# Journal of Visualized Experiments

## BtM, a low-cost open-source datalogger to estimate water content of non-vascular cryptogams.

--Manuscript Draft--

| | |
|---|---|
| **Article Type:** | Invited Methods Article - JoVE Produced Video |
| **Manuscript Number:** | JoVE58700R2 |
| **Full Title:** | BtM, a low-cost open-source datalogger to estimate water content of non-vascular cryptogams. |
| **Keywords:** | conductance measurement, cryptogam, water content, bryophyte, lichen, conductivity, impedance |
| **Corresponding Author:** | Nagore Medina<br><br>SPAIN |
| **Corresponding Author's Institution:** | |
| **Corresponding Author E-Mail:** | ngmedina@gmail.com |
| **Order of Authors:** | María Leo |
| | Ángel Lareo |
| | Carlos Garcia-Saura |
| | Joaquín Hortal |
| | Nagore Medina |
| **Additional Information:** | |
| **Question** | **Response** |
| Please indicate whether this article will be Standard Access or Open Access. | Open Access (US$4,200) |
| Please indicate the **city, state/province, and country** where this article will be **filmed**. Please do not use abbreviations. | Madrid, Spain |

Madrid, 30th August of 2018

Dear Editor,

Attached please find a revised version of our manuscript entitled “BtM, a low-cost open-source datalogger to estimate water content of non-vascular cryptogams.” (María Leo, Angel Lareo, Carlos Garcia-Saura, Joaquín Hortal, Nagore G. Medina) which we submitted for publication in *Journal of Visualized Experiments* about two months ago.

In the actual version we have addressed the editorial comments. We have removed the commercial names and typos present in the text, added the required references and clarified some parts of the text as suggested. As a consequence, we believe we are submitting a clearer version of the manuscript that meets the requierements of *Journal of Visualized Experiments*.

To our knowledge, we are providing a first and original open-source low cost data logger to measure impedance in non-vascular cryptogams.

The work has been led by María Leo and Ángel Lareo so we suggest signing the manuscript as coauthors in the first place followed by the rest of the authors who significantly worked in designing and writing the manuscript.

Thank you for considering the potential of our manuscript for publishing in *Journal of Visualized Experiments.*

Best regards,

María Leo and Ángel Lareo, on behalf of all the authors.

**TITLE:**

# BtM, a Low-cost Open-source Datalogger to Estimate the Water Content of Nonvascular Cryptogams

**AUTHORS AND AFFILIATIONS:**

María Leo[1] *, Angel Lareo[2] *, Carlos Garcia-Saura[2], Joaquín Hortal[3,4], Nagore G. Medina[5]

[1]Real Jardín Botánico (CSIC-RJB), Madrid, Spain
[2]Grupo de Neurocomputación Biológica, Dpto. de Ingeniería Informática, Escuela Politécnica Superior, Universidad Autónoma de Madrid, Madrid, Spain
[3]Department of Biogeography and Global Change, Museo Nacional de Ciencias Naturales (MNCN-CSIC), Madrid, Spain
[4]Centre for Ecology, Evolution and Environmental Changes (cE3c), Faculdade de Ciências da Universidade de Lisboa, C2, Lisboa, Portugal
[5]Department of Botany, Faculty of Science, University of South Bohemia, České Budějovice, Czech Republic

* These authors contributed equally to the paper.

**Corresponding Authors:**
María Leo (leo@rjb.csic.es)
Angel Lareo (angel.lareo@uam.es)

**E-mail Addresses of the Co-authors:**
Carlos Garcia-Saura (carlos.garciasaura@uam.es)
Joaquín Hortal (jhortal@mncn.csic.es)
Nagore G. Medina (ngmedina@gmail.com)

**KEYWORDS:**
conductance measurement, cryptogam, water content, bryophyte, lichen, conductivity, impedance

**SUMMARY:**
We present a simple and cost-effective method to build an open-source datalogger that measures the conductance of nonvascular cryptogams together with the environmental temperature and humidity. We describe the hardware design of the datalogger and provide step-by-step assembly instructions, the list of required open-source logging software, the code to run the datalogger, and a calibration protocol.

**ABSTRACT:**
Communities of nonvascular cryptogams, such as mosses or lichens, are an important part of the Earth's biodiversity, contributing to the regulation of the carbon and nitrogen cycles in many ecosystems. Being poikilohydric organisms, they do not actively control their internal water content and need a humid environment to activate their metabolism. Therefore, studying water

relationships of nonvascular cryptogams is crucial to understand both their diversity patterns and their functions in the ecosystems. We present the BtM datalogger, a low-cost open-source platform for the study of the water content of nonvascular cryptogams. The datalogger is designed to measure ambient temperature, humidity, and conductance from up to eight samples simultaneously. We provide a design for a printed circuit board (PCB), a detailed protocol to assemble the components, and the required source code. All this makes the assembly of the BtM datalogger accessible to any research group, even to those without previous specialized knowledge. Therefore, the design presented here has the potential to help popularize the use of this type of device among ecologists and field biologists.

**INTRODUCTION:**

Communities of nonvascular cryptogams are a ubiquitous and an often-neglected part of terrestrial ecosystems[1]. They are made up of an aggregate of very different small-sized organisms among which bryophytes and lichens are the outstanding primary producers. These two groups of organisms share a physiologic characteristic that makes them unique: poikilohydry, or the inability to actively control their internal water content. This has profound implications for their physiological processes since the metabolism ceases when the cells are dried out in response to low levels of humidity and resumes when the environment is humid again[2]. As a consequence, nonvascular cryptogams avoid drought instead of coping with it[2], which allows these communities to survive in a wide range of environments from cold and hot deserts to the tropics[3,4].

Besides, they also show relatively simple structures and have low nutrient requirements. These characteristics make them highly sensitive to microclimatic conditions. In fact, nonvascular cryptogams often occupy a niche space that is unavailable to vascular plants of a larger size, forming ecosystems in miniature that constitute an important part of the world's diversity. Bryophytes and lichens alone include almost 40,000 species (*ca.* 20,000 bryophytes *sensu lato*[5,6] and *ca.* 20,000 lichens[7]). Furthermore, their contribution to the Earth's biodiversity is even larger since their communities offer shelter for a vast number of species of fungi, including a diverse flora of free-living and mycorrhizal fungi, N-fixating cyanobacteria growing as epiphytes, and a myriad of micro-invertebrates, such as tardigrades, collembola, myriapods, insects, and mites that take advantage of the water retention capacity and buffered conditions inside these miniature ecosystems.

Communities of non-vascular cryptogams also contribute to the regulation of biogeochemical carbon cycles. In dry ecosystems, the so-called biological soil crusts cover up to 40% of their surface[8] and play a major role as carbon sinks. A recent review estimated that biological soil crusts of dry environments could be fixing 7% of all carbon fixed by terrestrial vegetation. Besides, in other ecosystems where either bryophytes or lichens or a combination of both are the primary producers—like some boreal forest systems or peat bogs—they produce between 30% and 100% of the total net primary productivity[10,11]. They are also important in ecosystems in which these organisms are not dominant, such as temperate forests. Indeed, forest floor bryophytes had an annual carbon uptake equivalent of about 10% forest floor respiration in a New Zealand temperate rainforest. Further, they are also important for nitrogen fixation, since the cyanobacteria living as epiphytes in these communities could be fixating almost 50% of the global amount of biological nitrogen[4].

Due to the dependence of their physiologic activity on the availability of water in the surrounding environment, both the diversity of nonvascular cryptogam communities and their functions in the ecosystems are strongly dependent on water content[2]. Note that, since they cannot actively control the water content in their tissues, their roles in carbon balance and nitrogen fixation are coupled with the hydration and desiccation cycles and, therefore, depend on the interval and periodicity of the dry-wet cycles. Thus, knowing the water content status of these organisms in real-time is key to understand the functions performed by cryptogams in the ecosystems.

Despite its importance, the development of methods to measure the water content and physiological activity in poikilohydric organisms has been relatively slow. In 1991, Coxson[12] made a first approach to directly measure the water content of lichens. After that, there was a gap in this kind of study until a recent development, when several works have provided methods to approximate measures of the physiologic status of nonvascular cryptogams[13-16]. Nevertheless, such knowledge is still scarce and scattered, and these works are mostly focused on soil crusts[4, 8]. However, bryophytes and lichens also play a relevant role in many other ecosystems, particularly at temperate, boreal, and polar regions[1], and their importance is significant not only in soil communities but also for epiphytic communities growing on trees and saxicolous communities on rocks. This lack of research is partially linked to the absence of commercially available measurement dataloggers, which forces research groups to build their own equipment. Developing a datalogger requires specific knowledge that most ecologists do not have, so it substantially increases the cost of implementing the relatively large measuring networks needed to gather representative data on the performance of nonvascular cryptogams along environmental and habitat gradients.

In this paper, we present a simple and cost-effective method to build a datalogger capable of measuring the conductance of nonvascular cryptogamic organisms simultaneously with the ambient temperature and humidity. It is programmed to record autonomously for relatively extended periods of time (up to two months) and is rugged enough to withstand harsh outdoor field conditions. Due to its simplicity, it will be a useful tool for ecologists and field biologists without specialized training in the development of dataloggers or those research groups that lack specialized staff. Therefore, this datalogger has the potential to help popularize the use of this type of device.

