

# **TECNIPLAST**



DVC®Analytics Instruction Manual ver. 4.0



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# **Revision History**

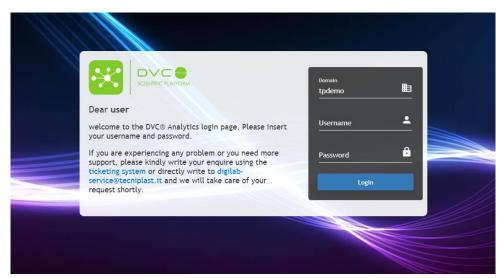
Date	Version	Author	Summary of Changes
15/05/17	V1.0	Giorgio Rosati	First Draft
16/05/17	V1.1	Giorgio Rosati	Explanation of how to download raw data
27/07/2018	V2.0	Giorgio Rosati	DVC Analytics version 2
19/09/2019	V3.0	Giorgio Rosati	DVC Analytics version 3
01/01/2024	V4.0	Giorgio Rosati	DVC Analytics version 4

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# 1 Login page

To login into the system, go to: <a href="https://analytics.dvc.tecniplast.it/login/XXX">https://analytics.dvc.tecniplast.it/login/XXX</a> (XXX provided to you by Tecniplast or got during the registration) and enter your Username and Password that have been previously created.



# 2 Home Page

The top part of the home page (dark blue background) displays general information about the Facility (e.g., Facility name). The lower part of the home page (white background) displays the DVC® Analytics menu.

The home page varies based on the user role, whether Facility Manager or Researcher (the User Setting button is hidden to the Researcher(s) that will have access only to the cages assigned previously by the Facility Manager)



DVC RACKS: how many DVC® Racks are connected to the DVC® Analytics platform.

CAGES: how many cages:

- Running (green): # of registered cages in the DVC® system currently inserted in the DVC® Rack.
- Out of Rack: (yellow): # of registered cages in the DVC® system currently removed from the DVC®
   Rack (but still registered)

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- **Terminated (grey):** # of cages properly terminated in the DVC® system.

USERS: how many registered DVC® Analytics Users are registered (enabled and disabled)

**RESEARCH PROTOCOLS**: how many DVC® Research Protocols have been currently received by the DVC® Analytics (sent by the DVC® System)

The lower part of the home page shows different available buttons depending upon your role (Facility/Researcher) and  $DVC^{\otimes}$  Analytics type (enable per Facility/Rack or by credits)



Through those buttons, you can analyse data and set different Facility rules.

# 2.1 Facility Manager and Researcher Roles

The facility Manager role has been designed to perform any task and have access to all functionalities of the DVC® Analytics system. The Researcher's role has been designed to provide limited access to only a subset of information (only to the Researcher's data) to increase privacy and limit any possibility of "mixing" data.

More specifically, these are the different enabled functionalities for the various roles:

#### **FACILITY MANAGER**

(Has full access to the DVC® Analytics menu)

# RESEARCHER

(Limited access to the DVC® Analytics menu)

- is enabled to view all DVC® cages connected to the
- DVC® Analytics.

   is enabled to register new DVC® Analytics users
- and associate them with DVC® cages.
  is enabled to group DVC® users and associate
- is enabled to set and change the facility settings (e.g., dark hour).
- is enabled to view only DVC® cages previously associated with her/him.
- Is enabled to create cage and mice groups using only his/her cages

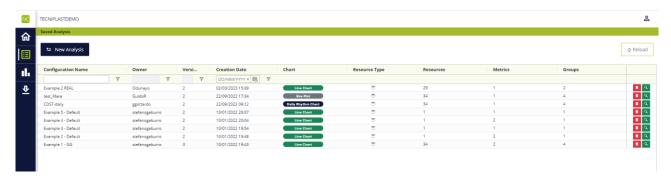
#### 2.2 Data Analysis - V2

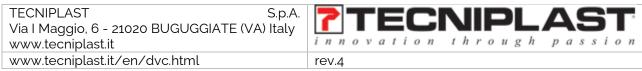
them with the DVC® cages.

In this section, it is possible to analyse an individual cage or group of cages in depth by selecting multiple choices from a temporal and data presentation perspective.

#### 2.2.1 Saved Analysis

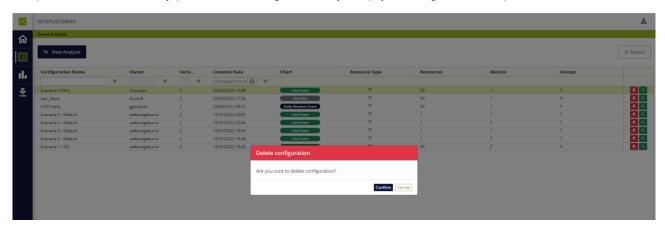
Just clicking on the "Data Analysis V2" icon, you enter a menu section where, if any, all the previously saved configurations are displayed:







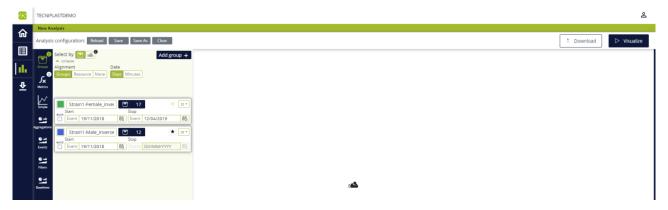
It is possible to delete any pre-saved configuration by simply clicking the correspondent icon



By confirming, the configuration is deleted.

On the other hand, it is possible to load the pre-saved configuration by clicking the correspondent icon

By doing so, the entire configuration is loaded with all the previously selected settings (Cages, groups, metrics, charts, etc.)



Please remember that the "Stop" date is calculated when the configuration has been saved. If you recall the configuration days after, the "Stop" date is still the original one that should be updated in case the cages were running cages.

#### 2.2.2 New Analysis

The "New Analysis" button is needed to start a new analysis where all the fields must be filled out step-by-step based on the experiment.





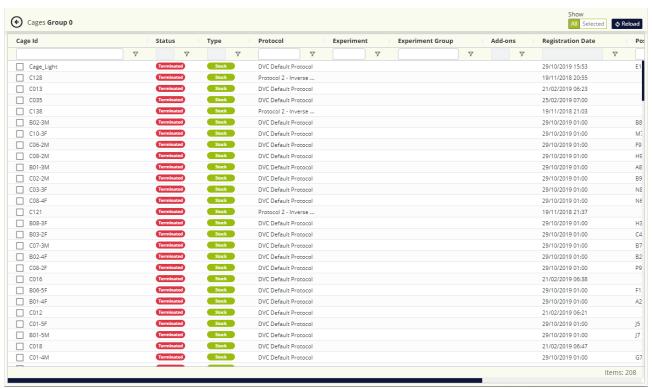


#### 2.2.3 Groups

The first step to start any analysis is to select one or more cages/animals and create one or more groups. To do so, first, decide if the element of the analysis is the cage or the animal. By default, the preferred resource is the cage:

Whatever is described below is valid if the selection is the animal. Pay only attention to the fact that, in the case of using Animal ID as selection, this retrieves all the correspondent cage data where the animal is inserted.

By clicking the corresponding icon , a cage list appears on the right side:



This is the complete list of all the cages created in the Facility. Each cage is displayed by its Cage Id (name assigned during the cage registration), status (Terminated, Running, Out of Rack), Type (Stock, Breeding, Experiment), Protocol (if any, by default "DVC Default Protocol"), experiment (if any), Experiment Group (if any), Add-ons (Running Wheel), Registration Date, Position (coordinate in the DVC Rack), Rack (Name assigned if any, otherwise the SN), Animals (the current number of animals in the cage), DVC Owner (if any, otherwise Anonymous).

You can select one or multiple cages. Depending on this selection, features are enabled in the subsequent sections. For instance, multiple cages in a group allow the display of the SEM or the interquartile and the selection of BoxPlot as a chart type.

You can rename the group by clicking in the corresponding area, "Group o".

You can change the color of the group by clicking the Color Palette

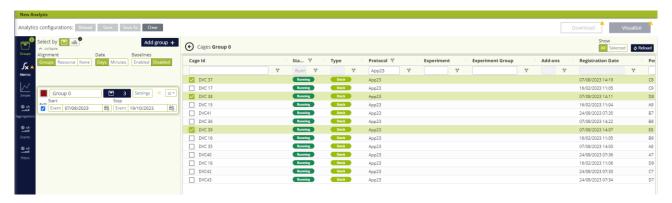
If the "Auto" button is clicked, once more cages are selected, the "Start" and "Stop" fields are automatically filled with the first data received from these cages, and the "Stop" date is the last one.





