DASCOR™
DATA LOGGERS

DASCOR LRCpH User's Guide


Figure 1, Original body design (left, Series-3), New design as of May 2007 (Right, Series-4)

For Software and Header Versions
In the "**06xx**" series, and
"**JN09**" Firmware

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Contents and logger specifications are subject to change without notice.

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Table of Contents

List of Figures

1 Conventions and Images

Please be aware that the screen images appearing throughout this document may not be identical to the ones appearing on your system. Colors and shapes of the various windows will change depending on the operating system and how your desktop is set up. The basic content, however, should be similar.

Many of the operations involving the logger are repetitive, usually involving only changes to the scan period, or the name of the data file being saved. Once a particular sequence of steps has been presented, setting up and initializing the logger for example, further procedures will either reference the prior procedure, or give an abbreviated version.

Throughout this manual, the following conventions apply:

- Tabs or control buttons will be in *bold italics*.
- "Tab" implies that one of the nine tabs across the main screen has been selected.
- "Screen" refers to any display for the active tab, or any special displays such as the real-time chart, that is visible and has been brought to the foreground.
- "Message," "Box," or "Message Box" refer to small pop-up boxes that appear as needed to advise the operator of important conditions. Frequently, the message box only requires a single click on ***OK*** to confirm that the operator has read the contents of the box. However, where corrective operator action is required (or suggested), additional options are presented, including ***Cancel***, ***Abort***, or ***Ignore***. In each case the subsequent actions of the software will be explained in the box.

Where practical, screen images will include circles around key commands or areas of interest.

The M1b-V6.10 release of the DASCOR Data Logger Software is designed to work specifically with DASCOR **M1b Series** Data Loggers with **JN09 Firmware**. Operation with older loggers that have not been upgraded is not guaranteed, and may cause loss of data. With this hardware and software release, all known faults as of this release have been resolved. The M1b-V6.10 release of the software is considered to be the **final release** for this Hardware and Firmware combination.

NOTE: the ***Data Logger*** tab will clearly identify the logger as using ***Firmware*** Release JN09.
After the first initialization of a logger, the displayed ***Header/Software version*** should be "0600/0610".

The DASCOR Software Guide is intended to be the final authority on the software.

2 Forward

Several years ago, DASCOR was contacted by Greg Penner, a graduate student of the University of Saskatchewan. Greg was conducting the research portion of his degree at the Lethbridge Research Centre regarding the development of an autonomous data logger that could be used for logging pH and temperature in cattle. The idea was to develop a ruminal pH measurement system that did not require animals to be tethered. DASCOR originally provided their basic data logger in a small (2.5" cube) box. Greg fabricated a housing using PVC pipe and connections around the data logger forming the original version of the LRCpH. This version was used during his research and was the basis for several published papers[1], including a thorough validation study.

Figure 2, Original Logger

Over the intervening years, DASCOR became directly involved with designing new housings and new sets of logger electronics modules specifically for use in ruminal pH research. At the present time there are two variants of the logger in circulation. The units produced after May, 2007, can be identified by the white section in the middle and will have serial numbers 100 and up. The two styles are very similar in size and function. The mechanical differences primarily involve accessing the battery and I/O connector. Electronic differences are subtle and mostly centered on improving noise reduction and accuracy, as well as providing additional protection to the electronics from inadvertent contamination with rumen contents.

Future systems will continue to shrink in size and increase in performance. Enhancements are expected to include sensors for pressure and ammonia, use of removable memory modules, and a high speed USB interface.

[1] Penner, G.B., K.A. Beauchemin, and T. Mutsvangwa. 2007. The severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. J. Dairy Sci. 90: 365-375.

Penner, G.B., K.A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132–2140.

3 System Overview and Components

The Lethbridge Research Centre Ruminal pH Measurement System (LRCpH) is a stand-alone, submersible data logger that can be used to record pH, temperature, and oxidation-reduction potential (ORP) in the rumen of cattle over many days. The LRCpH system measures ruminal pH providing nearly continuous data in ruminally cannulated cows without restricting animal movement. The system is placed in the cow's rumen (stomach) through a rumen fistula. The unique design makes it water tight and impervious to the harsh conditions of the rumen environment. The system comes complete with data logger, a 9-volt battery, and a long-use pH electrode (ordered separately). The capsule is weighted down to keep the sensor positioned in the ventral sac of the rumen. A cable connects the capsule to the rumen plug for easy retrieval.

The DASCOR LRCpH system is modeled after the original system designed and used by Agriculture & Agri-Food Canada researcher Dr. Karen Beauchemin and University of Saskatchewan student Greg Penner. For a full description of the accuracy and precision of the LRCpH system, please consult their full-length paper[2]. An example of how the system can be used in nutrition research is given in their subsequent paper[3]. The accuracy and precision of the LRCpH, relative to manual sampling, has been evaluated by Penner et al. (2006) and the LRCpH has been used to measure ruminal pH in transition Holstein heifers (Penner et al., 2007).

Although it is not a requirement for animals to be restrained, regular animal handling is required and, therefore, it is wise to select animals with a calm temperament.

3.1 LRCpH System Components

3.1.1 Data logger (Case Series 3 and 4)

The LRCpH Data Logger is powered by a 9-V battery, either Alkaline or Lithium-Ion types. For normal short term tests, a good quality alkaline battery (e.g. Duracell brand) is adequate. Lithium-ion type (Ultralife brand, non-rechargeable) will give about twice the life. Details on battery life and power consumption are detailed in the Software Manual. The key point is that batteries are very inexpensive compared to the cost of running a test, and it is always a good idea to start critical tests using NEW batteries. Batteries should always be replaced at the start of a test if the voltage is less than 8 Volts.

The data logger measures pH using an external replaceable sensor on Channel-1. A Platinum temperature sensor is included and is read on Channel-2. Battery voltage is read on Channel-3. Channels 4-8 are available for optional sensors. ORP (REDOX) has been installed on most loggers and appears on Channel-4. Pressure and Ammonia Ion are being developed and will appear on Channels 5 and 6.

The logger includes a LED to signal when the logger is functioning (see Figure 3). The logger will flash briefly at high intensity every time data is logged. It turns on all the time when the logger is plugged into the interface cable and a PC with the software running. Very dim flashing at one second intervals indicates that a test is complete (memory is filled) and waiting download, <u>or</u> that an error condition such as low battery has occurred. The amount of installed memory in a given logger will be shown on the ***Data Logger*** tab. Each style of logger includes a RS232 computer interface connection (see Figure 3).