We developed a low-power and low-cost datalogger able to measure the conductance from up to eight different sources and record the environmental temperature and relative humidity simultaneously. The device is designed after Coxson's design[12] and implemented on an open-source platform (**Table of Materials**). The aim was to prioritize the ease of assembly and power efficiency and to facilitate the maintenance of long-term installations. The design is derived from an article by *Open Source Building Science Sensors* (OSBSS)[17]. This design was modified by incorporating additional circuitry to read out the impedance of cryptogams and making it more compact and easier to manufacture.

The result is BtM board (Bryolichen Temperature Moisture board), an open-source printed circuit board[18]. Each board is controlled by a high energy-efficient microcontroller (**Table of Materials**). Environmental temperature and relative humidity data are gathered through a temperature and humidity sensor that comes precalibrated and, aside from its low power consumption, has an adequate price-performance ratio.

The board uses a digital communication protocol (standard SPI serial) to manage the measurement cycle. A real-time clock (DS3234) mounted on each board provides accurate timing. In order to reduce energy consumption, the processor remains in standby mode most of the time. Each time data needs to be collected, the real-time clock activates the processor and triggers the logging process. The real-time clock is also used to accurately record the date and time of each data case.

Up to eight moss and/or lichen samples can be logged in parallel using a single BtM board. When the experiment is set up, two crocodile-clip electrode probes are applied to each moss/lichen sample. Then, a voltage divider between each electrode and a resistor reference with a known value (330 KΩ in this case) are used. This resistor value was selected through calibration and based on previous measures of the cryptogams. It provides a resolution of one order of magnitude around the reference value (100 - 1,000 KΩ). The voltage drop is buffered and then read with the microcontroller using its analog ports (A0 - A7)[18]. The voltage is calculated by applying the following formula.

$$V_i = (ADC_i \times VCC) / 1023$$

Here, $ADC_i$ is the raw value from the ADC (Analog-to-Digital converter) of channel i, VCC is the power supply voltage (3.3 V in this case), and 1023 is the range of the ADC output. The resulting voltage $V_i$ is then used in combination with Ohm's law to calculate the resistance ($R_i$, Ω) and conductance (G, S) of each moss sample.

$$R_i = (VCC \times R_L) / V_i - R_L$$

$$G = 1 / R_i$$

Here, $R_L$ is the value of the resistor reference (330 KΩ in this case). The microcontroller's onboard software incorporates all these equations, so it can directly register the values of resistance and conductance.

The board also collects measurements of the ambient temperature and humidity using sensors. Then, each data point is written to a log file on a microSD card. A microSD TransFlash breakout board was mounted on each BtM board for this purpose. Finally, the microSD card can be manually collected after the experiment. All data points can be transferred to a computer for further analysis.

**PROTOCOL:**

**1. Assembly of the Datalogger**

1.1. Prepare a soldering iron and a spool of solder wire. Wait for the soldering iron to heat up and moisten the cleaning sponge.

1.2. Cut the pin header strips to the desired length and solder them into the sockets for the temperature and humidity sensor, the microcontroller, and the RTC clock and microSD breakout modules.

1.2.1. To solder, preheat the desired join with the tip of the soldering iron.

1.2.2. Then, apply a small amount of material from the solder wire, enough to fill up the junction.

1.2.3. Finally, remove the soldering iron and wait for the junction to cool down.

1.3. Assemble the components to the circuit board using the same procedure as in step 1.2, following the markings of the PCB and the component references specified in the **Table of Materials** (see **Figure 1** for an assembly scheme).

1.3.1. First, solder the resistors. Then, solder the sockets for the operational amplifiers, the SHT7X sensor, and the RTC clock and microSD breakout modules.

1.3.2. Next, solder the two transistors. The board also needs to be soldered now, using pin headers. Finally, solder the connectors to the board.

1.4. Solder the SHT7X humidity/temperature sensor into a pin header or extension cable to reinforce the leads.

1.5. Prepare a multimeter in the continuity testing or conductivity testing mode. Use the multimeter to verify that there are no short circuits between any of the pins or connections.

1.5.1. Doublecheck the +ve and –ve terminals of the power supply. Also, verify that each solder joint creates a stable connection between the component pins and the copper tracks of the circuit.

Note: This step is very important; do not skip it.

1.6. Connect the battery terminals and crocodile cable clips to the board using a Phillips screwdriver.

1.6.1. First, use any cutting tool to strip ~4 mm of each wire end, exposing the conductive core. Next, introduce each cable into the appropriate terminal and tighten the screw with the Phillips screwdriver.

1.6.2. Ensure and doublecheck the correct polarity of the cables, especially those of the power supply. Test the strength of the connection by pulling the cables slightly, verifying that everything is firmly connected.

1.7. To further reduce power consumption, remove the power LED of the microcontroller board by either desoldering or cutting off the LED diode from the board.

1.8. Finally, mount the BtM board in a weatherproof enclosure to keep moisture away from the electronics.

1.8.1. Fit the enclosure with the battery pack, connecting it to the +ve and –ve terminals. Mount the humidity/temperature sensor outside of the box, leaving it connected to the BtM board.

1.8.2. Route the eight pairs of crocodile clips needed for conductance measurements to the outside of the weatherproof enclosure. Last, clip each moss strand with the crocodile clips.

**2. Loading the Software**

2.1. Download and install the integrated development environment (IDE) 1.0.6 from the website[19]. The microcontroller used is an open-source physical computing platform and it comes with its own IDE. It is important to download the adequate version since there are known compatibility problems with some of the required libraries.

2.2. Download the necessary libraries from the GitHub repository[18]: DS3234, DS3234lib3, PowerSaver, SdFat, and Sensirion.

2.3. Download the main source code for the datalogger from the GitHub repository[18].

2.4. Open the clock.ino file to set up the current time and date. Edit the parameters for the function **RTC.setDateTime** with the current time and date using the following format:

```
RTC.setDateTime(DD,MM,YY,hh,mm,ss); // Date: DD/MM/YY hh:mm:ss
```

Here, DD is day, MM is month, YY is year, hh is hour, mm is minutes, and ss is seconds.

2.5. Then, upload the clock program to the BtM board, plugging the USB-to-Serial adapter (FTDI breakout) into the microcontroller programming ports and using a mini-USB-to-USB cable to connect the board to the computer. Finally, first press **Verify** and, then, **Upload** in the IDE.

2.6. Open the datalog project in the IDE and modify the datalog.ino file. Set up the start time for the logger editing the following variables:

```
int dayStart = DD, hourStart = hh, minStart = mm
```

Here, DD is the number of the day, hh is the starting hour of the measurements, and mm the minute of the start.

Note: The code to set up a specific time should look like this:

```
RTC.setDateTime(DD,MM,YY,hh,mm,ss);// Date 01/12/17 12:00:00
```

2.7. Set the interval between measurements (in seconds) modifying the value of the variable **interval.**

**3. Set-up of the Measurement Probes**

3.1. Place the crocodile clips at a central position of the communities in the cases of fruticose lichens and bryophytes (**Figure 2**). For fruticose lichens, attach the clips in the thallus and, for mosses, directly on the stem of an individual. In the case of foliose lichens, place the clips on the border of the thallus.

3.2. Keep a minimum distance of *ca*. 5 mm between electrodes. Ensure that the clips are not easily detached before starting measurements.

**4. Calibration for Conductance Measurements**

4.1. To ensure that the specimens are dry, perform the calibration at noon, on a day with low air relative humidity, preceded by at least one, and preferably two, dry days.

4.2. Select a community of moss or lichens that is healthy and well-structured.

4.3. Connect the datalogger to the moss or lichen, following the steps in section 3 of this protocol.

4.4. Start the measurements (turn on the datalogger) and leave the BtM board running for approximately 3 min to stabilize the recorded values.

4.5. Perform a precalibration test to estimate the amount of water required in each watering event. Connect the clips to the sample and add water until the conductance reaches a value that does not increase with the addition of water. This is the maximum conductance value of that sample. This value will be used to establish the watering steps of the calibration (see step 4.7.1).

4.6. Wait until the conductance measures return to the initial values (the samples are dry).

4.7. Then, add water sequentially with a small spray.

4.7.1. Moisten the samples with a quantity of water equivalent to 1/10 of the amount of water required to achieve the maximum conductance (see step 4.5) in the sample.

4.7.2. Wait until the moss or lichen fully absorbs the water and the conductance measurements are stable before watering again (~1 min between each watering event).

4.7.3. Repeat until the conductance reaches the maximum value (saturation) and the moss or lichen is fully hydrated.

Note: Each calibration test should take around 15 min, depending on the interval between the waterings, which should be 1 - 2 min.

4.8. After finishing the calibration, take the microSD card from the BtM board and copy the data file to a computer.

Note: The logged values can then be used as a baseline for the experiments. It is also necessary to do this step to verify that the set-up is correctly registering the conductance of the samples, just before running the actual experiment.

