the cursor to highlight all or a portion of the contents of the full Output table and copy the contents by simultaneously hitting the Control key and letter "C." In Excel, place the cursor in a destination cell and simultaneously hit the Control key and letter "V" to paste the table.

Figure 15. Sample full table output corresponding to the two simulations described in Section II (Quick Start Guide).

Table 2. Description of column headings from full output table.

Column heading	Description
SPECIES	name of user-selected species
FLEDGLINGS	mean # of fledglings/female/year
F L95	lower 95% confidence limit for FLEDGLINGS
F U95	upper 95% confidence limit for FLEDGLINGS
BROODS	mean # of successful broods/female/year
B L95	lower 95% confidence limit for BROODS
B U95	upper 95% confidence limit for BROODS
NESTS	mean # of nest attempts/female/year
N L95	lower 95% confidence limit for NESTS
N U95	upper 95% confidence limit for NESTS
SUCC.	mean overall nest success rate
S L95	lower 95% confidence limit for SUCC.
S U95	upper 95% confidence limit for SUCC.
APPS	# of pesticide applications/year
APP 1	date of pesticide application #1
RATE 1	rate (lbs/A) of pesticide application #1
AID 1	adult initial dose for pesticide application #1
JID 1	juvenile initial dose for pesticide application #1
APP 2	date of pesticide application #2
RATE 2	rate (lbs/A) of pesticide application #2
AID 2	adult initial dose for pesticide application #2
JID 2	juvenile initial dose for pesticide application #2
APP 3	date of pesticide application #3
RATE 3	rate (lbs/A) of pesticide application #3
AID 3	adult initial dose for pesticide application #3
JID 3	juvenile initial dose for pesticide application #3
APP 4	date of pesticide application #4
RATE 4	rate (lbs/A) of pesticide application #4
AID 4	adult initial dose for pesticide application #4
JID 4	juvenile initial dose for pesticide application #4
APP 5	date of pesticide application #5
RATE 5	rate (lbs/A) of pesticide application #5
AID 5	adult initial dose for pesticide application #5
JID 5	juvenile initial dose for pesticide application #5
crop	user-defined name for crop
pesticide	user-defined name for pesticide

Sort table: This sub-menu choice provides functions to sort the displayed Output table using any of the table headers as sort keys (Figure 16). The sort function does not work while viewing the full table.

Figure 16. Example of the "Sort" window for sorting simulations in the "Output table."

Save table: This sub-menu choice allows the entire table to be saved to disk. For this option the user is prompted to provide a name for the table. The user is responsible for changing the default name of "*.mat" to a unique name with a "mat" suffix. It can later be reopened in MCnest, at which point it would be appended to the bottom of any existing simulations in the Output table.

IV. Systematic description of MCnest controls

The descriptions below contain minimal background and justification for the design of MCnest. For more details about the algorithms underlying MCnest and the technical justification for programming choices, please see the MCnest Technical Manual. MCnest has three basic categories of user controls, drop-down menus, window controls, and the table context menus. Not all windows contain all types of controls. For example, only the main MCnest window has drop-down menus, which are located on the standard menu bar at the top of the window (Figure 18). Similarly, context menus are restricted to the two output tables (one on the main MCnest window, the other a separate window accessed using the context menu for the first). Finally, most MCnest windows include various controls that will be familiar to users of windows driven programs, including push buttons, radio buttons, check boxes, list boxes, and text boxes. Our presentation below is organized by window, beginning with the main MCnest window.

A. *MCnest window*

MCnest opens to its main window upon launching and most of the controls are accessed through this window. The main menu bar contains three choices, "MCnest," "Clear," and "Season."

1. *The MCnest menu*

Of the three drop-down menus on the main MCnest window, the first (MCnest) is by far the most important, giving access to virtually all of the functionality of MCnest (Fig. 17).

Figure 17. The main MCnest window with drop-down menu under the MCnest tab.

Load Species Library: Although MCnest opens up to the default species profile library, the menu option allows the user to select other species profile libraries in the MCnest working directory, if they exist. There are many reasons why a model user may want to create additional species libraries, including: 1) the user may have one or more species of particular interest to their location; 2) the user may want to explore a series of alternative dates for defining the start and end of the egg-laying period related to climate change, regional variation, etc.; or 3) the user may want to test a series of alternative values for other life-history parameters (e.g., changes in the background daily rates of nest failure as a surrogate for habitat quality). With the current version of MCnest we recommend using the default species library; however, if a user wants to create a new species library file, it must use exactly the same column headings and exist in the same directory as the default library. Further details on creating custom species libraries will be provided in a future release.

Life History: Choosing this menu option loads the Life History window. Controls for the Life History page are described in Sub-section B (Life History window), below.

Set Pesticide: Choosing this menu option loads the Pesticides page. Controls for the Pesticides page are described in Sub-section C (Pesticides window), below.

Batch Mode: Choosing this menu option loads the Batch mode page. Controls for the Batch mode page are described in Sub-section D (Batch window), below.

Random Numbers: Choosing this menu option loads the Random Numbers window. Controls for the Random Numbers page are described in Sub-section E (Random Numbers window), below.

Load MCnest file: Choosing this menu option loads the results of previous simulations into the output table on the main MCnest window. Previous results will be appended to the end of any currently displayed results.

Exit: Choosing this option exits MCnest. MCnest can also be closed by clicking the upper right hand corner of the main MCnest window.

2. *The Clear menu*

The Clear menu is used to remove existing results. It has only three choices (Figure 18).

Summary Table: Choosing this menu option clears the summary table from memory and from display. It cannot be recovered unless the data were first saved to a file.

Figures: Choosing this menu option closes all open figures. They can be regenerated by repeating the appropriate commands.

All: Choosing this menu option clears the summary table and closes all open figures. Neither the table nor figures can be recovered unless the data were first saved to file.

Figure 18. The Clear menu.

3. *User-input Controls*

In addition to the menus described above, the main MCnest window also contains a number of user controls. Some of these overlap with or are completely redundant with choices in the drop-down menus. For example, clicking any empty check box within the Batch mode block calls up the Batch mode window, which is also accessed under the Batch mode menu described above. Below we describe all of the controls on the main MCnest window.

Run: The Run button initiates a simulation.