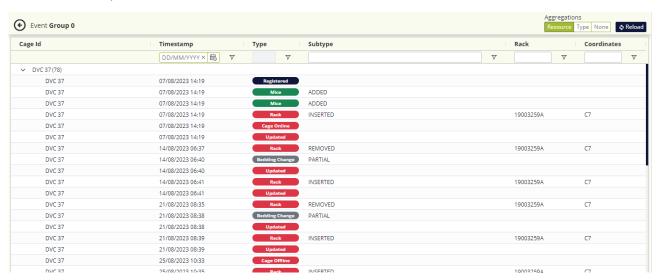


The "auto" button is deselected whenever any date is locally modified.



These dates can be modified manually by clicking the calendar icon 🛅 and selecting the right one.

Alternatively, the timestamp can be selected by looking at the "Event" table generated by the DVC®. This is accessible by clicking the correspondent icon and then by choosing the cage to select the event and its timestamp:



The current DVC® events to be considered are the following:

Туре	Subtype	Description
Registered The cage has been registered in the DVC® system.		The cage has been registered in the DVC® system.
Mice	Added	A new mouse has been added to the cage
	Moved	An already existing mouse has been moved from a cage to this one.
	Culled	A mouse has been culled from the cage.

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Rack	Inserted	The cage has been inserted in the DVC® Rack
	Removed	The cage has been removed from the DVC® Rack.
Update		A field of the cage (Owner, Protocol, etc.) has been updated in the cage
		information.
Bedding Change		When the cage undergoes a bedding change, this is tracked as an event.
Cage Offline		The cage went offline (for instance, the network was down for a while)
Cage Online		The cage is back online (for instance, the network is restored)

All the selected cages can be easily retrieved by selecting the in the corresponding section the button "Selected."

Now, it is time to decide how to align the selected cages. By default, the "Alignment" is by "Groups", and the "Date" field is set in "Days".



This default selection means that all the cages of a group maintain their relative distance in time. Any calculation that will be applied to the group will consider such. But if you select more groups, they are all aligned to the relative day 0 as the starting point.

Differently, by selecting the "Resource" button, the "Start" and "Stop" dates disappear, and the "Duration" button appears.



The "Duration" button sets the length of the overall analysis applied to multiple groups, if any. By default, the starting point of any cage within the same group is determined by the first timestamp associated with the first data/event collected by the DVC® system. All cages within the group are aligned to the relative group day 0. And any calculation that will be applied to the group will consider such.

If you create multiple groups, all the cages of each group are internally realigned to day 0, and all the groups are then realigned to day 0.

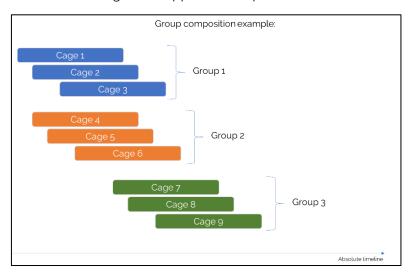
Lastly, by selecting "None" in the Alignment section, no within or between groups alignment is applied. The analysis is performed by considering day o as the "Start" date for all the groups' cages, and they maintain their absolute dates. The length of this analysis is set in the "Duration" box.

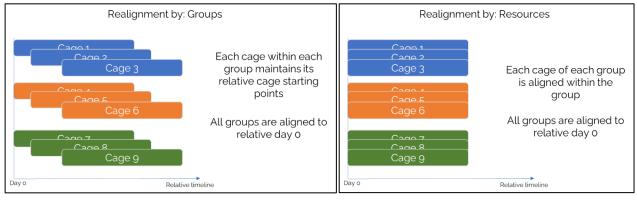
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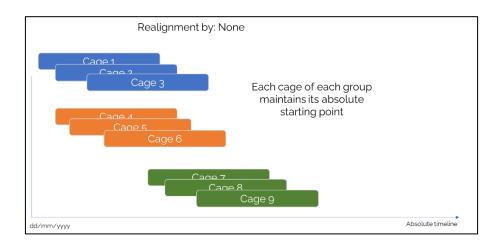
As a visual recap of the different realignment opportunities, please refer to the below pictures:





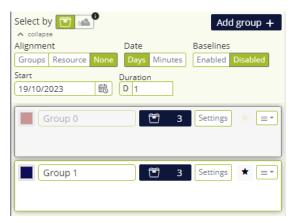
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By selecting in the "Date" section the button "Minutes" Days Minutes, all the previously detailed fields about temporal variables can be set considering "hh: mm."

Then, inserting as many new groups as needed is possible by clicking the corresponding icon Any new group is displayed below the first (primary) one.



All the previously described features are available also for the following groups except for the "Stop" date in the "Alignment: Groups," which cannot be modified manually because the duration of the first group length sets the length of the whole analysis.

Lastly, by clicking the specific icon , it is possible to select different topics:

Item	Comment
Resources	Open the side menu to select cages/animals.
Import Group Resources	Open the side menu to select groups already created in the past.
Primary	Set this group as the primary one.
Save	Save this group for future retrieval.
Split	Split the group into the individual correspondent cages/animals.
Clone	Clone the group with the same cages.
Delete other groups	Delete all the other groups (only if this is the primary group)
Delete	Delete itself (only if this is not the primary group)

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## 2.2.4 Baseline setting

There is the opportunity to create a baseline period for each group cage by clicking the corresponding button (by default, it is disabled).



When enabled, it enables the corresponding fields in the "Setting" section:



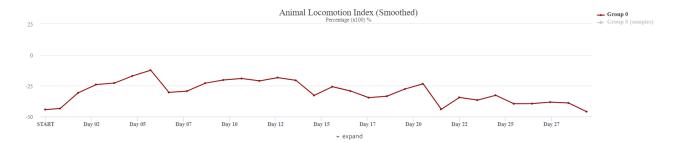
For every cage, assigning a "start Baseline" date and the length of the "Baseline duration in days" is possible. The baseline period will be calculated (based also on the following settings, such as the aggregation and filters) and applied to the remaining days of the analysis period to normalise the data.



The formula we apply is the following:

$$Data - bas = \frac{data - baseline}{baseline} \%$$

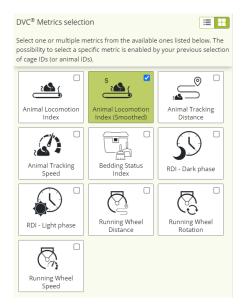




#### 2.2.5 DVC® Metrics Selection

Once the cage groups have been created, it is possible to move forward and select the DVC® metrics to apply to the calculation. The availability of the DVC® Metrics is based on the *cage content* assigned while registering the cage in the DVC® system. Some metrics are only available if the cage contains one animal. These are the "Animal Tracking Distance" and "Animal Tracking Speed." Some other metrics are available only if the cage is registered with a Running Wheel as an add-on (the "Running Wheel Distance/Rotation/Speed").

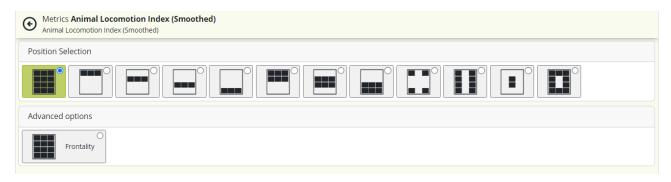
The remaining metrics are available for any *cage content*. Please refer to the specific section to discover how each metric is calculated.



Some metrics consider the DVC® board electrodes as individual data points. Therefore, for these metrics, it is possible to further refine the analysis by selecting only the electrodes to be considered in the calculation:

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By default, all the electrodes are always selected.

Moreover, the advanced option called "Frontality" applies the following calculation:

$$avg(front)/[avg(front) + avg(rear)]$$

The average of the frontal electrodes is divided by the sum of the average of the frontal ones + the average of the rear electrodes.

#### Where the:

- Frontal electrodes are the electrodes # 7-8-9-10-11-12
- Rear electrodes are the electrodes # 1-2-3-4-5-6

#### 2.2.6 Charts Selection

After the chosen metric (s), it is time to select how to visualise the corresponding data. There are several different possibilities:



The **Line plot** also has several other options that can be selected.

## Subtype:

- **Simple**: displays a continuous line in the selected interval. Every point is the data point collected by the DVC® system.
- **Cumulative**: all data are summed up and show an incremental progression of the selected data along the specified interval.
- **Urination**: This selection is designed when the previously selected metric is the "Bedding Status Index". It calculates the difference between two consecutive samples and inverts the sign of the





results. Remove the bedding change event data point. Then, the cumulative curve is applied to display the overall "urination" curve.

- **SEM:** displays a continuous line in the selected interval, overlaid by the Standard Error of the Mean. This option is available only if the group has multiple cages.
- **IQR:** displays a continuous line in the selected interval, overlaid by the Interquartile values (2<sup>nd</sup> and 3<sup>rd</sup>) as limits. This option is available only if the group has multiple cages.
- **Light/Dark** displays two simple continuous lines simultaneously, one for the light phase and one for the dark phase of the selected period. Each data point represents the value of the selected metric in the aggregated time interval.
- **Daily Rhythm:** displays the single 24-hour cycle, minute by minute, of the selected metric in the overall selected analysis period. Each data point is the smoothed average of all the same data points in terms of the time of the day of all the days included in the selected analysis period. This chart describes an animal's circadian pattern in a specified period.