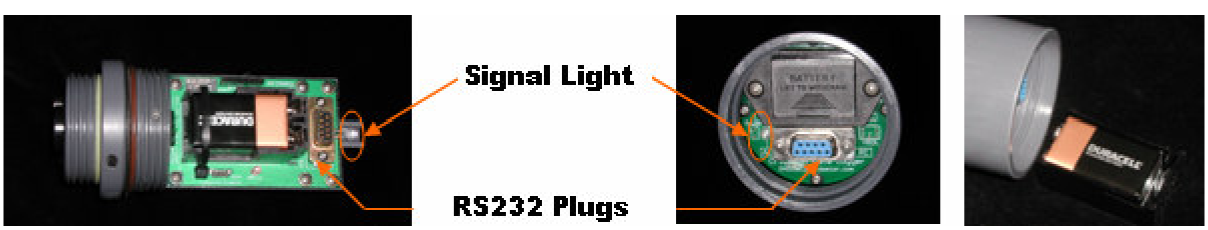


Figure 3, Data logger (left: original model, right: new design)

2 (G.B. Penner, K.A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. Journal of Dairy Science 89:2132-2140)

3 (G.B. Penner, K.A. Beauchemin, and T. Mutsvangwa. 2007. Severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. Journal of Dairy Science 90: 365-375)

3.1.2 Waterproof PVC Case

The logger electronics and battery are enclosed in a PVC case fitted with a Viton o-ring to provide waterproof protection. Cases have been tested to approximately 15 PSI, equivalent to a submersion of about 33 feet. To open the case, cross holes are provided for use with small screw drivers or T-wrenches to apply torque to remove the case or cap (see Figure 4).

With the original system, the case should be tightened on just so the numbers on the case and the head align (the o-ring should not bulge out of the seal). With the newer version, the cap can be tightened easily by hand. Note the use of the cross hole in the main body, and lanyard hole on the cap/cover.

Never over-tighten!

Figure 4, Removing logger case (left: original model, right: new design)

3.1.3 pH Sensor (electrode)

The loggers are designed to use with a pH sensor which is manufactured by Sensorex, Inc. for rugged applications requiring operation over extended periods in any orientation. Additional features include a special electrolyte that has proven to resist decomposition in the rumen and provide extended life, water-proof seals to protect the quick disconnect BNC style connector, and a rugged PVC case. Two part numbers are available: S655CD-HT, and 97176, both available thru DASCOR, Inc. The two models are functionally identical except for the shape of the glass. The first is flat, but more fragile; while the second is hemispherical, which is stronger, but more exposed. Both include the reference electrode.

Both styles are designed to work best with fluid "flowing" past the end of the sensor which provides a limited cleaning action. During storage, the sensors must be kept moist (place protective black cap with sensor storage solution over the sensor end when not in use).

Numbering sensors with permanent marker or a vibratory etcher as they are received to keep track of usage (see Figure 5) is strongly recommended. There is a date stamp on each sensor which represents the week of the year and the year in which the sensor was manufactured (eg. 2507 = 25th week of 2007; see Figure 6)

Figure 5, Numerical numbering of pH sensors for record keeping purposes

Figure 6, Location of date stamp on sensor

3.1.4 Other Sensors (ORP/REDOX, Temperature, etc.)

Other sensors are normally mounted permanently to the logger. Figure 9 shows the temperature sensor (stainless steel part, upper right) and the ORP sensor (black or clear epoxy around a glass electrode, upper left). Other optional sensors will be mounted in similar locations.

3.1.5 Electrode Shroud

Four large holes in the shroud allow free movement of fibrous rumen material past the electrode (sensor) (see Figure 5 & 7). The shroud also keeps the sensor end from pressing against the rumen wall and prevents fibrous material building up at sensor end. It also serves as a handy stand and protector for the fragile glass portion of the sensor. The same shroud can be used with the original and new design data logger models. An extension ring is available to extend the shroud and provide additional space between the sensor glass and any surfaces.

3.1.6 Stainless Steel Weights (optional)

Each weight weighs 1 kg and should be connected to the electrode shroud with plastic cable ties – ¼" wide, 80 Pound test—as shown (see Figure 7). **Loop the ties around the vertical sides of the electrode shroud (see Figure 7) to restrict the weights from striking the glass part of the sensor!** This method of attachment should prevent any breakage of the sensor glass due to contact with the steel weights, especially with the new 971761 sensors. Optional Plastisol coatings are being considered for additional protection of the sensor glass. See Section 6.3 for more discussion on sensor glass breakage issues.

Attach the weights as shown using ¼" (80-pound test) wire ties (see Figure 7). If insertion and removal through the cannula is a problem, attach the weights so that they are staggered slightly (approximately 2.5 and 5 cm from the bottom of the electrode shroud). Be sure that the wire ties are short enough to keep the weights from hitting the sensor glass! When properly placed in the rumen, weights keep the LRCpH system in one location (preferably the ventral sac).

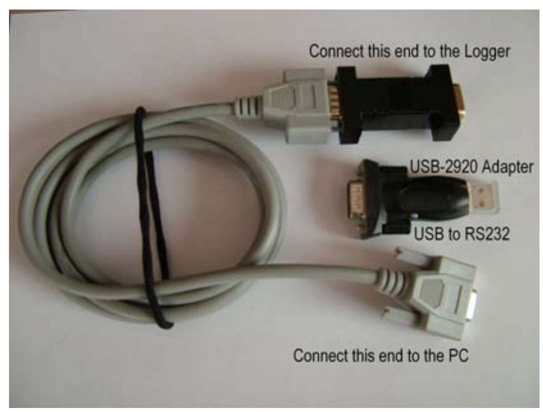


Figure 7, Electrode shroud with attached weights, SIA Cable & Dongles

3.1.7 RS232 Cable

A serial cable, with an SIA module (dongle) at the logger end, is used to send and receive data between the data logger and computer. An optional USB interface for computers without a DB-9 RS232 Serial COMM port is included. (See Figure 7.)

3.1.8 DASCOR LRCpH Software

The latest version of software (V-6.1.0) will be included on a CD with the system, or is available for download from the DASCOR Web Site.

NOTE - older loggers used with previous versions of software (V-5.xx) will be updated automatically by the new version (v-610) during their first initialization. Please see the Software Manual for more details.

3.1.9 Pre-formatted Microsoft Excel file

The "**LRCpH Barn Worksheet_2007.xls**" file, included with this documentation, consists of several worksheets. These include:

- A worksheet used to record the LRCpH unit number, cow number, sensor number, mV readings in buffers 7 and 4 for starting and ending standardizations, time of LRCpH deployment and removal, and downloaded data filenames for 1one cow when standardization method 1 is used.
- A worksheet used to input starting and ending mV readings in pH buffers 7 and 4 for the calculation of the slope and y-intercept.
- A sample data file.
- A sample calculation for converting mV readings to pH units using values from the starting and ending standardizations.