**5. Alternative Calibration for Lab Experiments**

5.1. Fully hydrate the community of moss or lichen until an excess of external water is observed. To ensure that the community is fully hydrated, keep the community moist for 30 min.

5.2. Connect the datalogger to the moss or lichen, following the steps in section 3 of this protocol.

5.3. Start the measurements and leave the BtM board running for approximately 3 min to stabilize the recorded values.

5.4. Wait until the conductance reaches the minimum value (desiccation) and the moss or lichen is no longer conducting electricity.

Note: Each calibration could last at least 1 h, but the duration is highly variable depending on the species. Measurements should be taken until a minimum conductance value is achieved.

**REPRESENTATIVE RESULTS:**

We analyzed the changes in conductance in two species of mosses, *Dicranum scoparium* Hedw. and *Homalothecium aureum* (Spruce) H. Rob. (**Figure 3**), during the calibration process in lab conditions. Mats of the two mosses were kept for 24 h in silica gel and placed in an artificial substrate (*i.e.*, wadding) that kept their original structure (**Figure 2**). Then, the samples were watered 15x to 20x with a spray in 1 min intervals. Each watering event consisted of *ca.* 0.1 mL of water. In both species, a high correlation between the water added and the sample conductance

(*D. scoparium* $r_S = 0.88$, $p < 0.001$; *H. aureum* $r_s = 0.87$, $p < 0.001$) was observed. There was a high increase in the conductance (from 0% to 25% at least) just in the first water addition, and the measures reached their maximum conductance at 4 mL for *D. scoparium* and 10 mL for *H. aureum*. It is important to remark that the relationship between the quantity of water and conductance is logarithmic. Therefore, the values of conductance need to be transformed to have a linear relationship between both variables, and their relationship should be modeled using nonlinear regression.

We found some variability among the samples (see the different colors in **Figures 3a** and **3b**), although all samples belonging to the same species drew a similar curve. The variation between samples can be attributed to differences in biomass and morphology of the patches. Samples in the field are very likely to show this type of variability, so taking several measures of each community type is recommended. Not surprisingly, the highest variability was found among species, since species differ in several fundamental traits (*e.g.*, the aggregation of the mats or morphology). To control for intra- and interspecies variability, we recommend calibrating each clip until achieving the maximum conductance values and, then, rescaling the results for each clip so that the values go from 0 to 100. Consider that absolute conductance values depend on the distance between clips and the basal conductance of the stems, so the values they provide are not directly comparable.

The amount of water added in each watering event of the calibration process is crucial and will strongly affect the results. Here, the aim was to have several watering events in the range of maximum accuracy of the BtM. We present an example of a calibration curve when too much water is added in each step (**Figure 4**). If the sample is overwatered in the first watering event, the increase in the conductance cannot be appreciated and the calibration will be inaccurate. This may lead to biases in the range where nonvascular cryptogams are active, which are the most interesting measurements taken with the BtM.

We also analyzed the desiccation curve of the same two species (*H. aureum* and *D. scoparium*), to provide an alternative calibration procedure. Mats of the two mosses were watered overnight to ensure they were fully saturated. Then, a representative stem of each mat was extracted and placed in a stable, controlled environment and the conductance was recorded continuously. As for the other calibration measure, the values of conductance need to be transformed to have a linear relationship between both variables, and their relationship should be modeled using nonlinear regression.

**Figures 5a** and **5b** show the dessication curves of *H. aureum* and the *D. scoparium* variability among samples of the same species. The intra- and interspecies variability found were quite large and, as in the other calibration procedure, could be attributed to differences in biomass and morphology of each stem. To control for it, we recommend performing at least three measurements per species. Absolute conductance values are not directly comparable in this calibration procedure, as they also depend on the distance between clips and basal conductance of the stems.

We present an example of field data after a rain event occurred between June 23 - 26, 2014. We show the daily variation in the percentage of conductance (**Figure 6a**), relative humidity (**Figure 6b**), and precipitation (**Figure 6c**) for one species of moss (*Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr). There was a strong relationship between the conductance of the moss, the precipitation events, and the relative humidity of the air. During the period analyzed, there were two peaks in the conductance and humidity as a consequence of two precipitation events. The first one occurred just before midnight of June 23 and the second one after the midday of June 24. About 8 h after the first rain event, we observed a decline in the relative humidity of the air, followed by a sudden drop in the moss conductance that goes below 25%. The second rain event was smaller and, consequently, produced a smaller peak in conductance. After this rain event, the moss did not dry out immediately but stayed hydrated while the humidity was above 75%.

**FIGURE AND TABLE LEGENDS:**

**Figure 1: Assembly schematic of the BtM datalogger.** The schematic includes a picture of the BtM board and the placement of each component on the board.

**Figure 2: Correct placement of the clips in a moss (*Homalothecium aureum*).** The image shows how to place the clips to maintain a minimal distance between the clips without damaging the bryophyte.

**Figure 3: The response of conductance to water addition.** These panels show the response of conductance to water addition in (**a**) *Dicranum scoparium* and (**b**) *H. aureum.* The colors show the different replicates. The data points are the average of the log-transformed conductance in an interval between 10 and 30 s after the watering event. The error bars represent the standard deviation of the data in that interval.

**Figure 4: Response of log-transformed conductance to water addition in *D. scoparium* when the amount of added water is too large to allow calibration.** The error bars represent the standard deviation of the data in that interval.

**Figure 5: Desiccation curves.** These panels show the desiccation curves of (**a**) *D. scoparium* and (**b**) *H. aureum*. The data points are the average of the log-transformed conductance measured every 30 s. Black points show the mean of the three replicates and the error bars represent the standard deviation of the data in that interval.

**Figure 6: Daily variation in a moss' (*Syntrichia ruralis*) conductance, precipitation, and relative humidity.** The measures were taken in soil communities of the Cantoblanco, Campus of the *Universidad Autónoma de Madrid*, Spain. The conductance and relative humidity were measured with the BtM prototype, while the precipitation data comes from a weather station placed a few meters away from the measurement location.

**Table 1: Example of the BtM output.**

**DISCUSSION:**

To our knowledge, this is the first time that a datalogger to measure temperature, humidity, and conductance simultaneously as a proxy of the water content of poikilohydric organisms has been designed based on an open-access platform. The BtM datalogger is easy to build and cost-effective, and also provides high-quality measurements of air humidity, temperature, and impedance data using minimal power.

The simple assembly is one of the main advantages of this datalogger. As it is an open-source project, we provide the data-logging software and a detailed scheme of its structure, together with a nontechnical manual for building a ready-to-use BtM datalogger. This makes the method accessible to any research group, even to those that do not work with an engineer or specialized technicians. Besides, the assembly of each datalogger requires just about 1 hour if the printed board circuit is used and about 4 hours if the circuit is mounted by the researchers. Additionally, the BtM datalogger is highly cost-efficient. The estimated cost of the components of each unit is approximately 100 euros, a fairly low price that can be reduced even further in large-scale projects by assembling batches of several dataloggers.

Although there have been several recent methodological developments aimed at implementing devices that measure different aspects related to the physiological activity of nonvascular cryptogam communities, the BtM fills an important knowledge gap. Raggio *et al.*[15] employ Moni-Da, a monitoring system that obtains physiological and microclimatic information. The physiological activity is collected through chlorophyll *a* fluorescence, a method widely used in the laboratory to estimate the activity of photosynthetic organisms. Although this method is highly accurate, it is significantly more expensive than the BtM datalogger. Besides, the monitoring system is a private company product, which cuts back the autonomy of the research group.

The two other methods that have recently been published are also based on estimating water content of nonvascular cryptogams. The first is based on thermal measurements (a dual-probe heat pulse (DPHP) method). Although promising results have recently been shown by Young *et al.*[16], the lack of any specific scheme in the paper makes assembling it without specialized knowledge highly challenging. Lastly, Weber *et al.*[14] presented a sensor called the biocrust wetness probe (BWP), which is very similar to the BtM datalogger. However, they do not provide any scheme for its construction, which hinders the possibility of building the datalogger without the assistance of a specialist. We overcome this issue by providing not only the construction scheme but also the circuit board to assemble the datalogger. Interestingly, the BtM can be easily modified to measure biocrusts, isolated individuals, or cushions, just by changing the crocodile clips (for lichen or bryophyte individuals/cushions) to copper alloy electrode pins (for the biocrusts). If necessary, only part of the crocodiles can be replaced, allowing direct comparisons between the two measurement probe types.

When interpreting the results, the relationship between activity and water content should be carefully addressed, because the BtM does not directly measure photosynthesis. Photosynthesis and activity are closely related in nonvascular cryptogams since a dry poikilohydric organism is in metabolic cease and a wet one is active. However, the degree of photosynthetic activity cannot be inferred directly from the water content, even though a higher metabolic activity—and, thus, a higher photosynthetic activity—can be expected in a well-hydrated organism.