Some subtype offers different "Time Interval Visualization" options:

- Continuous: the line is continuously showing data along the selected time interval
- **Daily**: seven different graphs corresponding to the seven days of the week are displayed. If the interval is longer than 1-week, multiple lines will be created for each weekday.
- **Weekly**: one graph showing multiple lines corresponding to the selected period's multiple weeks (if any).
- **Custom**: multiple weekly graphs based on the number of selected weeks.

The **Bar Plot** presents data with rectangular bars with heights proportional to the values in the selected interval.

The **Box Plot** represents the standard five-number summary of a data set in the selected aggregated interval.

**Heatmap**: data are aggregated day by day (each line is 24-hour data), and every point corresponds to the "Data Time Aggregation" selection (hour, minute, custom). The data are colour-coded (blue is a low value, red is a high value)

**Actogram**: data are aggregated daily (each line is 24h data), and every point corresponds to minute aggregation, and the line height is proportional to the data value collected.

The **Live data** is a simple line plot where the last 15m/30m/60m/custom minutes are displayed and updated every minute from the data coming if the selected cage is currently inserted in the DVC® system.

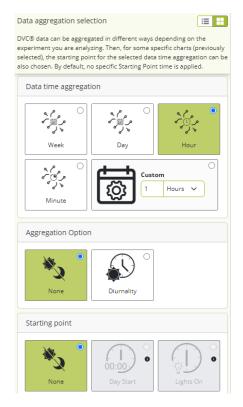
Lastly, the **Board Heatmap** is a data visualisation technique that shows the magnitude of a metric as colour-coded for the corresponding electrode of the DVC® board. Every DVC® board is a different time interval (week, day, or custom)

#### 2.2.7 Data aggregation selection

Depending on the previous selections, data can be aggregated in different ways. Then, for some specific charts (previously selected), the starting point for the selected data time aggregation can also be chosen. By default, no Starting Point time is applied.

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More specifically, it is possible to aggregate the data by minute, hour, day, week or custom aggregations.

The diurnality is a new functionality available only if the "Day" or "Week" aggregation is selected, and it applies the following formula:

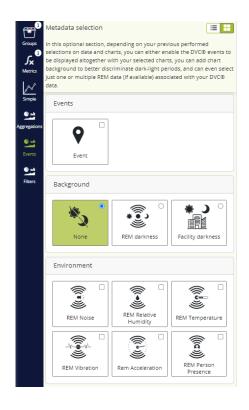
$$avg(light)*nsamples(light)/[avg(light)*nsamples(light)+avg(dark)*nsamples(dark)]$$

Lastly, the Starting point selection depends on the previously selected chart. For instance, if the Heatmap visualisation has been chosen, it is possible to either select the "Day Start" (i.e., midnight) or the "Lights On" (set in the Facility). In contrast, if it is chosen, the Simple Line chart, no starting point selection is mandatory.

#### 2.2.8 Events Selection

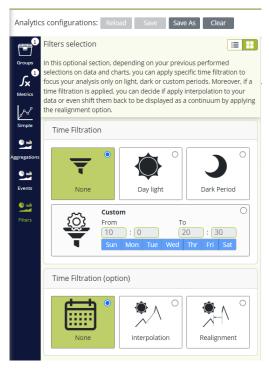
This is an optional section. Depending on your previously performed metrics, charts, and data aggregation selections, you can enable the DVC® events to be displayed with your selected charts (as a continuous line) or add a chart background to discriminate dark-light periods. You can choose even just one or multiple REM data (if available) associated with your DVC® data.





#### 2.2.9 Filter selection

This is an optional section. Depending on your previously performed metrics, charts and data aggregation selections, you can apply specific time filtration to focus your analysis only on light, dark or custom periods. Moreover, if time filtration is applied, you can decide if you use interpolation to your data or even shift them back to be displayed as a continuum by applying the realignment option.







## 2.2.10 Save configuration

Once you are satisfied with your selections, to quickly retrieve these in the future, it is possible to save your specific settings by clicking the corresponding "Save As" button:

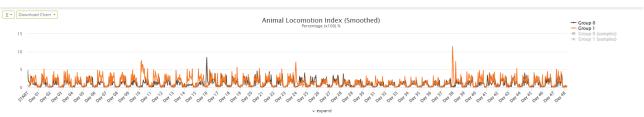


You must name and assign it to a specific Owner who can only use it (or delete it).

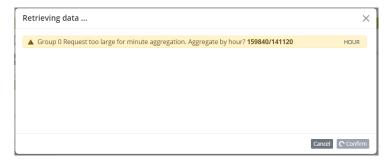


# 2.2.11 Visualize the analysis.

To run the analysis based on your specific multiple settings, click the icon and the corresponding data and charts are displayed:



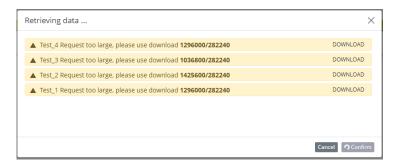
Suppose the request is too big to be displayed (for instance, aggregation by minute of several months). In that case, the system automatically proposes to switch to a more considerable aggregation period (for example, by hour).



Or even directly suggest downloading the data:







In some cases, for some charts, it is possible to resize the Y-axis by clicking in the corresponding area and then inserting the value:



For any displayed chart, it is possible to download it in high resolution by clicking and selecting the proper format:



Lastly, for some charts, it is possible to zoom in by clicking on any chart position and pressing the left mouse button until the end of the area you want to zoom in. To reset the zoom, click on the corresponding icon of the zoomed graph

Reset zoom

Moreover, if you want to analyse any graph better, you can magnify it by clicking on the corresponding icon

below the chart.

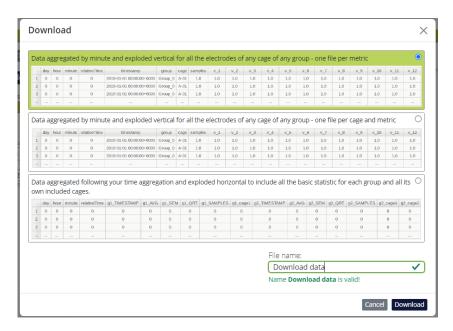
#### 2.2.12 Prepare Download

When all the selections have been performed, it is also possible to prepare the download of the corresponding selected data.

Clicking on the icon tis is then possible to choose between three different options:

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## Type 1 (upper choice):

Reporting data aggregated in rows by minute or hour (or custom aggregation, depending on your specific selection) for any group cage's electrodes in vertical sequential order. One file for each metric selected with all the cages included in the global selection.

Each column is the following:

Day	Day from date selected in the observation time	
Hour	Hour of that day 1-24	
Minute	Minutes of that hour 1-60	
relative time	Absolute value (in seconds) of the timing from the previous midnight of the starting date	
Timestamp	Absolute Time Stamp in UTC (https://en.wikipedia.org/wiki/Coordinated_Universal_Time)	
Group	Name of the Group	
Cage	Cage name within the selected cage group	
Samples	The number of collected samples detected in the specified time frame. Such information is helpful to understand if the cage has been removed from the rack, thus estimating how much data has been lost due to this event.	
V_1/2/3/4/5/6/7/8/9/10/11/12	Value of the selected metrics for each electrode 1/2/3/4/5/6/7/8/9/10/12 of the DVC® board	

#### Type 1-bis (middle choice):

It is the same as the previous Type-1, with the difference that there are as many files as cages (and metric). This approach is preferred when the selection includes a very long time interval (several months or years)

#### Type 2 (lower choice):

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Data are aggregated following your time aggregation and exploded horizontally to include all the essential statistics (average, quartile, SEM) for each group and its included cages.

Day	Day from the date selected on the observation time		
Hour	Hour of that day 1-24		
minute	Minutes of that hour 1-60		
relative time	Absolute value (in seconds) of the timing from the previous midnight of the starting date		
g1_TIMESTAMP	Absolute Time Stamp in UTC for group 1 (https://en.wikipedia.org/wiki/Coordinated_Universal_Time)		
g1_AVG	Metric average of group 1		
g1_SEM	SEM Group 1		
g1_QRT	Quartile Group 1 [Minimum, Lower Quartile, Median, Upper Quartile, Maximum]		
g1_SAMPLES	Collected samples, detected in the specified time frame and for the entire group. Such information is helpful in understanding if the cage has been removed from the rack, thus estimating how much data has been lost due to this event.		
g1_cage1	Metric average of the first selected cage within group 1		
g2_TIMESTAMP	Absolute Time Stamp Group 2		
g2_AVG	Metric average of group 2		
g2_SEM	Standard Error Group 2		
g2_QRT	Quartile Group 2 [Minimum, Lower Quartile, Median, Upper Quartile, Maximum]		
g2_SAMPLES	Amount of information and samples detected in the specified time frame and for the entire group. Such information is helpful in understanding if the cage has been removed from the rack, thus estimating how much data has been lost due to this event.		
g2_cage1	Metric average of the first selected cage within group 2		
g2_cage2	Metric average of the second selected cage within group 2		

Filling in the File name section to activate the Download button is mandatory to start preparing the download.