3.2 Other Requirements

3.2.1 Computer System

Most computers will operate the LRCpH software without problems. However, the system was programmed using Windows 2000Pro and XPpro, and is known to work with these operating systems. Older versions of windows are not supported, but the software should work on most systems. Operation with Vista is untested at this time. Regretfully, operation on DELL laptops is known to be problematic, and is not guaranteed. Toshiba and IBM laptops have a history of excellent performance with the software and their serial ports.

A built-in serial port (COMM) is optimum. Otherwise a USB 2.0 to serial port adapter is provided for use on laptops that do not have a built in port.

It is preferable to use a laptop computer with battery power as the power supply should be disconnected from the computer during standardization (see Method 1, page 16) for the older style loggers.

Complete details of computer system requirements, hardware, and software installation and operation are included in the Software Manual.

3.2.2 Standard pH Buffer and Storage Solutions

pH 4 and 7 are in the physiological range of rumen pH. The loggers are set for either 0-14 pH nominal (typically SN<113), or 3-9 nominal (SN >=113). Shipments of five or more sensors include a set of packets of powder to make calibration solution for pH 4, 7, & 10 when added to 100 mL of deionized water. Additional packets may be ordered from DASCOR. Any standard pH calibration solution will work, however. Also, at least one to three minutes should be allowed for the readings to stabilize once the sensor is placed in the calibration solution. pH 4 buffer may be used for storing the electrodes, or special buffer solution may be purchased from DASCOR.

Several excellent links to tutorials and other information on pH sensors and measurements are included in the reference section.

3.2.3 Calibration Kit for ORP Sensor (optional)

Packets to make two ORP calibration solutions are included. When mixed with 100 mL of deionized water, the solutions will create ORP values of approximately 90 and 225 mV. These solutions are NOT exact and should be used for system verification rather than calibration.

3.2.4 Water (deionized or distilled)

Deionized or distilled water should be available for cleaning the sensor ends during calibration and making calibration solutions.

3.2.5 Soft Tissues

Soft tissues (such as Kleenex) are useful to wipe off the sensor end between calibration steps and when cleaning the electrodes for storage.

3.2.6 Detergent Solution

Sensors should always be cleaned before storage and as needed during use. A protein film can build up on the glass and cause partial plugging of the reference electrode filter. The sensor manufacturer recommends Terg-A-zyme (Alconox), a standard laboratory cleaner for protein materials on glass. Follow the manufacturer's recommendations for use. Use warm, NOT hot, cleaning solution. See Section 6.2 for additional information.

3.2.7 Rumen Cannula Stopper

A Cannula stopper, such as Bar Diamond 1EZ[4], shown below in Figure 8 with a nylon or polyester cord (~ 40 cm) is used to connect to the LRCpH system for easy retrieval. Stoppers are available for purchase from DASCOR or Bar-Diamond.

Figure 8, Example of connection of system cap to rumen stopper

[4] Contact: http://www.bardiamond.com/index.html

4 Preparation of the LRCpH for Ruminal pH Measurement

Preparation of the LRCpH system for ruminal pH measurement entails 4 steps: 1) pre-warming the LRCpH system, 2) standardization, 3) logger initialization, and finally 4) insertion into the rumen.

4.1 Pre-Warming

Prior to standardization, the complete LRCpH system (including the pH sensor) and buffer solutions should be warmed to the approximate temperature of the rumen (35-40°C). This is done to minimize the temperature effect for standardization relative to the ruminal environment.

Lightly lubricate the O-rings and mating surfaces with a lubricant suitable for use in cattle. Plumbers grease or Vaseline should be acceptable.

For the pH sensor, be very careful to avoid getting any contaminants on either half of the connector!

Assemble the whole system by carefully positioning the cap (new body) into the data logger body and thread together lightly—hand tight is adequate.

DO NOT OVERTIGHTEN!

For older style loggers, hand tighten the case on to the logger, taking care not to over tighten. **Note: the o-ring should be snug at the junction but should not be pinched and bulge out as this may allow fluid to leak into the logger unit** (See Figure 1).

Attach the pH sensor, leaving the protective black cap on, by aligning the pins of the BNC connector on the sensor with the notches in the BNC receiver on the bottom of the logger body (see Figure 9). The black cap is filled with storage solution, so don't push on it all the way.

Press the sensor straight in towards the body until both of the brown o-rings on the sensor are within the logger body, then turn the sensor clockwise until the fit of the black seal on the neck of the sensor is tight. There should be a slight snap or click when the pins seat completely.

DO NOT FORCE! With properly lubricated O-rings, the sensor should slip into the body and lock into place without excess force.

Figure 9, Proper alignment for connection of pH sensors to the LRCpH system

Suspend the intact LRCpH system in clean water that is 35-40°C for 10-20 minutes to allow the system to come to rumen temperature. Depending on the container size used, it may be necessary to attach the electrode shroud with weights to keep the complete LRCpH system submersed in the warm water.

Meanwhile, place containers filled with standard solutions of pH 7 and 4 into water of the same temperature (35-40°C; Figure 10). Straight-walled 30-ml plastic vials work well if they are 27-30 mm in diameter and 80–85 mm in height (such as Nalgene 6250-9028). Ensure the plastic vial will allow the electrode to remain in solution without touching the bottom of the container (Figure 15 & 46).

DO NOT allow the full weight of the logger to rest on the glass surface of the electrode!

DO NOT bump the glass surface of the electrode against anything hard, including the weights or the glass bottom of a beaker. Impact breakage is NOT covered by warranty.

Figure 10, Example of a warm water bath to keep cal-solutions at approximate rumen temperature

4.2 Pre-Measurement Standardization of the LRCpH System

During standardization, measure the mV readings in pH buffers 7 and 4 in order to calculate the slope and y-intercept. The regression data from the starting and ending standardizations is then used to calculate the pH values from mV readings while accounting for changes in mV readings from the starting and ending standardizations.

The absolute mV reading in buffer 7 and 4 can vary with each LRCpH system and sensor pair and, therefore, it is recommended to standardize each system prior to and after removal from the rumen. There are three methods for standardization; while the preferred method utilizes the ***Real-time*** tab (Method 1), in some instances there can be a large variation in mV readings when the LRCpH system is attached to the computer. When large deviations are recorded, the standardization protocol should be performed without the logger attached to the computer (Method 2 or 3).

4.2.1 Method 1: Standardizing the system using the *Real-Time* Tab

This method of standardization allows the real-time recording of mV readings in buffers 7 and 4. The user is able to visually observe the readings to ensure the sensors are functional.

Connect the supplied communication cable to the serial or USB-Serial adapter on the computer (see the supplied installation and driver guide for proper port designation). Turn on the computer and load the DASCOR LRCpH software (M1b Ver610.exe file). The screen below should appear (Figure 11). Click on ***Log On*** as shown. The screen will immediately change to the ***Waiting*** tab (see figure 13). Note the box in the lower left corner will indicate ***no logger is connected*** when the software, COMM port, and cable are correctly set up, prior to connecting a logger.

