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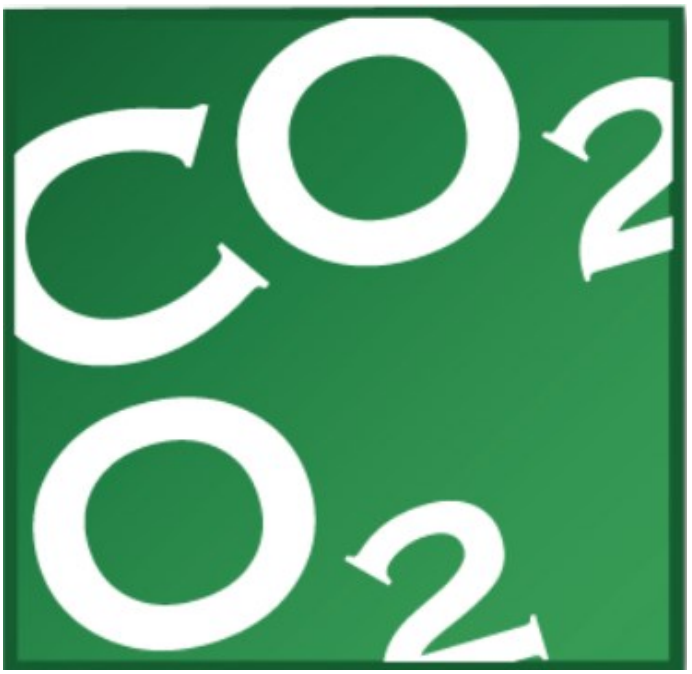

USER MANUAL

METABOLISM 3.0.01

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1. INTRODUCTION

METABOLISM is a software platform that is paired with our Oxylet system. This modular system allows recording and analyzing O2/CO2, food/liquid consumption, spontaneous activity, including an option for rearing, and treadmill exercise in rodents.

METABOLISM 3.0 software is the latest development in the evolution of the chain of components of the PANLAB FOOD and DRINK and PHYSIOCAGE system.

METABOLISM allows the extraction of the data obtained from these Panlab devices as well as the calculation of important parameters for physiological studies.

A combined evaluation of the respiration metabolism, food/drink intake, spontaneous activity and rearing is possible through the use of this user-friendly software.

The METABOLISM software platform offers three different software modules:

- METAOXY, for O2/CO2 metabolism studies involving either our Oxylet Home Cages or Single Lane Airtight Treadmills.
- METAINT, for food/liquid intake studies.
- METAACT, for activity studies (including rearing).

Data can be analysed using different time intervals for calculation. The analysed data table can be saved in Excel or CSV format for further analysis.

1.1. What's New in METABOLISM 3.0?

METABOLISM 3.0 has the most user-friendly interface ever! The new Launch Assistant and Experiment Assistant bar will guide users through the experimental steps.

The new Subjects-Database has been restructured and follows the logical working procedure of an experiment. (see chapter 5.1).

The new Protocol Organizer tool allows users to edit protocols and apply changes during data acquisition (see chapter 5.2).

METABOLISM 3.0 allows easy importation and exportation of protocols between different experimental files.

Now, users can store acquired data in a single experiment file, together with subjects' information and protocols.

Software is provided via USB flash key.

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2. INSTALLATION OVERVIEW

Before installing METABOLISM 3.0, the user MUST have administrative rights on the PC or laptop in which the software or device is to be installed. Contact your IT department in order to confirm **before** initiating the installation procedure.

2.1. Requirements

METABOLISM is very basic and easy-to-use, however, the processing of the data generated consumes a large number of computer-resources. It is important that the computer meets the minimum recommended requirements.

METABOLISM 3.0 software requires:

- A fully compatible computer with a minimum:
  - 3 GHz Pentium® processor (Celeron/Turion-based processor cannot be used).
  - 2 GB of RAM.
  - 150 MB of free hard disk space.
  - Graphics: 1024x768 pixels and 32-bit color depth.
  - One available USB port for the USB flash key.
- Connection interface:
  - For all equipment except for the Touchscreen Treadmill
    - One free USB port for the RS-232-USB converter for interfacing the hardware to the computer.
  - For the Touchscreen Treadmill
    - 1 free USB port.
- Operating system supported:
  - Microsoft® Windows® 11 64 bits
  - Microsoft® Windows® 10 32/64 bits
- External Software needed:
  - Microsoft Excel ®

WARNING: If Microsoft Excel® is not available, some analysis reports cannot be generated and an "Excel/Word OLE Server not found" error will be generated. Please contact your IT staff in order to install Microsoft Excel ® before analyzing data.

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2.2. Installation steps

The steps to follow for the installation of the software associated drivers are different depending on the equipment used and Windows operative system. Here a summary of the steps to follow for each case please refers to the corresponding chapter indicated in each step:

- Physiocage and Standard Treadmill
    1. Check that the control unit or the USB-adapter are **not** connected to the computer (very important!).
    2. Install the USB-adapter (see chapter 2.4).
    3. Install the software (See chapter 2.3).
- Touchscreen Treadmill
    1. Check that the control unit is **not** connected to the computer (very important!).
    2. Install the software (See chapter 2.3).

2.3. Software Installation

METABOLISM 3.0 software is provided on a USB flash key, which contains the application and license for use.

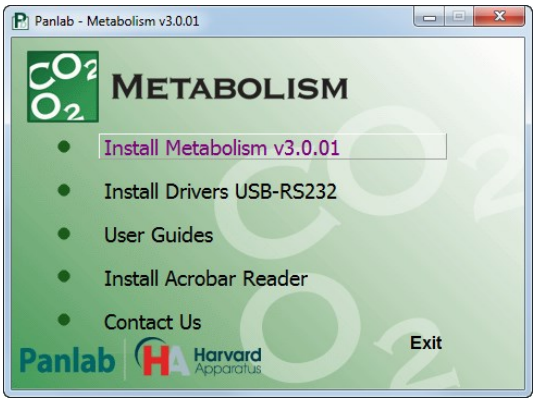
The USB flash drive contains the software installation tool, User Manual in PDF format and other components required to work in specific conditions.

IMPORTANT NOTE: Install the software provided with your devices before connecting the device to your computer.

For Windows ® operating system security reasons, administrative rights are required to install the software and other components. Please contact your IT staff before installing the software.

To install the software:

- Plug the USB flash drive in a USB port of your computer and wait until Windows® installs it as a new removable drive.
- Access the new removable drive detected and execute the PANLAB.EXE file. The following window will be displayed:



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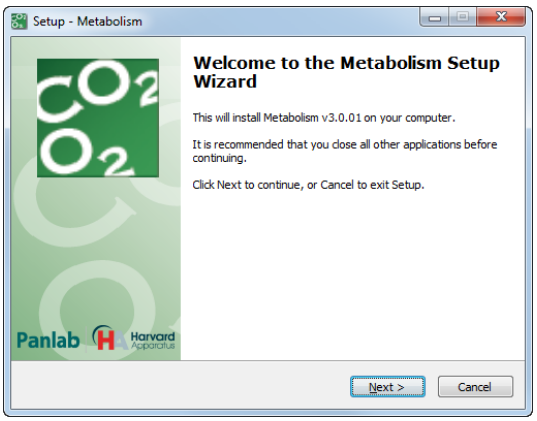
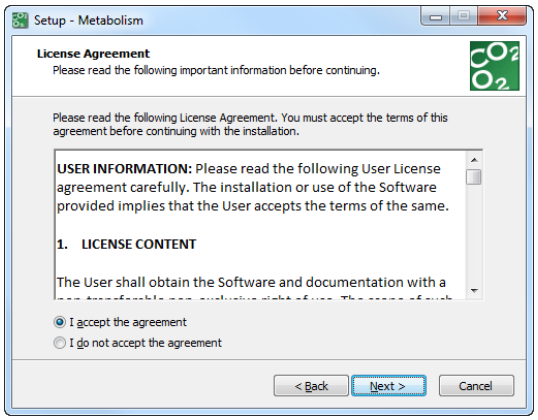
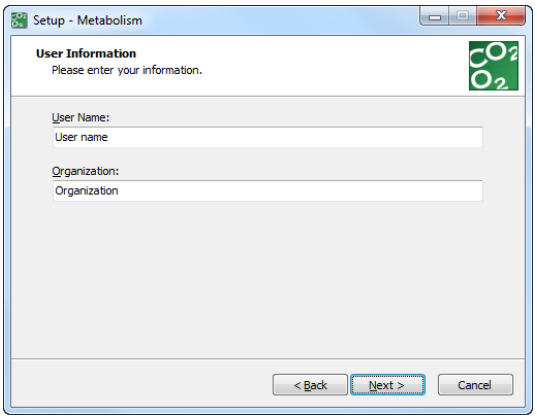
- Select the option [Install METABOLISM v3.0.01] from the main screen assistant.
- An installation wizard will appear. Press the [Next] button to start the software installation.



- Read the **License Agreement** and check the **"I accept the terms of the license agreement"** option to continue the installation of METABOLISM. Then press the [Next] button to start the installation.



- In the next window, introduce the name of the user and the company in the correct field. Press [Next] button to continue.



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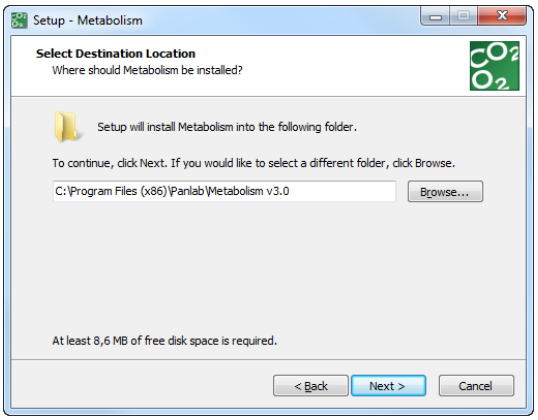
- During the installation, the software is installed in a new folder called [Panlab\METABOLISM 3.0] created under the Programs Files (x86) folder. If desired, the installation program allows choosing another folder to locate the software. The location of the software is independent of the data folder, which is defined by the user using the "Options" feature in the program.



- Press the buttons [Next] and [Install] following the Install Wizard until reaching the [Finish] button.
- The installation is complete when the Finish installation screen is shown. At this point press the Finish button to close the installation wizard.

A new shortcut to Metabolism v3.0 will appear on the Windows ® desktop. At this point, the Metabolism software is completely installed.

2.3.1. USB flash license key management

The following points must be taken into consideration regarding the use of the USB flash license key in order to enable data acquisition, protocol configuration, and session analysis properly:

- Data acquisition (session registering) from connected hardware can only be performed when the USB flash license key plugged in to the computer running the METABOLISM 3.0 software.
- Protocol configuration, protocol validation, and data analysis (data report generation) does not require the license key and can be performed on as many computers as needed by the user. That is to say, the experimental file registered from the computer used for data acquisition can be copied and opened on another computer for protocol configuration, protocol validation and data analysis. To install METABOLISM on another computer and use it for all of these functions, the following is required:
  - METABOLISM software must be installed on the computer used for analysis.
  - The USB flash license key must be plugged into the computer being used for analysis only the first time METABOLISM is being used. From that point, METABOLISM will not require the USB flash key in order to analyze data.

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2.4. CONRS232USB-HS converter (high speed mode)

This step is not needed for the Touchscreen Rotarod, Touchscreen Treadmill and Incapacitance Test devices.

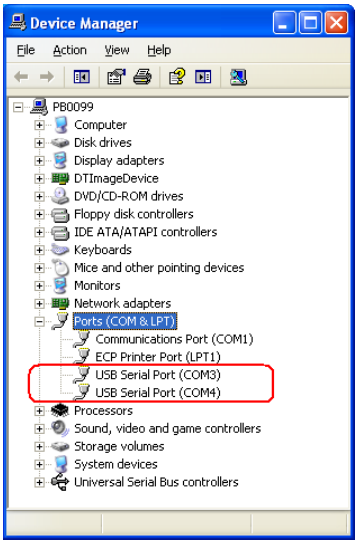
SEDACOM requires the use of the high-speed converter from RS232 port to USB port. A USB – Serial adapter will allow you to set 2 serial ports in your PC or laptop.

IMPORTANT NOTE: do not use a direct connection between the device and the computer RS232 serial port (if any).

We recommend the use of a specific model of converter. We cannot guarantee the correct functioning of the system with any other USB-serial converter. The converter includes an extension cable just in case.

To Install the converter:

- Connect the converter to the computer.
- Windows 8, 10 and 11 will automatically install the drivers.
- If working with Windows 7 or previous, please refer to the notice provided in the box of the converter.
- Once connected and installed, two serial ports will appear in the [Device Manager] window on the Windows Operative System. Usually, the numbers assigned by Windows are sequential.



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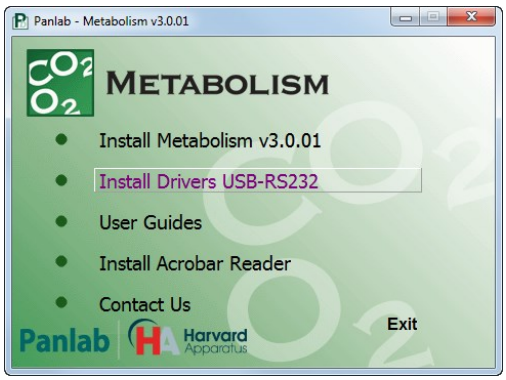
2.5. CONRS232USB (blue) converter (Legacy mode)

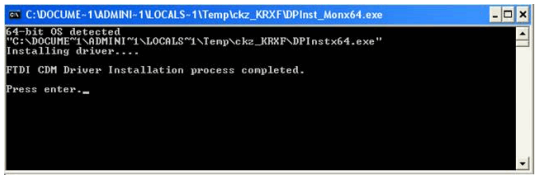
The blue RS232/converter was included in older METABOLISM software packages.

METABOLISM is still compatible with the use of the system in a Legacy mode (not high-speed). In case you need to re-install this device, please follow the below procedure:

- Administrator privileges are required to install any new drivers. Please contact your IT staff in order to clarify this issue before installing the device.
- The drivers should be installed prior to hardware installation. Do not connect the blue adapter to the USB port of your computer before you finish driver installation.
- Insert the METABOLISM software USB flash key into a free USB port of your computer and access its content.
    - If your PC is running Windows XP, a manual installation is required: go to folder Files\USBCom and execute file USBCom-CDM_20824.exe.
    - Otherwise, for the rest of Windows versions, execute the installation assistant (Panlab.exe). The following installation window will be shown. Press the [Install Drivers USB-RS232] option to start the software installation process.



- The USB COM installation program will auto-detect the OS type and install the driver automatically. In some operating systems, a dialog box may appear requesting you press [ENTER] to end the installation.



- After the message "FTDI CDM Driver installation process completed" appears, press [Enter] to complete the driver installation.

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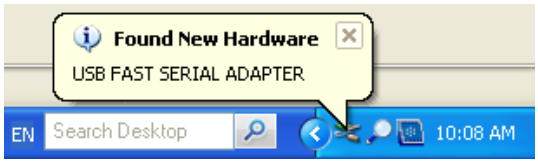
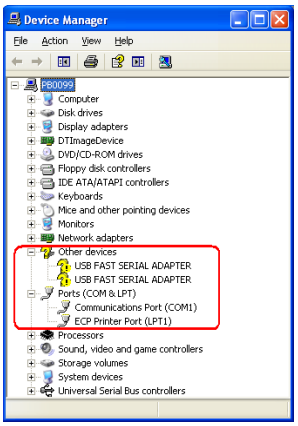
- Plug the blue adapter in any USB port of your computer. Windows will finish installing the driver files.

- In the lower right corner of the screen the next message will be automatically shown:



- At the same time, two devices will appear in [Device Manager]. The ports provided by the new [USB FAST SERIAL ADAPTER] will be shown under [Other devices] with a warning sign attached.



- Please wait while the wizard locates the previously installed drivers. This process may require some time, depending on the computer.

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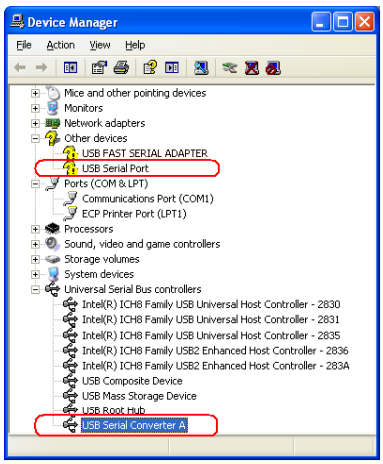
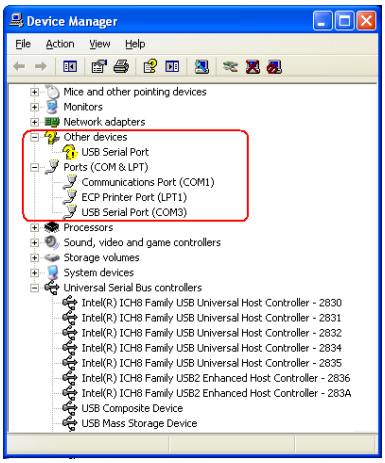
- The process of activating the device (that is, when the PC or laptop recognizes the new serial port), is done one at a time.

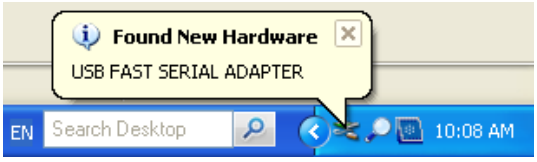


- The following image illustrates how the ports are assigned after they are activated.



- When the wizard is finished, the user will be prompted to press the [FINISH] button.

IMPORTANT NOTE: Until now, only one serial port has been correctly installed. The process must be repeated for the second port on the blue USB adapter to be installed. Please wait while your PC or laptop finds another COM port. Once again, the next message will appear in the lower corner of the screen:



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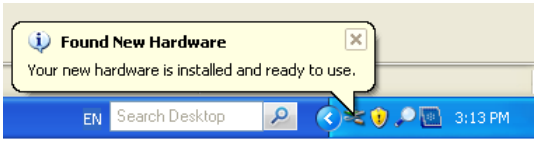
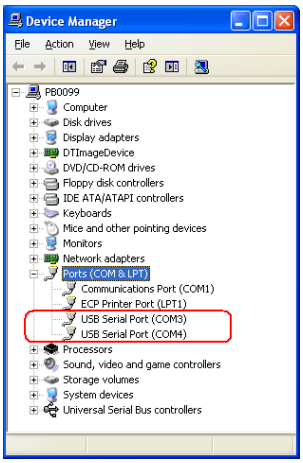
- The adapter will be correctly installed when all previous steps have been repeated. Finally, the message below will appear in the lower right corner of your screen.