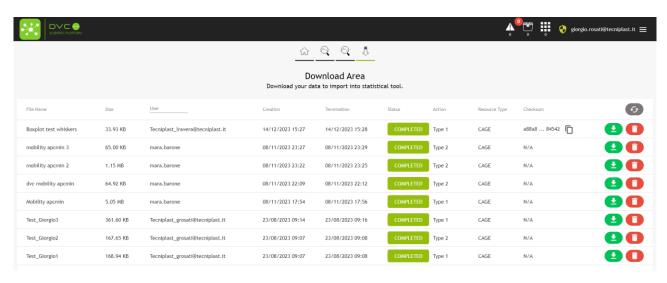
PLEASE NOTE: you will find the "Downloaded data" in the dedicated section called Download Area



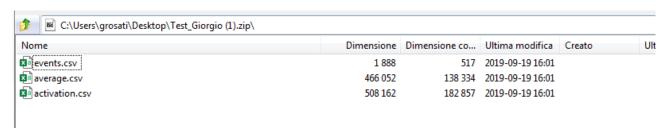
You can finally download the data on your PC by clicking the 😉 icon.

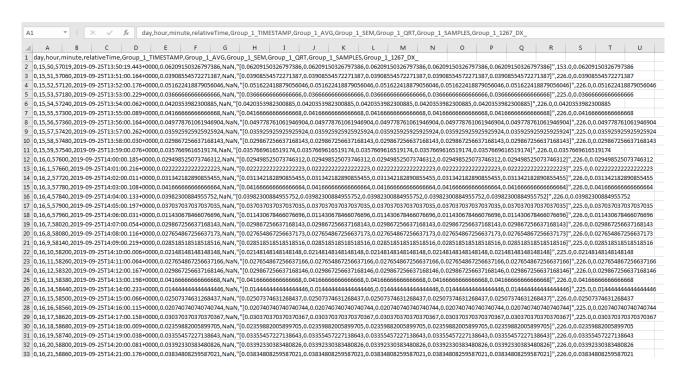
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The file you download is a .zip file that contains .csv files (comma-separated file), one for each metric you have previously selected (in this case, average = Bedding Status Index, activation = Animal Locomotion Index, events = DVC events)





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**PLEASE** NOTE: TIMESTAMP are produced in UTC (<a href="https://en.wikipedia.org/wiki/Coordinated\_Universal\_Time">https://en.wikipedia.org/wiki/Coordinated\_Universal\_Time</a>). If you use MS Excel to open these files, remember to set the "." as the decimal separator.

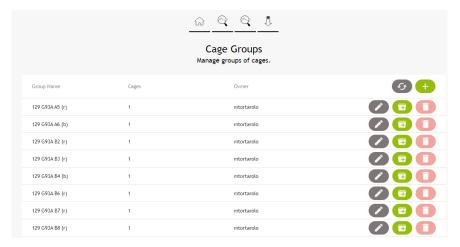
# 2.3 Manage Experiment

Clicking on the icon "Manage Experiment", other choices appear:

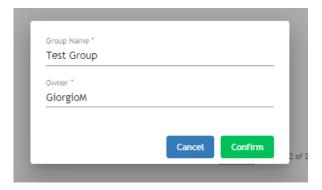


## 2.3.1 Cage Groups

In this section, it is possible to create specific groups of cages and assign them to (already) existing DVC® Analytics users (and also list the Cage Groups created in the Data Analysis – V2 section)

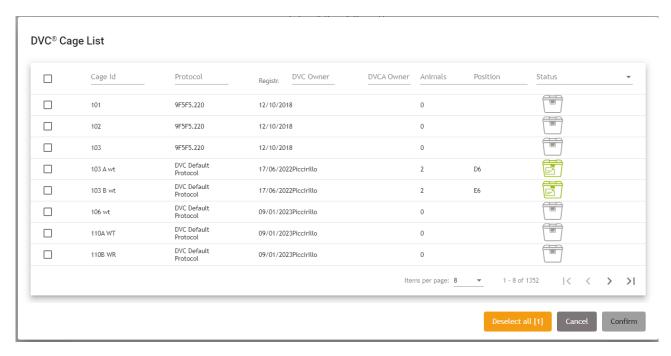


The first step is creating the group by clicking the corresponding icon. A specific pop-up area appears, and you are requested to insert the name of this group as well as the owner of the group.



Then, you can add cages to this group by clicking on the icon / and selecting the cages to be included.



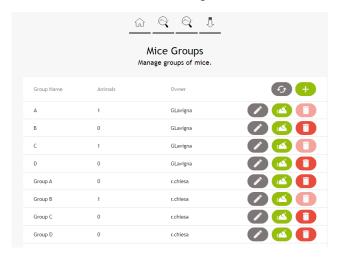


PLEASE NOTE: The same cage(s) can be assigned to different groups.

**PLEASE NOTE**: to delete a group, you must deselect all the cages before being able to delete the Cage Group with the corresponding icon .

#### 2.3.2 Mice groups

To create a Mouse group, please follow the previous workflow, with the only difference being that you must choose between available mouse IDs (instead of cage IDs).



#### 2.3.3 Alerts

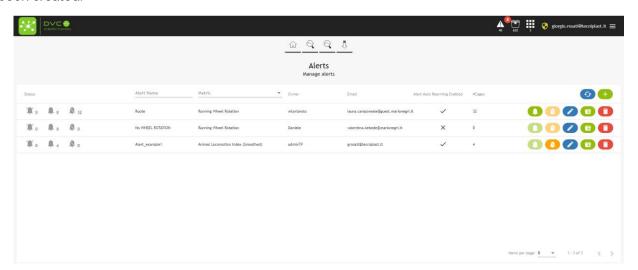
The Alert functionality has been designed to support the possibility of being automatically notified by the system if a specific condition is accomplished.

It is possible to create unlimited conditions applied to any running cage of the system,





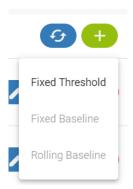
By clicking on the corresponding button, a specific page opens with the list of the alerts that have already been created.



A new pop-up menu is opened by clicking the button , where the User is guided through seven specific steps to create the Alert's properties.

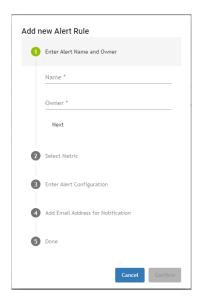
**Step 1:** The Alert can be created by looking at:

- Fixed threshold (currently available)
- Fixed Baseline (not available yet)
- Rolling Baseline (not available yet)

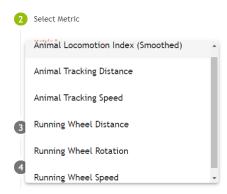


**Step 2**: Insert Name of the Alert and Owner of the Alert (if the User is a "Researcher" profile, only this User is available). Then click "Next."





**Step 3:** Select the Metric for the Alert. Currently available are:

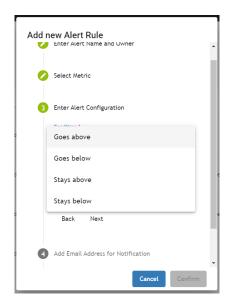


**Step 4:** Select the "Condition" to trigger the Alert. Currently, there are four different conditions:

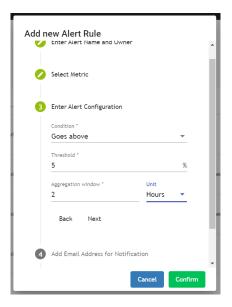
- Goes above (metric data goes above a fixed threshold)
- Goes below (metric data goes below a fixed threshold)
- Stays above (metric data stays above a fixed threshold)
- Stays below (metric data stays below a fixed threshold)

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**Step 5:** Now, set the threshold to refer to (in this case, 5% of the specific selected metric). Then, the temporal window is considered for the condition (in this example, 2 hours).



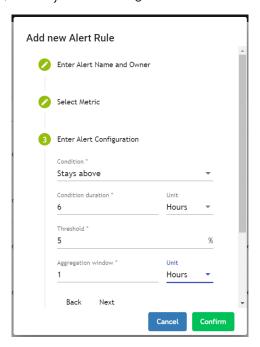
**Step 5 bis**: Depending on the condition chosen, different parameters are requested to be set. In this second example, if the User chooses "Stays above" or "Stays below," other temporal parameters are requested. More specifically, the User must insert:

- Condition duration (6) and corresponding Unit (hours)
- (fixed) Threshold (5%)
- Aggregation window (1) and corresponding Unit (hours).