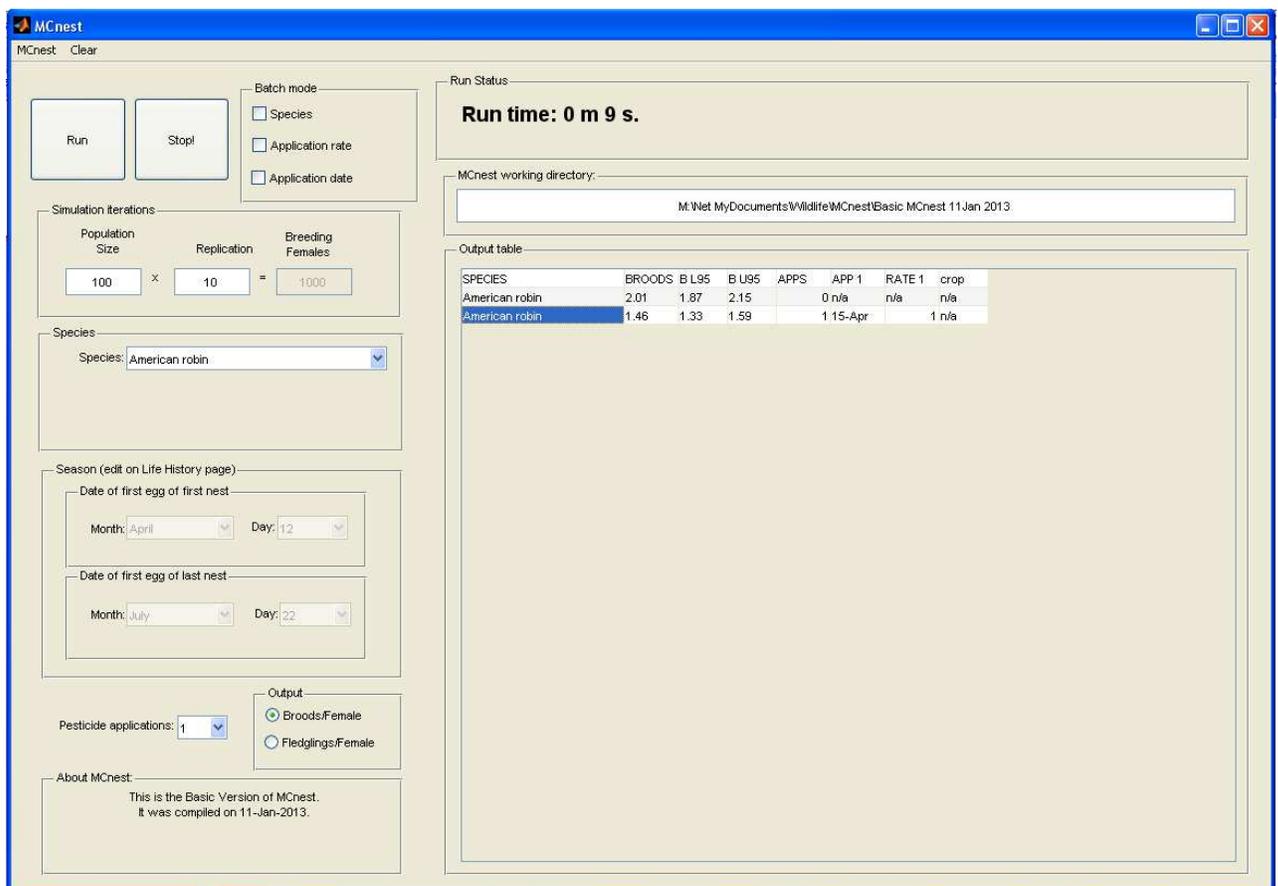Stop! The Stop button terminates a simulation currently in progress.

Batch mode: This form contains three check boxes indicting the three types of batches that can be run. Selecting any unchecked box will load the Batch mode window. This window is described in detail in Sub-section E below and is best understood after covering both the life-history and pesticide information (Sub-sections B and C below).

Simulation Iterations: This form contains two user-input boxes labeled "Population Size" and "Replication," respectively. The "Population Size" box allows the user to enter a population size of females. The default parameter is 100. The second box allows the user to enter a replication parameter describing the number of separate identical populations that will be simulated. The default number of replicates is 10. The total number of breeding females in a simulation is the product of the "Population Size" and "Replication." This structure allows the simulation routine to develop a distribution of population-level outcomes and calculate 95% confidence limits for output parameters.

Output table: This frame contains the outputs of individual runs of MCnest. When no previous runs are available it is empty.

In Figure 19, the Output table shows the results for the two simulations run in Section II with the second row highlighted. The first column gives the species name (American robin). The second column gives the global average (across all populations) of the number of successful broods produced per female (2.02). The third column gives the lower confidence limit for a 95% confidence interval (1.87) constructed around the global average number of successful broods per female. The fourth column gives the associated upper 95% confidence limit (2.18). The confidence limits described above were generated using the replication parameter specified in the second text box in the Simulation Iterations form. Thus, MCnest simulated 10 populations of 100 females (1,000 females in total) and the confidence limits are generated from the variance in the number of successful broods per female across the 10 replicates. Increasing the number of replicates will not, in general, affect the width of this confidence interval. However, the precision with which the confidence limits are estimated will improve with the number of replicates. In contrast, increasing the Population size will reduce the width of the confidence limits.

Figure 19. Main MCnest window showing output of two simulations from Section II (Quick Start Guide).

MCnest working directory: The default working directory for saving or loading MCnest output files is the same as the directory containing the MCnest program files. To aid in file management, the model user can change the working directory to a different directory or a subdirectory within the MCnest directory for saving and retrieving MCnest output files.

To change the MCnest working directory, right-click on the file path name in the box. After a couple of seconds a new Browse for Folder window will pop-up, and the user will be able to navigate through their directories to select a different folder name to use as the working directory until changed. When MCnest is closed and later reopened, the program returns to the default working directory.

Species: The species block contains a listbox menu of species names for which life-history profiles have been pre-coded for use in MCnest. For additional information about the species and their profiles, see the companion document on Avian Life History Profiles for Use in the Markov Chain Nest Productivity Model (MCnest). The default is the first species in the list, i.e., mallard. Specific details about the life history of species in the library, and functions to edit life-history parameters to create new species are found on the Life History window, which can be accessed under the main MCnest menu and is described in Section IV.B (Life History window) below.

Season: The dates of first egg in the first and last nests of the breeding season determine the length of the breeding season. They cannot be edited on this page, but are shown here for reference. They can be changed on the Life History window, described in Section IV.B (Life History window) below.

Pesticide applications: This list box ranges from 0-5. Changing its value to anything but 0 will display the Pesticides window, described in Section IV.C below.

Output: Once simulations have been run, this box allows the user to toggle between the number of successful broods/female/year and the number of fledglings/female/year in the Output table. Results for both metrics are captured in the Log file.

Run Status: This is not an interactive frame. This space is used to provide information to the user on the current run status of the program.

B. The Life History window

The life history window is invoked by selecting the "Life History" option under the "MCnest" menu.

The Life History window has five control frames and a push button for returning to the main MCnest window (Figure 20). The default species profiles contain default parameters for all of the controls on the Life History page. Thus, if you are using default species you need not use this window. If you invoke this window with a default species selected, it will display the default parameters for that species. This can be useful at runtime to see what those values are. However, changing any parameter on this page will automatically change the default species to "-new-." It will also make visible a new box on the main MCnest window for naming the new species. The default name for the new species is "Bird."

Figure 20. The Life History window with default data for American robin.

Season: This frame allows you to change the length of the breeding season, which is defined in MCnest by two dates: the date of the first egg in the first and last nests for the season of each species. Implicit in this definition is the ability of females to complete any nesting attempts initiated by this later date, assuming the nest is not lost due to ecological or pesticide-induced failure.

The end date (date of first egg in last nest) must be at least two weeks later than the beginning date (date of first egg in first nest). If you try to set a breeding season shorter than two weeks, you will get an error and the end date will be automatically set to two weeks after the beginning date.

Diet & body weight (exposure): You do not need to use this frame unless you are simulating pesticide effects. Dietary information is only used to estimate dose, based on residue analyses of different food types. Body weight must be provided in grams. Dietary proportions must be between 0 and 1 for all categories and they must sum to 1 (down columns). Specifying proportions outside the range (0,1) will generate an immediate warning and that category will be set to 0.33. However, specifying sums of dietary categories that do not sum to 1 will not generate errors until you attempt to push "Return to Main Screen."