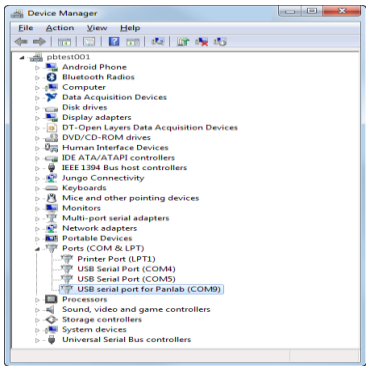
- At this time, two serial ports will appear in [Device Manager]. Usually, the numbers assigned by the system are sequential.



NOTE: A yellow label with the text [Port 1] is attached to the adapter device to identify the first port recognized for the computer system. Therefore, if [Device Manager] displays two ports (COM3 and COM4), then the port label [Port 1] corresponds to COM3.

2.6. Installing the Treadmill Touchscreen

In order to install the Treadmill Touchscreen device, ensure you have already installed the Metabolism software as explained in chapter 2.3.



Then you can just plug the device using the USB cable in a free USB 2.0 port and let the operating system to install the recognized device.

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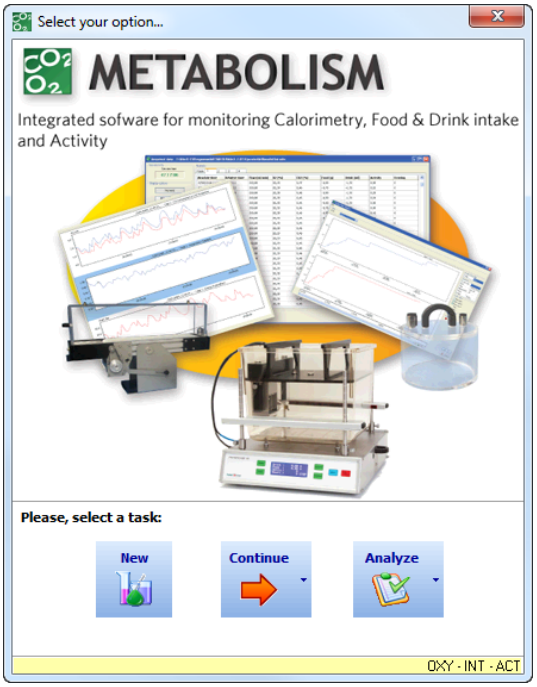
3. STARTING WITH METABOLISM

3.1. METABOLISM Launch Assistant

Each time METABOLISM is launched, the Starting Assistant is automatically initiated to help the Experimenter with:

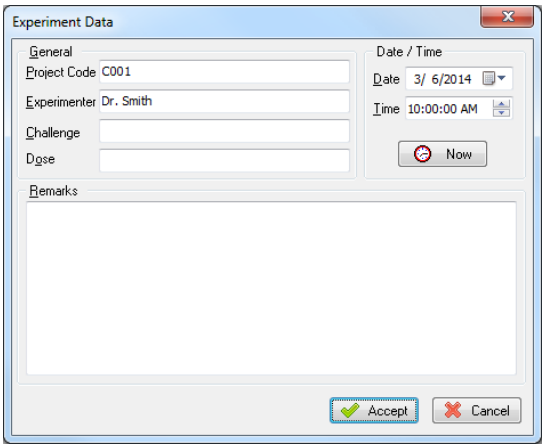
- Creating a new experiment.
- Continuing a previously saved experiment.
- Analyzing the data acquired in a previously saved experiment.



3.1.1. Creating a new experiment file

To create a new experiment file:

- Select **New** task
- Choose a name and location for the new experiment file
- Enter details in **Experiment Data** dialog



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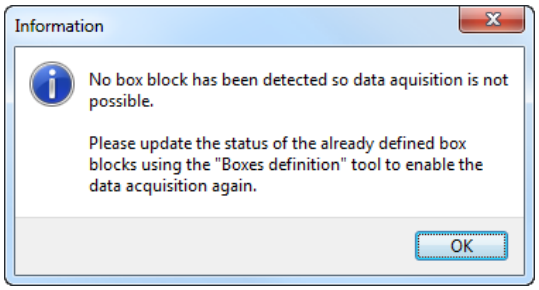
This panel can be completed at a later time by selecting the [Experiment data…] option of the [Configuration] menu.

- Press the **Accept** button.

3.1.2. Continuing an Experiment

To continue with already existing experiment files:

- Select **Continue** task.
- Locate and select the desired folder and experiment file.
- Press the **Open** button to load the experiment file.
- If there are no box blocks online (see section 4.1.2.3) the acquisition cannot start, the system will then warn the user showing this message box:



The system will redirect the user to the "Boxes definition" task, by opening the "Boxes definition" dialog box.

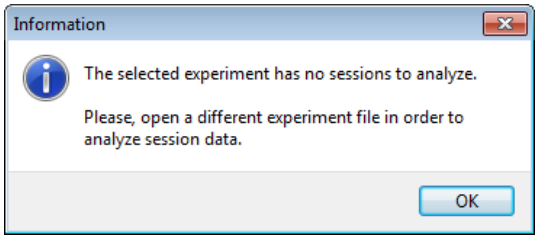
3.1.3. Analyzing an Experiment

To analyze the session contained in an existing experiment file:

- Select **Analyze** task.
- Locate and select the desired folder and experiment file.
- Press the **Open** button to load the experiment file.

The application will open the "Session explorer" section.

If the selected experiment file has no data to analyze, the system will warn the user displaying the message below.



The Analysis task will not be selectable. The experiment assistant bar will remain without any task selected.

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3.2. METABOLISM Main Window

Once a new experiment is initiated, the main window of METABOLISM is shown with the following elements:

3.2.1. Title Bar

The title bar shows the name of the application and the name of the current experiment file.

3.2.2. Main Menu Bar

The main menu bar contains all of the functions that are available within the application arranged in the following sections:

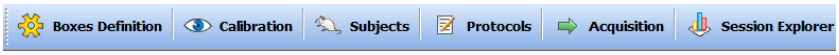
File: managing (opening, saving, etc.) experiment files.

Configuration: adjusting the parameters as needed for the session execution.

Window: managing (tile, cascade, show) child windows.

Help: obtaining on-line support and licensing information.

3.2.3. Experiment Assistant Bar



The **Experiment Assistant bar** provides the user with a means to quickly access the main experimental operation. Each button in the bar corresponds to an operation.

This bar will have only the permitted operations active.

The main operations and the suggested order in which these operations should be executed are as follows:

1		Detect and define the boxes included in the experimental configuration
2		Define the calibration
3		Define the experiment subjects and groups database
4		Define the experiment protocol
5		Execute and register experiment sessions
6		Obtain analysis reports of finished sessions

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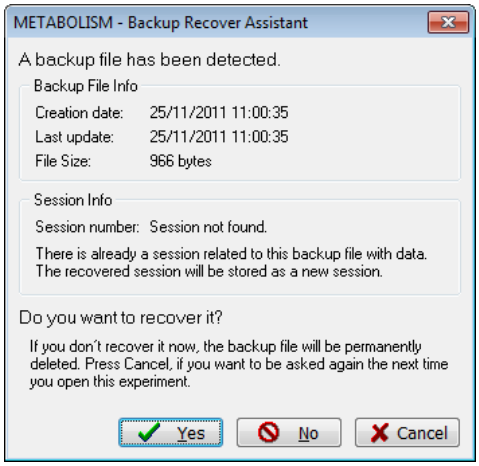
3.3. Automatic Backup System

The METABOLISM software has an automatic backup system. During acquisition, the data are recorded directly to the hard disk; therefore, if the system fails for any reason, the data recorded up to that moment will be stored in a temporary backup file.

When the software is restarted after a system failure, the backup recover assistant displays the information regarding the backup file and asks the user how the file should be handled. This provides the user a chance to retrieve the file.



Depending on the selected option, the software can do one of the following:

- By clicking [Yes], the backup file is retrieved. The system will append the session data as a new session into the experiment file.
- By clicking [No], the backup file is deleted. The data will be lost permanently.
- By clicking [Cancel], the backup file is not retrieved, and the same window will reappear each time the user opens this experiment.

3.4. Importing Sessions

It is possible to import session files generated with previous version of the program (Metabolism v2.2.02 or above). This functions just as if retrieving a backup file of a session.

To import a session file, click the option File\Import Session File. A "Select session file to import" dialog will be displayed. Select the session file (with the extension .mtb) to import and press Open button.

METABOLISM will display a message dialog box if there are any problems importing the file. A success message will be shown once the importation is complete.

If the importing process will take more than a few seconds, a progress dialog will be shown.

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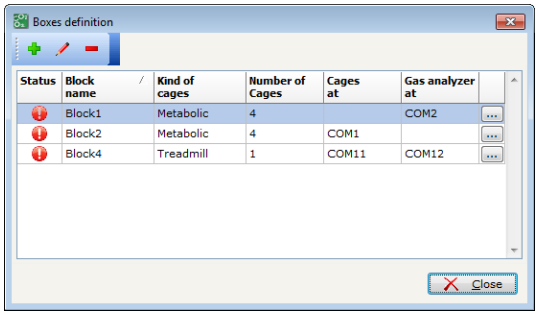
4. BOXES DEFINITION

4.1. Boxes Definition

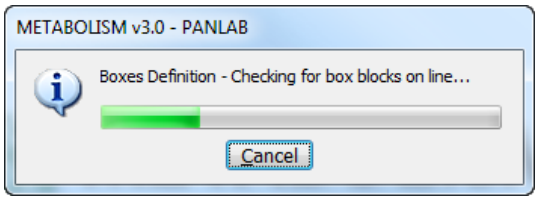
This section allows detecting and defining the boxes connected to the system.

Pressing the Boxes Definition button, the following dialog is shown.



Box blocks can be added, edited and removed in the system configuration.

If the Boxes definition task is selected and there are no boxes on line, the system will automatically try to detect them.



This task can be canceled by pressing the [Cancel] button.

4.1.1. Box Block Definition

METABOLISM works with box blocks in order to communicate with the hardware.

A box block is the way the user identifies which hardware is included in the system and how it is connected, (which serial port is used) to the computer.

A box block contains the following parameters:

- block name, determined by the user
- type of cage, determined by the detection process (see below)
- number of cages auto-detected/configured
- serial port where the first cage or the treadmill unit is connected
- serial port where the gas analyzer is connected.

4.1.1.1. Add a new box block

To add a new box block, press the [Add] button in the toolbar, the "Add block" dialog is shown.

A default block name is entered. Press the Ok button to close the dialog.

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4.1.1.2. Edit a box block

In order to edit a box block, double-click the row to be edited or select the row and press the [Edit] button in the toolbar, the "Edit block" dialog will appear.

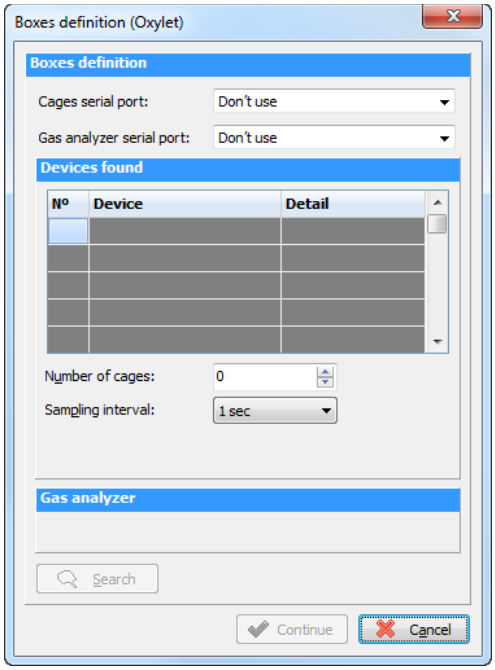
The name of the box block must be unique.

4.1.1.3. Remove a box block

In order to remove a box block, select that row and press the [Remove] button on the toolbar. A confirmation dialog box will appear. Accept to remove the box block.

4.1.2. Boxes Definition

In order to establish which serial ports are reserved for a box block and identify what kind of cages are installed, a box block must be configured. To do this, press the "…" button. The "Boxes definition" dialog will appear.



If metabolic cages will NOT be used, leave "Cages serial port" as "Don't use".

If the configuration has a treadmill unit, select the serial port in the "Cages serial port" combo box.

If the system configuration includes a gas analyzer, select the serial port in the "Gas analyzer serial port" combo box. If the system will not be monitoring gas, leave it as "Don't use".

Be sure all of the devices are connected and powered on and then press the [Search] button to start the auto-detection task.

After a brief time the message "Devices found" will appear and indicate the number of cages that were detected.

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4.1.2.1. Number of cages

If a TREADMILL unit has been detected, then just one cage is allowed, but if METABOLIC CAGES have been detected you must define the number of cages for the current box block. The system is able to handle up to 32 metabolic cages.

4.1.2.2. Sampling interval

The sampling time can be set in metabolic cage experiments with activity and/or intake options enabled. However, experiments with the OXYMETRY option enabled will force the sampling time to 1 second automatically.

The switching time is also important for OXYMETRY experiments. You must select the same value as you selected on the front panel of LE400x.

If the configuration has only metabolic cages neither gas analyzer nor treadmill unit), the sample interval can be set.

If the detection is successful, accept the dialog and close the dialog box by pressing the Continue button.

4.1.2.3. Box block online status

Updating the status of the box block will prevent issues with the serial ports.

Once a box is added and configured to a box block, its online status will be set to "Ready".

The next time METABOLISM runs, all the box blocks that have been defined will have their online status set to "off-line" until the status has been updated.

In order to update the status of a box block, press the […] button, and then press "Search" on the "Boxes definition" dialog and finally press "Continue".

The red icon will change to a green icon.

4.1.2.4. Configuring the devices

The airflow and switching value can be set on the OXYLET Air Flow & Switching device (LE-400x).

Airflow is controlled by adjusting the flow regulator dials on the airflow device's front panel (one for each cage). The switching value is adjusted via the digital selector. The Flow Rate Viewer tool can be used for fine adjustment (see chapter 6.4) of the air flow rate.

For treadmill configurations, the shock intensity is configured from the Treadmill Control Unit by using the [Intensity] Settings on the touchscreen main panel. The speed of the belt can be configured directly from METABOLISM (refer to the section 6.3.6 for more details).

For food and drink monitoring, put the food and the water in their respective containers for each cage. Calibrate each station by pressing the [BAL] button on the front panel of the transducer platform.

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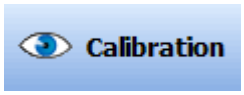
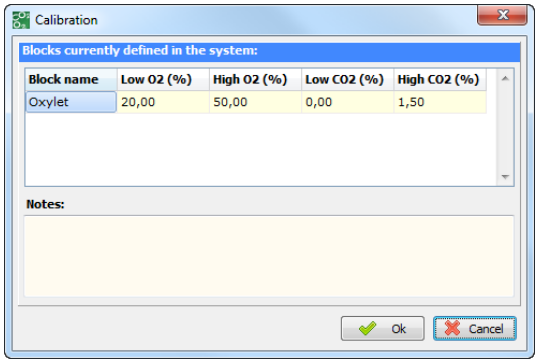
4.2. Calibration



METABOLISM can annotate the coefficients of the gas analyzer calibration. Please refer to the corresponding chapter in the LE405's manual for more details.

Pressing the Calibration button opens the Calibration dialog box.

For each online oximetry box block, a row will show its current calibration coefficients.



Modify the coefficients in the grid and press the Ok button to accept the changes.

If the coefficients are not within the advisable concentration values, the system will display a warning message.

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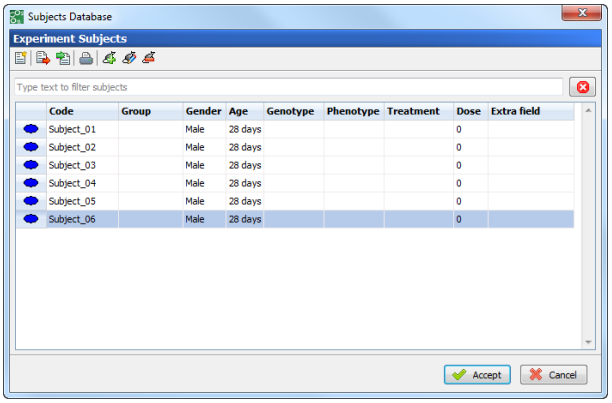
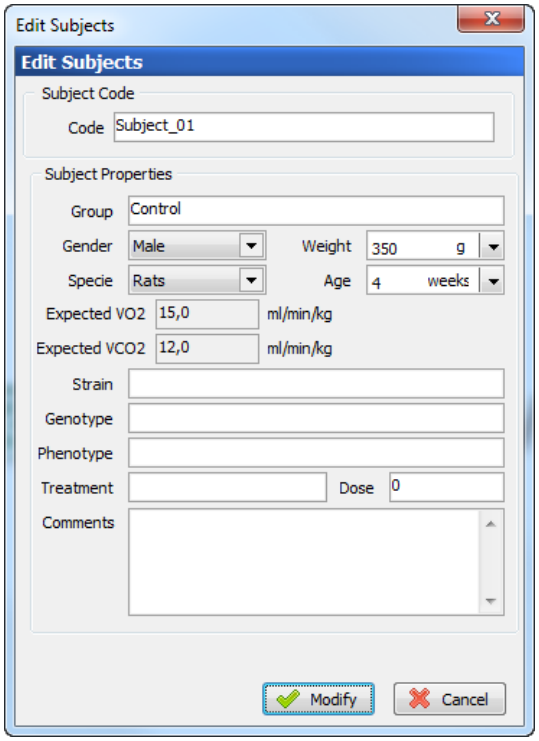
5. EXPERIMENT CONFIGURATION

5.1. Subjects Database

The METABOLISM **Subject** Database collects all the characteristics of the subjects used in the experiment (name, gender, group, age, weight, etc.).



5.1.1. Subjects Data



The following fields are provided for user input:

- **Subject Code**: name of the subject
- **Subject Group**: experimental group related to the subject
- **Gender**: gender of the subject
- **Weight:** weight of the subject in grams (g)
- **Specie:** specie of the subject (rats or mice)

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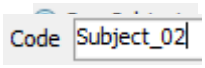
- **Age**: age of the subject and the units (days / weeks / months / years)
- **Strain**: strain of the subject
- **Genotype**: Genotype of the subject
- **Phenotype**: Phenotype of the subject
- **Treatment**: treatment used, if any
- **Dose**: dose of the treatment used, if any
- **Comments**: adding notes, comments or any other useful characteristics that would be important to be included within the analysis report

5.1.2. Adding New Subjects

5.1.2.1. Adding one new subject



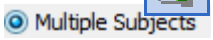
1. Press the [**+**] button to add new subjects to the database.