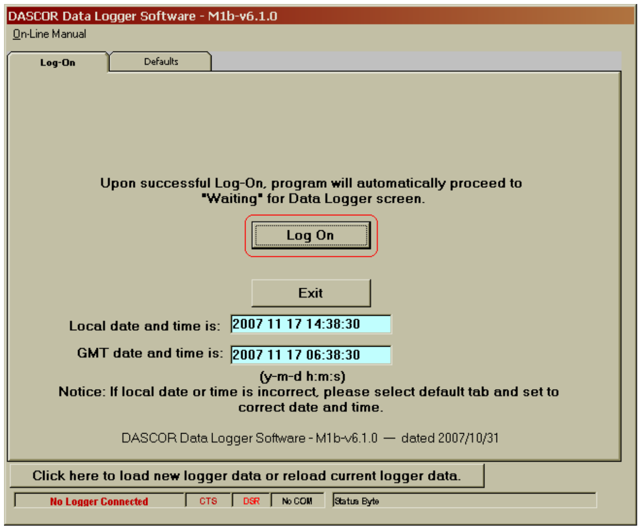


Figure 11, Logging on to the LRCpH

Remove the LRCpH system from the warm water bath, drying it off with towelling to prevent moisture from contacting the internal data logger

Remove the LRCpH cover (Type 3) or cap (Type 4) using a screwdriver or T-wrench in the logger head and carefully turn counterclockwise (see Figure 3). Now connect the communications cable to the exposed connector on the logger.

When the logger is properly connected, the LED indicator at the top of the logger head (see Figure 3) should emit a solid red light. If the LED is not on, check to ensure that the cables are securely and properly attached, the battery is fresh, and the software is running.

In Figure 12, the ***Waiting*** screen on the left should appear when no logger is connected. When disconnecting a logger, be sure that the software returns to this screen as it indicates that the software memory has been completely cleared in preparation for accepting the next logger.

The ***Waiting*** screen will be followed by the screen on the right a few seconds after a logger is connected. The progress bar will indicate that the header memory data is being downloaded to the PC. The header memory includes all of the setup information for the logger, including calibration information, but does not include test data. This step should take just a few seconds.

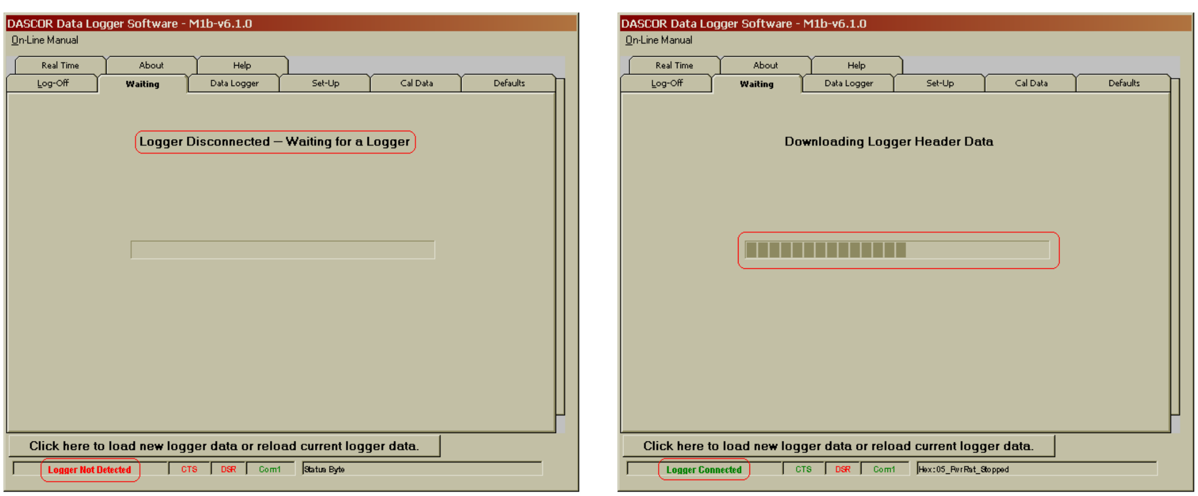


Figure 12, Logging on to the LRCpH: downloading header data

Once fully loaded, the next screen will appear, giving information on the attached logger system (Figure 13).