Transition Probabilities: This frame contains three text boxes for model parameters. The first, p, is the daily probability that a female in the pair formation stage initiates rapid follicle growth for her first egg of her first nest of the season. This parameter applies only to a female's first nest attempt of the year. The second two parameters are the daily probabilities of nest failure in egg-laying and incubation (m_1) and nestling rearing (m_2) periods of the nest cycle. These failure rates should include all causes of failure except for failures arising from the pesticide algorithms in MCnest. Pesticide failures are

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handled separately, as described below in Sub-section C and in the MCnest Technical Manual. All parameters in this frame are restricted to the interval (0,1).
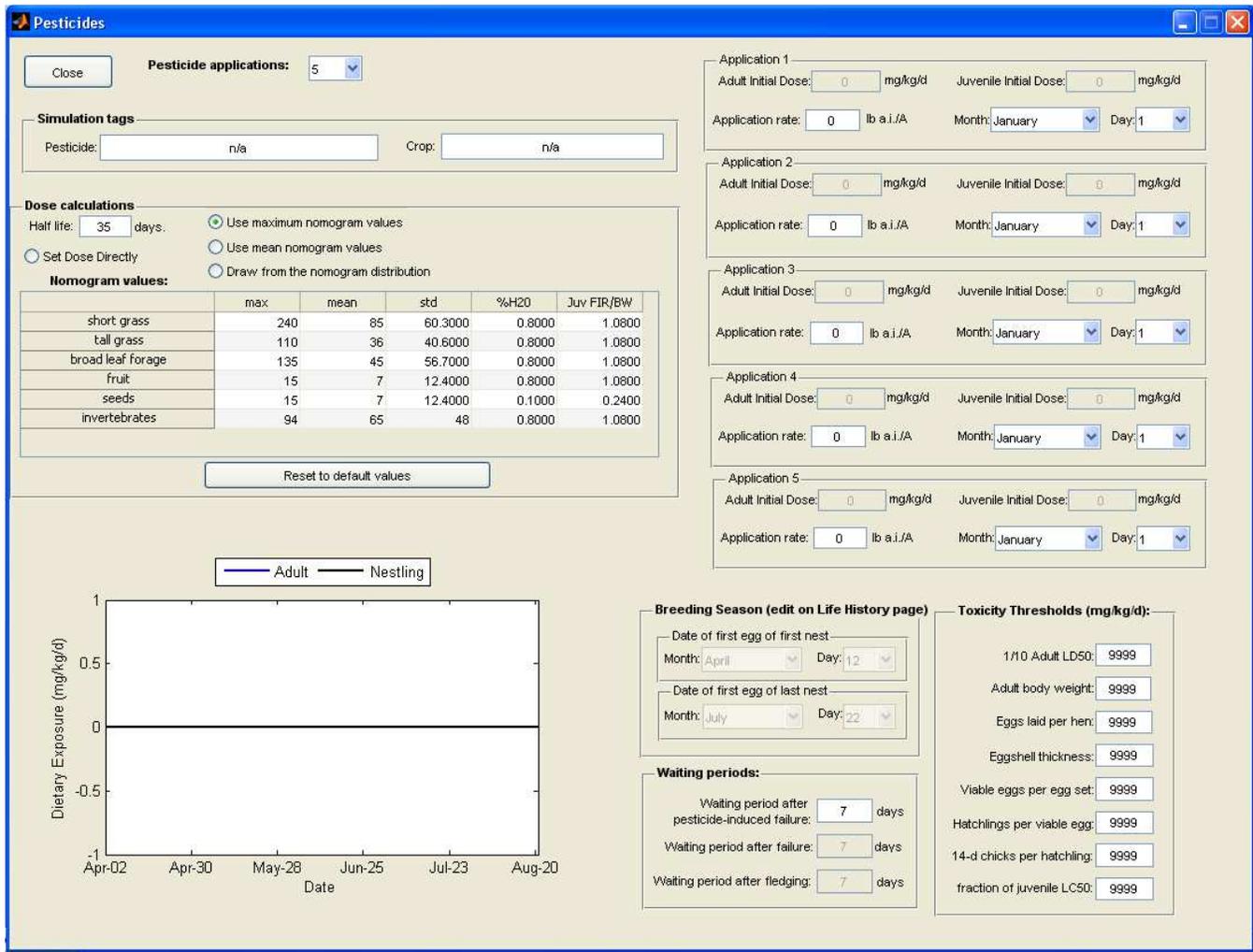
Other Life History: This frame contains two text boxes and two radio buttons. The first text box is for average clutch size, the average number of eggs laid in a nest. It must be an integer. The two radio buttons indicate whether the species generally begins incubation with the penultimate versus the final egg. Whichever button is selected, all females are assumed to follow the same pattern. The second text box is for an estimate of the mean number of fledglings per successful brood. It does not need to be an integer.

Phase Durations (days): This frame contains eight text boxes for entering the phase durations. These durations describe the time required to complete various processes in the nesting cycle and are described in greater detail in the MCnest Technical Manual. With the exception of the rapid follicle growth period and the egg-laying interval, these parameters must be specified as integers.

C. The Pesticides window

The Pesticides window is invoked by selecting the "Set Pesticides" option under the "MCnest" menu. The Pesticides window also will open automatically when the Pesticide applications listbox on the main MCnest window is changed to any value other than 0. The Pesticides window has a list box for indicating the number of pesticide applications, a variable number of control frames (depending on how many applications are specified) and a push button for returning to the main MCnest window (Figure 21).

Figure 21. The default Pesticides window when selecting five pesticide applications.

Simulation tags: This frame contains two text boxes for entering the name of the crop and the pesticide being considered for simulations. These tags are used only as identifiers in MCnest outputs to help the model user describe the pesticide-use scenario for the simulations.

Pesticide applications: This listbox is largely redundant with the Pesticide applications listbox on the main MCnest window. A second copy is placed here mostly for convenience. Choosing "0" will automatically close the Pesticides window. Choosing any other number changes the number of applications frames that are displayed to match the number of applications selected.

Dose calculations: The controls in this frame include four radio buttons and two text boxes. The first text box is for setting the half-life of pesticide residue degradation of food items (expressed in days). The default is 35 days, but the value can be set to any positive value. The radio buttons are mutually exclusive (only one can be checked) and indicate how pesticide application rates are translated into exposure doses. The first choice "Set Dose Directly" allows the user to manually set the adult and juvenile initial dietary dose of the pesticide on application day. The final three choices translate the application rate into estimates of adult and juvenile initial dietary doses at application using the

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approach in OPP's Terrestrial Residue EXposure model or T-REX (USEPA 2012). The T-REX approach is based on the estimated mean or maximum residue concentration on each of the six food type categories immediately following an application of 1 pound active ingredient (AI) per acre. The user can choose "Use maximum nomogram values," "Use mean nomogram values," or "Draw from the nomogram distribution," using a log-normal distribution based on the mean and standard deviation. Residue concentrations on food types are translated into dose using body weight and dietary information on the Life History window. Additional detail is provided about these calculations in the MCnest Technical Manual. Whichever method is used to determine the initial dietary dose on the day of application, subsequent exposure will decay according to the specified half-life. When two or more applications are simulated, it should be noted that the initial dietary dose presented for each application represents only the contribution from that application, and does not add the contribution from earlier applications. The additivity of overlapping decay curves from multiple applications can be observed in the Seasonal Exposure Profile figure, even though this is not reflected in the adult and juvenile initial doses shown for each application.

The second text box, labeled "Nomogram values," lists the default maximum and mean nomogram concentrations on each of the six food type categories used in T-REX Version 1.5, plus the standard deviation. It also includes the default percent moisture value from T-REX and the juvenile food ingestion rate per body weight (i.e., FIR/BW) used in the calculation of juvenile doses, as described in the MCnest Technical Manual. All of these values are editable by the user. If changes are made to any values within the "Dose calculation" box, there is a button to restore all default values.