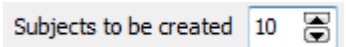
With the **One subject** option already selected, enter the subject's code.

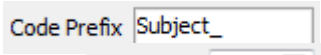
2. Fill the rest of the subject's information in the **Subject Properties** section.
3. Press the **Create** button to add the new subject.

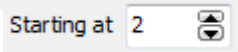
IMPORTANT NOTE: The new subject to be created cannot have the same code of another subject already belonging to the same group. The code or the group must be changed in this case.

5.1.2.2. Adding a batch of multiple subjects









1. Press the [**+**] button to add new subjects to the database.
2. Select the **Multiple subjects** option.
3. Enter the number of subjects to be created between 2 and 999.
4. Every new subject created will have a code composed by three parts combined in sequence:
    a. A code prefix.
    b. A code number: starts at the number specified in the field **Starting at**.
    c. A code suffix.
5. Fill the rest of the subject's information in the **Subject Properties** section. Every subject will have the same properties.
6. Press the **Create** button to add the new subject.

IMPORTANT NOTE: New subjects cannot have the same code of subjects already belonging to the same group. If that happens, METABOLISM automatically adds a suffix "(n)" to the subject's code, "n" being an incremental number used to differentiate them (e.g. "Subject_10" is renamed by "Subject_10 (2)").

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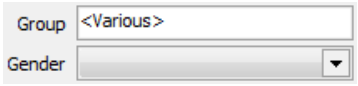
5.1.3. Editing the properties of the subjects

5.1.3.1. Editing the properties of one subject

1. Double click over the subject or select it and press the **Modify selected subjects** button.
2. Edit the subject's code or any of the rest of the properties.
3. Press the **Modify** button.

5.1.3.2. Editing the properties of multiples subjects

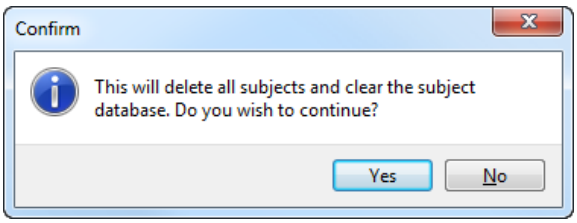


1. Select the subjects to be edited. The combination of the [SHIFT] and [CTRL] keys and the left button of the mouse allow the user to select a variety of ranges very easily.
2. Press the Modify selected subjects button.
3. The **Edit Subjects** panel shows the properties of all the selected subjects. The value of the common properties is shown directly but heterogeneous properties are shown as "<Various>" or as an empty field.
4. Edit the subject's properties. Enter the values to apply to all the selected subjects at the same time and leave the remaining properties unchanged.
5. Press the **Modify** button.

5.1.4. Deleting Subjects

1. Select the subjects to be deleted. The combination of the [SHIFT] and [CTRL] keys and the left button of the mouse allow you to select a variety of ranges very easily.
2. Press the Delete selected subjects button.
3. If multiple subjects are to be deleted, then a confirmation message is displayed. Accept the message to definitely delete the subjects.

Press the **Clear Subjects** button and accept the confirmation message for deleting all the subjects at once from the database.



IMPORTANT NOTE: As with other changes made within the subjects database, deleted subjects can be recovered only if the **Subjects Database** panel is closed by means of the **Cancel** button. If the panel is accepted, changes made in the subjects database cannot be undone.

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5.1.5. Saving the Subjects Database

To save the changes done within the subjects database, press **Accept** button of the **Subjects Database** panel.

If the changes made are not saved, press the **Cancel** button.

IMPORTANT NOTE: All the changes made within the subjects database can be recovered only if the **Subjects Database** panel is closed by means of the **Cancel** button. If the panel is accepted, changes made in the subjects database cannot be undone.

5.1.6. Other tools regarding subjects management

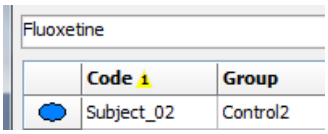
5.1.6.1. Sorting the subjects database

Left click column headers for the main sort index. Click again on the header to change the sorting direction. Add secondary sort indexes with [SHIFT] + left click.

Code 2	Group 1	Gender
Subject_03	Control	Male
Subject_04	Control	Male

Place the cursor on a column-separator line in order to change the width of the columns.

5.1.6.2. Searching for subjects in the database



The subjects' database includes a useful tool to facilitate searching for subjects with properties matching a specific text.

1. Enter the text to search for in the filter box located just under the subject's table. As keys are being pressed, the subject's table is automatically filtered to show only those subjects with the text inside any of their properties.
2. Press the **Clear search** button located at the right side of the filter box to cancel the filter.

5.1.6.3. Exporting and importing the subjects

The complete subjects database can be exported to a Microsoft Excel® file.

1. Press the **Export subject list** button.
2. Select the destination folder and file name and press the **Save** button.

This file can be edited manually and also imported in a different experimental file.

A2 | Subject_01

	A	B	C	D	E	F	G	H	I	J
1	Code	Color	Group	Sex	Age	Genotype	Phenotyp	Treatment	Dose	ExtraField
2	Subject_01	16744448	Control	Male	2	+/+	Hyperactiv	Fluoxetine	0	
3	Subject_02	16744448	Control	Male	2	+/+	Hyperactiv	Fluoxetine	0	
4	Subject_03	16744448	Control	Male	2	+/+	Hyperactiv	Fluoxetine	0	
5	Subject_04	16744448	Control	Male	2	+/+	Hyperactiv	Fluoxetine	0	
6	Subject_05	4227327	Exp	Male	0	+/+	Hyperactiv	Fluoxetine	0	
7	Subject_06	4227327	Exp	Male	0	+/+	Hyperactiv	Fluoxetine	0	
8	Subject_07	4227327	Exp	Male	0	+/+	Hyperactiv	Fluoxetine	0	
9	Subject_08	4227327	Exp	Male	0	+/+	Hyperactiv	Fluoxetine	0	

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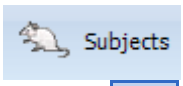
Some versions of Microsoft Excel® may warn you that the Excel file generated by exporting a subject list has a different format than the type specified by its extension. In that case, accept the warning message to open the file normally.

Although manual manipulation can be done in the exported file, please keep in mind that the new information must fulfill the rules of a coherent subject database (i.e., two or more subjects belonging to the same group cannot have the same code; gender must be "Male" or "Female" only; age and dose fields must be numbers equal or greater than zero).

In order to import a previously exported subject list:

1. Open the experimental file in which the subject list is to be imported.



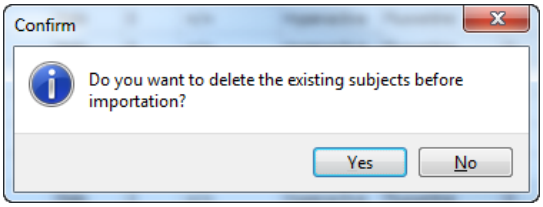
2. Access the **Subjects Database** manager.

3. Press the **Import subject list** button.
4. Locate the folder and file in which the subject list is stored. Then press the **Open** button.

   The subjects within the file are automatically inserted in the subject's database of the experimental file.

5. Accept the **Subjects Database** tool.

   If the current subject's database already contains subjects, a warning message is displayed before importing the new list:



   If the subject's database must be cleared before importing the new list, click the Yes button. If the subject database is not cleared before importing the new list, the subject's code of the imported subjects will be automatically renamed to avoid a duplication of codes in the same group.

5.1.6.4. Printing the subjects database

1. Press the **Print subject list** button.
2. Select a printer device to be used to print the list and press the **Accept** button.

3. Navigate through the pages of the report using arrow buttons in the **Report Preview** window.

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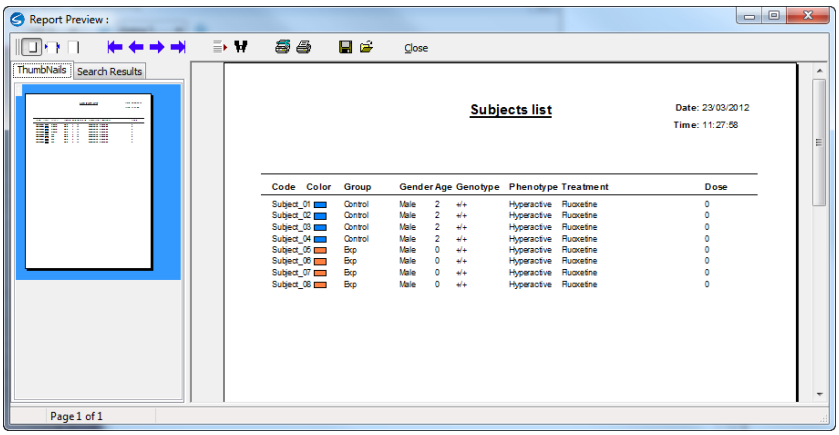
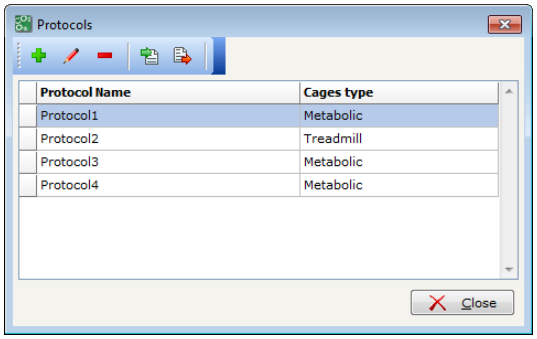


4. Prepare your printer for printing the report.
5. Press the **Print** button to print the report.

5.2. Protocol Organizer

METABOLISM provides a specific tool for the management of protocols contained in the current experiment file.



The Protocols Organizer main window is comprised of a protocol organizer table and an assistant toolbar.

5.2.1. Protocol Organizer Table

The summary table provides useful information about the protocols available in the experiment file. The following information is displayed:

Name: the user defined protocol name

Cage type: type of cages compatible with the protocol

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5.2.2. Assistant Toolbar

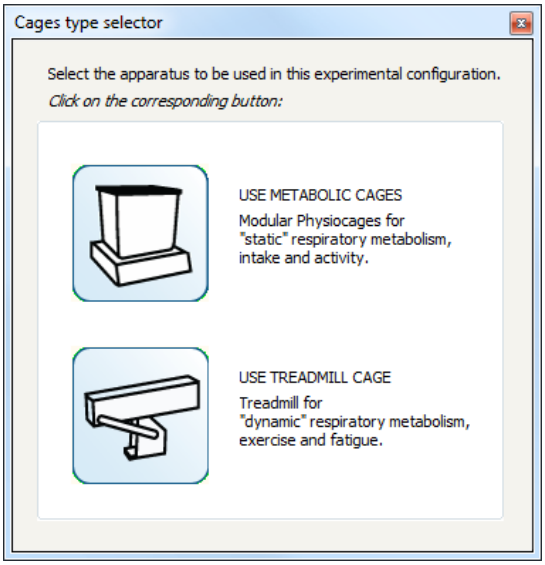
The assistant toolbar contains buttons for Creating, Editing, Deleting, Importing and Exporting protocols.

5.2.2.1. Create a new protocol

Press the **New** button. The application will then require the user to choose between two kinds of cages:

Metabolic cages: "static" respiratory metabolism, intake and activity.

Treadmill cages: "dynamic" respiratory metabolism, exercise and fatigue.



5.2.2.2. Edit a protocol

Select the protocol to be edited from the protocol organizer table.

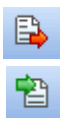
Press the **Edit** button.

5.2.2.3. Delete a protocol

Select the protocol to be deleted from the protocol organizer table.

Press the **Delete** button.

5.2.2.4. Export and Import a protocol



The Export/Import option is available for facilitating the transfer of protocols from one experiment file to another.

This option is particularly useful when the user intends to use the same protocol in a different experiment without having to define it again in the newly created experiment file.

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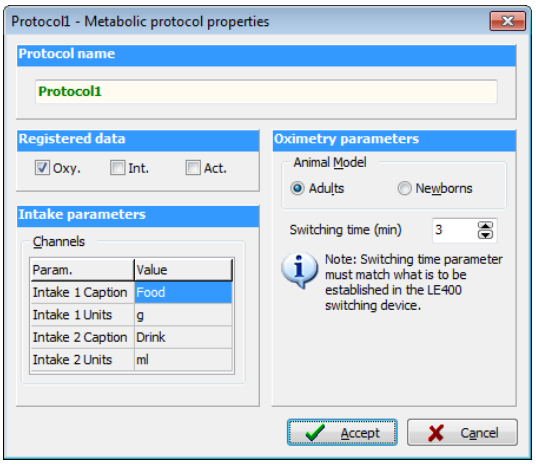
1. First, open the experiment file containing the file to be exported.
2. Select the protocol to be exported from the protocol organizer table.
3. Press the **Export** button. Choose a destination folder to which the exported protocol will be stored and select accept.
4. The selected protocol will be exported with the ".mtbp" extension.
5. Open the target experiment file in which the user wants to transfer the exported protocol.
6. Press the **Import** button in the corresponding Protocol Organizer panel.
7. Select the protocol file to be imported and select accept.

The selected protocol will be imported with the prefix "Import of…"

5.2.3. Metabolic Protocol

A Metabolic protocol acquires data for metabolic/calorimetric intake experiments.

The registered data section shows which data is acquired. The different options available depend on the active license information, which of the software modules are ordered and activated within the license.



The title and units of each data channel can be configured for food and drink intake parameters.

The sampling time can be set in metabolic cages experiments with activity and/or intake options enabled. However, experiments with the OXIMETRY option enabled will force the sampling time to 1 second automatically. (See 4.1.2.2).

The switching time is also important for OXYMETRY experiments. This value **must** be the same value as selected on the front panel of LE400x.

By default, the [Animal Model] setting is for the [Adults] option. If using Newborns, (for Newborns, oxygen measurements are in cl/min), must be set to the [Newborns] option in this parameter screen.

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5.2.3.1. Experiment modules: OXIMETRY

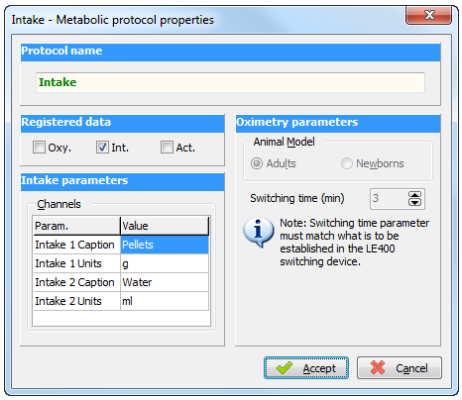
OXIMETRY experiments will focus on the Energy Expenditure rate (EE). To calculate this value, the OXYLET system will measure the oxygen consumption and the carbon dioxide production. As a sub product of the results, the Respiratory Quotient (RQ) will be determined, which gives the substrate of the metabolic energy generation mechanism, (i.e., the amount of food that the animal is burning in order to generate energy). It also serves as a diagnostic measurement, indicating experimental complications.

5.2.3.2. Experiment modules: INTAKE

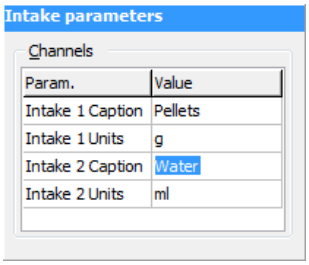
Intake experiments will focus on food and/or liquid consumption. The system measures the weight of food and liquid, second by second, detecting the consumption and identifying episodes of feeding and drinking events. Hardware and software considerations are in place to adjust for the effects of large vibrations, such as the animal touching the intake components.

NAMING THE INTAKE CHANNELS

Users can define names for the intake channels. This is done by selecting [Intake parameters - Channels] while editing a metabolic protocol (see picture below):



To change the names of the channels just click over the Value field and type the desired name:



5.2.3.3. Experiment modules: ACTIVITY

Activity is an option that will provide data reflecting the general activity of the animal and the number of rearing events that occur during a specified time interval. It is an auxiliary measurement, used in conjunction with an OXIMETRY and/or INTAKE data to offer an activity framework where the Energy Expenditures and/or intakes can be compared.

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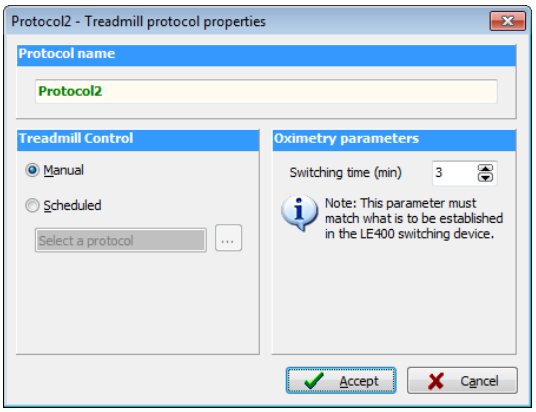
5.2.4. Treadmill Cage Protocol

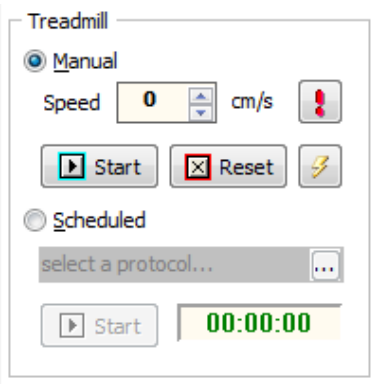
The TREADMILL cage allows a special kind of OXIMETRY experiment: respiratory metabolism during exercise. These experiments are generally short and are performed primarily in manual mode. There are correlative registers of the OXIMETRY and treadmill data in order for the results to be comparable.

METABOLISM can manage a treadmill control unit, offering the user two working modes: Manual and Scheduled.

The Acquisition window provides a treadmill section where the user can control the treadmill unit.



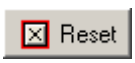
In Manual mode, controls on the touchscreen panel of the Treadmill Control unit are disabled except for the intensity settings and all controls are now displayed on the computer screen:



Manual mode allows the user to:

- Set the belt speed by pressing the update button after introducing a speed in cm/s

Enable/disable the shocker by pressing the shock button



- Reset the counters by pressing the counter [Reset] button

- Start/stop the motor and the counters by pressing the [Start/Stop] button

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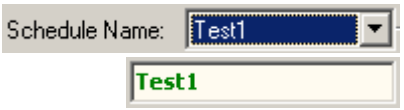


WARNING: the use of the METABOLISM software with the touchscreen treadmill is not compatible with the new stop conditions available from the touchscreen. This means that they would not be taken into consideration even if the user configures them on the touchscreen control unit.