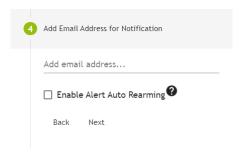
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This example means that the Alert is triggered if the selected metric data, expressed in minimum aggregation time of 1 hour each, is always above the 5% of the fixed threshold for six consecutive hours.



**Step 6:** Last, optionally, you can enter an email address to send the correspondent alert if triggered. Users can add multiple email addresses by simply adding one after the other.



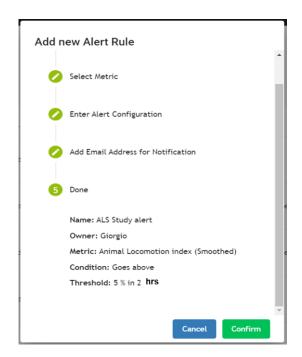
Moreover, in this step, "Enable Alert Auto Rearming" is also possible, meaning that if the specific cage in Alert is physically removed from the DVC® Rack and reinserted, the Alert is automatically enabled again.



**Step 7:** Clicking Next, the entire alert information is recapped. In this specific example, an alert (named ALS Study Alert) will be created and sent to <a href="mailto:grosati@tecniplast.it">grosati@tecniplast.it</a> and <a href="mailto:ggottardo@tecniplast.it">ggottardo@tecniplast.it</a> in case the metric "Animal Locomotion Index (smoothed)" goes above a fixed threshold of 5% for 2 consecutive hours (considering the hour aggregation time for the corresponding metric).

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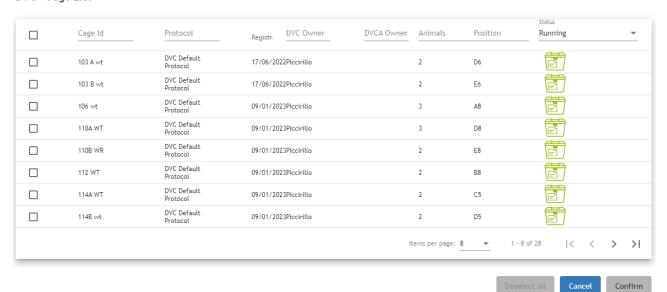


Once a new alert is created, it is listed on the recap page. But there are no cages yet assigned to this new Alert:

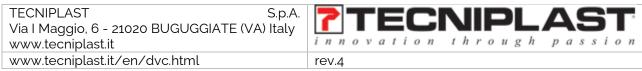


Clicking on the button, the User can add all the cages to the alert specifications applied (only "Running" and "Out of Rack" cages can be selected).

#### DVC® Cage List



From now on, all the selected cages will be monitored to check the alert specifications:







The area related to the status of the Alert recaps:

- How many cages triggered the alert 🌲 0
- How many cages are enabled for Alert management
- How many cages are turned off for Alert management  ${}^{igwedge}$   ${}^{_0}$

In case the Alert is triggered, several different events are generated:

Firstly, there is a quick recap of all the cages in Alert. Just click on the alert line, and it expands to detail all the cages in Alert (3 cages in our example):



Users can discover the condition to trigger the Alert (in this example, 1 h data goes below the 4% fixed threshold during the dark period), Cage ID, the status of the Alert, and the date and time the Alert has been triggered:



In case the User wants to reset the Alert for a specific cage, click on it:



And then click on the button to reset it.

If the User wants to disable or delete only a specific cage from the Alert, this one must be in an "Enabled" mode (not triggered). Click on it:





Then, click on the button to turn off this cage or click on the button to disassociate this cage from the Alert.

Users can also enable all the cages in Alert again by clicking on the corresponding icon:



Conversely, the User can turn off all the cages currently under the management of the Alert:



In this latter case, all the cages under the management of the Alert are set to status Disabled and not monitored anymore:



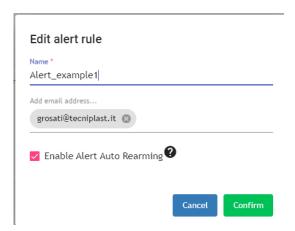
Then, the User can modify the name of the Alert and the email addresses by simply clicking on the button.







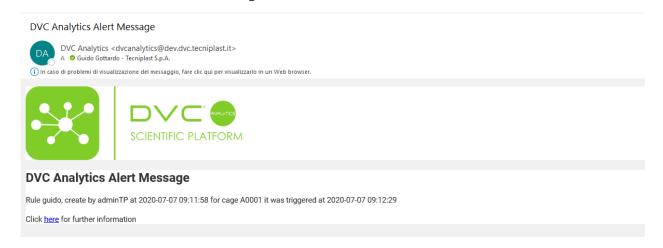




Moreover, the home page has an icon recapping how many alerts have been triggered and how many cages are enabled and disabled.



Finally, below, the User can find an example of the email that can be received in case an alert is triggered. Users can find the Alert's name, the cage's name, and when (date and time) the Alert was created.

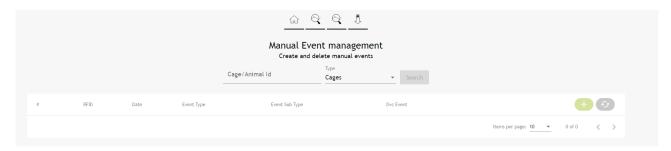


# 2.3.4 Manual Event Management

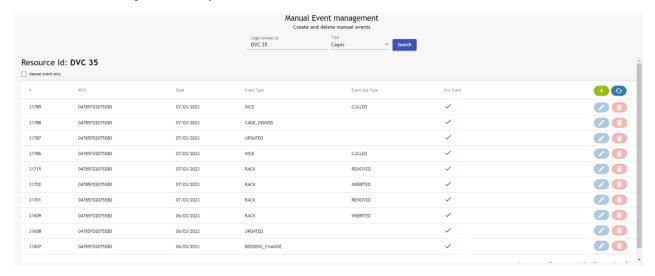
The DVC® events are generated automatically by using the DVC® system in the animal room. But now, generating "custom" events by entering them into this section is also possible.

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Step 1 is to Search for the specific cage to apply the manual event; it is possible to create it manually (and look for all the ones generated by the DVC®):

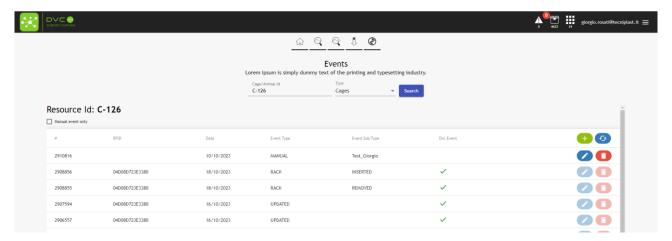


By clicking the button, a specific pop-up appears where you can provide the name of the manual event and its timestamp.

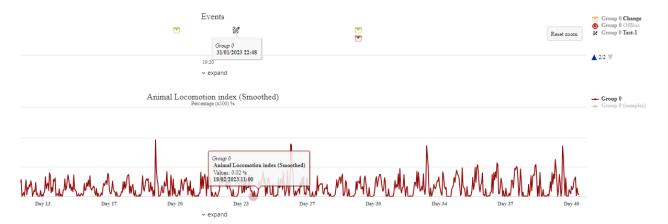


**PLEASE NOTE**: you can only edit or delete Manual events (not the DVC®'s ones):



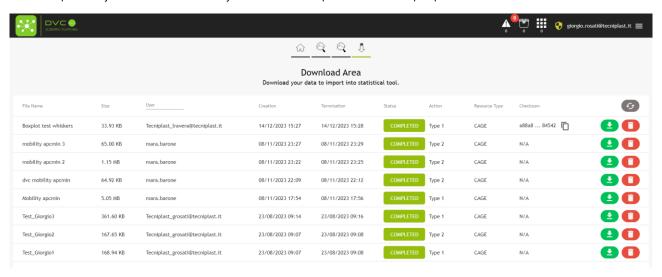


This also generates the corresponding icon on the timeline of the events in the Analysis of the data



# 2.4 Download Area

As anticipated, you can immediately find all the requested-to-be-prepared data in this section.



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Additionally, you have other information such as the status of the requested task (Running, Failed, Completed), who requested it, the size of the file and the date of starting (Creation) and Termination. By clicking the corresponding icon it is possible to download it locally.