Toxicity Thresholds: This control frame contains eight text boxes for entering toxicity thresholds (values which, if exceeded by exposure, will trigger nest failure or prevent a bird from initiating a new nest). These values come directly from standard toxicity tests and can be set to any positive value. Toxicity threshold values are expressed as daily dose (mg AI/kg body wt/day), but the surrogate endpoints from the avian reproduction test and the dietary LC50 test are typically reported as dietary concentrations (mg AI/kg food). Dietary concentrations need to be converted to daily doses by the model user for use in MCnest. The conversion from concentration to dose is accomplished using information on body weights and daily food ingestion rates (FIR) from the toxicity test:

$$\text{Daily dose} = \frac{\text{Dietary concentration x FIR (g food/day)}}{\text{Body weight (g)}}.$$

The conversion of dietary concentrations to daily doses is an approximation because body weight and food ingestion rates are changing during the course of both the reproduction test and the LC50 test. Also, studies vary in the degree to which they quantify food spillage during the tests. For converting dietary concentrations to daily doses, the model user can calculate the average daily food ingestion rate per bird and the average body weight at the beginning and end of the test period for each bird. The model user should consult the MCnest Technical Manual for more background information about the selection of toxicity threshold values prior to use.

Breeding Season: The controls in this frame are redundant with the same controls on the Life History window. They are not operational on the Pesticides window, but are provided for reference when setting the dates of pesticide applications.

Waiting Periods: This frame lists the three waiting periods after nest success or failure before the first egg is laid in a subsequent nest. The two lower periods are redundant with waiting periods on the Life History profile for each species. The top period is the "Waiting period after pesticide-induced failure."

The default value for this waiting period is set equal to the "Waiting period after ecological failure" for each species. The waiting period after pesticide failure must be an integer and can be set to a longer duration than for ecological failure, but it cannot be shorter.

Application 1: All of the pesticide application frames operate identically to Application 1. These frames each contain three text boxes and two list boxes. The top two text boxes are for the adult and juvenile initial dose (i.e., the dose on application day), respectively. These boxes are not editable when any of the final three radio buttons in the Dose calculations frame are selected. In this case, initial doses are calculated using the application rate and the dietary information and body weight information for the species selected on the main MCnest page, as described above and in more detail in the MCnest Technical Manual. The Application rate text box can be set to any non-negative value. It will not be visible if the "Set Dose Directly" radio button is selected in the Dose calculations frame, above. Finally, the two listboxes allow the user to specify the date on which the pesticide is applied to the environment for each application (i.e., the day on which the initial doses will be experienced).

The Application frames operate independently from each other, though they all use the same half-life for defining the decay curve. Applications can be specified in any order (e.g., Application 2 could be specified as occurring prior to Application 1), and they need not occur within the window defined by the Breeding Season (i.e., an application may occur prior to or after the breeding season of some species). Regardless of how many pesticide applications are chosen on the Pesticides window, the adult and juvenile initial doses shown for each application are calculated based only on information for that application. However, when more than one application is specified, the decay curves for each application may overlap. Consequently, the estimated initial doses at application for birds in the simulation may reflect the combination of the pesticide residues produced by each application plus any carry-over residues from previous applications. The additivity of overlapping decay curves from multiple applications can be observed in the Seasonal Exposure Profile figure, even though this is not reflected in the adult and juvenile initial doses shown for each application.

Seasonal Exposure Profile: As information on application rate, application date, and residue half-life are modified in the Pesticides window, a Seasonal Exposure Profile will be generated automatically. The model user can use this to visualize the exposure profile during the breeding season of a species and/or to examine the degree of residue carry-over from one application into the next. After a simulation, the Seasonal Exposure Profile also is available by right-clicking on a highlighted simulation in the Output table and under "Row," click "Exposure series."

D. The Batch window

The Batch window is invoked by selecting "Batch Mode" under the main MCnest window, or by selecting any of the Batch mode checkboxes on main MCnest window. This window offers the ability to set up multiple simulations in advance according to three different types of progressions (Figure 22). Depending on which check box is selected, different control forms may be displayed.

Set & Return: This pushbutton closes the Batch window and returns to the main MCnest window.

Parameter to batch: This frame contains three radio buttons for specifying the kind of progression you wish to set up. If you invoke the Batch window by checking one of the boxes under Batch mode on the main MCnest window, that radio button will already be selected. It may be subsequently changed within the Parameter to batch frame.
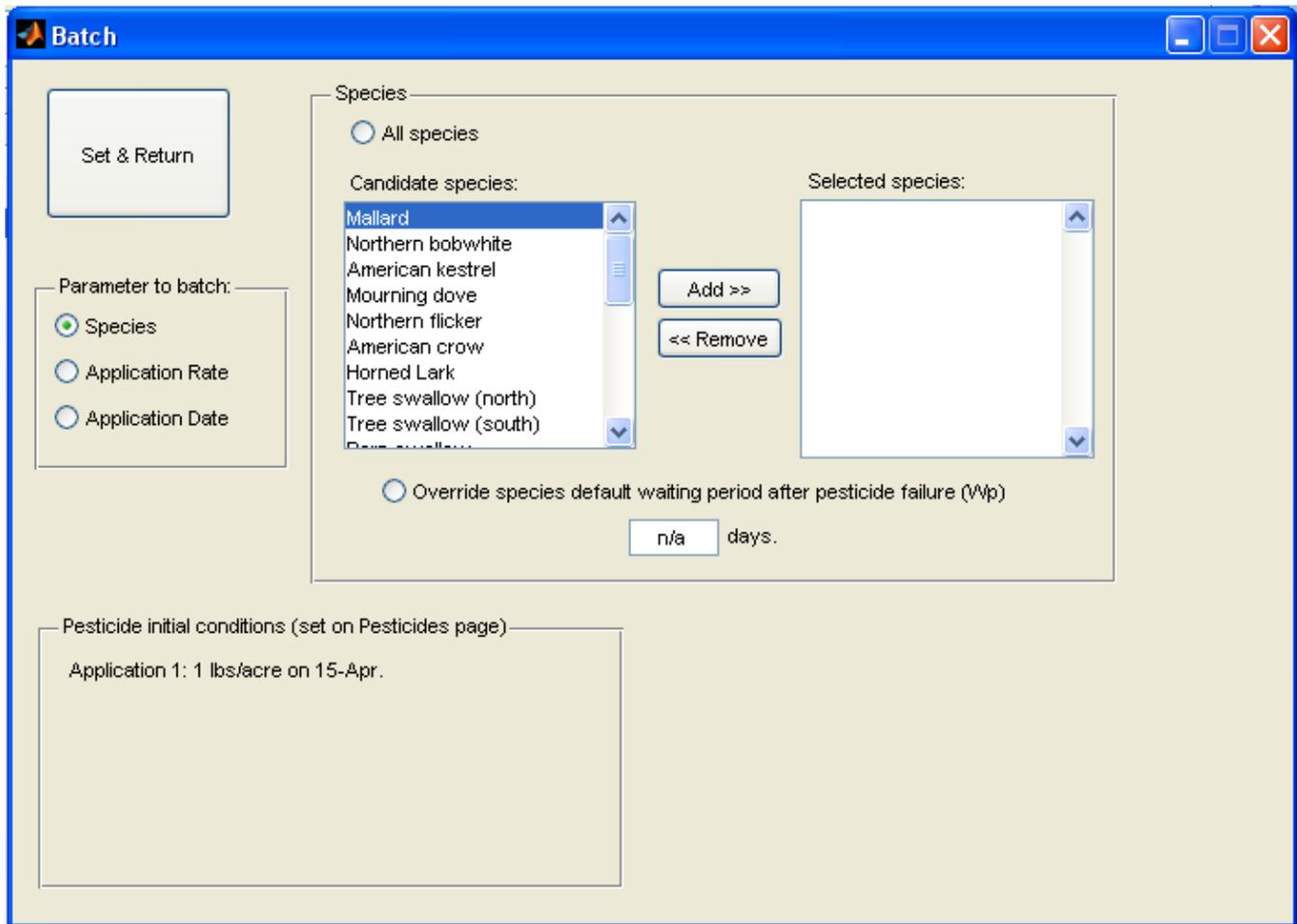
Note that if you select one of the three radio buttons and then hit <u>Set & Return</u> without making any further inputs as to which species, rates, or dates to batch, the MCnest model will not run.