Scheduled mode allows the user to select a schedule or protocol to be run during the OXYMETRY acquisition session.

In order to select a protocol, press the selection button. It will display the Treadmill schedule editor. Then, select an already defined protocol from the dropdown menu and press the Select button. The dialog box will close and display the name of the selected protocol in the text box.

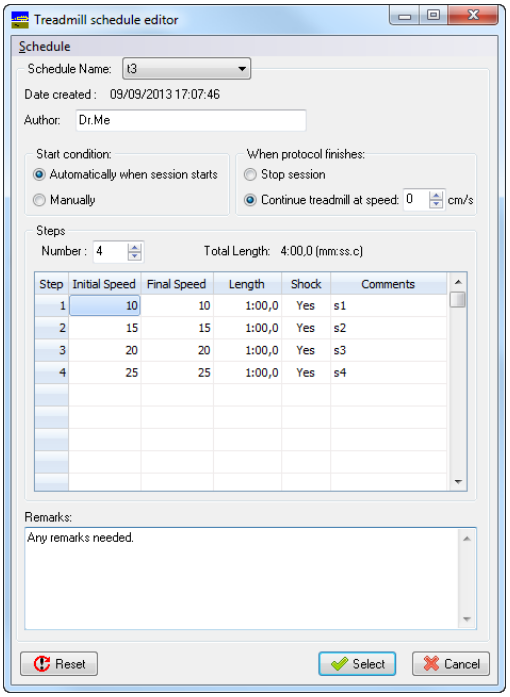
The software will prepare the execution of the protocol, locking the front panel of the control unit and send the required commands.

For a complete explanation of how to edit protocols, refer to the section 5.2.4.1

5.2.4.1. Treadmill schedule

METABOLISM allows the user to edit schedules for use during an OXYMETRY session with a treadmill cage.

To edit a treadmill schedules, select the menu option Setup-Treadmill schedule in the main window and the Treadmill schedule editor window will be shown.



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METABOLISM allows very flexible control of the treadmill belt speed in the range of 5 to 150 cm/sec from the Protocol definition procedure. A value of 0 cm/sec halts the belt movement.

A treadmill schedule is defined by:

- name
- starting condition
- protocol steps
- stop condition

The protocol steps must contain:

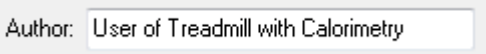
- a starting speed (in cm/sec)
- a final speed (in cm/sec)
- the time range (in mm:ss.d format) over which the belt's speed will change from the start to final speed
- shock enabled option
- optional comments

5.2.4.2. New scheduler

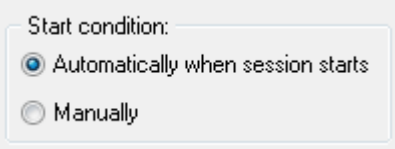
To create a new scheduler, press the New option of the Schedule menu and enter the name of the schedule.

5.2.4.3. Author name

Enter the name of the author in the Author section (optional).



5.2.4.4. Start condition



The start condition section defines the condition under which the schedule will begin.

- Select the "*Automatically when session starts"* option for initiating the treadmill schedule simultaneously with the Oximetry session (i.e., when the Oximetry Start button is pressed).
- Select the "*Manually"* option for initiating the treadmill schedule independently from the start of the Oximetry session (Oximetry session typically initiates prior to the treadmill start).

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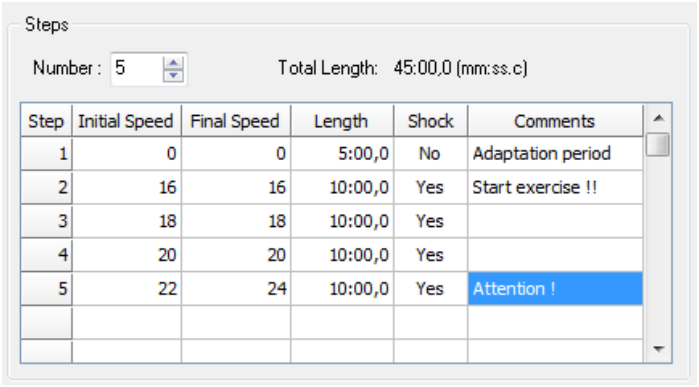
5.2.4.5. Create a schedule



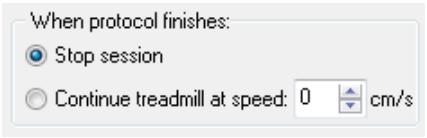
Select the number of steps in the *Number* section.

Define the characteristics of each step. The following settings are available:

- Initial speed: from 5 to 150 cm/sec (a special value of 0 cm/sec can be set to stop the belt movement)
- Final Speed: from 5 to 150 cm/sec (a special value of 0 cm/sec can be set to stop the belt movement)
- Length: (duration of the step in in mm:ss.d format, meaning the duration elapsed to go from the Initial speed to the Final speed). The set value must be higher than 0.
- Shock status option: *No* means inactivated; *Yes* means activated (the animal will receive a mild shock any time it will contact the grid).
- Optional comments

The schedule editor will calculate the total duration of the protocol and display the value in the *Total Length* section.

5.2.4.6. Stop conditions



The Stop condition section defines the condition under which the treadmill schedule will terminate.

- Select the "*Stop session"* option for stopping the treadmill schedule simultaneously with the end of the Oximetry session (i.e., when the Oximetry [Stop button is pressed]).

Select the "*Continue treadmill at speed [value]* cm/s" option for setting the speed of the treadmill to a user-defined value once the schedule ends. In this case, when the schedule ends, the "Scheduled mode" option is unselected in the Treadmill runtime acquisition panel and the Manual mode is automatically selected with the user-defined speed. The treadmill will be running in manual treadmill mode at the given belt speed until the [Stop] button is pressed. See section 3.5.6 for the details about the Treadmill runtime panel.

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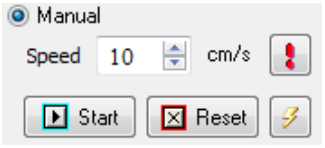

The software carries out the current protocol systematically, sending the required commands for belt speed, as set in the protocol, to the Treadmill control unit.

When executing a step, METABOLISM sends the Treadmill control unit the suitable commands to carry out this change at a constant rate. As a protocol can include many steps, a second step containing a definition such as"50, 5, 60" will make the belt speed reduce from 50 down to 5 cm/sec in one minute. In this way, a saw-tooth shape change in speed is possible.

If the starting and the final speeds are the same, the effect would be to keep the belt speed constant during all of the programmed time. Using these three kinds of effects, (constant speed, ramp shaped increase or decrease of speed) all desired protocols can be defined.

Protocol definitions can be stored and recovered as many times as necessary.

WARNING: the use of the METABOLISM software with the touchscreen treadmill is not compatible with the new stop conditions available from the touchscreen. This means that they would not be taken into consideration even if the user configures them on the touchscreen control unit.

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5.2.4.7. General protocol management

The schedules can be stored and recovered as many times as necessary. The program can have as many protocols as needed. To manage the schedules, the following options are available from the *Schedule* menu:

- New: create a new schedule
- Rename: rename the current schedule
- Save: save the changes made in the current schedule
- Save a: save the changes made with another schedule name
- Reset: apply the default values to the current schedule
- Delete: delete the current schedule. This option requires confirmation. **Warning**: this action is not reversible once confirmed.
- Close: close the schedule editor window

The Treadmill schedule editor provides three additional buttons:

- Cancel: close the window without saving the changes
- Reset: same as Reset menu
- Select: selects the current schedule to run during acquisition

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6. DATA ACQUISITION

Once a protocol is complete and the subjects defined, the experiment is ready to run, and the session can start.

This section details the execution of protocols in METABOLISM.

6.1. Requirements to Start Acquisition Process

Before starting the acquisition process:

- Ensure all equipment and devices are correctly mounted and connected. See the user manual of each component for details.
- The O_2/CO_2 Gas Analyzer calibration was done correctly (see chapter 4.2 for details) by using gas mixtures of:
  - High point: 50% O_2, 1.5% CO_2, 48.5% N_2.
  - Low point: 20% O_2, 0% CO_2, 80% N_2.

  The two reference gases must have a difference of at least 15% for O_2 and 1% for CO_2.
- Switching time must be the same on both the hardware and software (see 5.2.3 section).
- Determine the optimal flow for Oximetry. This depends on the animal size and weight. The following flows are ***suggested*** to work with the O_2/CO_2 gas analyzer:

Animal	Static (l/min) Metabolic cages	Dynamic (l/min) Treadmill
Mouse	0.2-0.4	0.3-0.7
Rat	0.5-0.8	0.7-1.2
Rabbit	1.5-2.0	1.8-2.4

6.2. Select the Protocols and Boxes

The **Select protocols and boxes to run** panel allows the assigning of a protocol to the experimental boxes configured during the **Box Definition** process.

The panel is composed of three sections:

- **Protocols** list (left): displays the defined protocols contained in the experiment file
- **Blocks** list (central): displays the available experiment box blocks
- **Assignations** grid (right): displays the associated protocols and blocks

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After selecting a Protocol and a Block, press the right-pointing red arrow for associating them together.

Use the left-pointing red arrow for disassociating the protocol and block listed in the Assignations grid.

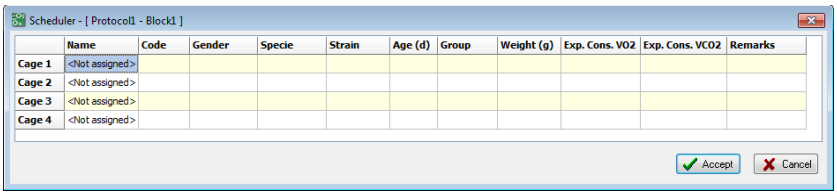
Press the ***Ok*** button when done.

Note 1: a protocol can be associated with several experimental blocks.

Note 2: a protocol can be run in each experimental block.

When pressing the OK button for each association there will appear a data acquisition window.

The system prompts for a subject to be assigned for each cage identified in the box block.

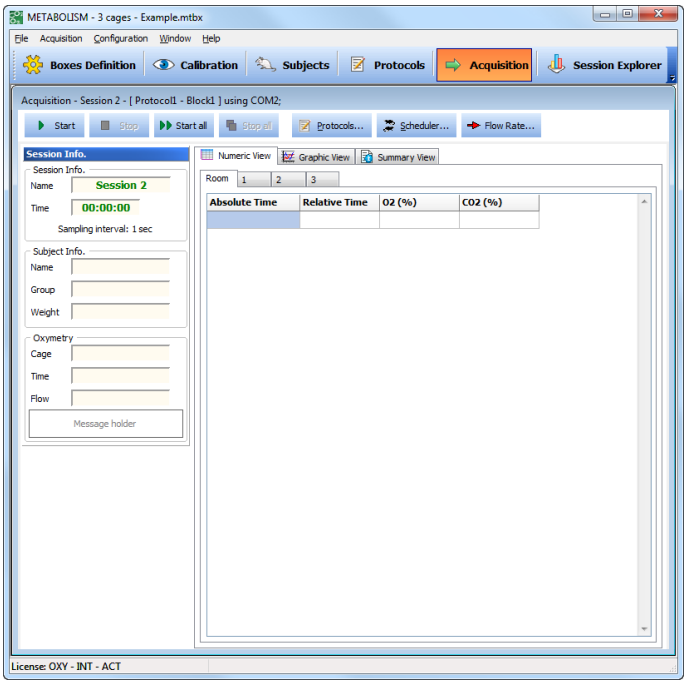


The image below displays the scheduler dialog for box block with four metabolic cages.

6.3. The Acquisition Window

The acquisition window contains an Acquisition menu, toolbar, Session Info., Subject Information, OXIMETRY panel, Display options, and View Panel.

The title of the acquisition window will show the name of the session, the name of the protocol and the name of the box block.



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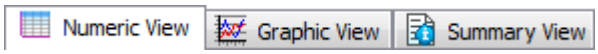
6.3.1. Toolbar

Start	starts acquisition of the current block
Stop	stops acquisition of the current block
Start all	starts acquisition of all the blocks ready to run
Stop all	stops acquisition of all running blocks
Protocols...	for changing the protocol association with the blocks
Scheduler...	for changing the subject association with the cages of the block
Flow Rate...	for assigning the optimal flow rate for each cage

6.3.2. Display Options

The Acquisition windows show acquired data in both numeric and a graphic format, as well as a summary view. To change between each option, select the desired tab control on the main region of the acquisition window:



6.3.2.1. Numeric view

The [Numeric view] tab shows the acquired data as a spreadsheet, arranging the data of each cage into different tab pages.

Each row represents a sample acquired over time and each column represents a parameter or data series.

6.3.2.2. Graphic view

The [Graphic view] tab shows the acquired data as a set of charts, illustrating the signal evolution over time. Charts are vertically oriented with a vertical scroll bar when the data exceeds the height of the acquisition window. Similarly, each cage has its own tab page.

There is a single common X-axis at the bottom of the charts and the acquisition time of the session is indicated in the format hh:mm:ss.

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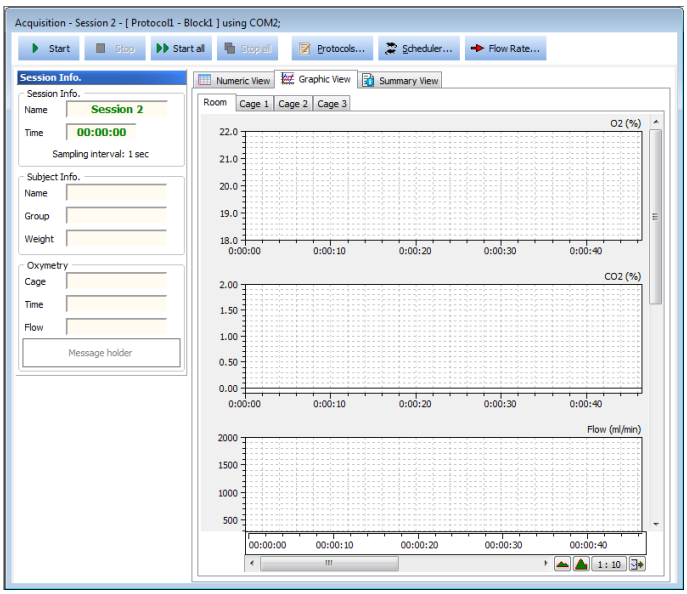
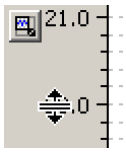


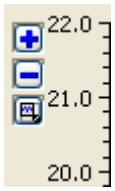
The graphic area allows the user to:

- Horizontal scroll: with a horizontal scroll bar located just below time axis.
- Automatic horizontal scroll (plot continuation) by pressing button  located at the end of the horizontal scroll bar.
- Time zoom-in and zoom-out: using  and  buttons located at the bottom right side of the horizontal scroll bar. The zoom list  may also be used.
- Vertical zoom-in and zoom-out: allows increasing / decreasing the visual data precision.

Y-axis features a drag-and-drop mechanism allowing users to move freely along the data range. Drag the zone near the axis values (when the mouse pointer changes to a double-arrow icon).



Also, a pair of buttons (+ and -) allow the user to zoom in and out to increase / decrease the data resolution. In addition, an auto adjust button permits scaling the Y-axis to best fit the current shown data.



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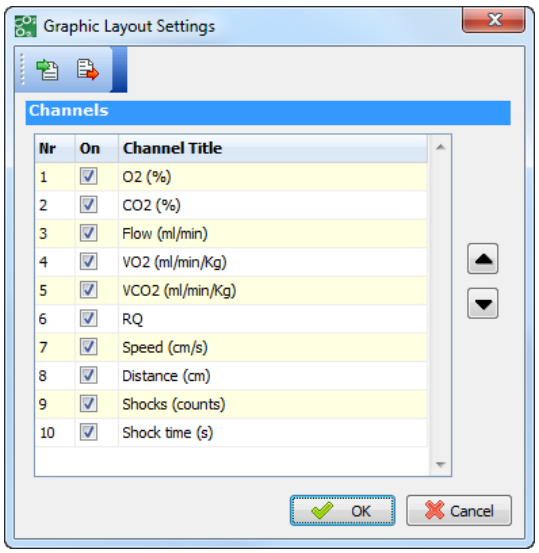
6.3.2.3. Graphic layout

By default, the graphic layout contains all the possible channels that the system is able to provide; however, it might not be necessary to see all of them. . METABOLISM allows you to define which channels you want to display through the "Graphic Layout Settings" tool.

In order to change the graphic layout, select the "Graphic Layout" option in the Acquisition/Settings menu. The dialog below will be displayed:



Check/uncheck the channels to see/hide.

Select a row/channel in the grid and use the up/down arrow buttons to change the order position.

METABOLISM allows users to export/import the graphic layout in order to facilitate to use of tool.

6.3.2.4. Summary view

The [Summary view] tab shows a panel for each cage. On each panel, the set of summary data displayed depends on the experiment options.

TREADMILL EXPERIMENTS

For treadmill experiments, the following data is shown:

Speed	Current belt speed in cm/s
Distance	Accumulated distance in cm
Shocks	Accumulated shocks count
Shock Duration	Accumulated shock duration in sec
VO2 max	Maximum VO_2 raised by the subject

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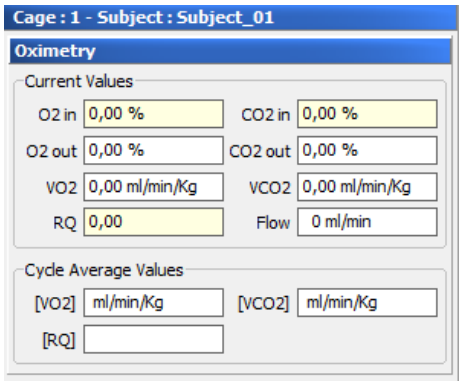
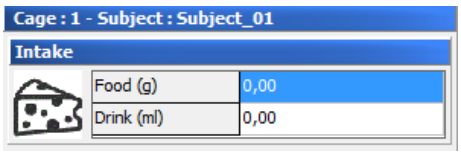


OXIMETRY EXPERIMENTS

For Oximetry experiments, the following data is shown:

Current Values	
O2 in	Percentage of O_2 measured inside the cage
CO2 in	Percentage of CO_2 measured inside the cage
O2 out	Percentage of O_2 measured outside the cage
CO2 out	Percentage of CO_2 measured outside the cage
VO2	Oxygen consumption in ml/min/kg
VCO2	Carbon Dioxide production in ml/min/kg
RQ	Respiration Quotient (also called RER, Respiration Exchange Ratio)
Flow	Mean flow inside the cage

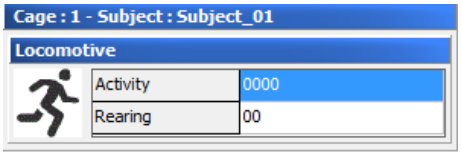
Cycle Average Values	
[VO2]	Oxygen consumption in ml/min/kg
[VCO2]	Carbon Dioxide production in ml/min/kg
RQ	Respiration Quotient (also called RER, Respiration Exchange Ratio)



INTAKE EXPERIMENT

For Intake experiments, the following data is shown:

Intake	
Food (g)	Accumulated food intake in g.
Drink (ml)	Accumulated drink intake in ml.