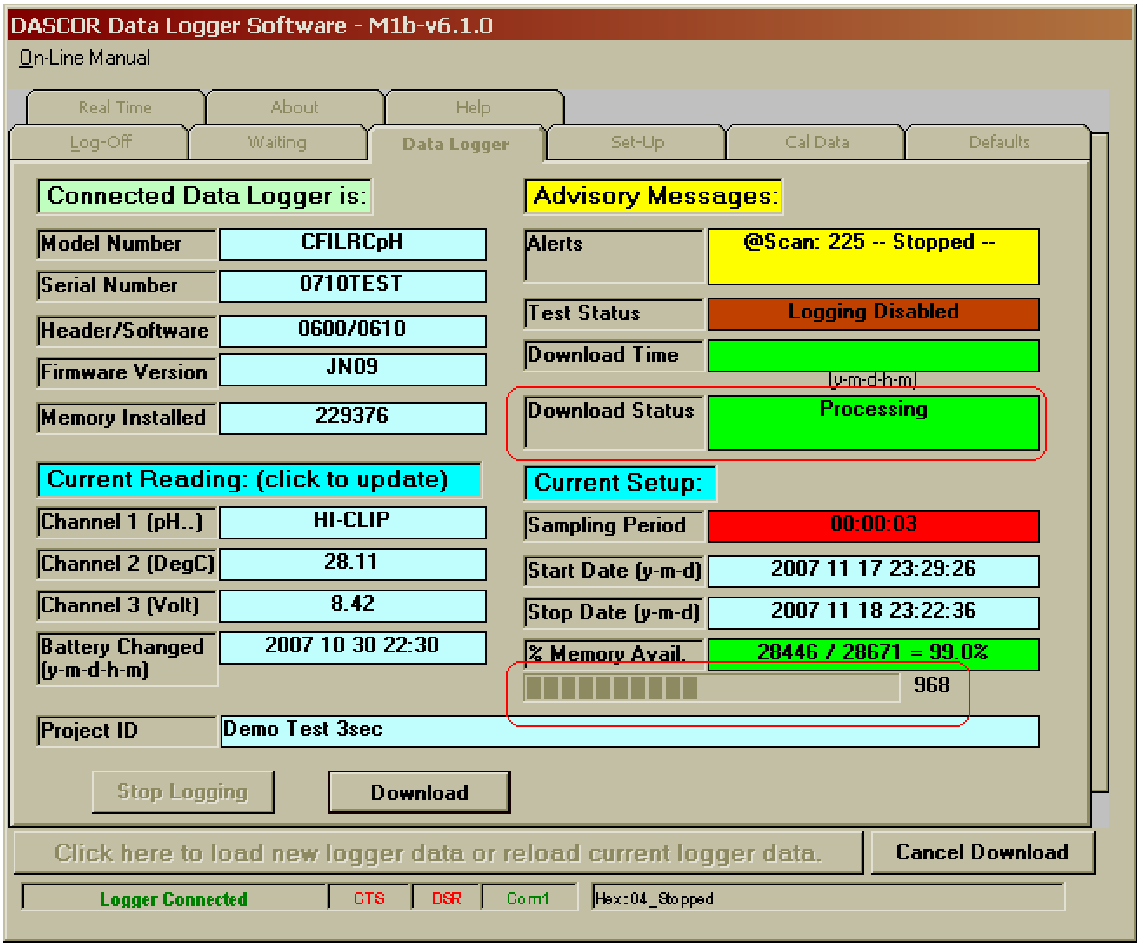


Figure 13, LRCpH logger status screen

Model Number- The model number should always be "CFILRCpH".

Serial number- The last numbers should correspond to the number engraved on the protective cover of the data logger system. The example above was from a DASCOR test unit without a complete numerical serial number.

Header/Software– These fields show the version numbers for the format of the Header (should be "**0600**"), and the software used to initialize the logger ("**0610**" in this case).

If the header version is older than "**0600**" or contains alphabetic characters, the first time the logger is initialized with the new version 610 software, the header format will be updated to the current level.

If the header and software version is not in the "**06xx**" series, the data in the logger was acquired using older software-most likely version 5.2. In this event, the logger should be disconnected and then the current software should be closed. Open the older version of the software and download the data to insure that the data format is correctly maintained. Once this has been done, the logger should be initialized for the next test using the new software version.

Firmware Version– These fields show the version numbers for the firmware ("**JN09**" for all loggers covered by this manual).

Memory Installed- This will vary between manufacturing runs, but should typically be 229,376 Bytes for the T4 series. This equates to 114,688 samples, or 28,671 Scans of all four channels.

Advisory Messages- These indicate the status of the logger (i.e. if logging is enabled or disabled and whether data has been downloaded yet). For the logger shown, data taking was stopped at scan #225, and the data is being downloaded. The Download Status box gives the file name for the downloaded file when download is completed.

Current Setup- This should be checked for the scanning interval (this is the length of time between each consecutive sensor reading, which determines the start and end dates that will fill the available memory) and the percentage of memory available in the data logger at the point of connecting the logger system to the computer. Note the Sampling Period is three seconds and is highlighted in red, which indicates that this period is less than the recommended 10-second minimum.

Channels 1, 2, 3, and 4- Basic LRCpH systems come with the capability to measure pH (in mV), temperature, and battery voltage using channels 1, 2, and 3, respectively. When the optional ORP sensor is included the data will appear in channel 4, which is not shown on this screen. Readings can be updated by clicking the ***Current Reading: (click to update)*** bar. The readings in channel 1 (pH) may be slightly different than the actual pH of the solution because the slope and y-intercept in the software has been set during the final manufacturing test to standard theoretical values. The **HI-CLIP** reading indicates that a pH sensor was not installed, or had failed. As mentioned previously, the actual readings differ with each pH sensor and LRCpH system pair—and with temperature. Therefore, it is suggested to use the readings in channel 1 as a guide to ensure the sensor is working, not as an absolute value. Different readings in channel 1 from the actual pH in the buffer solution will not affect in-vivo readings as you measure mV and convert them to pH units using the regression data derived from the starting and ending standardizations.

Record the battery voltage on a sheet similar to Figure 14. If the voltage drops below 6.7-V, the battery will deplete rapidly and possibly stop the data logger operation, or give erroneous data. Thus, ensure the battery voltage >8; otherwise, change the battery before standardizing by:

- Method 1) on the original system design: sliding the cable tie off the battery, carefully removing the old battery, replacing it with a new battery and then slide the cable tie back in to place; or
- by Method 2) on the new system design: pop open the battery holder by applying pressure to the black battery cover in the direction of the arrow, removing the old battery, placing a new battery back in the holder, and sliding it back into place in the logger head (see Figure 3 for battery locations).

Experiment, animal number, logger serial number, and sensor number should be recorded for later reference. See Figure 14 as an example of the log sheets used at Lethbridge; sample sheets are included in the Excel spreadsheet accompanying this User's Guide.

Experiment:						
Anim #			Logger#			
Date				Date		
	Start				End	
	7	4			7	4
Mean mV				Mean mV		
Max dev				Max dev		
3 sigma				3 sigma		
counts				counts		
battery volt				battery volt		
Initialize set up				Time probe out		
Time probe in				File Save as		
Date				Date		
	Start				End	
	7	4			7	4
Mean mV				Mean mV		
Max dev				Max dev		
3 sigma				3 sigma		
counts				counts		
battery volt				battery volt		
Initialize set up				Time probe out		
Time probe in				File Save as		

Figure 14, Sample data recording sheet

Remove the protective black cover from the pH sensor, rinse with deionized or distilled water, wipe the end with a soft tissue, and carefully suspend the pH sensor in the warm pH 7 buffer (see Figure 15). The sensor end should be totally submersed in solution but not sitting flat on the bottom of the container. If the shroud is installed, it will support the sensor off the bottom of the bottle. (See Figure 44)

Figure 15, Positioning of LRCpH system in calibration solutions

Click on the ***Real Time*** tab, then first click ***Display in milli-Volts***, then ***Start RT Display***. Allow the displayed readings for pH to stabilize, usually about one minute, then click ***Accumulate Statistics*** as shown in the example below (Figure 16). The scan interval on the real time display is set as fast as possible by default and 25 samples can be collected and averaged in less than 30 seconds.