<u>Pesticide initial conditions (set on Pesticides page).</u> This frame is not interactive. It is provided to give details about the initial application conditions and dose parameter set on the **Pesticides** window.

<u>Species:</u> When the "Species" radio button in the <u>Parameter to batch</u> frame is selected, the batch window displays the <u>Species</u> frame (Figure 22). This frame contains two radio buttons, two listboxes, two push buttons, and an edit box. The "All species" radio button moves all species from the "Candidate species" listbox to the "Selected species" listbox. Deselecting the "All species" button will restore them to the "Candidate species" listbox. The "Add" and "Remove" buttons will move selected individual species back and forth between listboxes. When batching on species, separate simulations will be run on the species in the "Selected species" listbox and species in the "Candidate species" listbox will be ignored. Selecting the radio button "Override species default waiting period after pesticide failure (Wp)" will allow you to set a unique value for the waiting period for pesticide failure in the associated edit box. This value will then apply to all species in the designed batch, unless the specified value is shorter than the species' value of We (the waiting period after ecological failure), in which case the value for We will be used for that species.
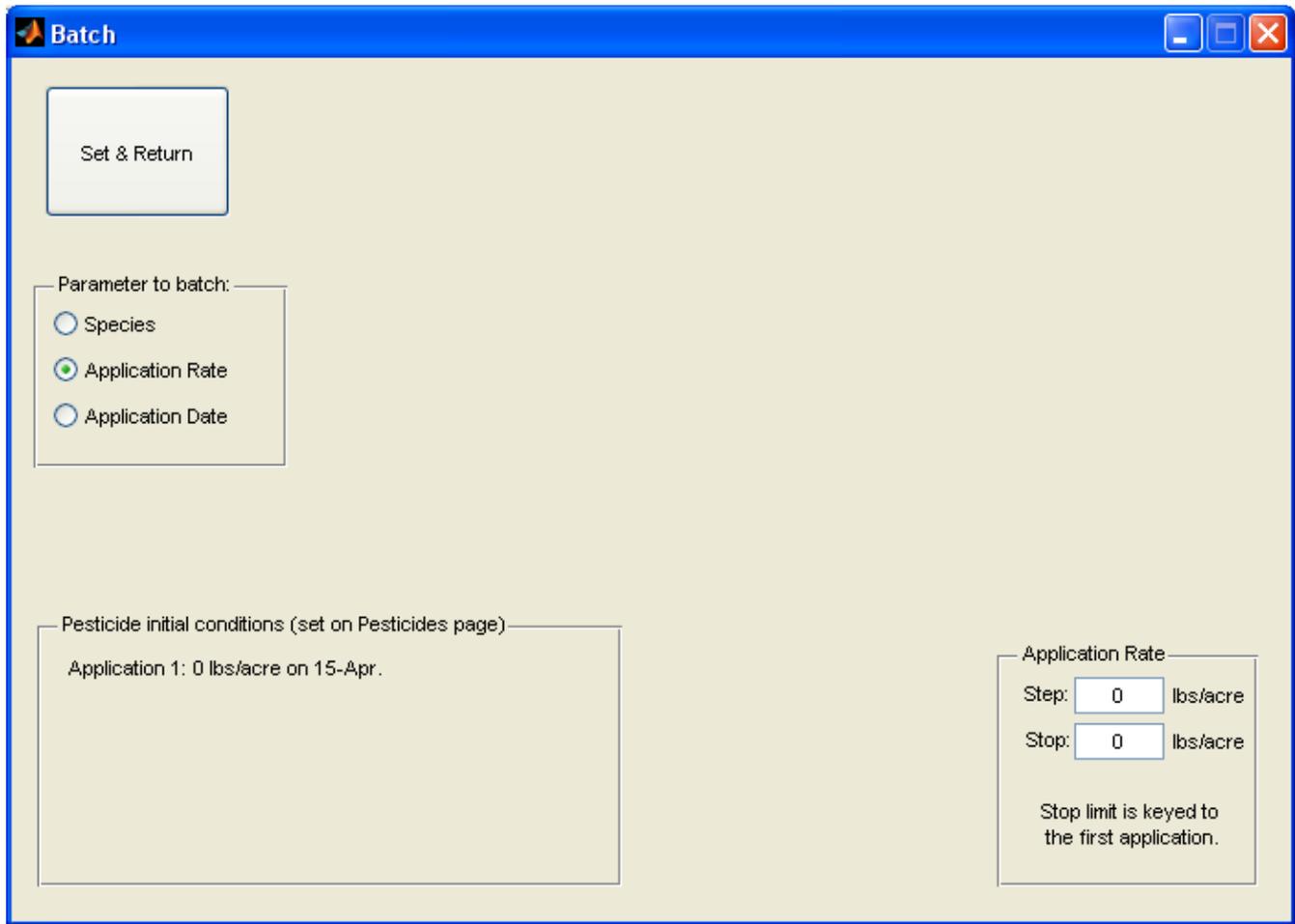
Note that is the "Override species default" radio button has been selected and the model user wishes to return to using the default values for Wp for each of the selected species, the "Override species default" radio button needs to be deselected and then hit <u>Set & Return</u>.

If the "Species" radio button is deselected, all species return to the "Candidate species" listbox. To view or edit the current list of "Selected species," select "Batch Mode" from the "MCnest" drop down menu on the main MCnest window.

Figure 22. The **Batch** window when the "Species" radio button is selected.