# 2.5 User settings

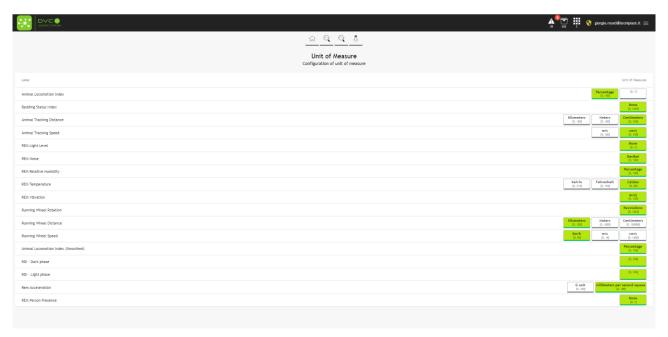
As described in section 2.1, this section is available only for the DVC® Analytics users registered as Facility Managers and not available to the users registered as Researchers.



There are different options you can set.

## 2.5.1 Unit of Measure

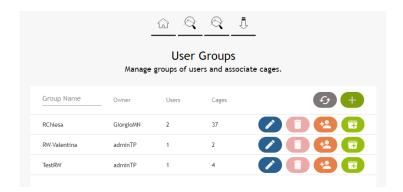
Clicking on the corresponding button, you can see your current settings set by default and change some of them if needed.



## 2.5.2 User Groups

This functionality allows a group of users to access a selected number of cages.

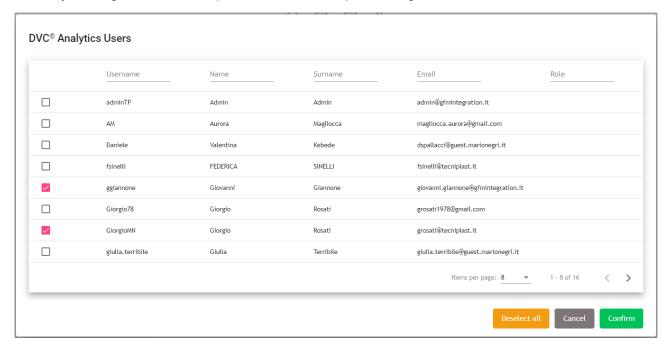




Everything starts by creating the new Group by assigning the name and the owner of this new group:

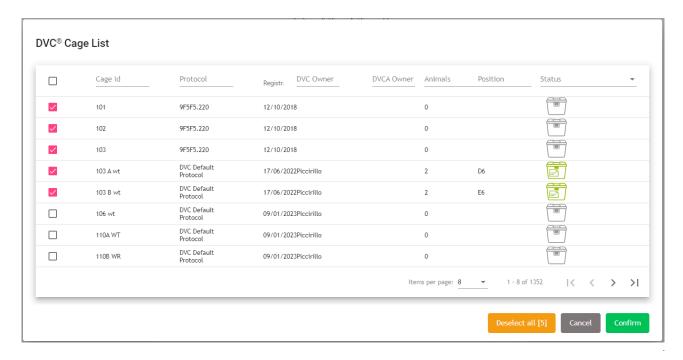


Then, by clicking the button, it is possible to add multiple existing users:



And finally, by clicking the icon 😐, the cages become available to the group:





**PLEASE NOTE**: to delete a user group, you must deselect all the users and all the cages from the User group.

### 2.5.3 DVC® Analytics Users

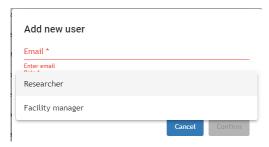
Leveraging the already existing AMAZON cloud service, we implemented a critical package to manage user credentials securely. It is called Amazon COGNITO.

 $(\underline{https://docs.aws.amazon.com/cognito/latest/developerguide/what-is-amazon-cognito.html}).$ 

Thanks to this functionality, now the process of a new user registration follows the below steps:

You can create unlimited users by clicking the corresponding icon .

In this window, you must enter the email address of the new User and the "Role" ("Researcher" or "Facility manager"):



Once you "Confirm" the information, an invitation email is sent to the new User.





The new User receives the email and must click "here" to properly register in the DVC® Analytics.



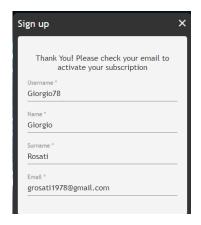
The new User is redirected to the DVC® Analytics registration page, where credential pieces of information are requested:



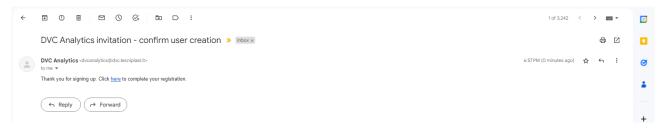
Remember that the password has some requirements: "Use min 8 characters or more, minimum one number and one symbol and minimum one special symbol as [@\$!%\*?&~()\_[]]:"<>,€]"

Once all the information is inserted, you can click "Confirm", and a confirmation pop-up appears.





It is time to validate the email entered for the new User (that can be different compared to the invitation email). A confirmation email has been sent to the new User, who must accept it to confirm the email address (this is important for password recovery).

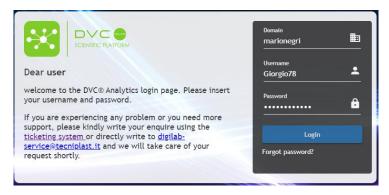


Clicking "here", the process of new user authentication is completed and confirmed.



The new User can now finally log in to the DVC® Analytics platform.

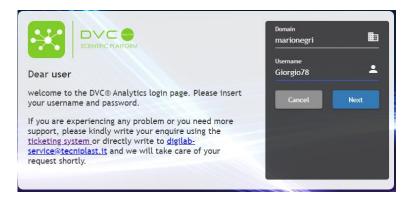
In case any user has lost credentials, it is possible to click on the corresponding section "Forgot password?" on the login page:



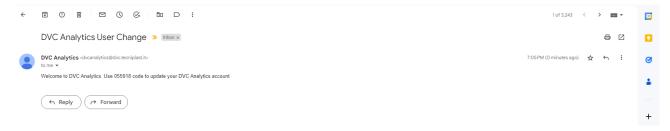
The User must enter the Username and then click "Next":



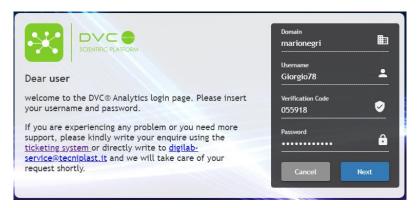




A "Verification Code" is sent to the email address of the User:

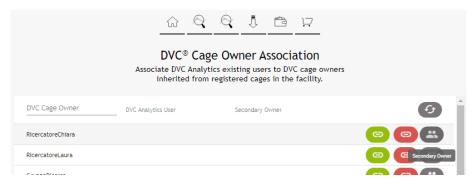


Inserting it in the corresponding field altogether with a new (valid) password, then clicking on "Next", the User can finally login again (with the new password):



## 2.5.4 DVC® Cage Owner Association

This section is fundamental, especially if you are (or have) "Researcher users" because it is the only way to analyse cages using this profile.



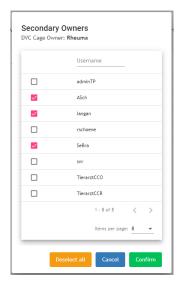




Every cage prepared in the DVC® system can have a DVC® Owner associated (it is not mandatory but highly suggested when combined with DVC® Analytics). If so, this DVC® Owner is pushed to the DVC®

Analytics and can be associated with existing DVC® Analytics users by clicking the icon. This will be set as the primary user.

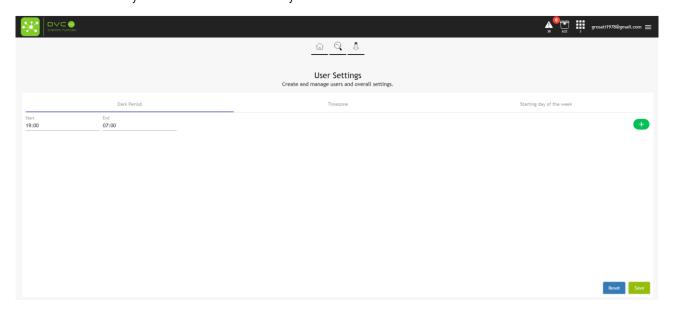
Then, by clicking the button, it is now possible to associate "secondary users" to the same cages created with this DVC® Owner:



The main difference between this feature and the "User Groups" described earlier is that, in this latter feature, any new cage created in the Animal room with the specific Cage Owner will become automatically available to all the primary and secondary users, while in the "User Groups" feature, only the selected ones and available to the entire group of users.

### 2.5.5 Settings

This button allows you to set different Facility information.







#### 2.5.5.1 Dark Period

This section is fundamental to properly setting your official night period in the Facility. This setting is used in the "Day Time Filtration" section.

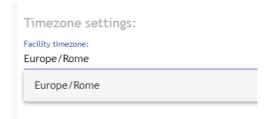
If you manage time-shift in your Facility because of summer-winter time, you can click on the then select the new time interval and the start date from this interval.