LOCOMOTIVE EXPERIMENT

For Locomotive experiments, the following data is shown:

Locomotive	
Activity	Accumulated activity in a.u.
Rearing	Accumulated rearing (counts).

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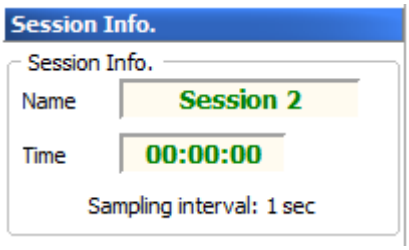
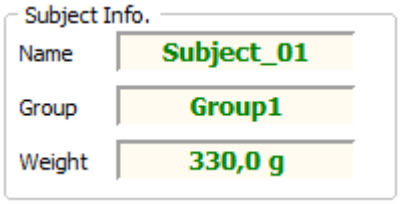
6.3.3. Session Info



The Session Info panel provides some information about the evolution of the current session run in each block:

- session name
- elapsed time from the beginning of the session
- sample interval

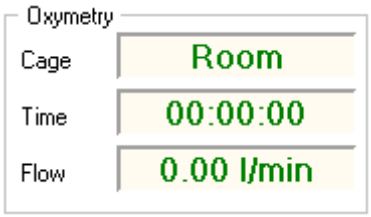
6.3.4. Subject Info



The Subject info section will show info related to the subject assigned to the selected cage through the Subject Scheduler.

- Subject name: the code of the subject assigned to the current cage selected.
- Group: the selected subject's group.
- Weight: the selected subject's weight.

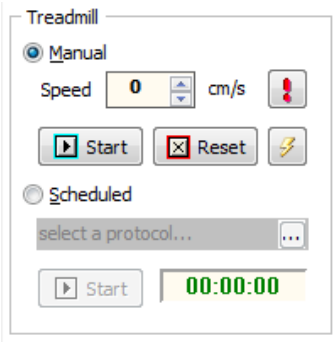
6.3.5. Oximetry



- Cage: Cage number active in the gas analyzer unit.
- Time: Elapsed time that air has been flowing into that cage.
- Flow: Airflow measured in the cage.

6.3.6. Treadmill Options

For treadmill experiments, the acquisition window will show the Treadmill control panel:



The treadmill can be controlled directly from the computer (see 5.2.4 section for more details).

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6.4. Adjusting the Air Flow

Although the flow could vary for each cage, it should remain stable for each respective cage for the duration of the acquisition session.

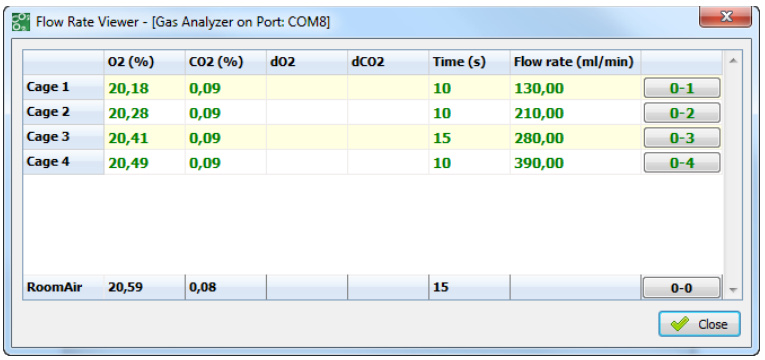
The software monitors the flow rate set in the Air Supply & Switching Unit(s) via the Flow Rate Viewer tool.

For each cage, the following info will be shown:

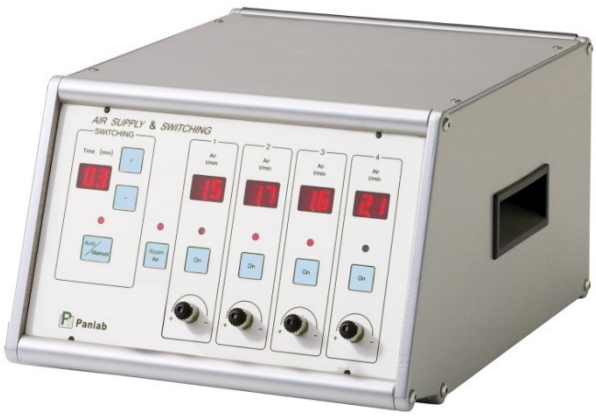
- Cage number, or Room Air.
- O_2 (%), oxygen concentration.
- CO_2 (%), carbon dioxide concentration.
- dO_2, oxygen difference between cage and room air.
- dCO_2, carbon dioxide difference between cage and room air.
- Time (s), measuring elapsed time in seconds.
- Flow rate (ml/min), cage incurrent flow rate.

To fine-tune the appropriate value of flow rate for each cage:

- Open the acquisition session window. Prior to starting acquisition, press the menu option Acquisition / Settings / Flow Rate Viewer. The Flow Rate Viewer window will be shown:



- Set the manual mode button in the [Air Supply & Switching] device.



- Set the cage 1 button on the Air Supply & Switching device. To view the flow value, refer to the third column on the data sheet within the Flow Rate Viewer form for the corresponding cage row.

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- The Air Supply & Switching device displays the flow in l/min, however, METABOLISM displays this value in ml/min for greater resolution.
- If the value of the flow rate (ml/min) does not correspond to the value pre-determined for the experiment, slowly adjust the corresponding cage dial on the Air Supply & Switching device until reaching the desired value in the software.
- Wait a few seconds for the value to stabilize in the software.
- Repeat the same steps to fine-tune the flow for each cage.

It is possible to switch to a different cage directly from the Flow Rate Viewer by clicking on the buttons [E-C] of each row, where "E" means "equipment" and "C" means "cage/channel".

Recommendation:

Observe the values for at least 5 minutes to ensure that the flows have been properly established.

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6.5. Start/Stop Acquisition

6.5.1. Start Acquisition

Press the [Start] button to initiate the acquisition process.

With OXIMETRY activated, the O_2/CO_2 Gas Analyzer takes control and synchronizes every other unit. Without OXIMETRY activated, the METABOLISM software controls the hardware. Raw data is acquired and visualized in real time on the acquisition screen.

For TREADMILL experiments, acquisition will disable speed and shock controls on the control unit.

The data listed below is provided along with the sampling time established by the protocol and is described further in section 5.2.2.2.

6.5.2. System Data

- Absolute Time:
  This is the system time, derived from the computer's clock.
- Relative Time:
  This is the time the sample was acquired relative to the beginning of data acquisition.

6.5.3. Oximetry Data

- Flow:
  OXYLET Air Flow & Switching Unit (ml/min).
- O2 %:
  Percentage of Molecular Oxygen measured inside the cage.
- CO2 %:
  Percentage of Carbon Dioxide measured inside the cage.
- Cage:
  The cage number indicated by the Air Supply & Switching Unit.

6.5.4. Intake Data

- Food:
  The weight of food as measured in the PHYSIOCAGE food container.
- Drink:
  The weight of liquid measured in the PHYSIOCAGE liquid container.

6.5.5. Activity Data

- Activity:
  General activity of the animal measured inside the PHYSIOCAGE.
- Rearing:
  Number of rearing events measured inside the PHYSIOCAGE.

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6.5.6. Treadmill Data

- Speed:
  Speed of the treadmill set on the Treadmill Control Unit (cm/s).
- Distance:
  Distance travelled by the subject on the treadmill (cm).
- Shock Time:
  Accumulated shock duration from the beginning of the session (s).
- Shocks:
  Accumulated number of shocks received by the subject from the beginning of the session.

IMPORTANT NOTE: Selecting the TREADMILL cage prevents the measurement of ACTIVITY and INTAKE. In this cage selection mode, only OXYIMETRY is possible.

6.5.7. Stop Acquisition

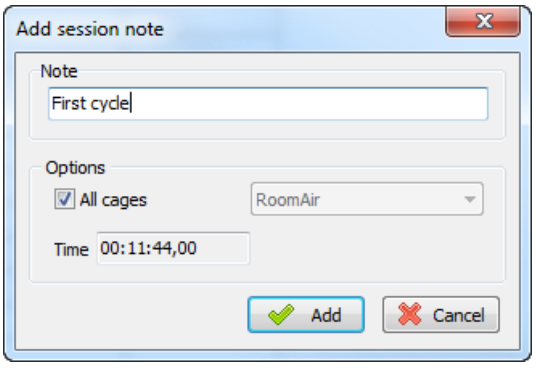
Pressing the [Stop] button terminates the acquisition process.

6.6. Session Notes

METABOLISM does allow users to enter notes for recorded data. The system adds automatic annotations at the beginning and end of the session.

Notes added by the system have the Type attribute assigned to SYS. However, notes added by the user has the Type attribute assigned to USR.

To add a note during the acquisition, press the "Acquisition \ Add note". The dialog "Add session note" will be displayed.



Enter the text of the note, up to 255 characters.

You can attach a note to the whole session leaving the option "All cages" checked or to a specific case by selecting it from the list. In calorimetry experiments, a special box "RoomAir" is also available.

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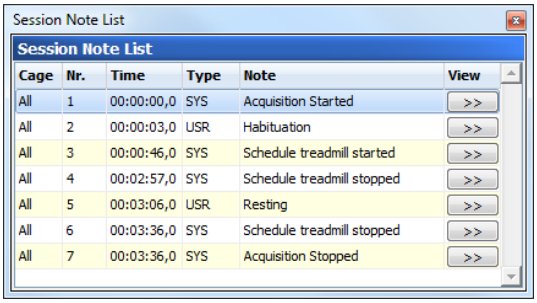
As each note is created, it is time stamped and can be seen under the time column. To see the complete list of notes at any time, press the "Acquisition\Note viewer" menu option. The window "Session Note List" is displayed.



The Notes window displays a table with the following columns for each note:

Cage: Cage number associated with the note. "All" when the note is not associated with a particular cage.

Nr.: Note consecutive number in the list.

Time: Time stamp of the note.

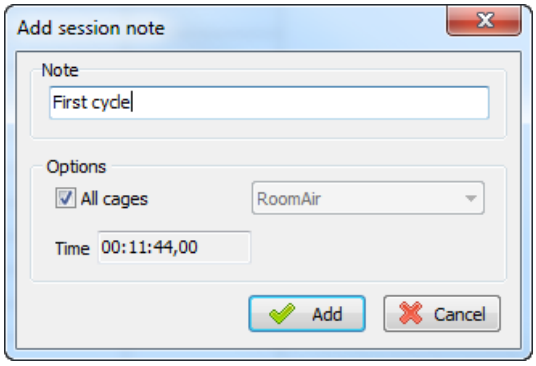
Note: Text of the note.

Vew: Button to return to the numeric view.

To return to the point where you made a note, press the ">>" button associated with that note. This will change the view mode to "Numeric View" and will scroll the grid to the time stamp of the note. If the note is associated with a particular cage, also the tab of that cage will be selected.

The notes are registered with the session and can be reviewed post-acquisistion. During analysis, it is possible to check and also add new notes to the list.

To add new notes during the analysis, select the numeric vew, locate the time stamp for which the note is required and press the combination keys CTRL + K. This also can be done by selecting the menu option "Acquisition\Add Note". The "Add Session Note" dialog will be displayed:



Enter the text of the note and press the button "Add". The new note will be added to the end of the current list of notes.

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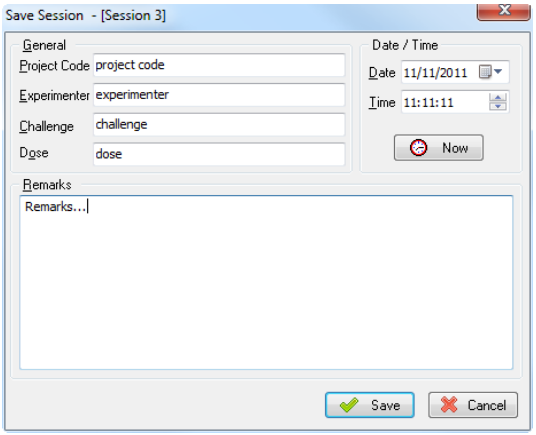
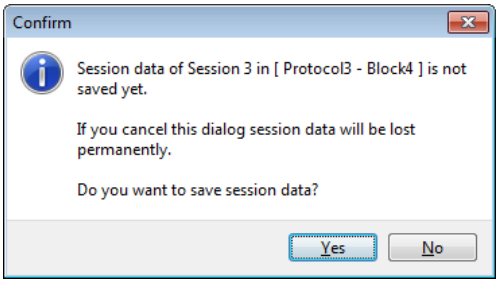
6.7. Save Session Data

Once a box block acquisition process is finished, the system prompts to save the session data. The dialog "Save Session" is shown, allowing the user to enter final remarks.



Press the "Save" button to confirm the action.

If the button "Cancel" is pressed, the system will show a confirmation message, in order to avoid the loss of data.



If the user presses the "No" button in the Confirm window, the session data will be lost permanently. Otherwise, the session data will be stored into the experiment file for future analysis.

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6.8. Gas Analyzer Monitoring

METABOLISM automatically monitors oximetry data.

This system is able to detect faults or anomalies during acquisition to avoid data loss or collection of incorrect results due to hardware issues.

Warnings detected by the software's automatic monitoring are displayed in the section "Oximetry". The following criteria are monitored:

- Null values of measured concentrations
- Reference values outside than the expected range
- Difference between the cage and reference concentration is insufficient
- Lack of reference for a measurements cycle in progress

All the generated notes are added to the notes list for the session (see section 6.6).

6.9. Change Protocol Association

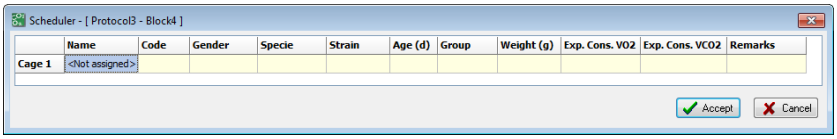
To change the protocol associated with a specific box block before starting a new acquisition session, press the [Protocol…] button and the "Select protocols and blocks to run" dialog will be shown.

Modify the associations as desired and press the Ok button.

The system will prepare new acquisition windows according to the user defined associations.

6.10. Change Subject Schedule

To assign a new set of subjects before starting a new acquisition session, press the [Scheduler…] button, and the Scheduler dialog will be shown.



Modify the subject assignment and press the Accept button.

6.11. Running Multiple Sessions Simultaneously

When more than one box block has been prepared for acquisition, it is possible to start a synchronized acquisition of all of these blocks.

Press the "Start all" button from the toolbar on any of the opened acquisition windows to start multiple sessions simultaneously. This will initiate all the sessions already prepared to run.

To stop all the running sessions, press the "Stop All" button from the toolbar on any of the opened acquisition windows. All the running sessions will stop and the confirmation dialog will appear to save the session data as described in section 6.7.

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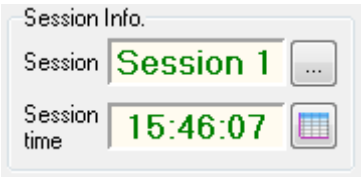
7. DATA ANALYSIS AND REPORTING

METABOLISM can analyze data acquired during the acquisition process or imported session.

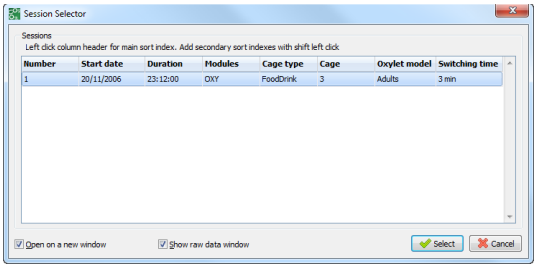
All the digital data are stored together in the same experiment file.

Each time the user selects the "Session Explorer" task, the program defaults to the last registered session and an "Analysis" window opens.

7.1. Session Selection



Click on the […] button from the Session Info to analyze a different session. The Session Selector dialog displays.



Select the desired session on the grid and press [Select] button.

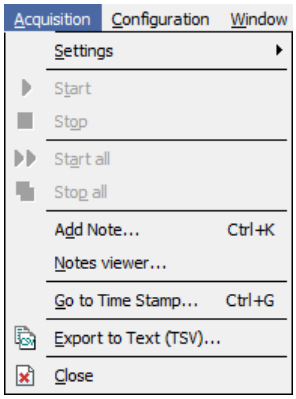
Uncheck the option "Open in a new window" in order to replace the current opened session for the new one, or keep the option checked to open a new analysis window.

There are two windows shown: the Acquired data window and Analysis window.

7.2. Acquired Data Window

The Acquired data window allows the user to review raw data in the same manner it was recorded.

7.2.1. Exporting Raw Data



Metabolism enables you to export all the samples of all the cages and all the columns of each tab in the Numerical View. Please note, that this option is only available when "Numerical View" is selected.

To export raw data:

- Choose Acquisition > Export to Text (TSV)…
- Select a location for the file and enter a filename.
- Your TSV file (Tab Separated Value file) saved in the location you selected.

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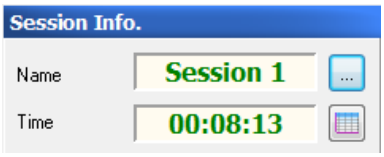
7.3. Analysis Window

The analysis window has four sections:

- Session Info
- Switching Settings
- Consolidation Parameters
- Numeric Result

7.3.1. Session Info

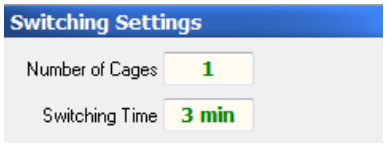
The Session Info section shows the name of the current selected session and the duration.