Application Rate: When the "Application Rate" radio button in the Parameter to batch frame is selected, the batch window displays the Application Rate frame (Figure 23). This frame contains two text boxes. The "Step" box indicates the incremental increase in application rate that should be run with each simulation. The "Stop" box indicates the maximum application rate desired for the first application. The minimum application rate desired is assumed to be that set on the **Pesticides** window and reported in the Pesticide initial conditions (set on Pesticides page) frame, directly above. With these parameters set, MCnest will run repeated simulations with each application rate, increasing the application rate by "Step" with each simulation until the maximum application rate (less than or equal to "Stop") for application 1 is reached. Thus "Stop" does not need to be an exact maximum; rather it operates as a ceiling beyond which MCnest will not raise the application rate for the first application. If more than one application is simulated, the same step increase applies to all applications, but the "Stop" does not apply to later applications even if they are higher than the ceiling value for the first application. For example, if an initial simulation has two applications with the first being 1 lb/acre and the second is 3 lbs/acre and the batch is set up with a "Step" of 1 and a "Stop" of 3, the last simulation in the batch will use a first and second application of 3 and 5 lbs/acre, respectively.
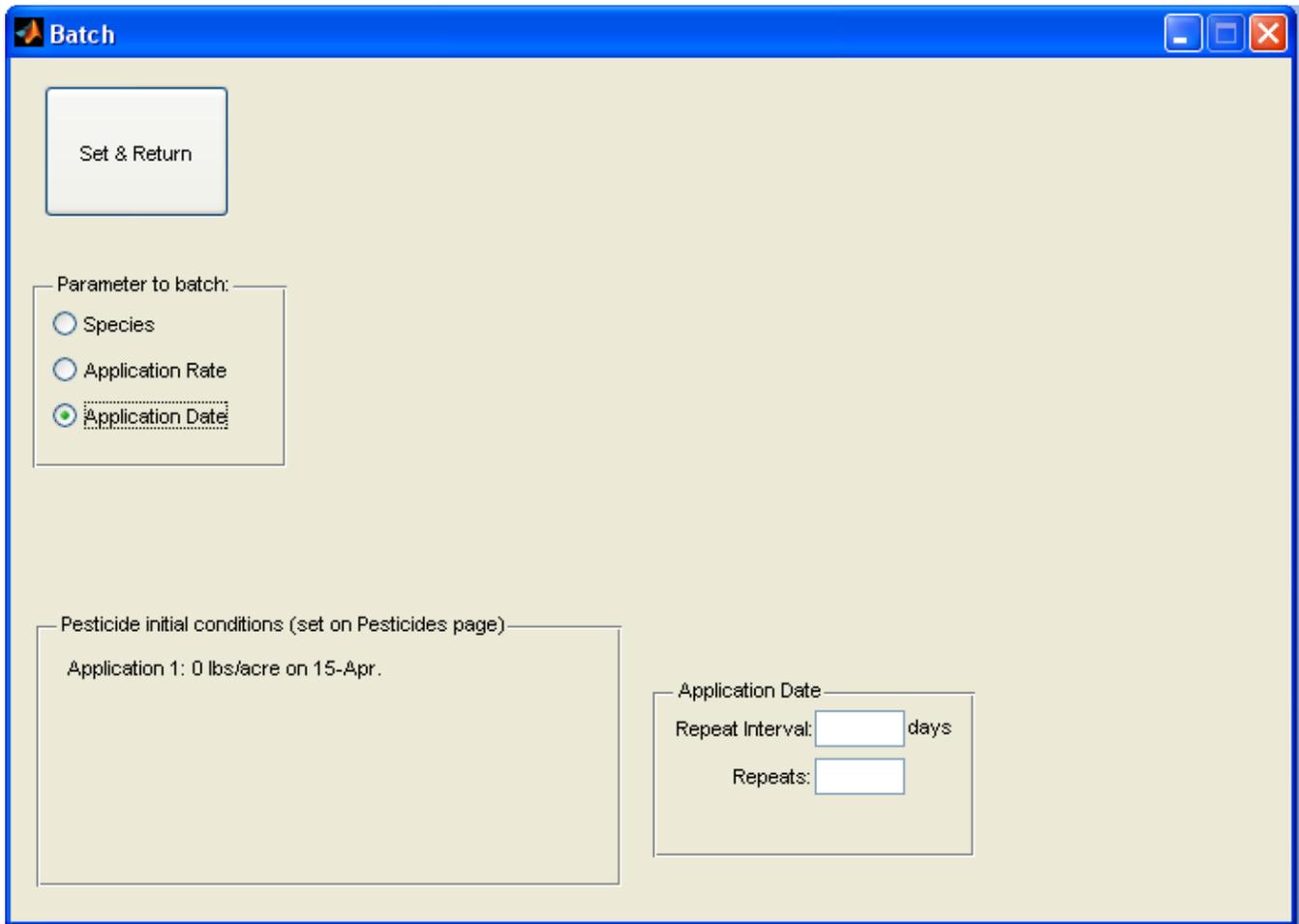
Figure 23. The **Batch** window when the "Application Rate" radio button is selected.

Application Date: When the "Application Date" radio button in the <u>Parameter to batch</u> frame is selected, the batch window displays the <u>Application Date</u> frame (Figure 24). This frame contains two text boxes. The first text box, "Repeat interval" allows the user to specify the desired number of days between applications. The second text box "Repeats" specifies the total number of repeat applications to simulate. Using these two pieces of information, MCnest simulates the first application date according to the date reported for Application 1 in the <u>Pesticide initial conditions (set on Pesticides page)</u> frame. It then repeats the simulation, shifting the application date by "repeat" days each time until the total number of desired repeats is reached.

Note that, although the repeat applications are pegged to Application 1, the same time shift is applied to every pesticide application specified on the **Pesticides** page.

Figure 24. The **Batch** window when the "Application Date" radio button is selected.

E. The Random window

The Random window is invoked by selecting "Random Numbers" under the main MCnest window. This window offers the ability to choose among different types of random number generators and to set the seed for random number generation in MCnest. This may be useful for exactly repeating a particular simulation in MCnest. The Random window contains one push button and a control frame (Figure 25).

Figure 25. The Random window in MCnest, without a seed specified.

Return to Main Screen: This pushbutton returns the user to the main MCnest screen

Random Numbers: This control frame contains a listbox and a text box. The "Random Numbers" listbox allows the user to specify which algorithm is used to generate random numbers in MCnest simulations. There are six choices (Table 3). We have no reason to believe that any of the algorithms below would offer significantly better performance than any other, and we recommend using the MATLAB and MCnest default option called the Mersenne twister (listed as "mt19937ar") based on its computational efficiency (speed). The "Seed" textbox allows the user to specify a numeric seed for the random number generator. This should be a positive number. To exactly duplicate a previous simulation, find the seed value at the bottom of the Log file and enter it on "Random Numbers" listbox. If no seed is specified, MCnest will use the computer's system clock to generate a seed. In this case, the seed is still recorded, so the simulation may be recreated even when no seed was specified.

Table 3. Random number generators available in MATLAB and MCnest[1].

Option	MATLAB description	Reference[2]
mt19937ar	Mersenne twister (default)	Matsumoto and Nishimura 1998
mcg16807	Multiplicative congruential generator	Park and Miller. 1998
mlfg6331_64	Multiplicative lagged Fibonacci generator	Mascagni and Srinivasan 2004
mrg32k3a	Combined multiple recursive generator	L'Ecuyer et al. 2002
shr3cong	Shift-register generator summed with linear congruential generator	Marsaglia 1999
swb2712	Modified subtract with borrow generator	Marsaglia and Zaman 1991

[1]Adapted from MATLAB online documentation (Mathworks 2012).
[2] References:
L'Ecuyer P, Simard R, Chen EJ, Kelton WD. 2002. An Objected-Oriented Random-Number Package with Many Long Streams and Substreams. Operations Research 50:1073–1075. 2002.
Marsaglia G. 1999. Random numbers for C: The END? Usenet posting to sci.stat.math. Available online at: http://groups.google.com/group/sci.crypt/browse_thread/thread/ca8682a4658a124d/
Marsaglia G, Zaman A. 1991. A new class of random number generators. Annals of Applied Probability 1:462–480.
Mascagni, M, Srinivasan A. 2004. Parameterizing Parallel Multiplicative Lagged-Fibonacci Generators. Parallel Computing. 30:899–916.
Matsumoto M, Nishimura T. 1998. Mersenne Twister: A 623-dimensionally equidistributed uniform pseudorandom number generator. ACM Transactions on Modeling and Computer Simulation 8:3-30.
Park SK, Miller KM. 1998. Random Number Generators: Good Ones Are Hard to Find. Communications of the ACM 31:1192–1201.

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Bennett RS, Dewhurst IC, Fairbrother A, Hart ADM, Hooper MJ, Leopold A, Mineau P, Mortensen SR, Shore RF, Springer TA. 2005. A new interpretation of avian and mammalian reproduction toxicity test data in ecological risk assessment. *Ecotoxicology* 14: 801-815.

Bennett RS, Etterson MA. 2007. Incorporating results of avian toxicity tests into a model of annual reproductive success. *Integrated Environmental Assessment and Management* 3: 498-507.

Etterson MA, Bennett RS, Kershner EL, Walk JW. 2009. Markov chain estimation of avian seasonal fecundity. *Ecological Applications* 19: 622-630.