**Critical Steps:**

Despite the ease of assembly, there are some critical steps in the protocol that should be carefully addressed by researchers when mounting the sensor. First, as emphasized in the protocol, it is quite easy to produce short circuits when soldering, which, in the worst case, could result in serious damages to the microcontroller. It is very important to check for their presence with a multimeter and to remove them before connecting the batteries. We recommend using the provided PCB design since it significantly simplifies the process and may be the best option to overcome this issue. Second, not all IDE versions are compatible with the libraries required for this datalogger. It is important to download the proper one (1.0.6) to avoid any compatibility issues. Third, it is important to notice the polarity of the batteries. A polarity inversion could result in serious damages to the hardware. Fourth, calibration is a critical step. The BtM datalogger is designed so that the higher resolution coincides with the moment in which the cryptogam goes from dry to wet state. This implies that the conductance values saturate long before the sample is saturated in water. However, if the study at hand requires a higher accuracy around other values, it can be modified. Measures beyond one order of magnitude from this reference require the resistor to be changed and a recalibration process (see below). As the environmental temperature can affect the accuracy of the measurements, we recommend taking into account this factor when calibrating. To do so, the calibration should be done at low temperatures to check for changes in the measurement accuracy and stability (see Coxson[12] for temperature effects).

**Modifications:**

Although most of the components of the BtM are fixed, some can be easily modified in the field without resoldering. The simplest modification is to replace the crocodile clips for other probe or measurement systems. For example, instead of the crocodile clips, a probe with two pins, such as the one suggested in Weber *et al.*[14], can be used.

In remote environments, where changing the batteries may not be possible within the needed frequency, batteries could be complemented with a solar panel to power the BtM datalogger for longer periods.

By changing the reference resistors employed to measure the conductance, the rank of higher resolution can be easily modified to higher or lower values. If modified, we highly recommend a precise recalibration. Also, in the source code, the **RValue** variable, which is programmed for a resistor value of 330 KΩ, must be assigned to the new corresponding value (datalog.ino).

**Conclusion:**

Nonvascular cryptogam communities are highly diverse and play a number of different key ecological roles, so understanding their relationships with the abiotic environment is a crucial issue. The BtM datalogger has several applications that will help advance the knowledge of these relationships. For example, it will help deepen insights about the conditions where these organisms are acting as either carbon sinks or carbon sources. The fluctuations between these two roles are strongly related to abiotic conditions such as temperature and moisture[3], but large amounts of data are needed to describe and understand the variations of that relationship at a global scale. This requires dense sensor networks that are possible only if they rely on low-cost and easy-to-implement equipment.

To summarize, this device is a useful tool for ecological research groups and overcomes the technical constraints of designing and building a datalogger. The combination of these two factors may lead to a popularization in the use of dataloggers to measure the water relations of nonvascular cryptogams *in situ*. This, in turn, can boost the establishment of medium and long-term monitoring networks. Developing these networks is essential to assessing the response of nonvascular cryptogams to local and regional environmental factors, as well as to determine their role in ecosystem processes (*e.g.*, nutrient cycles, community assembly) and their most likely response in light of the changes on climatic and anthropic factors associated with global change.

**ACKNOWLEDGMENTS:**

The authors are thankful to Manuel Molina (UAM) and Cristina Ronquillo (MNCN-CSIC) for the help provided during the calibration tests, and to Belén Estébanez (UAM) for her help during the sampling campaigns.

**DISCLOSURES:**

The authors have nothing to disclose.

**REFERENCES:**

1. Fontaneto, D., Hortal, J. Microbial Biogeography: Is Everything Small Everywhere? In *Microbial Ecological Theory: Current Perspectives*. Edited by Ogilvie, L.A., Hirsch, P.R., 87-98, Caister Academic Press. Norfolk, UK (2012).

2. Proctor, M.C.F. *et al.* Desiccation-tolerance in bryophytes: a review. *The Bryologist*. **110** (4), 595-621 (2007).

3. Lindo, Z., Gonzalez, A. The Bryosphere: An Integral and Influential Component of the Earth's Biosphere. *Ecosystems*. **13** (4), 612-627 (2010).

4. Elbert, W. *et al.* Contribution of cryptogamic covers to the global cycles of carbon and nitrogen. *Nature Geoscience*. **5**, 459-462 (2012).

5. Magill, R.E. Moss diversity: New look at old numbers. *Phytotaxa*. **9** (1), 167-174 (2014).

6. Söderström, L. *et al.* World checklist of hornworts and liverworts. *PhytoKeys*. (59), 1-828 (2016).

7. Lücking, R., Hodkinson, B.P., Leavitt, S.D. The 2016 classification of lichenized fungi in the Ascomycota and Basidiomycota – Approaching one thousand genera. *The Bryologist*. **119** (4), 361-416 (2016).

8. Bowker, M.A. Biological Soil Crust Rehabilitation in Theory and Practice: An Underexploited Opportunity. *Restoration Ecology*. **15** (1), 13-23 (2007).

9. Wilske, B. *et al.* The CO2 exchange of biological soil crusts in a semiarid grass-shrubland at the northern transition zone of the Negev desert, Israel. *Biogeosciences Discussions*. **5** (3), 1969-2001 (2008).

10. Wardle, D.A. *et al*. Linking vegetation change, carbon sequestration and biodiversity: insights from island ecosystems in a long-term natural experiment. *Journal of Ecology*. **100** (1), 16-30(2012).

11. Lindo, Z., Nilsson, M.-C., Gundale, M.J. Bryophyte-cyanobacteria associations as regulators of the northern latitude carbon balance in response to global change. *Global Change Biology*. **19** (7), 2022-2035 (2013).

12. Coxson, D.S. Impedance Measurement of Thallus Moisture Content in Lichens. *The Lichenologist*. **23** (1), 77-84 (1991).

13. Raggio, J. *et al.* Continuous chlorophyll fluorescence, gas exchange and microclimate monitoring in a natural soil crust habitat in Tabernas badlands, Almería, Spain: progressing towards a model to understand productivity. *Biodiversity and Conservation*. **23** (7), 1809-1826 (2014).

14. Weber, B. *et al.* Development and calibration of a novel sensor to quantify the water content of surface soils and biological soil crusts. *Methods in Ecology and Evolution*. **7** (1), 14-22 (2016).

15. Raggio, J. *et al.* Metabolic activity duration can be effectively predicted from macroclimatic data for biological soil crust habitats across Europe. *Geoderma*. **306**, 10-17 (2017).

16. Young, M.H., Fenstermaker, L.F., Belnap, J. Monitoring water content dynamics of biological soil crusts. *Journal of Arid Environments*. **142**, 41-49 (2017).

17. Accurate low power temperature/relative humidity data logger. http://www.osbss.com/tutorials/temperature-relative-humidity/ (2015).