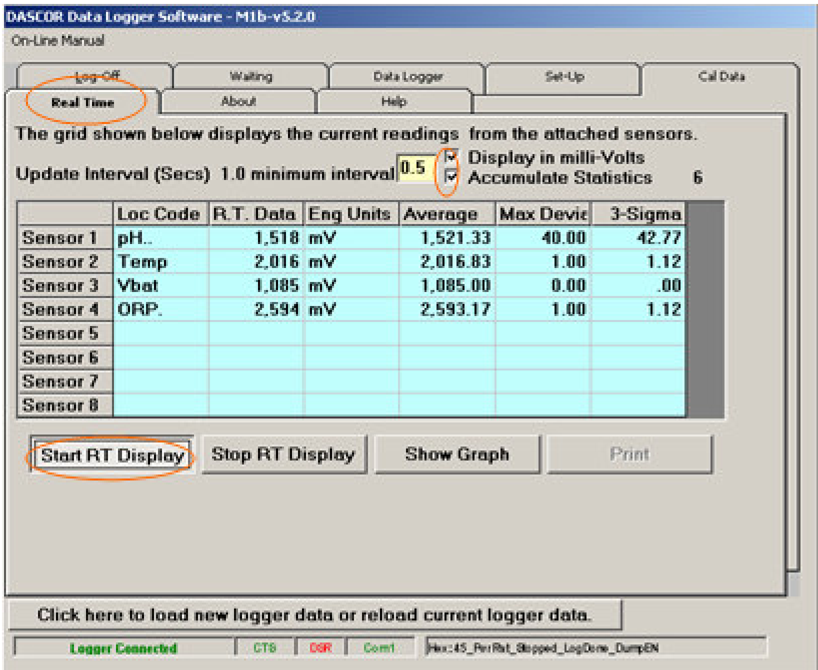


Figure 16, Real Time menu to record standardization mV readings

Allow for a <u>minimum</u> of 25 readings to allow the average to stabilize (this number appears to the right of ***Accumulate Statistics***), then click on the box to the left of ***Accumulate Statistics*** to stop the accumulation of further readings. If the readings bounce a lot, be sure to average more (50 to 100) reading!. **Note: the real time (R.T.) data will continue to change.**

Record the average mV numbers for pH 7, the deviations, and the number of readings on the data sheet (see figure 14). In figure 16, the values are 1521.33, 40.00, 42.77, and 6 for average mV, max deviation, 3-sigma deviation and number of counts, respectively.

Remove the sensor from the pH 7 buffer, rinse with deionized or distilled water, wipe with a soft cloth, and place in the pH 4 buffer solution. Allow for the readings to stabilize as before. Click ***Accumulate Statistics*** to reset the statistics calculations and obtain the readings for pH 4 and record on the data sheet.

With older loggers that have not been upgraded to the newer electronics, noise may appear on the readings. Note that the deviation recorded in figure 16 on channel 1 (pH) is 40 because the computer was connected to the electrical outlet. When disconnected, the deviation should drop to less than 10. Click the ***Accumulate Statistics*** box to reset the readings. During the following steps, if noise on the pH channel is a problem, DISCONNECTING THE COMPUTER FROM THE ELECTRICAL OUTLET will allow the computer to run on battery power and should reduce noise in the readings significantly, resulting in lower deviation for the average mV recorded.

Note: Readings that take more than 30 seconds to stabilize indicate the sensor is aging. Loggers set for 3-8 pH full scale will also take longer to settle. In this case, two minutes for an older but still usable sensor is normal. Similar mV readings in buffers 7 and 4 indicate that the pH sensor may have failed. In either case, replace the sensor and restart the standardization procedure from the beginning.

4.2.2 Method 2: Standardizing the LRCpH system by Initializing Logging

As stated previously, this method of standardization should only be used when the deviation for mV readings using Method 1 are >10 mV over 25-50 readings. This method allows the user to standardize the LRCpH system and sensor pair without interference from the computer thus minimizing deviation. However, the user must be more diligent to ensure that mV readings in pH buffers 7 and 4 are realistic to ensure that the sensor is functional.

Standardization method 2 requires the user to run a short measurement program exclusively for obtaining the mV readings for pH buffers 7 and 4. The reading interval used for this example is three seconds and the user must note the time the sensor was placed in each buffer. Following the readings, the user must download the standardization data and manually calculate the mean mV readings in each buffer solution. A scan period of 10 seconds or greater can also be used, but it will take longer to get the same number of readings to average. However, the short scan period warnings illustrated in the following steps will be avoided.

Place the logger so that the sensor is in buffer 7 solution with the logger connected to the PC.

Click on the ***Set-Up*** tab (see figure 18).

Leave the number of channels set to the maximum (four in this example). Type in a "3" for the scan period, followed by clicking on the ***Secs*** button.

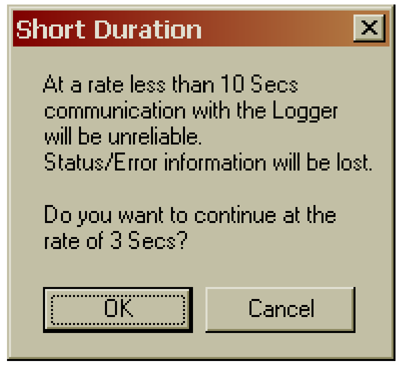


Figure 17, Short Duration Scan Period Warning

A box (Figure 17) warning that the scan period is below the recommended minimum of 10 seconds will be displayed. Click ***OK***. Note that various boxes are automatically completed with the projected date and time that memory will be filled, the number of scans available, and the total duration available for taking data. Notes of up to 64 characters long may be entered in the ***Project Identification is:*** box.

Click ***Initialize Logger***. Note: initialization will erase any data currently recorded on the logger. Ensure you have downloaded data prior to initialization. If data has not been downloaded, you will have an opportunity to download and return to the ***Set-Up*** tab, or initialize without downloading.

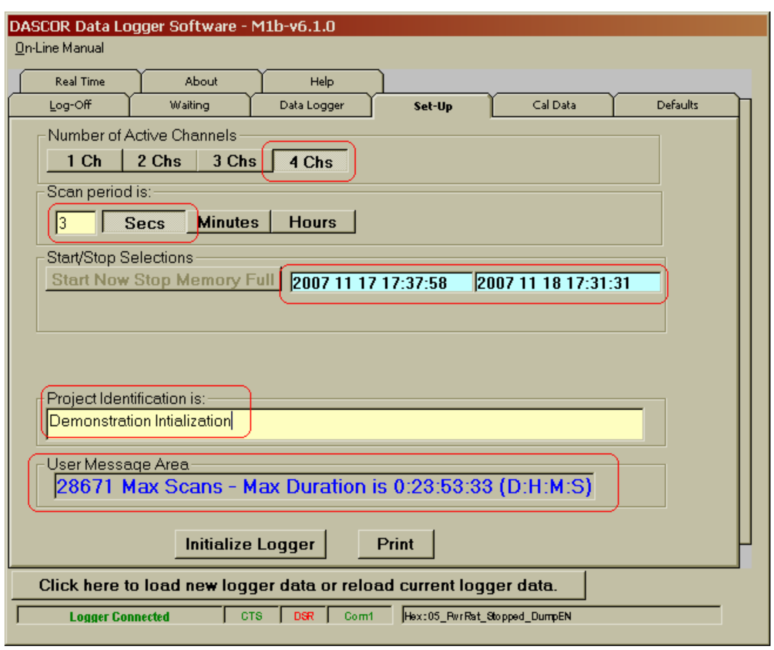


Figure 18, Set-Up tab for the initialization of the short program (Method 1)

After pressing the ***Initialize Logger*** button, the following warning will appear (see Figure 19). Click ***OK*** to approve the values, and again on the next box to start the upload.