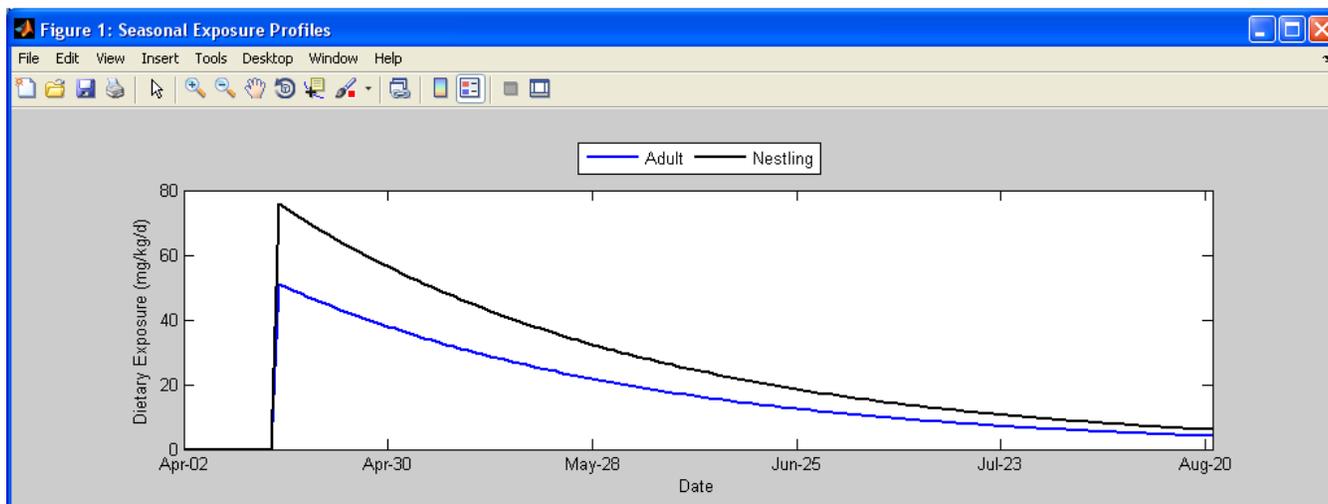MathWorks 2012. Matlab v2012a. MathWorks, Inc. Natick, MA, USA.

[USEPA] U.S. Environmental Protection Agency. 2012. User's Guide T-REX Version 1.5 (Terrestrial Residue EXposure model). Office of Pesticide Programs. Washington, DC, USA. (http://www.epa.gov/oppefed1/models/terrestrial/trex/t_rex_user_guide.htm#app_a   Last accessed 11 January 2013).

Appendix A. Additional Guidance for Interpreting Phase Diagrams

In this appendix, we provide additional guidance for the interpretation of information in the phase diagrams. As mentioned above, the phase diagram shows the proportion of females in each of the 11 phases (defined in Table 1 above) on each day of the breeding season. The phase diagram is a visual tool for understanding how the simulated pesticide exposure is projected to affect the breeding season of the species of interest. It is especially useful as a comparative tool to compare one pesticide-use scenario with a no-pesticide scenario or alternative pesticide-use scenario. Rather than following the progression of each female through various breeding phases during a season, the phase diagram shows the proportion of the females of the population in each phase with the phases stacked on each other. The outcomes may not be immediately obvious, so this appendix will describe further some of the patterns commonly observed in MCnest simulations.

All phase diagrams below are generated using the exposure scenario outlined in the Quick Start Guide (Section II above) with 1 lb AI/acre applied on 15 April with a half-life of 35 days (Figure A1, equivalent to Figure 11 above). In addition, Table 2, which provides a key to the phases of the nest cycle modeled in MCnest, may prove useful as a reference for the following material.
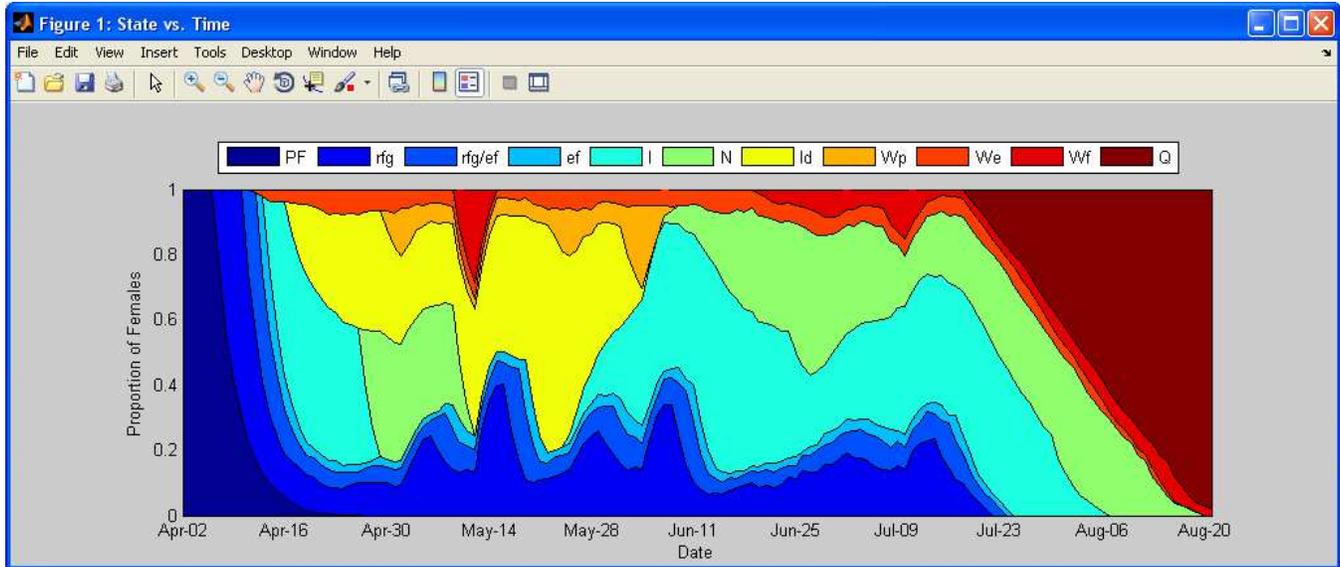
Figure A1. Example Seasonal Exposure Profile for American robins with pesticide parameters set as described in the pesticide example of Section II (Quick Start Guide).