18. GitHub - united-ecology/btmboard. https://github.com/united-ecology/btmboard (2018).

19. Arduino - Software. https://www.arduino.cc/en/Main/Software (2018).

Figure 1

Click here to access/download;Figure;Fig 1.pdf

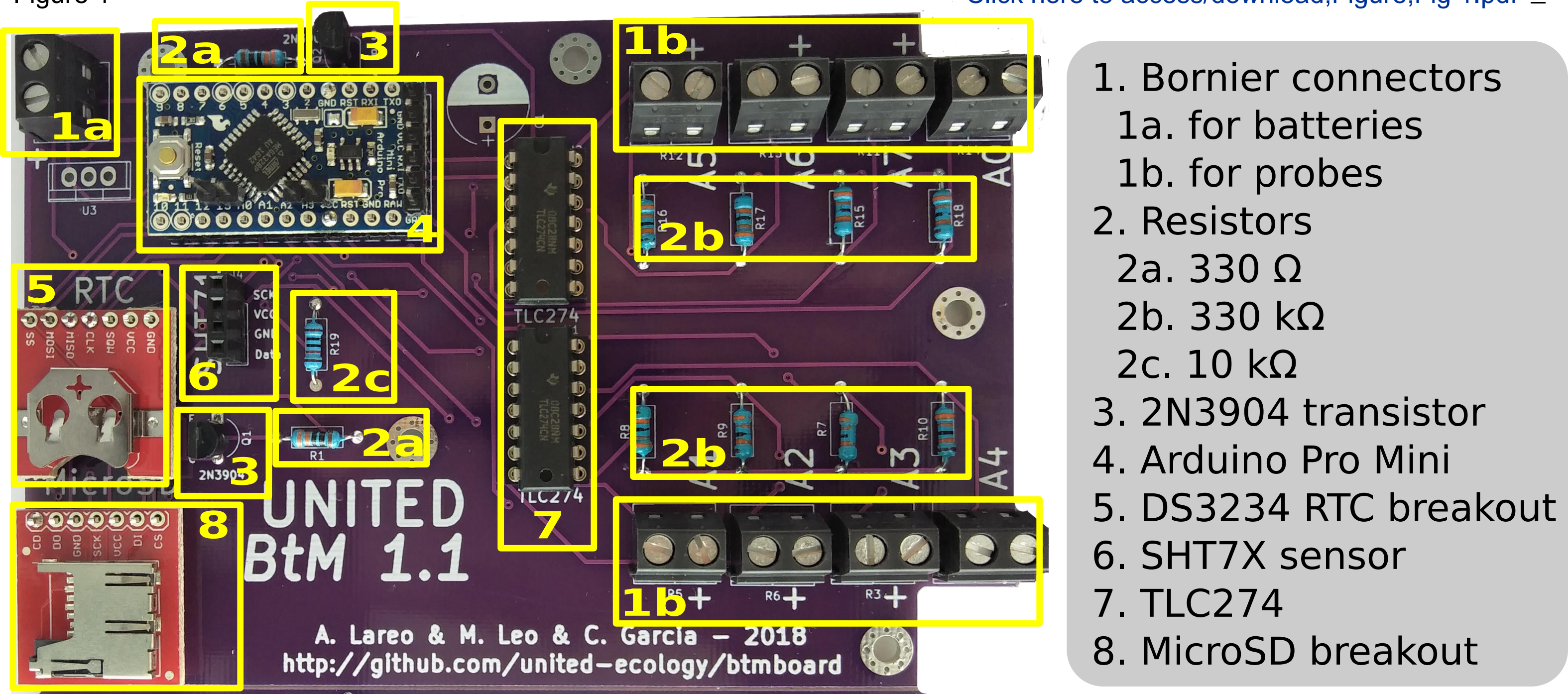


Figure 2



Figure 3



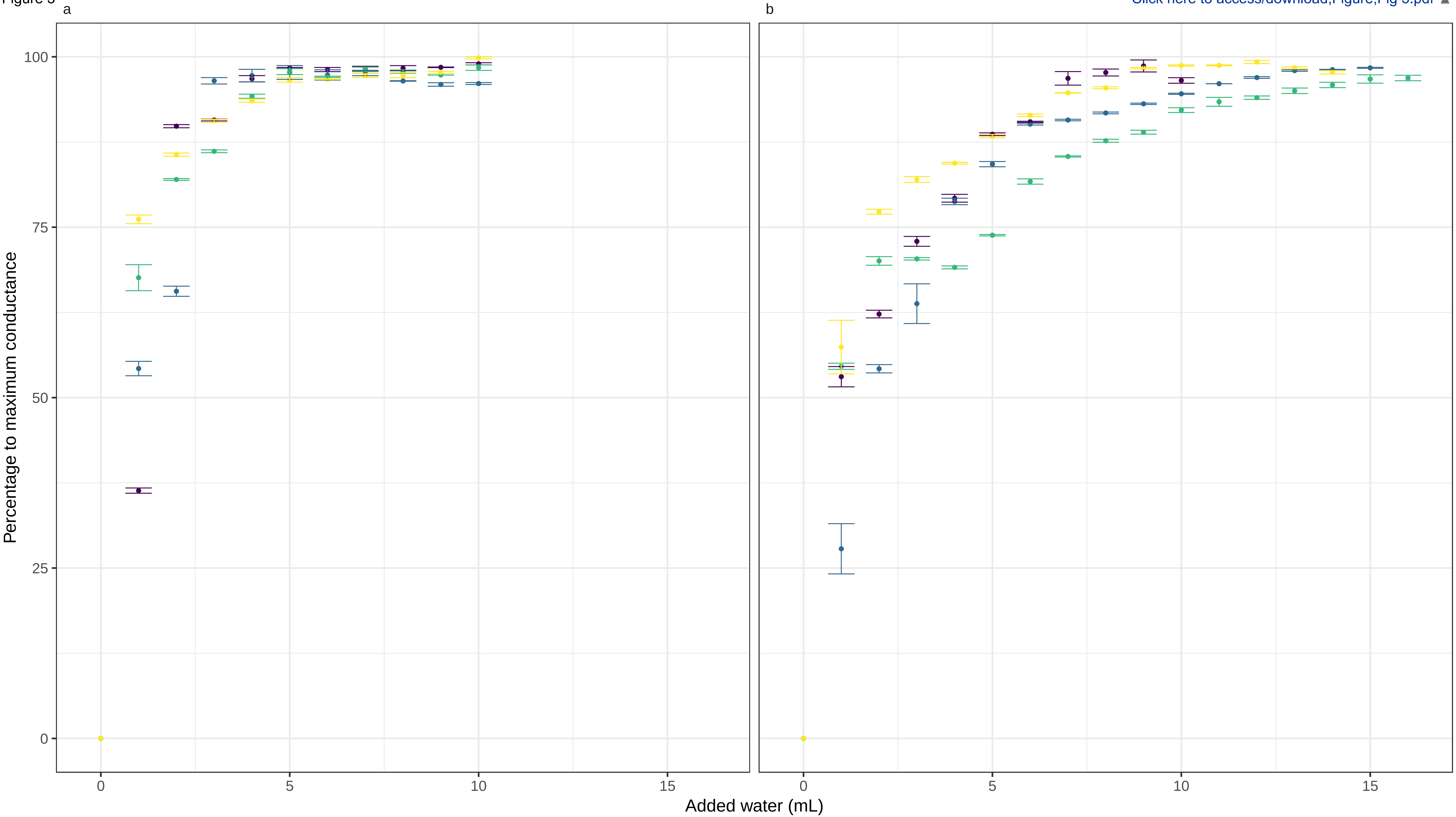

a
b
100
75
50
25
0
Percentage to maximum conductance
0
5
10
15
0
5
10
15
Added water (mL)

Figure 4



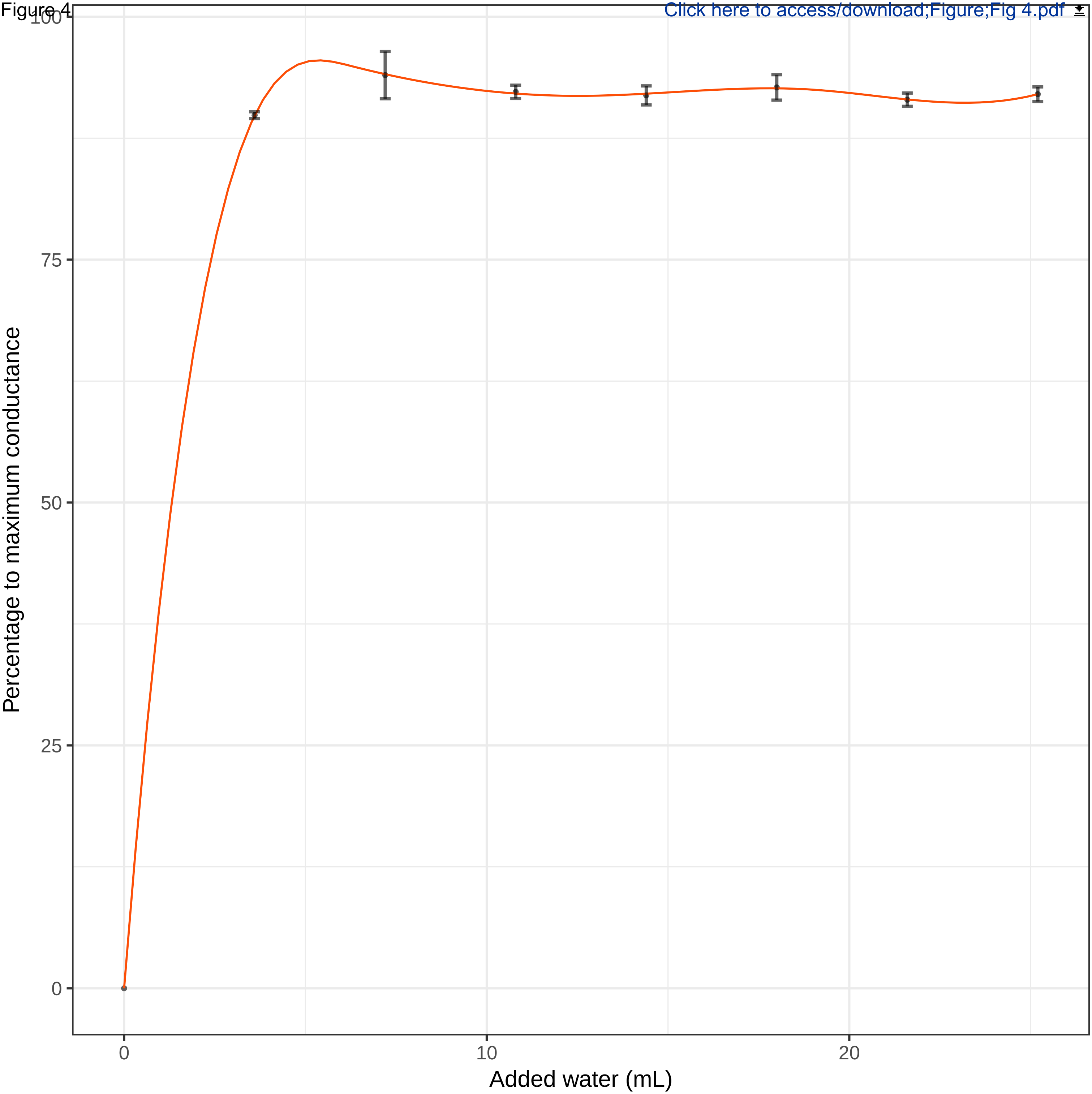
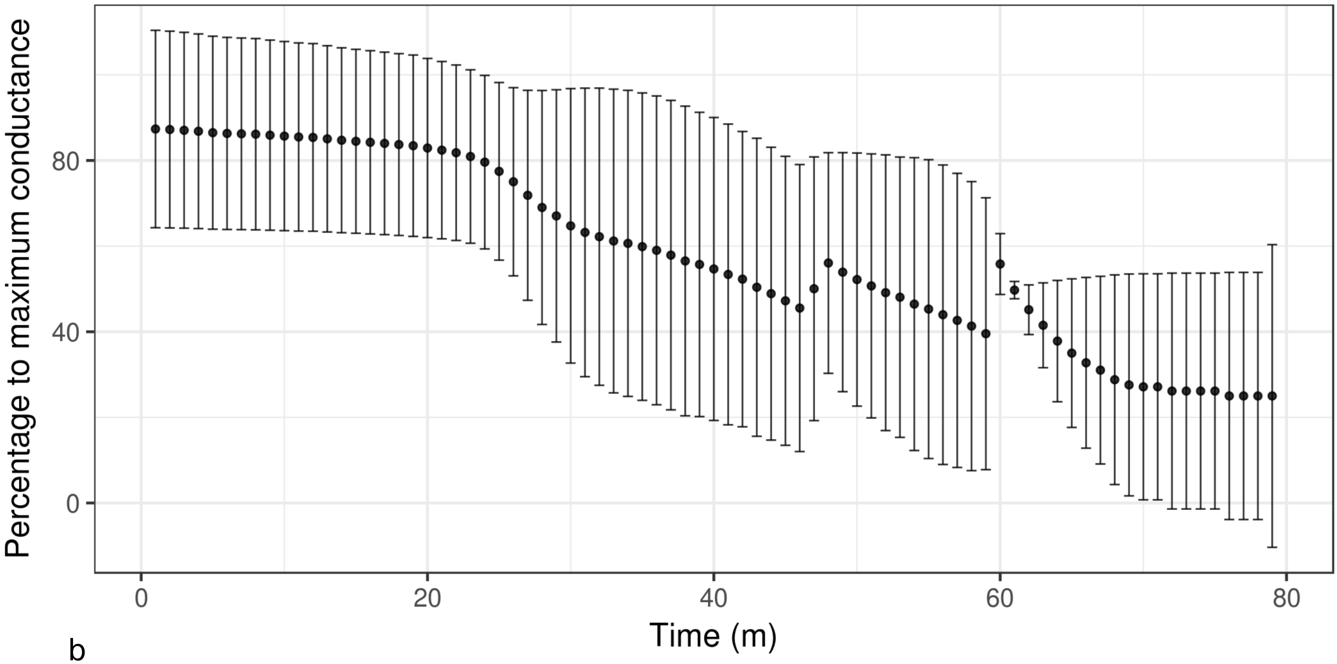