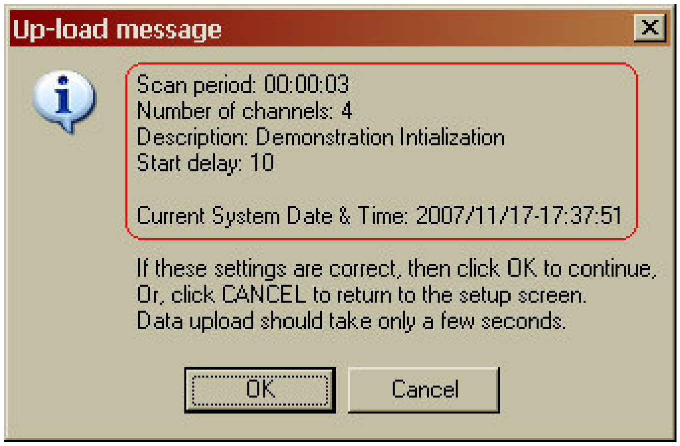


Figure 19, Verification of settings during system initialization

Figure 20, Upload Acknowledge Message

Once the system has been initialized the following screen will appear (Figure 21).

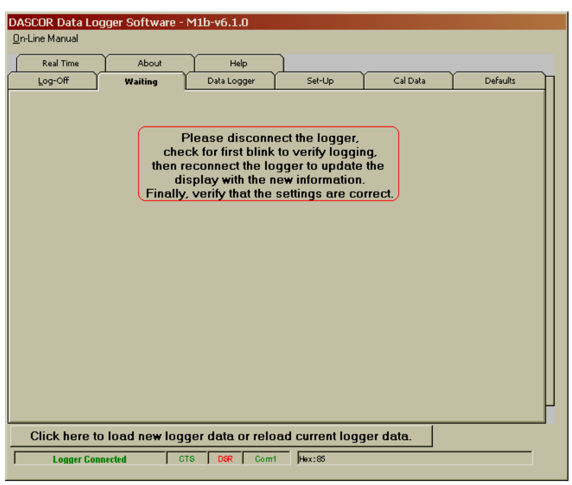


Figure 21, Screen indicating system initialization complete

Disconnect the LRCpH system from the computer interface cable to isolate the LRCpH system. After a short delay (set on the ***Defaults*** tab), the LED in the LRCpH system will flash every three seconds corresponding to a reading being logged. Since this test is to be short, and the short scan period might cause difficulty communicating with the logger, do not reconnect the logger to validate the setup.

Allow the logger to take readings for <u>at least</u> one minute (time for the sensor to stabilize, followed by 30 seconds for data). Rinse sensor with distilled water, wipe dry, and place in pH buffer 4. Allow the sensor to stabilize for at least two minutes to allow for the longer settling time caused by the step change going straight from pH 7 to pH 4. Be sure to note the times for each of these steps. Use the data recorded for the last 30 seconds (10 data points) for each pH value for your standardization.

Reconnect the LRCpH system to the computer and reload the logger (Figure 22). On Figure 24, note the red, yellow, and green highlighted boxes giving the status of the logger. Since the Scan Period was set to three seconds, communications with the logger may cause problems. Even if the initial download of the header information is not successful, click on the ***Data Logger*** tab and then the ***Stop Logging*** button and confirm the stop by clicking ***OK*** on the next message box. (see Figure 23)

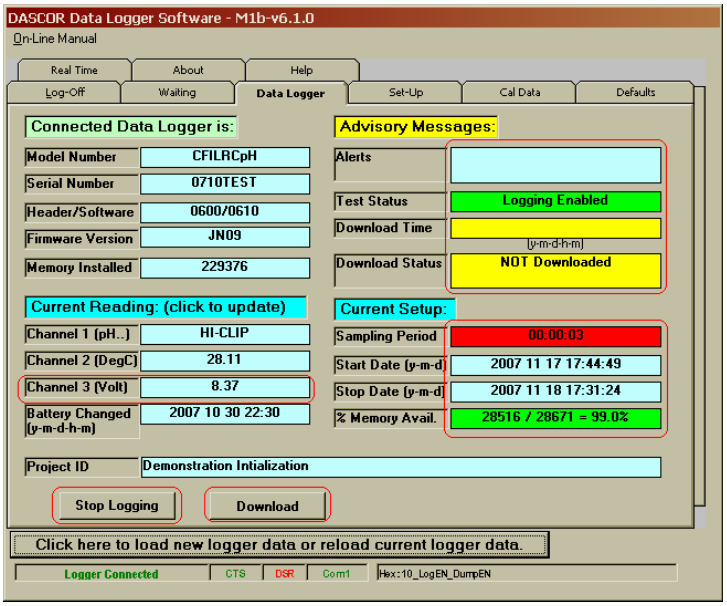


Figure 22, Checking logger status, and stopping the logger

Figure 23, Confirming the Stop Logging Command

When the logger is stopped successfully, the green Test Status box will turn dark red and say "Logging Disabled" (Figure 24). At this point, it is a good idea to disconnect the logger, wait for the ***Waiting*** screen to appear, and reconnect the logger to be sure the header is downloaded completely and correctly. A successful stop and header download is shown below. Notice the status box colors have changed, and the ***Stop Logging*** tab is disabled. At a scan interval of one or two seconds, these steps may need to be repeated, or the two pins identified as "PAUSE" near to the LED should be shorted, which will allow the command to be received and processed successfully. Please see the Software Manual for more details on utilizing the Pause pins.