In Section III.B above, the simulation of a pesticide with primary effects on egg hatchability (Figure A2) illustrated that during the period when exposure levels exceed the toxicity threshold for effects on hatchability, clutches of eggs are completed and incubated but are doomed to fail (i.e., doomed incubation), but the nest failure occurs at the point when the eggs should have hatched. In this figure, the pesticide application occurs several days after the first clutches are initiated so the earliest nest attempts are not affected by the exposure and result in the pulse of successful nests observed at approximately May 10. During May and early June there are small pulses of new nest attempts such as the pulse near May 14 following the first successful nests and the pulse in early June after a pulse of failed nests. The pulse of new nests around May 14 occurs when exposure levels remain higher than the toxicity threshold, so as soon as those clutches are completed, they enter the doomed incubation phase. It is not until late May that exposure levels have dropped below the toxicity threshold so that nest
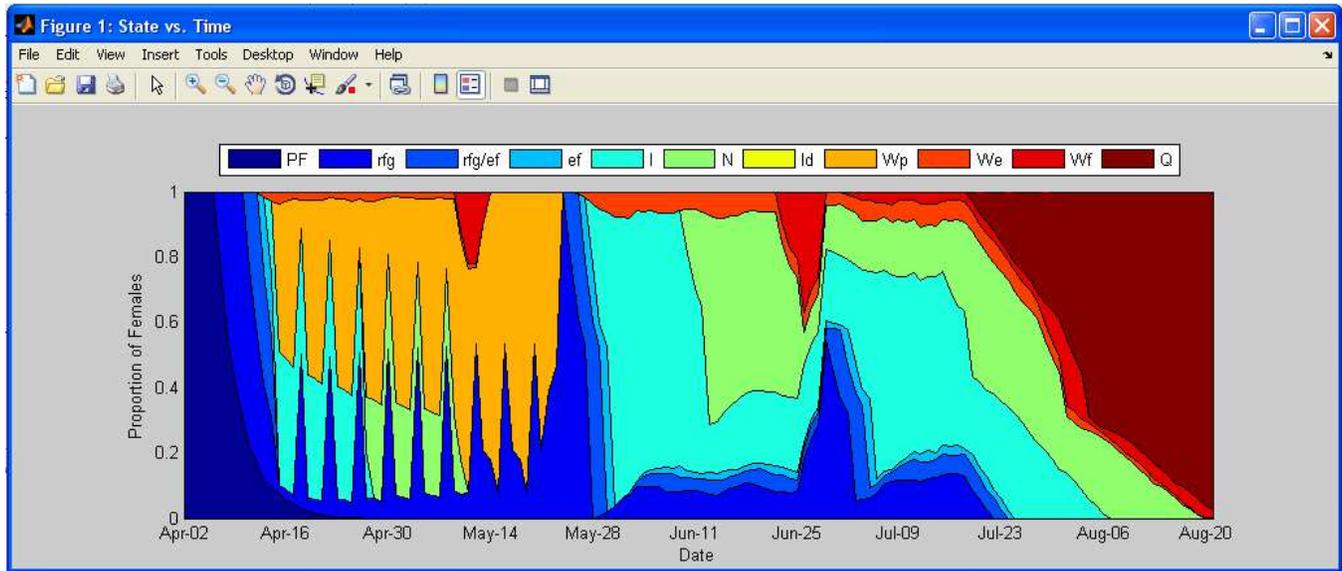
attempts proceed. It is important to remember that this simulation was run using the maximum nomogram value, so the exposure dose on each day was the same for every female in the population.

Figure A2. Phase diagram for a simulation with a short period of effects on egg hatchability (Same as Figure 9 above).

If the pesticide-use scenario is changed so that the effect of the pesticide is on egg production instead of hatchability (with the same toxicity threshold of 25 mg/kg/d), the overall model outcome is similar to that observed for the simulation in Figure A2. However, the phase diagram changes significantly (Figure A3). Immediately after the application on April 14, nest attempts that are still in egg production immediately fail and transition to the orange waiting period after pesticide failure. At the point in the waiting period after pesticide failure where the female would return to the rapid follicle growth phase, if the exposure level still exceeds the toxicity threshold for egg production, the female returns immediately to the waiting period after pesticide failure. The transition into the rapid follicle growth phase and immediate transition back into the waiting period gives pesticides that primarily produce effects on egg production this characteristic jagged profile. As in Figure A1, many females completed their first clutch prior to the April 15 application and were not affected, with a pulse of successful nests about May 10. However, as females finish the waiting period after a successful nest, the exposure level is still higher than the toxicity threshold, so all new nest attempts fail until about the end of May when exposure drops to a level that no longer causes failures.
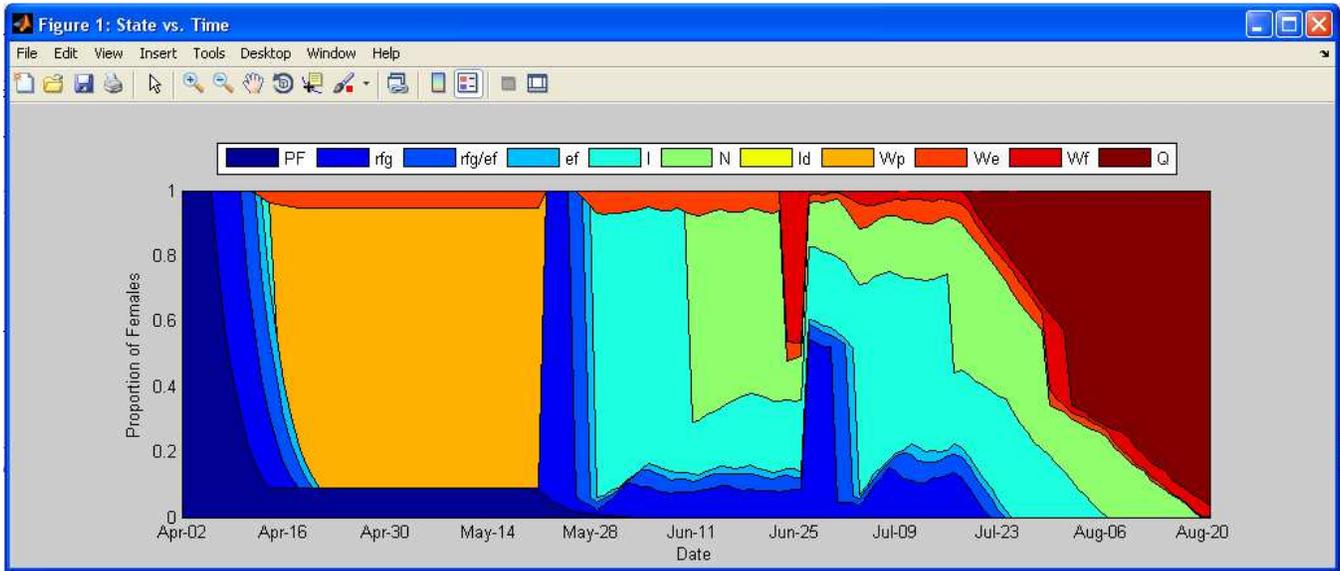
Figure A3. Phase diagram for a simulation with a period of effects on adult behavior.

If the pesticide-use scenario is changed so that the effect of the pesticide is on adult behavior (i.e., a change in prelaying adult body weight) instead of hatchability, the overall model outcome is similar to that observed for the simulation in Figure A2; however, the phase diagram changes significantly (Figure A4). Immediately after the application on April 14, all active nest attempts fail in or after entering the incubation phase and transition to the orange waiting period after pesticide failure. They remain there until exposure levels drop below the toxicity threshold values for adult behavior surrogate endpoints. Females from the few nests that had already failed due to other ecological causes remain in the waiting period after ecological failure, and approximately 10% of the females that had not initiated their first nest attempt remain in the pair formation phase. Once the exposure level drops below the toxicity threshold for adult behaviors, nearly all females initiate a new nest attempt. This level of synchronicity in the timing of renesting is undoubtedly very simplistic, but there are no data on which to base a more realistic pattern for the onset of renesting after a pesticide-related failure.

Since the waiting periods after success and failure are defined as the number of days until the first egg is laid in the next nest attempt, this overlaps with the rapid follicle growth phase. In the phase diagrams, when females are in this overlap period they are shown in the phase diagram as being in the rapid follicle growth phase (rfg), which has the effect of making the waiting periods seem shorter than is indicated in the life history profile. For example, the waiting period after success for the American robin is 7 d and the rapid follicle growth period is 4 d, so the pulse of successful nests at about June 25 shows females in the waiting period for only 3d rather than 7 before transitioning to rapid follicle growth.

Figure A4. Phase diagram for a simulation with a period of effects on adult behavior.

If the pesticide-use scenario is changed so that the effects of the pesticide are on juvenile survival via direct dietary exposure (i.e., fraction of the LC50), the exposure level for juveniles is high enough to result in nest failure for all nest attempts until early June (Figure A5). Because the exposure period for this surrogate endpoint is based on a 5-d time-weighted average, nest attempts fail after nestlings have five full days of exposure.

Figure A5. Phase diagram for a simulation with a period of effects on juvenile survival from direct dietary exposure.