PLEASE NOTE that you can change/delete this interval; the original data are unaffected.

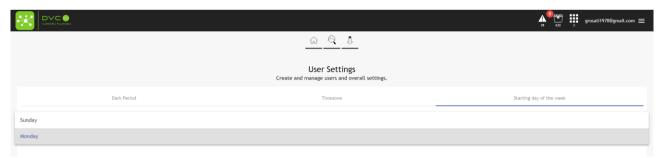
## 2.5.5.2 Time Zone

It is also important to "see" your data in the graph/charts considering your Facility Time zone. Start typing in the corresponding area; the field will autocomplete with your timezone.



## 2.5.5.3 Starting of the week

To allow you maximum flexibility, it is possible to set the "first" day of the week that will be then used to display data in the daily and weekly graph selection:



PLEASE NOTE: Sunday and Monday are currently the only available "first" days of the week.

#### 3 Useful information

In this section, we would like to provide some tips and information that would be important to understand better how the DVC® Analytics works and get the most from it.

# 3.1 DVC® board

The DVC® board is the core of the DVC® system. Twelve different electrodes map the entire base of the cage. These electrodes are numbered in the following way:







For some metrics (Animal Activity Index and Bedding Status Index), selecting ONLY some electrodes (corners, walls, etc.) can analyse specific patterns deeply.



For some other metrics, such as Running Wheel or Animal Tracking, it is impossible to select specific electrodes because the data are calculated using the Running Wheel or the entire DVC® board.

## 3.2 DVC® working principle and derived metrics.

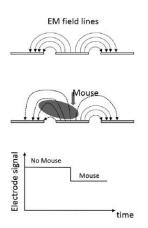
The DVC® board comprises twelve electrodes connected to an integrated circuit, continuously measuring their electrical capacitance every 250 msec (roughly). Since the matter influences capacitance in each electrode's surroundings, its measurements are affected by the presence of, e.g., water and animals. Note that materials with high water content are characterised by large values of relative permittivity (concerning air), which directly affects capacitance (high relative permittivity means higher capacitance). Since mice are characterised by high water content, their movements are performed. At the same time, close to an electrode induces significant capacitance changes, and thus, by properly tracking these changes over time, it is possible to monitor animal activity. Note that capacitance remains unchanged when material compositions around an electrode are unvaried.

Additionally, the capacitance readings are affected by the presence of water (due to, e.g., bottle leakage) or urine. However, animal activity occurs on a time scale different than water leakage or urine; thus, the two variables can be easily distinguished. Furthermore, the system's capability to discern animal movements is unchanged even when water/urine is present in an electrode surrounding (not a flooded cage, but a typical amount of water/urine in a dirty cage). Water/urine can change absolute capacitance readings but not capacitance variations due to animal movements.

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Keeping in mind this working principle, some important metrics can be applied. The currently available metrics in the DVC® Analytics systems are:

- Animal Locomotion Index
- Animal Locomotion Index (smoothed)
- Bedding Status Index
- Animal Tracking Distance
- Animal Tracking Speed
- RDI Dark Phase
- RDI Light Phase
- Running Wheel Rotation
- Running Wheel Distance
- Running Wheel Speed

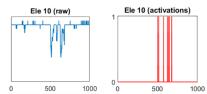
**PLEASE NOTE**: Every element (cage or animal) has specific capabilities assigned by the DVC® system that enable or do not enable the correspondent DVC® metrics (a cage without Running Wheel does not enable the Running Wheel metric).

#### 3.2.1 Animal Locomotion Index

This DVC® metric is highly robust because it uses the so-called "Activation Density" metric that has been extensively validated in the field across different experiments and validation processes (you can find detailed information in this publication: <a href="https://www.heliyon.com/article/e01454">https://www.heliyon.com/article/e01454</a>).

An electrode is activated when its measurements are significantly perturbed over a limited time interval, occurring when a mouse performs activity sufficiently close to an electrode (see below). Density indicates that the total number of activations is divided by the duration of the time interval and the number of electrodes of interest (up to twelve).

Any drop/increase of the signal higher than the electronic noise (1 count) is considered accurate activation.



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$$ACT_e = |\mathbf{e_k(t)} - \mathbf{e_k(t-1)}| > 1 \rightarrow 1$$
 (activation), otherwise 0

Where  $e_k(t)$  is the kth electrode at time t

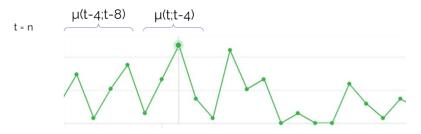
The Animal Locomotion Index is expressed in % of arbitrary units, and it is normalised between 0% and 100%

## $\sum_{e=1}^{12} ACTe / \#Electrodes / \#samples$

#### 3.2.2 Animal Locomotion Index (smoothed)

This is an improved version of the historical metric "Animal Locomotion Index" (ALI). The statistical reason is that the smoothed process increases robustness to noise and provides more sensitivity to the intensity of mice movements.

More specifically, to calculate the minute data aggregation, instead of performing a sample-by-sample difference (remember that every sample is collected every 250 msec), the ALI<sub>smoothed</sub> considers the moving difference between two groups of 4 consecutive samples each. If the module of this difference is above a minimum threshold, the current group of samples is counted as a valid activation.



 $ACT_{smoot} = |\mu(e_k(t;t-4)) - \mu(e_k(t-4;t-8))| \ge 1,25 \rightarrow 1$  (activation), otherwise 0

Where  $\mu(e_k(t;t-4))$  is the average of 4 consecutive samples of the kth electrode.

#### $\sum_{\ell=1}^{12} ACTsmoot/\#Electrodes/\#samples$

The Animal Locomotion Smoothed Index is expressed in % of arbitrary units, and it is normalised between 0% and 100%

The main benefits of this (smoothed) metric are:

- **Electrode 2** of the DVC® board was considered generally "less sensitive" because most of its area is used as the RFID antenna to read the below cage. Applying this new mathematical method and a dedicated threshold (1.01) allows us to assess its activity better for up to 50% more activations. This helps to evaluate activity, e.g., in the rear of the cage, with a better resolution.
- It reduces "background noisy baseline activity" and emphasises "spikes in activity" over a longer time window. Since the primary behaviour of mice, especially at baseline levels, does not change in time windows below 1 second, this new metric calculation will not affect the behaviours identified via DVC®-based algorithms to date.

### 3.2.3 Bedding Status Index

This metric has been designed initially to evaluate the level of moisture in the bedding. Indeed, this metric is the core metric of the DVC® Bedding Algorithm and the DVC® water flooding algorithm.

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Its mathematical formula is straightforward and robust. It considers the absolute values (i.e., the capacity-related-measurements or arbitrary Units) of each electrode and performs an average of these values among the selected time interval:

$$\sum_{e=1}^{12} El \ value / \# Electrodes$$

Where *El<sub>value</sub>* is the absolute value of the electrode

### 3.2.4 Animal Tracking Distance and Speed

The distance walked accounts for the total distance covered by the mouse within a given time interval, while the average speed is the distance walked divided by the duration of the time interval considered. We assume that the mouse position on the cage floor is identified in terms of its centroid, while the distance walked is computed via the sum of the Euclidean distances of the mouse centroid in successive frames within the time interval of interest. The distance walked is defined as follows. Let  $\mathbf{p}(t) = [px(t), py(t)]$  be a 2 ×1 vector of coordinates on the plane (cage floor) representing the position of the centroid of the mouse at time t. Then, the distance walked within the time interval t, and t2 can be computed as:

$$S(t_1, t_2) = \sum_{t=t_1+1}^{t_2} d(t)$$

Where:

$$d(t) = \sqrt{(p_x(t) - p_x(t-1))^2 + (p_y(t) - p_y(t-1))^2}$$

is the Euclidean distance between two positions in adjacent frames.

The average speed is instead defined as the ratio between the cumulative walked distance and the duration of the time interval:

$$V(t_1,t_2) = \frac{1}{t_2 - t_1} S(t_1,t_2)$$

#### 3.2.5 RDI (Regularity Disturbance Index)

This is a derived metric based on the ALI<sub>smoot</sub>. RDI has been developed to capture irregular animal activity patterns. To quantitatively capture these patterns, we designed this metric based on the sample entropy mathematical formula (Richman and Moorman, 2000) as the core metric.

RDI is a metric that measures irregularities of a time series (e.g., home cage activity), and the absolute amount of the activity itself does not influence it.

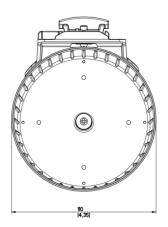
Currently, the DVC® analytics provides one data point per light or dark period.

#### 3.2.6 Running Wheel Rotation, Distance and Speed

Using the product DVC® Running wheel, it is possible to automate several metrics. The diameter of the plastic DVC® Running wheel is 110,4 mm (4,35 inch), which corresponds to a perimeter of about 33,75 cm (13,3 inch).