100
75
50
25
0
Percentage to maximum conductance
0
10
20
Added water (mL)

Figure 5

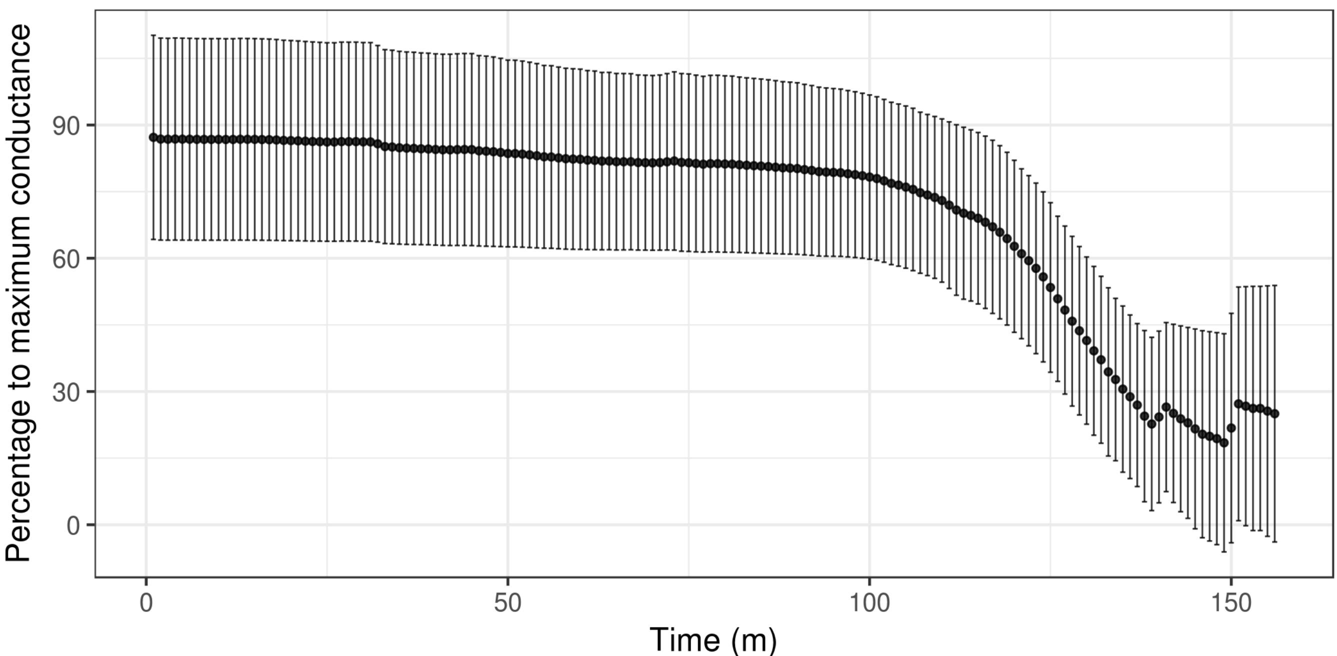


a



Percentage to maximum conductance
0
40
80
0
20
40
60
80
Time (m)

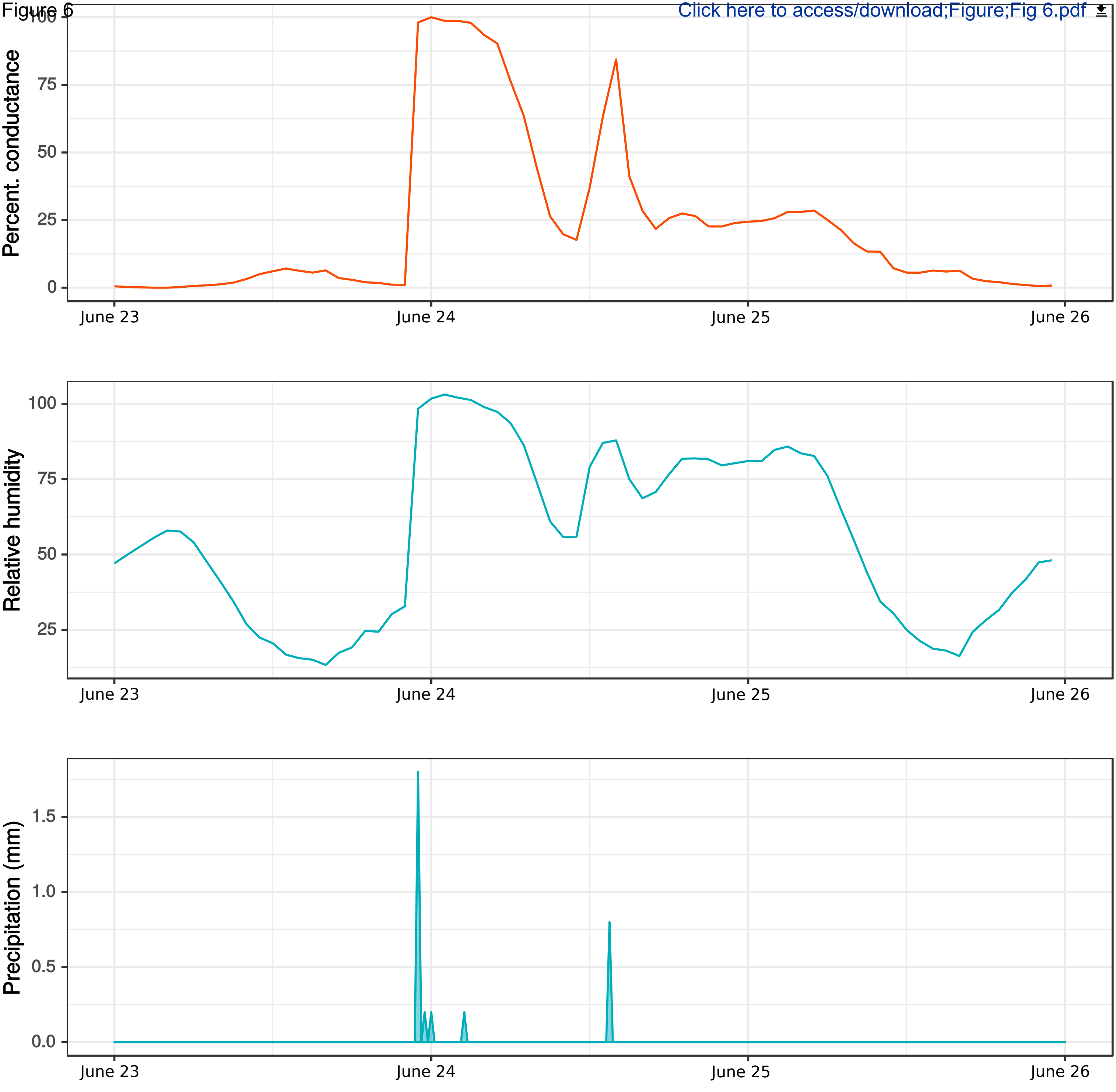

b



Percentage to maximum conductance
0
30
60
90
0
50
100
150
Time (m)