Users can switch between the Acquired window and the Analysis window through the switch button  .

7.3.2. Switching Settings

The Switching Settings section shows the number of cages and the switching time set during the acquisition process.



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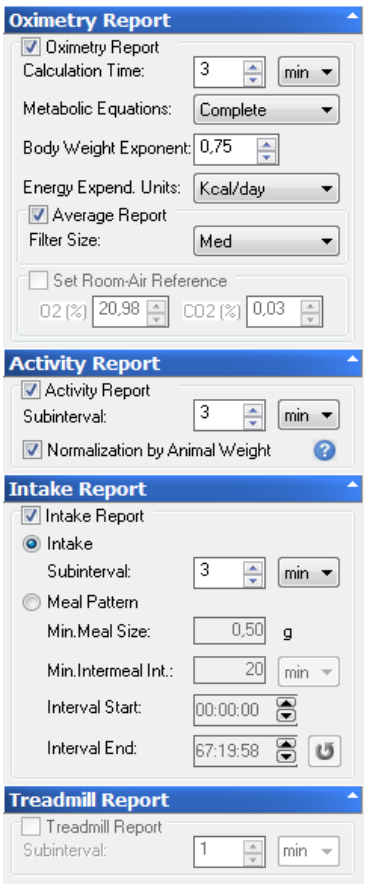
7.3.3. Consolidation Parameters

The section "Consolidation parameters" will allow the user to setup the parameters for each report.

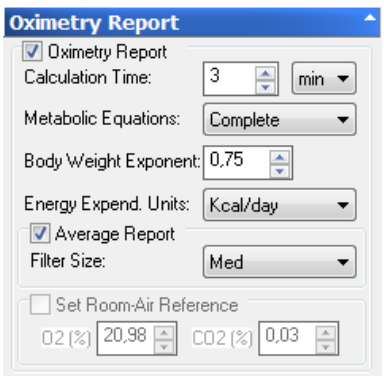


The user-defined time intervals can be in either units of seconds or minutes.

7.3.4. METAOXY Analysis Configuration

The Oxymetry section provides the configuration for analyzing data obtained with the METAOXY module.

7.3.4.1. Activate the report



Check the "Oximetry Report" option for requiring the associated report.

7.3.4.2. Oximetry calculation time

Settings for this parameter will depend on the switching time used in the experiment. The user-defined times intervals can be in either units of second or minutes.

The next paragraph explains how the selection of the oximetry calculation time will affect the data reported.

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Indirect calorimetry evaluation is sequential with a switching time set on each Air Flow & Switching unit. A maximum of four Physiocages can connect to each Air Flow & Switching unit. When working with more than four cages, the user will need multiple switching units. When the measurement completes on all cages, it begins again with the first cage.

Here is an example of measurement sequence with 8 cages:

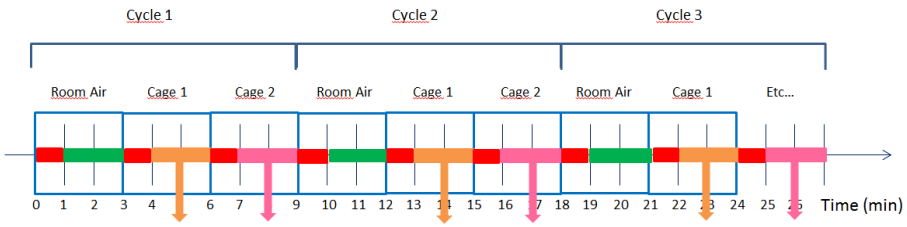
Measurement number	Cycle number	Source of the measured air
1	Cycle 1	Room air
2	Cycle 1	Cage 1 of switching unit 1
3	Cycle 1	Cage 2 of switching unit 1
4	Cycle 1	Cage 3 of switching unit 1
5	Cycle 1	Cage 4 of switching unit 1
6	Cycle 1	Room air
7	Cycle 1	Cage 1 of switching unit 2
8	Cycle 1	Cage 2 of switching unit 2
9	Cycle 1	Cage 3 of switching unit 2
10	Cycle 1	Cage 4 of switching unit 2
11	Cycle 2	Room air
12	Cycle 2	Cage 1 of switching unit 1
13	Cycle 2	Cage 2 of switching unit 1
14	Cycle 2	Cage 3 of switching unit 1
15	Cycle 2	Cage 4 of switching unit 1
16	Cycle 2	Etc…

The first minute of each measurement is discarded (in red) because it represents a mixture of air from the new cage and the previous cage that was analyzed from the switching process.

How are the calculations done?

Case 1: switching time = calculation interval of analysis.

Example: **3 min switching time** measurement and a calculation interval setting of **3 min analysis**:



In this case, each cycle will begin with a room air evaluation and each 3 minutes, the system will switch between the cages.

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If the calculations account for an interval time of 3-minute, one value will be given for each cage in each cycle.

Example of VO_2

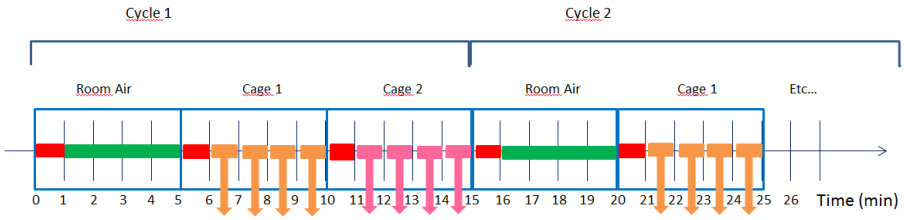
The calculation of VO_2 for one cage in one cycle uses a formula that takes into consideration the $[O_2]$ in the cage with respect to the $[O_2]$ in the room air in the same cycle.

In other words:

VO_2 cage 1 cycle 1 = $[O_2]$ in cage 1 in cycle 1 (taking the average of the 2-minute sample since the first min is discarded) with respect to $[O_2]$ in room air in cycle 1 (taking the average of the 2-minute sample again, since the first minute is discarded).

Case 2: switching time >>>> calculation interval of analysis.

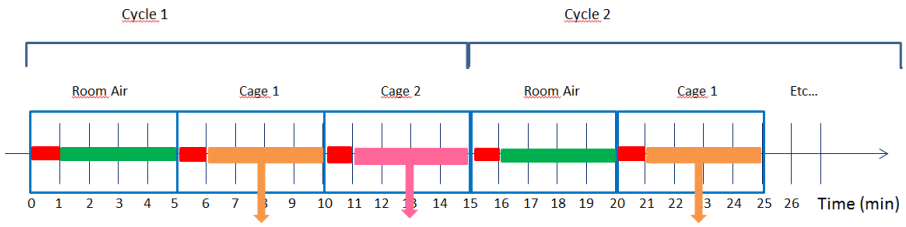
In this example, there is a **5 min switching time** and a calculation interval setting of **1 min analysis**:



For cage 1, when the first min is discarded, 4 minutes of samples are available for $[O_2]$. As the calculation interval of time of analysis is 1 minutes, the system will be able to calculate four values of VO_2 for each cage in each cycle. For these four calculations, the same $[O_2]$ in room air will be considered (average of all the available samples available after subtracting the first minute).

Case 3: (switching time -1) ≤ calculation interval of analysis.

For a **5 min switching time** measurement and a calculation interval setting of **6 min analysis:**



For cage 1, when the first minute is discarded, 4 minutes of samples are available for $[O_2]$. If the calculation interval of time of analysis is ≥ 4, the system will take all the available samples (in this case 4 minutes). The same data will be obtained whether the user selects an interval of calculation of 4, 5, 6 minutes or greater.

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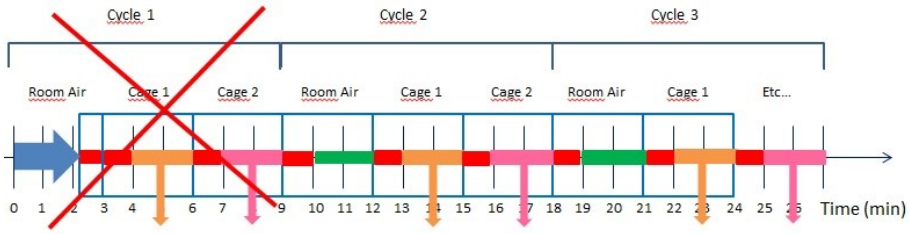
Case 4: Room air measurement time < 1 min in the first cycle.

An example of a **3 min switching time** measurement and a calculation interval setting of **3 min analysis** with a shorter room air measurement time in cycle 1:



If the room air measurement duration in the first cycle is < 1 minute, room air (nor VO_2 calculation in each cage) will not be calculated in the cycle because the first minute is discarded, so the system will have no room air sample to calculate the reference values. Therefore, the calculations will begin with the cycle 2.

7.3.4.3. METABOLISM equations

By default, METABOLISM will use complete metabolic equations (refer to APPENDIX

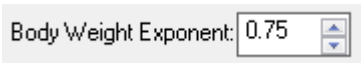
OXIMETRY equations) in order to calculate the OXIMETRY results.

Consequently, results will be unaffected by abnormally high concentrations of carbon dioxide in the room air. There is the option to use approximate equations that were employed in earlier versions of METABOLISM in the event the user wants to compare data with previously recorded data obtained with older versions of the software. When comparing data taken from different experiments, it is recommended that both analyses use the same equations.

If the user needs to compare previously calculated results with newly acquired data ones, the user can select the approximate equations option in the dropdown menu.

7.3.4.4. Body weight

The body weight will be used as a scaling factor in the metabolic equations (refer to the Annex 8.1 for detailed formula).



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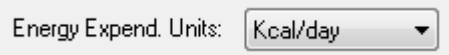
7.3.4.5. Energy expenditure unit

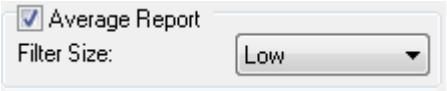
The "Energy Expenditure" calculation can by expressed in several units:



a) Kcal/day
b) Kcal/hour
c) Kcal/min
d) cal/min

7.3.4.6. Average report

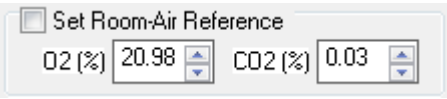
The option "Average Report" generates a more realistic and smoother result in the VO2, VCO2 and RQ curves in environments where there are considerable fluctuations of room air O2, CO2 concentrations during the experiment. It averages the specified amount of samples cycles of room air and cage to render the final result.



The filter size option determines how many calculation cycles will be rolling averaged. It will take 3, 5 and 7 calculation cycles for the Low, Medium and High filter size respectively.

7.3.4.7. Alternative room air reference

The "Set Room-Air Reference" option allows the user to set a gas reference for the whole session for the calculations. It is only available for TREADMILL cage experiments.



The user may activate this option if the first room-air reference cycle was not valid. Reasons that it might not be valid are if there was not enough time for its cycle completion or if the user prefers testing with different reference values.

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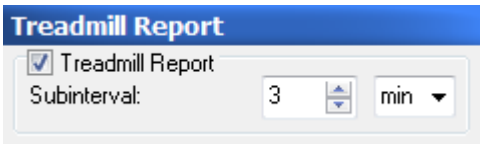
7.3.5. METAACT Analysis Configuration

The METAACT module provides two different reports depending on the experimental chamber used:

a) Activity report for Physiocage cage,

b) Treadmill report for Treadmill cage.



In both cases, the user-defined time interval can be in either seconds or minutes.

The system will provide the data of the animal activity or treadmill performance for each user-defined time interval.

7.3.5.1. Normalization by animal weight

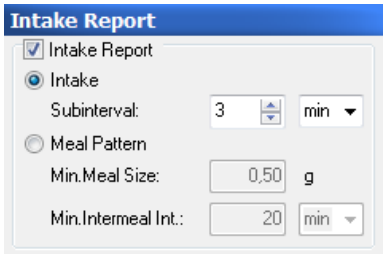
In order to facilitate the comparison of results from different weighted animals, METABOLISM allows the user to normalize the activity depending on the weight of the animal.

The normalized activity is the registered activity divided by the weight of the animal in Kilograms.

This is only an option if the user enters the weight of the animals.

7.3.6. METAINT Analysis Configuration

The Intake section provides the configuration for analyzing the data obtained with the METAINT module.



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Specific report parameters:

- Intake report:

  Subinterval: amount of time for accumulated/partial intake analysis.

- Meal pattern report:

  Minimum Meal Size: minimum quantity of food consumed to define a bout or part of a bout in a meal.

  Minimum Intermeal Interval: minimum interval time allowed between two bouts considered as part of the same meal.

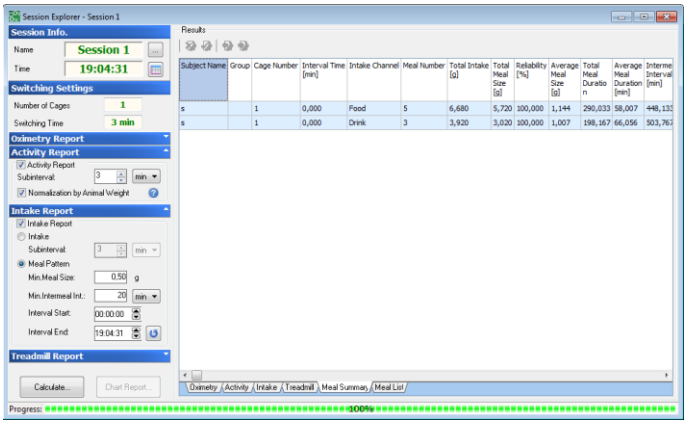
7.3.6.1. Intake report

The user-defined time interval can be in units of either seconds or minutes.

The system will provide the data of the animal intake for each user-defined time interval.

7.3.6.2. Meal pattern

Meal pattern analysis generates two additional sheets: Meal Summary and Meal List as shown below.



7.3.6.3. Meal summary

This report sheet shows a summary of meals pattern analysis for each association of channels and subject. The report contains the following columns:

- Subject's name
- Subject's group
- Cage Number
- Interval Time: Starting of the subinterval
- Intake channel association
- Meal Number
- Number of meals

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- Total Intake in grams:

  Quantity of food/drink consumed, calculated by the difference between last and initial weight values (independently of user-defined criterions of meal definition).

- Reliability:

  Reliability of the signal quality as a percentage, that allows detecting "anomalies" in the signal due to user error (for example: incorrect installation of the dispensers, dispenser manipulation during the acquisition, etc… see Annex 8.2) or hardware problems. Summary Table Data with reliability is < 95% are highlighted in grey.

- Total Meal Size:

  Cumulative quantity of food/drink consumed within the meals.

- Average Meal Size:

  Average quantity of food/drink consumed within a meal.

- Total Meal Duration:

  Cumulative time spent in meal intakes.

- Average Meal Duration:

  Average time spent in a meal intake.

- Total *Intermeal* Interval:

  Cumulative time spent between meals. When user-defined time interval contains one or no meals, the intermeal interval (time between two consecutive meals) cannot be calculated given that the next meal is not encountered. In this case, the corresponding Total intermeal interval cell indicates "None".

- Average Intermeal Interval:

  Average time spent between two meals. Shows "None" when Intermeal Interval cannot be calculated (number of Meals<2).

- Eating Rate:

  Average meal size divided by average meal duration, shows "None" when no Meals have been detected.

- First Meal Size:

  Quantity of food/drink consumed within the first meal.

- Latency First Mean:

  Latency for the beginning of the first meal intake. When no meals have been detected, the latency for the first meal corresponds to the entire time interval of analysis.

- Average Satiety Ratio:

  Average ratio between intermeal intervals and previous meal sizes. This shows "None" when the number of Meals detected is < 2.

7.3.6.4. Meal list

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The Meal List report shows a detailed list and analysis of each meal detected, grouped by subject and channel. The report contains the following columns:

- Subject name
- Group
- Cage Number
- Meal number
- Intake channel association
- Meal Initiation:

  Relative meal starting time
- Meal Duration:

  Defined as the time elapsed from the beginning of the first bout of the meal to the end of the last bout within the same meal.
- Meal Size:

  Quantity of food/drink consumed within the meal.
- Intermeal Interval:

  Time spent from the end of the meal to the beginning of the next one initiated from the same dispenser. For the last meal of each channel, the intermeal interval, defined as the duration between two consecutive meals, cannot be calculated. In this case, the corresponding cell indicates "None".
- Satiety ratio:

  Ratio between intermeal interval and meal size. For the last meal of each channel, the satiety ratio, depending on the intermeal interval, cannot be calculated. In this case, the corresponding cell indicates "None".

7.3.6.5. Pattern plot graph

The pattern plot graph shows the distribution of the bouts registered along the analyzed interval. Bouts are represented as small triangles aligned to the bout start time.

The plot graph also shows the meals detected within the analyzed interval according to the analysis parameters. Meals are represented as rectangles aligned to the start time of the first bout and with a size proportional to the duration of the meal.

When a meal cannot be completely drawn on a single row, the rest of the meal is drawn on the next row.

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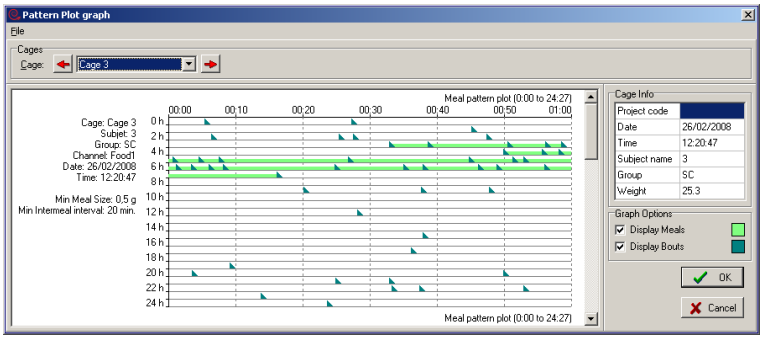


The user can hide/show the bouts and the meals. In order to show or hide bouts/meals, check/uncheck the corresponding graph options: ***Display Meals***; ***Display Bouts***.

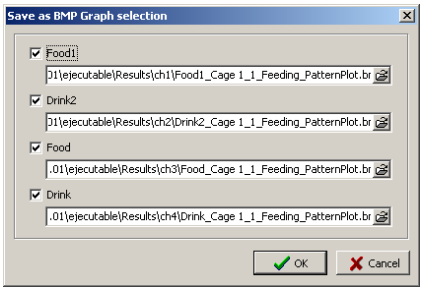
It is also possible to change the color used to draw bouts and meals. To do this, click on the color selector behind the corresponding option (*Display Meals, Display Bouts*). Then, select the desired color and close the dialog by clicking the ***OK*** button. The selected color is assigned, and the pattern plot updated.