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The minimum time resolution is the minute, and the metrics are expressed in:

- Running Wheel rotation: # Complete rotations in the selected time resolution (minute, hour, custom)
- **Running Wheel distance**: # complete rotations \* 33,75 cm (13,3 inch) in the selected time resolution
- Running Wheel Speed: expressed in cm/min (inch/min) or m/min (feet/min)

#### 3.3 How data are calculated and aggregated

Considering the different data and the different charts, it is fundamental to understand how these are calculated.

Considering all the available DVC® metrics, below you can find the recap of the aggregations:

Aggregation in the selected time interval
Average
Average
Sum
Average
Average
Average
Average
Sum
Sum
Average

# 3.3.1 Line Chart

Every point of the line is calculated in the following way:

**SPATIAL AGGREGATION**: average of the selected DVC® boards electrodes (by default, the twelve electrodes). The result is one single data point (if you select only the corners, the data point is the average of the four electrodes).

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**TEMPORAL AGGREGATION:** the default temporal window (minute and hour) is automatically calculated by the DVC® system while they are occurring. On the other hand, if you have selected a custom temporal window, the result is the average of all the minutes included in the customised temporal interval if the time is not a multiple of the hour.

Activation (3min) = [activation (min 1°) + activation (min 2°) + activation (min 3°)] / 3

If the custom temporal interval is a multiple of the hour:

Activation (3h) = [activation (hour 1°) + activation (hour 2°) + activation (hour 3°)] / 3

**GROUP AGGREGATION:** the data point in the graph is calculated as the average of the single calculated metric

Activation (3 cages) = [activation (cage 1°) + activation (cage 2°) + activation (cage 3°)] / 3

#### 3.3.2 Line chart cumulative

Spatial and temporal aggregation follow the abovementioned scheme, and in this specific case, every data point is the sum of the previous ones:

d(0) = A(0)

d(1) = A(0) + A(1)

d(2) = A(0) + A(1) + A(2)

d(n) = A(0) + A(1) + A(2) + ... + A(n)

#### 3.3.3 Line chart Urination

This specific line chart has been designed to support the analysis of the urination trend. By selecting the "Bedding Status Index" as a metric, this specific line chart applies the following calculations:

- Calculates the difference between two consecutive samples (aggregated per your choice).
- Inverts the sign of the previous result.
- Remove the data point associated with the event of the "Bedding Change" (tracked by the DVC® system)
- Cumulates the data points among the analysis interval and displays the result as a cumulative "urination" curve.

# 3.3.4 Line chart with SEM

As explained previously, SEM is enabled by selecting multiple cages in the same group. Everything is calculated the same as above in terms of spatial and temporal aggregation, and then, the SEM (Standard Error of the Mean) is calculated as:

$$\mathsf{SEM} = \mathsf{SD} \, / \, \sqrt{(\#samples)}$$

Where SD is the Standard Deviation.

The correspondent Line chart is average (central data point) ± SEM.

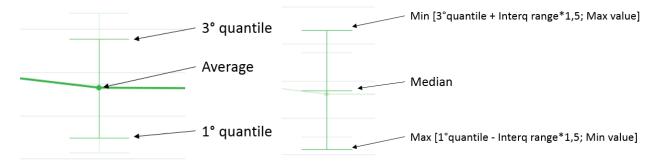
#### 3.3.5 Line chart with Interquartile

As explained previously, the INTERQUARTILE is enabled by selecting multiple cages in the same group. Everything is calculated the same as above in terms of spatial and temporal aggregation, and then, the INTERQUANTILE feature enables six different points per data sample:

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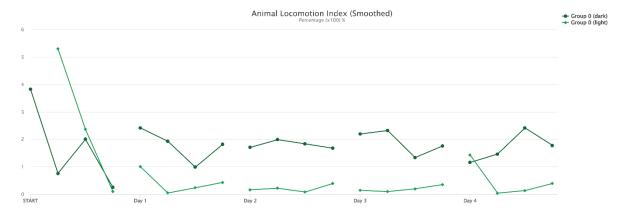


- Average
- Median
- Quantile
- 3\* Quantile
- Interquartile min range
- Interquartile max range



# 3.3.6 Line plot Light/Dark

This is a simple Line plot chart where light and dark data are simultaneously shown for each selected analysis day.

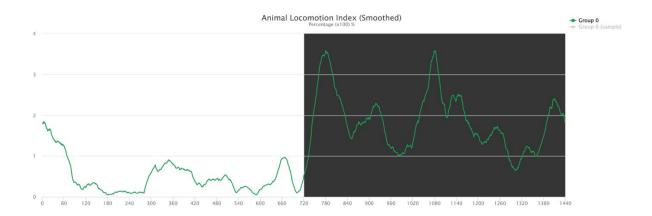


# 3.3.7 Line Plot Daily Rhythm

This chart is calculated by averaging and smoothing, minute by minute, all the data points of the 24-hour cycle of each selected interval day.

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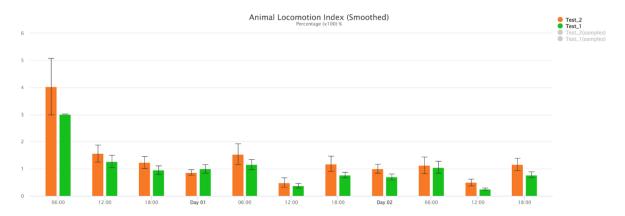




# 3.3.8 Bar plot

In the Bar plot chart, each rectangular bar represents the value of the selected metric in the aggregated time interval.

The height of the bar is proportional to the value of the metric.



## 3.3.9 Box Plot

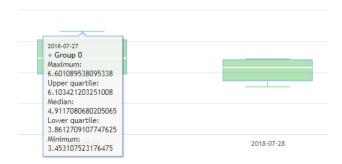
Spatial aggregation is the same as the Line Chart. Temporal aggregation is forced to be calculated within 24 hours (the day). The result is a single data point for each selected element (cage or animal). The Box Plot chart is available only when multiple elements (cages or animals) are selected.

The BOX PLOT feature enables five different points per data sample:

- Median
- 1<sup>st</sup> Quantile
- 3<sup>rd</sup> Quantile
- Min
- Max

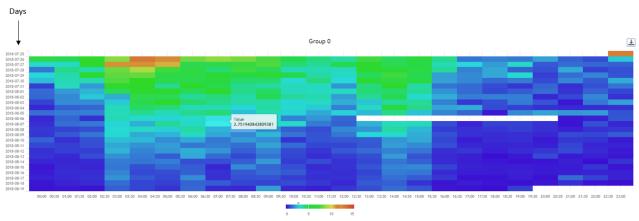
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## 3.3.10 Heatmap

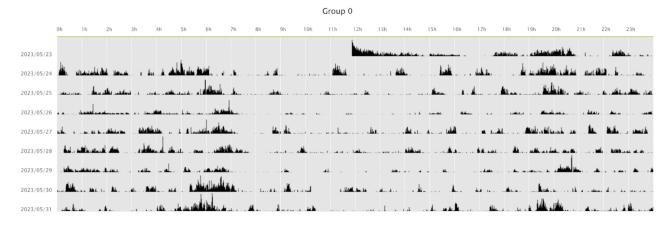
Spatial, temporal and group aggregation are precisely calculated as above. The only difference with the Line Chart is the chromatic visualisation (from blue as a lower value to red as a higher value). Every block is representative of the Data Time Aggregation chosen.



24 hours of the day, starting from midnight to midnight

## 3.3.11 Actogram

This chart is a simple graph showing the selected metric over a given period, day after day. The vertical line's length determines the metric's magnitude in a specific aggregated time.

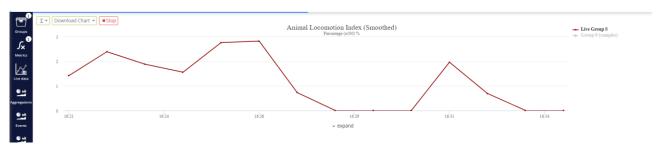


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## 3.3.12 Live data

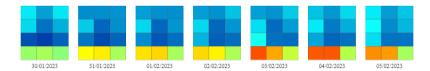
This chart can be applied only to "running" cage(s) (or animal(s)). You can visualise the last selected minutes of data (15-30-60 or custom) of the selected metric through a simple line chart. The data are updated every minute.



## 3.3.13 Board Heatmap

This chart can be applied to those metrics that consider the 12-electrodes as a data source (i.e., the ALI, the ALI smoothed and the Bedding Status Index). It is a colour-code representation of the DVC board with its 12 electrodes. The minimum granularity of the data aggregation is the day.

It can be helpful to discriminate, for instance, the latrine preference (by selecting the Bedding Status Index as a metric):



And the corresponding Animal Activity preference (by selecting the "ALI smoothed" as metric):