Figure 6

Click here to access/download;Figure;Fig 6.pdf



Sheet1

| Date/Time | Temp(C) | RH(%) | Conductance(KMho) |
|---|---|---|---|
| 11/03/18 12:00 | 26.6 | 66.6 | 139.53 |
| 11/03/18 12:00 | 26.6 | 66.4 | 167.92 |
| 11/03/18 12:00 | 26.8 | 66.4 | 199.14 |
| 11/03/18 12:00 | 26.9 | 66.4 | 212.75 |
| 11/03/18 12:00 | 26.6 | 66.6 | 217.15 |
| 11/03/18 12:01 | 26.9 | 66.7 | 218.93 |
| 11/03/18 12:01 | 27 | 66.8 | 139.53 |
| 11/03/18 12:01 | 27.1 | 66.9 | 164.28 |
| 11/03/18 12:01 | 27.1 | 67.3 | 194.21 |
| 11/03/18 12:01 | 27.3 | 67.3 | 209.28 |

| Name of Material/ Equipment | Company | Catalog Number | Comments/Description |
|---|---|---|---|
| BtMboard circuit (PCB) | | | 1 |
| Arduino Pro Mini 328 3.3 V (APM) | Arduino | | 1 |
| FTDI Basic Breakout | SparkFun | | 1 |
| MiniUSB to USB cable adapter | | | 1 |
| TLC274 operational amplifier | Texas Instruments | | 2 |
| 2.54 mm breakout pin strip | | | 1 |
| 330 KOhm resistor | | | 8 |
| 330 Ohm resistor | | | 2 |
| 10 KOhm resistor | | | 1 |
| 2N3904 Transistor | | | 2 |
| Bornier connector, 2x1 5.08 mm | | | 9 |
| 1.5 V AA battery | | | 3 |
| 3xAA battery holder with switch | | | 1 |
| Sensirion SHT71 | Sensirion | | 1 |
| DS3234 RTC Breakout (clock) | SparkFun | | 1 |
| CR1225 3 V Coin-cell battery | | | 1 |
| MicroSD Transflash breakout | SparkFun | | 1 |
| Crocodile clip connector | | | 16 |
| Weatherproof enclosure box | | | 1 |
| 12 AWG stranded cable spool | | | 1 |
| Cutting pliers | | | 1 |
| 30 W soldering iron | | | 1 |
| Solder wire spool | | | 1 |
| Arduino IDE 1.0.6 | Arduino | | 1 |
| Arduino library DS3234 | Arduino | | 1 |
| Arduino library DS3234lib3 | Arduino | | 1 |
| Arduino library Powersaver | Arduino | | 1 |
| Arduino library SdFat | Arduino | | 1 |
| Arduino library Sensirion | Arduino | | 1 |

1 Alewife Center #200
Cambridge, MA 02140
tel. 617.945.9051
www.jove.com

# ARTICLE AND VIDEO LICENSE AGREEMENT

Title of Article: BtM, a low-cost open-source data logger to estimate water content of non-vascular cryptogams.

Author(s): María Leo, Angel Lareo, Carlos García-Saura, Joaquín Hortal, Nagore G. Medina

Item 1 (check one box): The Author elects to have the Materials be made available (as described at http://www.jove.com/author) via: ☐ Standard Access ☑ Open Access

Item 2 (check one box):

☑ The Author is NOT a United States government employee.

☐ The Author is a United States government employee and the Materials were prepared in the course of his or her duties as a United States government employee.

☐ The Author is a United States government employee but the Materials were NOT prepared in the course of his or her duties as a United States government employee.

## ARTICLE AND VIDEO LICENSE AGREEMENT

1. Defined Terms. As used in this Article and Video License Agreement, the following terms shall have the following meanings: "**Agreement**" means this Article and Video License Agreement; "**Article**" means the article specified on the last page of this Agreement, including any associated materials such as texts, figures, tables, artwork, abstracts, or summaries contained therein; "**Author**" means the author who is a signatory to this Agreement; "**Collective Work**" means a work, such as a periodical issue, anthology or encyclopedia, in which the Materials in their entirety in unmodified form, along with a number of other contributions, constituting separate and independent works in themselves, are assembled into a collective whole; "**CRC License**" means the Creative Commons Attribution-Non Commercial-No Derivs 3.0 Unported Agreement, the terms and conditions of which can be found at: http://creativecommons.org/licenses/by-nc-nd/3.0/legalcode; "**Derivative Work**" means a work based upon the Materials or upon the Materials and other pre-existing works, such as a translation, musical arrangement, dramatization, fictionalization, motion picture version, sound recording, art reproduction, abridgment, condensation, or any other form in which the Materials may be recast, transformed, or adapted; "**Institution**" means the institution, listed on the last page of this Agreement, by which the Author was employed at the time of the creation of the Materials; "**JoVE**" means MyJove Corporation, a Massachusetts corporation and the publisher of *The Journal of Visualized Experiments;* "**Materials**" means the Article and / or the Video; "**Parties**" means the Author and JoVE; "**Video**" means any video(s) made by the Author, alone or in conjunction with any other parties, or by JoVE or its affiliates or agents, individually or in collaboration with the Author or any other parties, incorporating all or any portion of the Article, and in which the Author may or may not appear.

2. Background. The Author, who is the author of the Article, in order to ensure the dissemination and protection of the Article, desires to have the JoVE publish the Article and create and transmit videos based on the Article. In furtherance of such goals, the Parties desire to memorialize in this Agreement the respective rights of each Party in and to the Article and the Video.

3. Grant of Rights in Article. In consideration of JoVE agreeing to publish the Article, the Author hereby grants to JoVE, subject to **Sections 4** and **7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Article in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Article into other languages, create adaptations, summaries or extracts of the Article or other Derivative Works (including, without limitation, the Video) or Collective Works based on all or any portion of the Article and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. If the "Open Access" box has been checked in **Item 1** above, JoVE and the Author hereby grant to the public all such rights in the Article as provided in, but subject to all limitations and requirements set forth in, the CRC License.

4. Retention of Rights in Article. Notwithstanding the exclusive license granted to JoVE in **Section 3** above, the Author shall, with respect to the Article, retain the non-exclusive right to use all or part of the Article for the non-commercial purpose of giving lectures, presentations or teaching classes, and to post a copy of the Article on the Institution's website or the Author's personal website, in each case provided that a link to the Article on the JoVE website is provided and notice of JoVE's copyright in the Article is included. All non-copyright intellectual property rights in and to the Article, such as patent rights, shall remain with the Author.

5. Grant of Rights in Video – Standard Access. This **Section 5** applies if the "Standard Access" box has been checked in **Item 1** above or if no box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby acknowledges and agrees that, Subject to **Section 7** below, JoVE is and shall be the sole and exclusive owner of all rights of any nature, including, without limitation, all copyrights, in and to the Video. To the extent that, by law, the Author is deemed, now or at any time in the future, to have any rights of any nature in or to the Video, the Author hereby disclaims all such rights and transfers all such rights to JoVE.

6. Grant of Rights in Video – Open Access. This **Section 6** applies only if the "Open Access" box has been checked in **Item 1** above. In consideration of JoVE agreeing to produce, display or otherwise assist with the Video, the Author hereby grants to JoVE, subject to **Section 7** below, the exclusive, royalty-free, perpetual (for the full term of copyright in the Article, including any extensions thereto) license (a) to publish, reproduce, distribute, display and store the Video in all forms, formats and media whether now known or hereafter developed (including without limitation in print, digital and electronic form) throughout the world, (b) to translate the Video into other languages, create adaptations, summaries or extracts of the Video or other Derivative Works or Collective Works based on all or any portion of the Video and exercise all of the rights set forth in (a) above in such translations, adaptations, summaries, extracts, Derivative Works or Collective Works and (c) to license others to do any or all of the above. The foregoing rights may be exercised in all media and formats, whether now known or hereafter devised, and include the right to make such modifications as are technically necessary to exercise the rights in other media and formats. For any Video to which this Section 6 is applicable, JoVE and the Author hereby grant to the public all such rights in the Video as provided in, but subject to all limitations and requirements set forth in, the CRC License.

7. Government Employees. If the Author is a United States government employee and the Article was prepared in the course of his or her duties as a United States government employee, as indicated in **Item 2** above, and any of the licenses or grants granted by the Author hereunder exceed the scope of the 17 U.S.C. 403, then the rights granted hereunder shall be limited to the maximum rights permitted under such statute. In such case, all provisions contained herein that are not in conflict with such statute shall remain in full force and effect, and all provisions contained herein that do so conflict shall be deemed to be amended so as to provide to JoVE the maximum rights permissible within such statute.