As usual in METABOLISM's graphical reports, this report can be exported as an individual bitmap file.

To do this, click on ***File - Save as BMP*** menu option. It will display the *Save as BMP Graph selection* dialog.

Select the graphs you want to export into a bitmap file.

Introduce the path and the file name for each graph and click the OK button.



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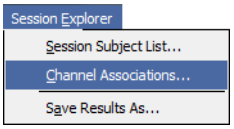
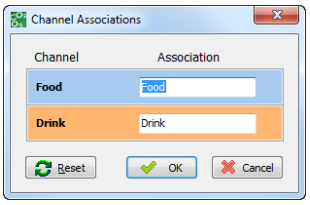
7.3.6.6. Channel Associations

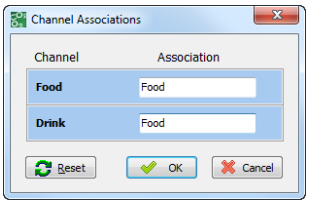
Different intake channels may be considered as one single intake channel during analysis process. The sample values (intake quantities) of the associated channels are added together to obtain the information of the new channel. This is helpful if there is more than one intake channel with the same food or drink.



To define the channel associations, open the channel associations dialog by using the "*Session Explorer* \ *Channel Associations…*" menu option.



To define associations, type the name of the association for each intake channel. Channels with the same association name will then be associated. While editing the associations, the associated intake channels will all have the same background color. All associated channels have the same color.



Upper and lower-case letters, as well as empty spaces at the beginning or at the end of the association are not considered.

Analysis reports are labelled with the name of the association, not the name of the channel.

To disable channel associations, use a different association name for each channel.

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7.4. Start Calculating

Press the button [Calculate] for starting the calculation process and report generation.

7.5. Results Given

The results provided will depend on the METABOLISDM modules used during the data acquisition.

Results are presented in spreadsheet format, one spreadsheet for each module.

The first rows of each spreadsheet show the same shared data for each report.

Listed below each of the parameters is shared among all the reports.

7.5.1. Session Data

- Experimenter: name of the experimenter entered in the Edit Header option in the main menu in the experimental file.
- Challenge: name of the challenge entered in the Edit Header option in the main menu of the experimental file.
- Dose: dose of the treatment entered in the Edit Header option in the main menu of the experimental file.
- Time: Time of the first registration of the experimental file and shown in the Edit Header option of the main menu.
- Calibration (Low O_2): Low point of O_2 used during calibration and entered in the Edit Header option of the Window main menu of the experimental file.
- Calibration (High O_2): High point of O_2 used during calibration and entered in the Edit Header option of the Window main menu of the experimental file.
- Calibration (Low CO_2): Low point of CO_2 used during calibration and entered in the Edit Header option of the Window main menu of the experimental file.
- Calibration (High CO_2): High point of CO_2 used during calibration and entered in the Edit Header option of the Window main menu of the experimental file.
- Remarks: Remarks entered in the Edit Header option of the main menu of the experimental file.

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7.5.2. Subject Data

- Cage n: Number of the experimental cage, as numbered by the METABOLISM software during the data acquisition.
- Subject name: Name of the subject entered by the user in the subject database.
- Code: Code of the subject entered by the user in the subject database.
- Genotype: Genotype of the subject entered by the user in the subject database.
- Group: Group name of the subject entered by the user in the subject database.
- Gender: Gender of the subject entered by the user in the subject database.
- Age: Age of the subject entered by the user in the subject database.
- Weight: Weight in grams of the subject entered by the user in the subject database.
- Remarks: Comments entered by the user in the subject database.
- Basal Activity: Activity threshold set in the Activity graphs (see Chapter 7.5.10.1. Basal activity level) for normalizing the activity calculations.

7.5.3. System Results

- Absolute time:

  Absolute duration of the experiment, from the beginning of the acquisition.

- Relative time:

  Elapsed time from the onset of the session.

7.5.4. METAOXY Oximetry Results

- VO_2 (ml/min/Kg^k):

  Volume of Oxygen consumed weighted by the animal's weight entered in the subject database (k is the body weight exponent specified in 7.3.4.4)*. See the formula in Annex 8.1.

- VCO_2 (ml/min/Kg^k):

  Volume of Carbon Dioxide produced weighted by the animal's weight entered in the subject database (k is the body weight exponent specified in 7.3.4.4)*. See the formula in Annex 8.1.

- EE (kcal/day/Kg^k):

  Energy Exchange weighted by the animal's weight entered in the subject database (k is the body weight exponent specified in 7.3.4.4)*. See the formula in Annex 8.1.

  To express the value in kcal/min, divide the value by 1440 (1440 is the number of minutes in one day).

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To express the value in cal/min, divide the value by 1440 (1440 is the number of minutes in one day) and multiply it by 1000 (1000 is the number of calories in 1 kcal).

- VO_2 (ml/min):

  Volume of Oxygen consumed (ml/min). See the formula in Annex 8.1.

- VCO_2 (ml/min):

  Volume of Carbon Dioxide produced in cage n (ml/min). See the formula in Annex 8.1.

- EE (u):

  Energy Exchange in cage n (u is the units specified in 7.3.4.5). See the formula in Annex 8.1.

  To express the value from kcal/day to kcal/min, divide the value by 1440 (1440 is the number of minutes in one day).

  To express the value from kcal/day to cal/min, divide the value by 1440 (1440 is the number of minutes in one day) and multiply it by 1000 (1000 is the number of calories in 1 kcal).

- RQ

  Respiratory Quotient (also called RER, Respiratory Exchange Ratio). See the formula in Annex 8.1.

- Room Air O_2 (%):

  Mean concentration of Oxygen content in room [Calculation time]. The value depends on the interval time configured for the calculation (see chapter 4.3.1.2 for more details).

- Room Air CO_2 (%):

  Mean concentration of Carbon Dioxide in room [Calculation time]. The value depends on the interval time configured for the calculation (see chapter 4.3.1.2 for more details).

- Cage O_2 (%):

  Mean concentration of Oxygen in cage [Calculation time]. The value depends on the interval time configured for the calculation (see chapter 4.3.1.2 for more details).

- Cage CO_2 (%):

  Mean concentration of Carbon Dioxide in cage [Calculation time]. The value depends on the interval time configured for the calculation (see chapter 4.3.1.2 for more details).

- Flow (ml/min):

  Mean Flow [Calculation time]. This value depends on the interval time configured for the calculation. The samples considered for the mean calculation are the same than the same considered for the VO_2 and VCO_2 calculation in each cage.

- Remarks:

  Text indicating the reasons for rejecting the value (see chapter 7.5.11 Rejecting Invalid data).

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* When the weight of the subject is not indicated in the subject database, the value in the data table is replaced by a blank.

7.5.5. METAINT Intake Results

The names of some calculations (listed below) are shown with default settings in the Intake Channels configuration panel (see Chapter 0 Naming the Intake channels). Depending on this configuration, a different name can then be shown in the report.

- Food (g):

  Weight of the remaining food measured in the feeder expressed in grams and calculated for each user-defined interval of time.

- Consumption (g):

  Weight of food consumed, expressed in grams and calculated for each user-defined interval of time.

- Food Cons. rate (g/g):
- Food consumption rate defined as the weight of food consumed by unit of animal weight.
- Drink (ml):

  Weight of remaining liquid measured in the drink container expressed in grams and calculated for each user-defined interval of time. When the liquid is water, there is a direct 1:1 correlation between the weight in grams and the weight in ml.

- Consumption (ml):
- Weight of liquid consumed expressed in grams and calculated for each user-defined interval of time. When the liquid is water, there is a direct 1:1 correlation between the weight in grams and the weight in ml.
- Drinking Cons. rate (ml/g):
- Liquid consumption rate defined as the weight of liquid consumed by unit of animal weight.
- Remarks n:
- Text indicating the reasons for rejecting the value (see chapter 7.5.11 Rejecting Invalid data).

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7.5.6. METAACT Activity Results

WARNING: The value of the calculations provided in the following list depends on the activity threshold set in the Activity graphs (see Chapter 7.5.10.1 Basal activity level) for normalizing the activity calculations. This normalization allows the user to compare data among different subjects in the experiment.

- Activity N:

  General activity of the animal in cage N where N represents the cage number.

- Rearing N:

  Number of rearing events in cage N where N represents the cage number.

- Remarks N:

  Rejection reason in cage N where N represents the cage number.

7.5.7. METAACT Treadmill Results

- Speed:

  Speed of the treadmill set on the Treadmill Control Unit (cm/s).

- Distance:

  Distance travelled by the subject on the treadmill device (cm).

- Shock Time:

  Accumulated duration of the shocks given from the beginning of the session (sec).

- Shocks:

  Accumulated number of shocks received by the subject from the beginning of the session.

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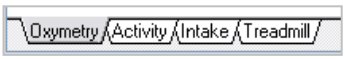
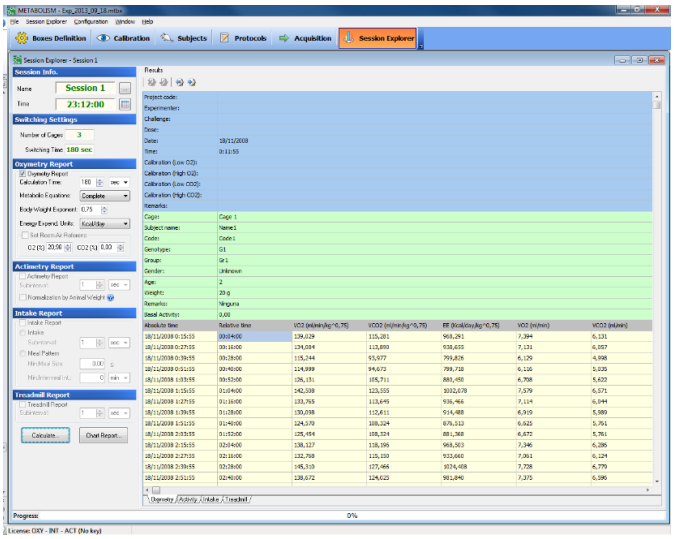


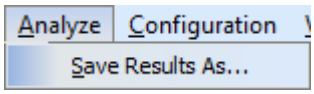
7.5.8. Numeric Results Report

The results obtained can be visualized by selecting the corresponding tab available on the bottom part of the screen.

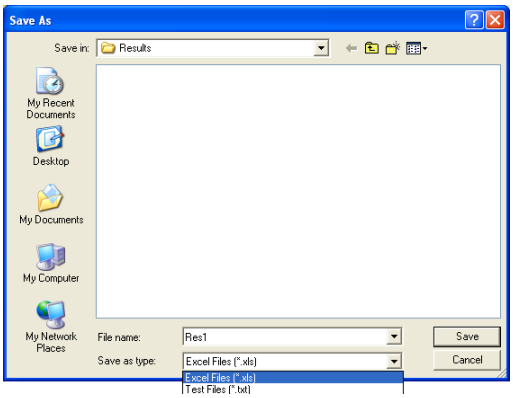


Data can be re-calculated using different user-defined intervals by specifying the appropriate information in the ***Consolidation Parameters*** box and clicking again on the [Calculate] button.

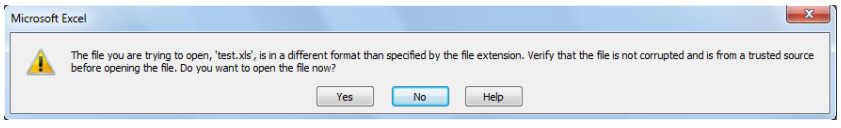
7.5.9. Saving Results



Open ***Session Explorer*** menu, select ***Save Result As***, enter the ***name*** of the file, choose the folder of destination, the format (Excel or text) and press ***Save***.



Note that when opening the report with Excel 2010, the following message may be shown and is dependent on the updates installed:



Ignore this message and click on the 'Yes' button to open the document.

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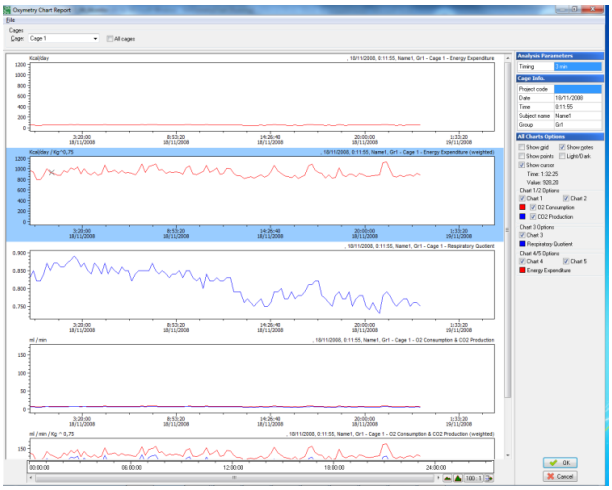
7.5.10. Graphical Analysis Reports

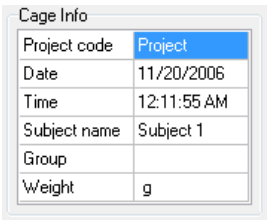
After the data generation, it is possible to create graphic reports for each cage. To do this:

- Select the data tab for the graph you wish to generate.
- Click [Chart Report] button to generate the graph.



- Select the desired cage through the cage list. Corresponding data will be displayed graphically.
- To save this graph as a BMP file, select File/Save as BMP menu option and choose a folder.

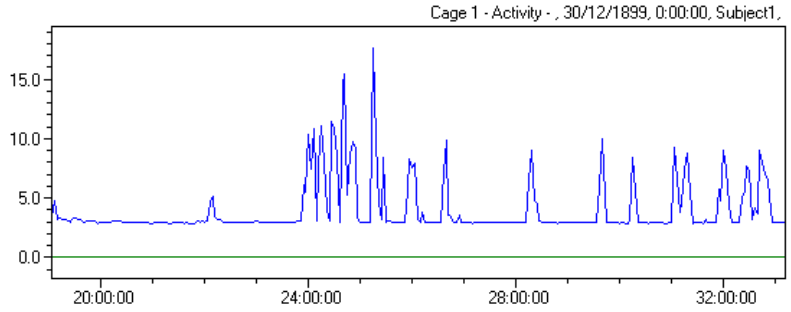
7.5.10.1. Basal Activity Level



In some situations, comparing two activity reports becomes very difficult due to the different vertical displacement of both signals. This displacement is the "Basal activity level".

In order to facilitate the comparisons and to normalize the Activity Analysis Report calculations, it is necessary to set the activity basal level. This is done through the *Baseline* tool of the graphic activity report.

A *Basal activity* field is shown in the "Cage info" section of this report. Initially, basal activity is set to zero but it can be changed so that the final signal is recalculated, subtracting this new basal activity level from the original value calculated by the analysis process.



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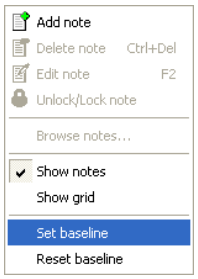
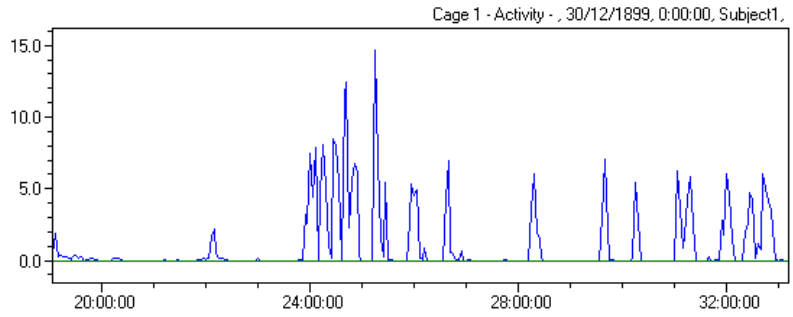
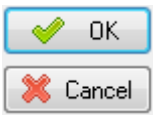


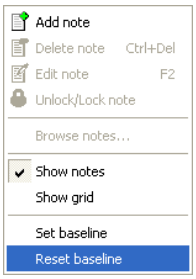
To set or change the basal activity level:

- Right click on a blank space of the activity graph.
- In the contextual menu, choose Set baseline option.
- A green line (baseline) indicates the current basal activity level.
- Drag the baseline and drop it in the new basal activity level.

When the baseline drops, the graph and the "Cage info" panel re-calculate, showing the effect of setting the new basal activity level.







Closing the graphic report with the [OK] button will cause the analysis numeric report to be recalculated. However, [Cancel] is used to close the report; the new basal activity level will be discarded.

The *Reset baseline* contextual menu option helps to set the basal activity level back to zero. It will update the graph using the original values calculated through the analysis process.

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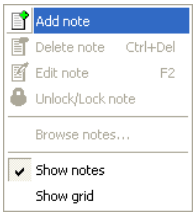
7.5.10.2. Graph notes

Experimental notes can be added to the graphic analysis report. Thus, plots can be documented directly from METABOLISM without editing the bitmap files with external applications not designed for this purpose.

To do that, there is a special contextual menu option in graphs: Add note.

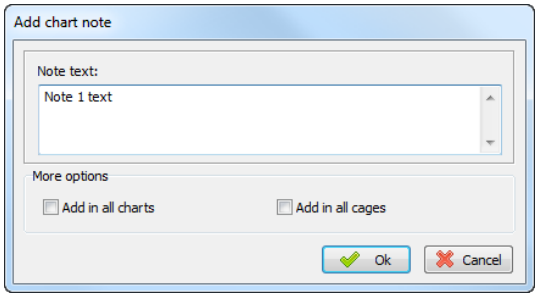
ADDING A NEW NOTE



Notes link to a time position. Right clicking on the graph will show a vertical line indicating the position in which the note will be inserted.

To add a new note, right click on the desired time position and select the contextual menu option "Add note".

The "Add chart note" dialog shown below will display:

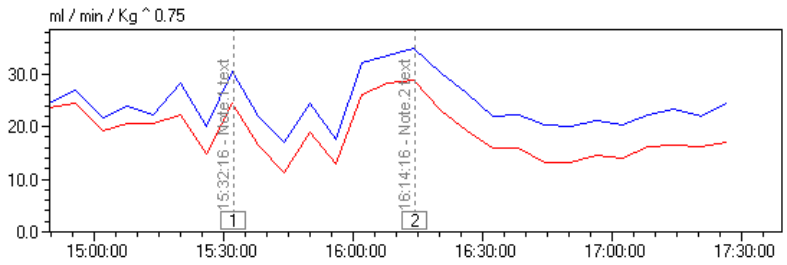


Enter the text to display in the "Note text" box and check the desired options whether you want to:

- Add in all charts: Insert the same note at the same time position of all the charts of the current cage.
- Add in all cages: Insert the same note at the same time position of the same chart of all the experimental cages.