8. Likeness, Privacy, Personality. The Author hereby grants JoVE the right to use the Author's name, voice, likeness, picture, photograph, image, biography and performance in any way, commercial or otherwise, in connection with the Materials and the sale, promotion and distribution thereof. The Author hereby waives any and all rights he or she may have, relating to his or her appearance in the Video or otherwise relating to the Materials, under all applicable privacy, likeness, personality or similar laws.

9. Author Warranties. The Author represents and warrants that the Article is original, that it has not been published, that the copyright interest is owned by the Author (or, if more than one author is listed at the beginning of this Agreement, by such authors collectively) and has not been assigned, licensed, or otherwise transferred to any other party. The Author represents and warrants that the author(s) listed at the top of this Agreement are the only authors of the Materials. If more than one author is listed at the top of this Agreement and if any such author has not entered into a separate Article and Video License Agreement with JoVE relating to the Materials, the Author represents and warrants that the Author has been authorized by each of the other such authors to execute this Agreement on his or her behalf and to bind him or her with respect to the terms of this Agreement as if each of them had been a party hereto as an Author. The Author warrants that the use, reproduction, distribution, public or private performance or display, and/or modification of all or any portion of the Materials does not and will not violate, infringe and/or misappropriate the patent, trademark, intellectual property or other rights of any third party. The Author represents and warrants that it has and will continue to comply with all government, institutional and other regulations, including, without limitation all institutional, laboratory, hospital, ethical, human and animal treatment, privacy, and all other rules, regulations, laws, procedures or guidelines, applicable to the Materials, and that all research involving human and animal subjects has been approved by the Author's relevant institutional review board.

10. JoVE Discretion. If the Author requests the assistance of JoVE in producing the Video in the Author's facility, the Author shall ensure that the presence of JoVE employees, agents or independent contractors is in accordance with the relevant regulations of the Author's institution. If more than one author is listed at the beginning of this Agreement, JoVE may, in its sole discretion, elect not take any action with respect to the Article until such time as it has received complete, executed Article and Video License Agreements from each such author. JoVE reserves the right, in its absolute and sole discretion and without giving any reason therefore, to accept or decline any work submitted to JoVE. JoVE and its employees, agents and independent contractors shall have

full, unfettered access to the facilities of the Author or of the Author's institution as necessary to make the Video, whether actually published or not. JoVE has sole discretion as to the method of making and publishing the Materials, including, without limitation, to all decisions regarding editing, lighting, filming, timing of publication, if any, length, quality, content and the like.

11. Indemnification. The Author agrees to indemnify JoVE and/or its successors and assigns from and against any and all claims, costs, and expenses, including attorney's fees, arising out of any breach of any warranty or other representations contained herein. The Author further agrees to indemnify and hold harmless JoVE from and against any and all claims, costs, and expenses, including attorney's fees, resulting from the breach by the Author of any representation or warranty contained herein or from allegations or instances of violation of intellectual property rights, damage to the Author's or the Author's institution's facilities, fraud, libel, defamation, research, equipment, experiments, property damage, personal injury, violations of institutional, laboratory, hospital, ethical, human and animal treatment, privacy or other rules, regulations, laws, procedures or guidelines, liabilities and other losses or damages related in any way to the submission of work to JoVE, making of videos by JoVE, or publication in *JoVE* or elsewhere by JoVE. The Author shall be responsible for, and shall hold JoVE harmless from, damages caused by lack of sterilization, lack of cleanliness or by contamination due to the making of a video by JoVE its employees, agents or independent contractors. All sterilization, cleanliness or decontamination procedures shall be solely the responsibility of the Author and shall be undertaken at the Author's expense. All indemnifications provided herein shall include JoVE's attorney's fees and costs related to said losses or damages. Such indemnification and holding harmless shall include such losses or damages incurred by, or in connection with, acts or omissions of JoVE, its employees, agents or independent contractors.

12. Fees. To cover the cost incurred for publication, JoVE must receive payment before production and publication the Materials. Payment is due in 21 days of invoice. Should the Materials not be published due to an editorial or production decision, these funds will be returned to the Author. Withdrawal by the Author of any submitted Materials after final peer review approval will result in a US$1,200 fee to cover pre-production expenses incurred by JoVE. If payment is not received by the completion of filming, production and publication of the Materials will be suspended until payment is received.

13. Transfer, Governing Law. This Agreement may be assigned by JoVE and shall inure to the benefits of any of JoVE's successors and assignees. This Agreement shall be governed and construed by the internal laws of the Commonwealth of Massachusetts without giving effect to any conflict of law provision thereunder. This Agreement may be executed in counterparts, each of which shall be deemed an original, but all of which together shall be deemed to me one and the same agreement. A signed copy of this Agreement delivered by facsimile, e-mail or other means of electronic transmission shall be deemed to have the same legal effect as delivery of an original signed copy of this Agreement.

A signed copy of this document must be sent with all new submissions. Only one Agreement required per submission.

**CORRESPONDING AUTHOR:**

Name: María Leo

Department: Department of Biodiversity

Institution: Real Jardín Botánico (CSIC-RJB)

Article Title: BtM, a low-cost open-source data logger to estimate water content of non-vascular cryptogams.

Signature: 

Date: 29 June 2018

Please submit a signed and dated copy of this license by one of the following three methods:

1) Upload a scanned copy of the document as a pfd on the JoVE submission site;
2) Fax the document to +1.866.381.2236;
3) Mail the document to JoVE / Attn: JoVE Editorial / 1 Alewife Center #200 / Cambridge, MA 02139

For questions, please email submissions@jove.com or call +1.617.945.9051

There are numerous scatterer grammatical and typographical errors. Please thoroughly review the manuscript and edit any errors.
We have thoroughly reviewed the manuscript and corrected the typos and errors, hoping not to have overlooked any of them.

References? (l. 66)
Added (Proctor et al. 2007).

References? (l. 120)
Added (Bowker 2007, Elbert et al. 2012).

References? (l. 122)
Added (Fontaneto & Hortal 2012).

This section fits better in the introduction due to JoVE's style requirements.
Actually, this section is the last part of the introduction. We wrote a general, more biological first part of the introduction to put into context the need and usefulness of this datalogger, and a second part focused on the design of the datalogger.

Please remove or replace (with "microcontroller") the recurring instances of the comercial name. 1 instance is okay in the introduction.
We initially kept the Arduino name in the text because one of the main strengths of this datalogger is that it is design in an open-source environment. However, according to your suggestions, we have replaced it by "microcontroller" in the following instances in the text.

The citation should be added to the reference list and cited using a superscripted number.
Done.

The citation should be added to the reference list and cited using a superscripted number, in the order of appearance.
Done.

This information includes overt comercial names, this sentence can be moved to the table of materials instead.
We have rewritten this sentence to avoid the use of comercial names.

A circuit diagram would be useful here. If this is published elsewhere please cite a reference here.
We have referenced a circuit diagram in the text.

To what length?
Depending on each component's size, the pin header strips will have different lengths.

BTM data logger board or Arduino?
It is the Arduino microcontroller, we have changed it in the text.

Table of material?

It is the table of materials, we have corrected it in the text.

Not defined so far.
We have replaced it with microcontroller.

Add to the table of materials.
Done.

Use superscripted citation number and moe this to the reference list.
Done.

This statement should be moved into the table of materials.
We have moved it to the table of materials.

Use superscripted citation number and moe this to the reference list.
Done.

Use superscripted citation number and moe this to the reference list.
Done.

Highlighted for continuity and because it is referenced in section 4.
Ok.

Where is this measured?
As it is seen in figure 3, in the calibration process the measurements reach a maximum at some point, which corresponds to the maximum conductance values.

Please describe what is done to start the measurements.
The only required actions is to turn on the datalogger. We have added a specification in the text to make this detail more patent.

Gel?
It was gel, we have corrected it in the text.

Example?
We have added an example in the text.

What is the moss weight or volumen?
It is the quantity of water added, a volume (0.1 mL).

There are no panels a and b on fig 4. The description does not match what is shown in fig 4 and its legend. It likley should be fig 6. Please list all figures in the order that they are referenced.
As noted, it was fig. 6 (now figure 5). We have corrected the reference in the text and renumbered the figures to list them in the correct order.

No labels on fig 5 and the ordering is incorect, e.g. the bottom panel shows precipitation.

We have corrected the numbering of the panels, and have added labels in the y axis.

Needs superscripted citation.
Although it may seem confusing, the citation is not needed, since *Syntrichia ruralis* (Hedw.) F. Weber & D. Mohr is the right way of citing the name of this species according to botanical nomenclature rules (I*nternational Code of Nomenclature for algae, fungi and plants, ICN*, https://www.iapt-taxon.org/nomen/main.php).

I cannot understand this statement. Please revise. The patterns follow precipitation but are not similar to it.
We have rewritten these sentences to make them easier to understand.

Please expand the legend to adequately describe the figures/table. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description. (Fig. 1)
Done.

Please expand the legend to adequately describe the figures/table. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description. (Fig. 2)
Done.

Please expand the legend to adequately describe the figures/table. Each figure or table must have an accompanying legend including a short title, followed by a short description of each panel and/or a general description. (Fig. 4)
Done.

Define error bars.
Corrected.

Please avoid the use of bullet points in this section.
Corrected in the text.

Citation number?
Added.