Pressing the [OK] button will add the new note in the graph, showing it as a vertical grey dotted line including:

- The note number
- The time position
- The entered note text



Notes are intended only for presentation purposes so they will not be saved into the experiment file and will be lost when closing the analysis window.

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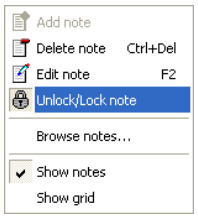
SHOWING AND HIDING NOTES

It is possible to hide inserted notes by unchecking the box "Show notes" of the "All Charts Options" section, in the right panel of the window.

Checking this option again will show all the notes.

This tool is useful for in including or omitting the notes in the exported bitmap file.

MOVING NOTES: LOCKING AND UNLOCKING



Once created, a note is automatically "locked" to the time position in order to avoid unintended movements.

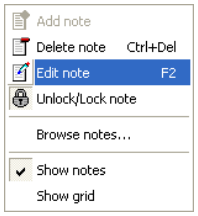
To move a note, it must be unlocked through the contextual menu option "Unlock/lock note".

A blue line indicates unlocked notes.

Once unlocked, dragging and dropping the note to another time position is possible.

Clicking in a different area of the graph will automatically lock the note.

EDITING NOTES TEXT

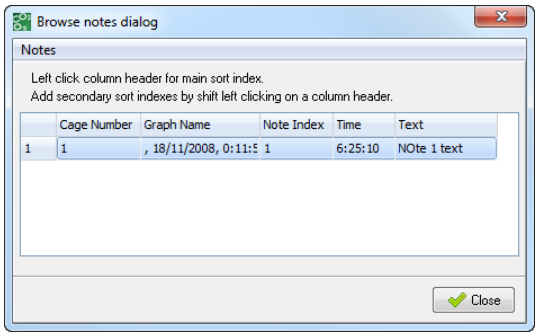


The note text can be modified at any time. To do this, double-click the note number, select the "Edit note" contextual menu option or press the F2 key once and the text is selected.

BROWSING ALL THE REPORT NOTES

Users can browse through all the notes created in the different graphs of a graphic analysis report using the "Browse notes" dialog.

To access the dialog, select the "Browse notes …" menu option of any graph.



[Browse notes] dialog permits the user to:

- Sort the notes by clicking on the column headers
- Change the column positions by dragging its headers
- Export the notes list, through the Notes – Export as TXT menu option.

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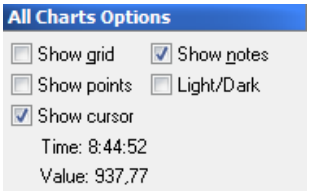


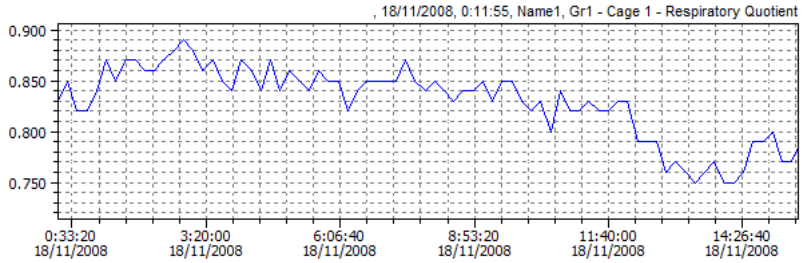
7.5.10.3. Graph cursor and grids

When viewing the graphic reports, knowing the exact values of the plots is very useful.

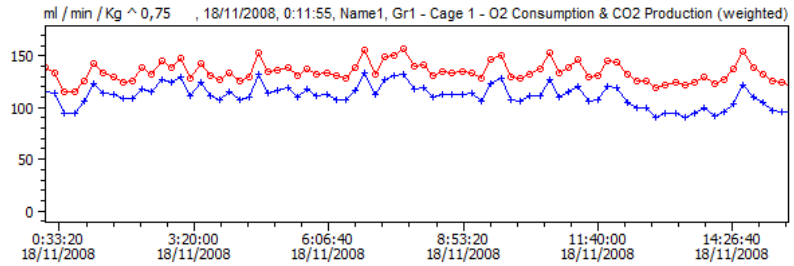
This information can be obtained by simply moving the mouse over the graph and looking for the "Time" and "Value" fields of the "All Charts Options" panel.

When several data series are in the same graph, the value displayed corresponds to the nearest series to the mouse and is indicated by the X on the graph.

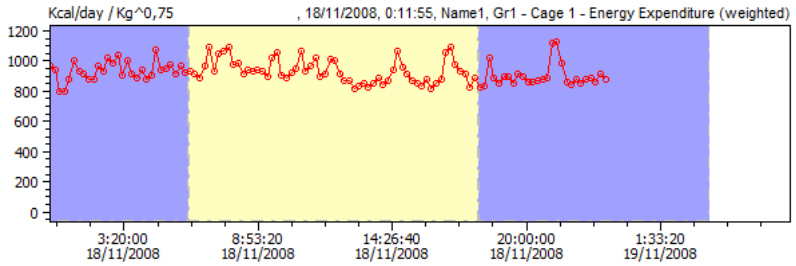
Grids are also a tool to facilitate this task and can be shown or hidden by means of the "Show grid" box in the same section.



Points are a tool to facilitate the task of seeing the exact points generated on the reports.



Light/Dark is a tool that uses two different colors to graph the range of time between 6 a.m. to 6 p.m. as shown in the image below.



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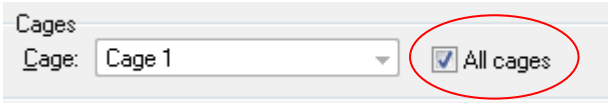
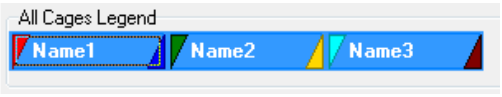
7.5.10.4. Multi-cage Graphic Report

When a session has several cages, it is interesting to compare the results graphically.

Checking the option "All cages", the system will show the same result of all the cages in the same chart.



In addition, a legend on the bottom of the window, allows the user to hide/show the chart of the related cage.



7.5.11. Rejecting Invalid Data

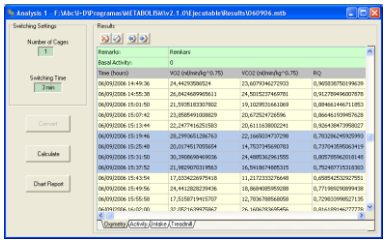
There are times when you may want to discard some specific blocks of calculated data. Some reasons for discarding analyzed data are:

- Data was acquired during a system-testing period.
- The conditions became temporarily unacceptable (e.g., invalid oxygen concentrations).
- A particular time period should not have been included in the data analysis.

This rejection task is selected in METABOLISM through the rejection toolbar located in the top side of the analysis main window (not in the graphic analysis report).

7.5.11.1. Selecting the period to reject

Through drag-and-drop, a set of adjacent time samples can be selected in the numeric analysis.



Selected rows display in blue, indicating the new status.

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7.5.11.2. Rejecting the data block

Once a time period is selected, all data included in this period can be rejected by pressing the button.

A "Reject samples dialog" is launched, showing the data section (Oximetry, Activity, Intake or Treadmill) and the final period to be discarded.

This dialog allows you to enter a rejection reason.

Accepting the rejection will cause the rejected period to display in red and the reasons for rejection are included with the existing ones in the column.

Rejecting data will not delete any acquired samples. It only causes the data to be ignored in following analyses.

Rejection reasons are intended for data documentation purposes only so they will not be saved into the experiment file and will be lost when closing the analysis window.

7.5.11.3. Recovering rejected data

Rejected data blocks can be recovered by means of the "Restore sample block" button.

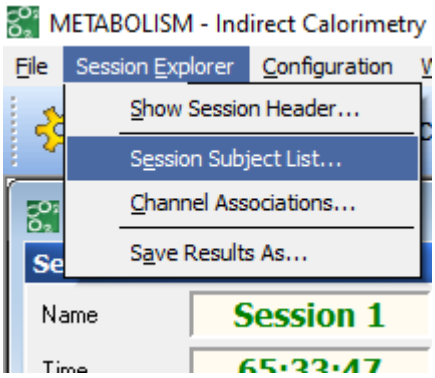
To do that:

- Select the set of rows you want to recover
- Press the button
- Confirm the recovery of the data

When data is recovered, the rejection reasons are cleared and will be lost.

7.5.12. Modify the weight of the animal

It is possible to modify the weight of the animal after the data acquisition and recalculate the new Oximetry and Activity values using the new weight. In order to do so, select **Session Subject List** from the **Session Explorer** menu.



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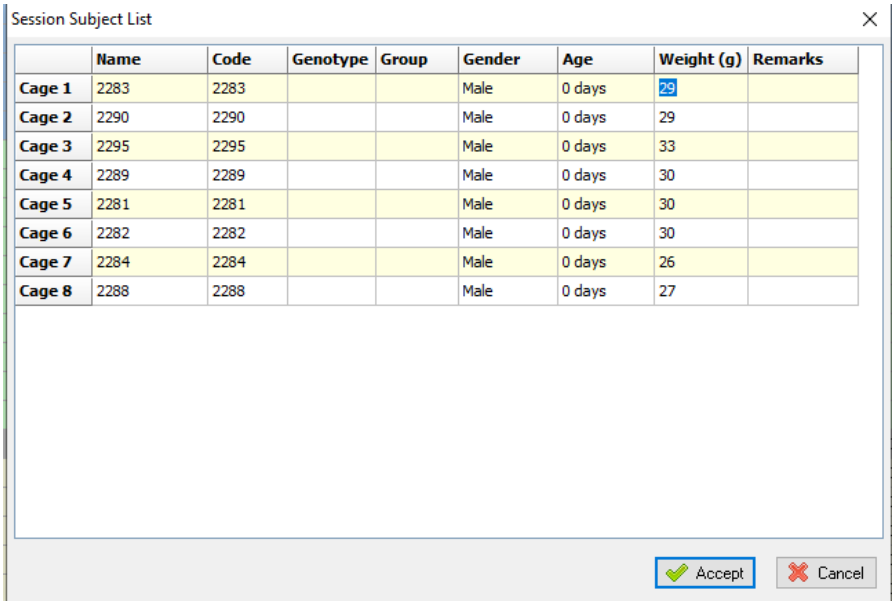
Double click on the cell containing the weight to modify. After editing, click on **Accept**.



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8. APPENDIX

8.1. OXIMETRY equations

Metabolism uses the following formulas to calculate VO_2, VCO_2, RQ and EE. Two versions for VO_2, VCO_2 and EE formulas are shown in the Oximetry report: considering the ¾ power of weight and without considering the weight of the animal.

- VO_2, VCO_2 weighted approximate equations:

$$VO_2 = \frac{\left(F \times \frac{[O_2]_e}{100}\right) - \frac{\frac{[O_2]_s}{100} \times F \times \left(1 - \frac{[O_2]_e}{100}\right)}{\left(1 - \frac{[O_2]_s}{100} - \frac{[CO_2]_s}{100}\right)}}{W^k}$$

$$VCO_2 = \frac{\frac{F \times \left(1 - \frac{[O_2]_e}{100}\right)}{\left(1 - \frac{[O_2]_s}{100} - \frac{[CO_2]_s}{100}\right)} \times \frac{[CO_2]_s}{100} - \frac{[CO_2]_e}{100} \times F}{W^k}$$

- VO_2, VCO_2 weighted complete equations:

$$VO_2 = \frac{\left(F \times \frac{[O_2]_e}{100}\right) - \frac{\frac{[O_2]_s}{100} \times F \times \left(1 - \frac{[O_2]_e}{100} - \frac{[CO_2]_e}{100}\right)}{\left(1 - \frac{[O_2]_s}{100} - \frac{[CO_2]_s}{100}\right)}}{W^k}$$

$$VCO_2 = \frac{\frac{F \times \left(1 - \frac{[O_2]_e}{100} - \frac{[CO_2]_e}{100}\right)}{\left(1 - \frac{[O_2]_s}{100} - \frac{[CO_2]_s}{100}\right)} \times \frac{[CO_2]_s}{100} - \frac{[CO_2]_e}{100} \times F}{W^k}$$

- RQ, EE weighted equations:

$$RQ = \frac{VCO_2}{VO_2}$$

$$EE = \left(3.815 + (1.232 \times RQ)\right) \times VO_2 \times 1.44$$

Where:

$[O_2]_e \rightarrow$ *oxygen concentration flowing into the cage*

$[O_2]_s \rightarrow$ *oxygen concentration inside the cage*

$[CO_2]_e \rightarrow$ *carbon dioxyde concentration flowing into the cage*

$[CO_2]_s \rightarrow$ *carbon dioxyde concentration inside the cage*

$F \rightarrow$ *air flow entering the cage*

$VO_2 \rightarrow$ *oxygen consumption*

$VCO_2 \rightarrow$ *carbon dioxyde production*

$RQ \rightarrow$ *respiratory quocient*

$EE \rightarrow$ *energy exchange rate*

$W \rightarrow$ *weight of the animal*

$k \rightarrow$ *body weight exponent*

1.44 → conversion factor to convert the cal/min unit into Kcal/day unit (1440 min/1000).

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8.2. Reliability

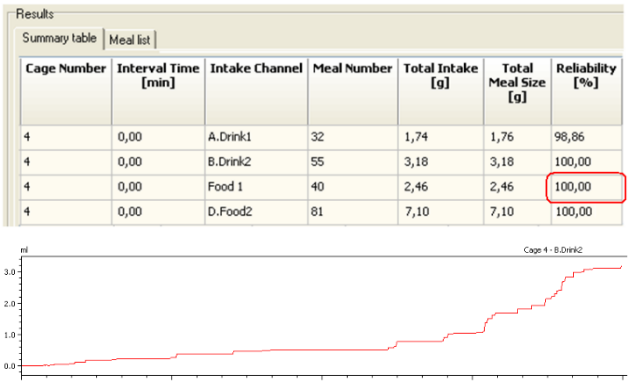
Reliability of the signal is given by the meal pattern analysis in the summary table. It indicates the percentage of confidence of the signal quality and, consequently, of the analyzed data.

Its calculation is based on the formula: 100-(a-b)/a*100, with "a" and "b" being "Total Intake" and "Total meal size" according to the conditions a > b, and with null criterions of meal definition ("Minimum meal size"=0 and "minimum meal interval"=0). This factor is independent of user-defined criterions of meal definition.

Analysis can be considered as acceptable when the Reliability factor is superior to 95%. Inversely, if its value is inferior to 95%, the corresponding raw in the summary table of the meal pattern analysis is highlighted in grey. In this case, the user is recommended to have a vigilant look at the corresponding signal graph of accumulated intake, in order to detect and identify causes resulting in these troubles. Here are several cases that can be encountered:

- Case 1:

  The following example gives an idea of a correct signal, as indicated by a Reliability factor of 100%. The graph shows a classical evolution of food intake for 24 hours.



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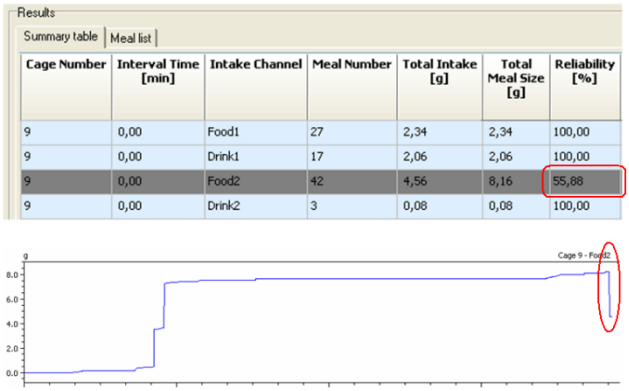
- Case 2:

  The following case gives an example of a low reliability percentage, due to an incorrect manipulation of the experimenter. During the last minutes of recording, an apparent abrupt decrease of food consumption appears, as a result of a premature addition of food by the experimenter in the corresponding canal before the end of the session. In this case, the user can work on an interval of time that does not include the trouble, with a reliability superior to 95%.



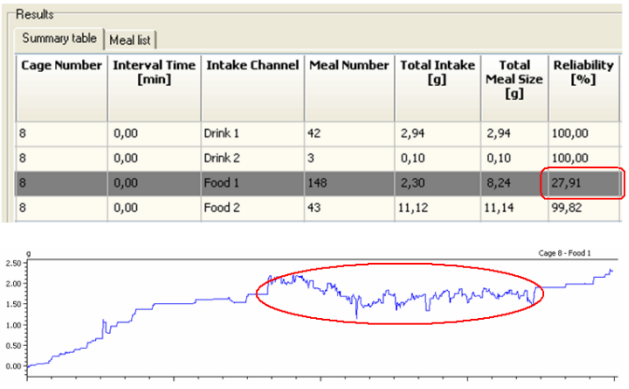
Results

Summary table | Meal list

Cage Number	Interval Time [min]	Intake Channel	Meal Number	Total Intake [g]	Total Meal Size [g]	Reliability [%]
9	0,00	Food1	27	2,34	2,34	100,00
9	0,00	Drink1	17	2,06	2,06	100,00
9	0,00	Food2	42	4,56	8,16	55,88
9	0,00	Drink2	3	0,08	0,08	100,00

- Case 3:

  Here is an example of a signal associated with a low reliability percentage. The graphical representation indicates that a part of the signal contains many negative bouts, which represent an improbable evolution profile of cumulated food intake. This trouble can be explained by an initial bad fixation of the dispenser corresponding to the recorded channel, resulting in a friction with the cage, thus in many weight variations independently of subject's food consumption.



Results

Summary table | Meal list

Cage Number	Interval Time [min]	Intake Channel	Meal Number	Total Intake [g]	Total Meal Size [g]	Reliability [%]
8	0,00	Drink 1	42	2,94	2,94	100,00
8	0,00	Drink 2	3	0,10	0,10	100,00
8	0,00	Food 1	148	2,30	8,24	27,91
8	0,00	Food 2	43	11,12	11,14	99,82

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9. Contact Information

We are available to help you with your questions and concerns. Should you hit a roadblock or need some additional training, please feel free to visit the HBIO Behavioral Support Center at https://support.behavior.hbiosci.com to find articles and helpful information in our knowledge base or submit a ticket. We are happy to help!

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