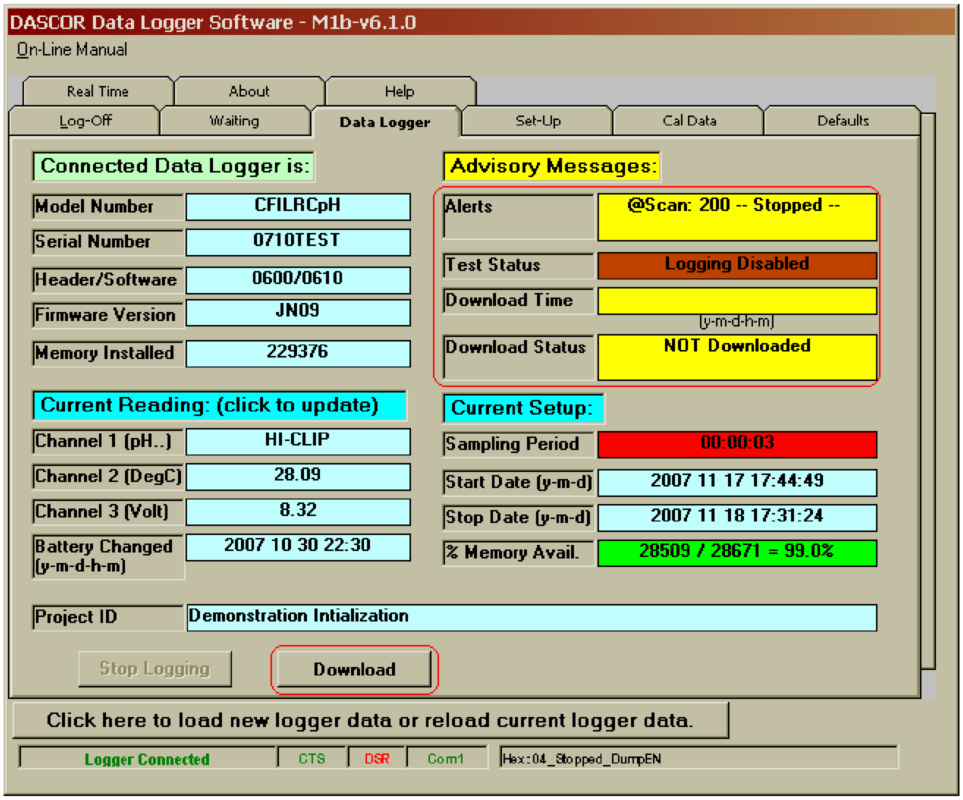


Figure 24, Stop Logging Successful; Downloading the data

Click on the ***Download*** button to initiate downloading of the test data from the logger.

If a small box appears saying "invalid Filename," Click ***OK***, then go to the ***Defaults*** tab and be sure that a valid default file path and folder has been selected. Otherwise, click ***OK*** to confirm moving to the next step (Figure 25).

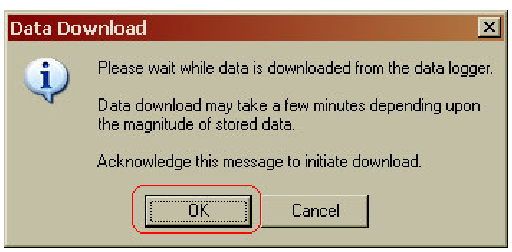
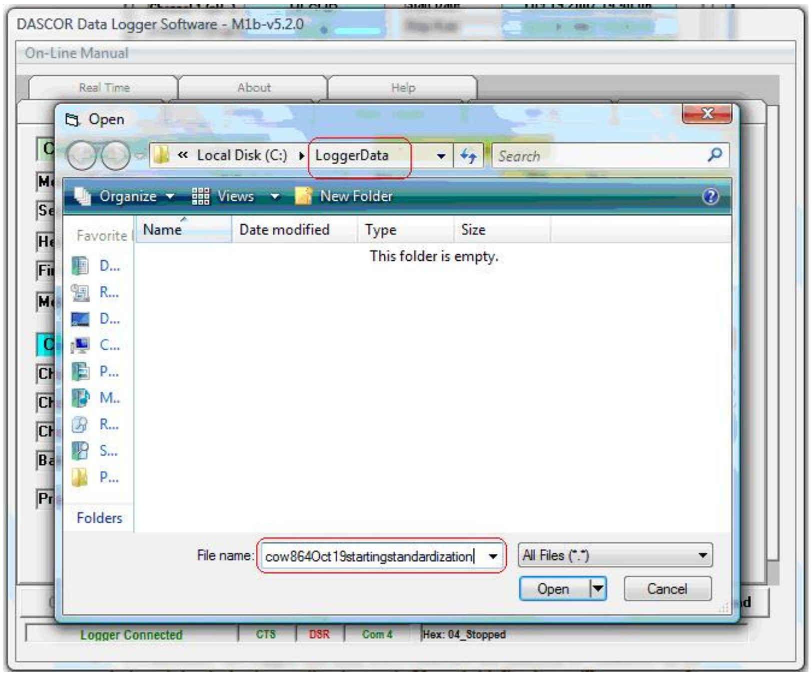


Figure 25, Confirm Download

The first step is to create a filename and save the file. The authors recommend including the cow number, date, and whether the file is the starting or ending standardization (Figure 26). Note that the software also provides a default file name which starts with the serial number of the logger, followed by the date and time of the download. You can overwrite this completely (as in the example below), or insert your information before or after default name. You can also choose to save the file to a completely different drive and folder. <u>HOWEVER, it is a good idea to establish a standard naming and filing protocol to avoid incorrectly identified, or lost files.</u>



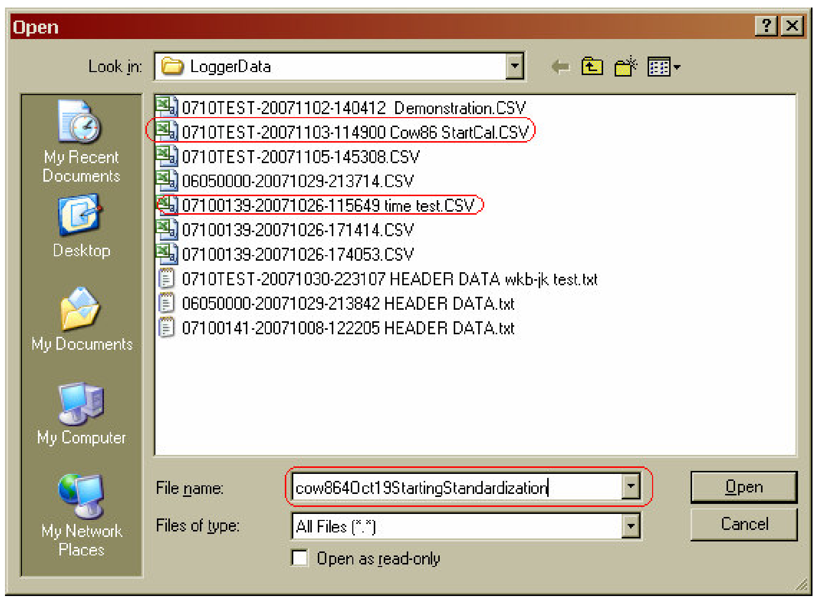


Figure 26, Naming a file after standardization

Once the file is opened and downloading starts, a progress bar and count-down counter will appear on the ***DataLogger*** tab. (see Figure 27) When download is complete, you will be presented with the opportunity to append notes to the data file, and then the option to save and return to the program, generate a report, or open the ".csv" file in Excel. The last two require that Excel be installed correctly and available for automatic call-up on your computer.

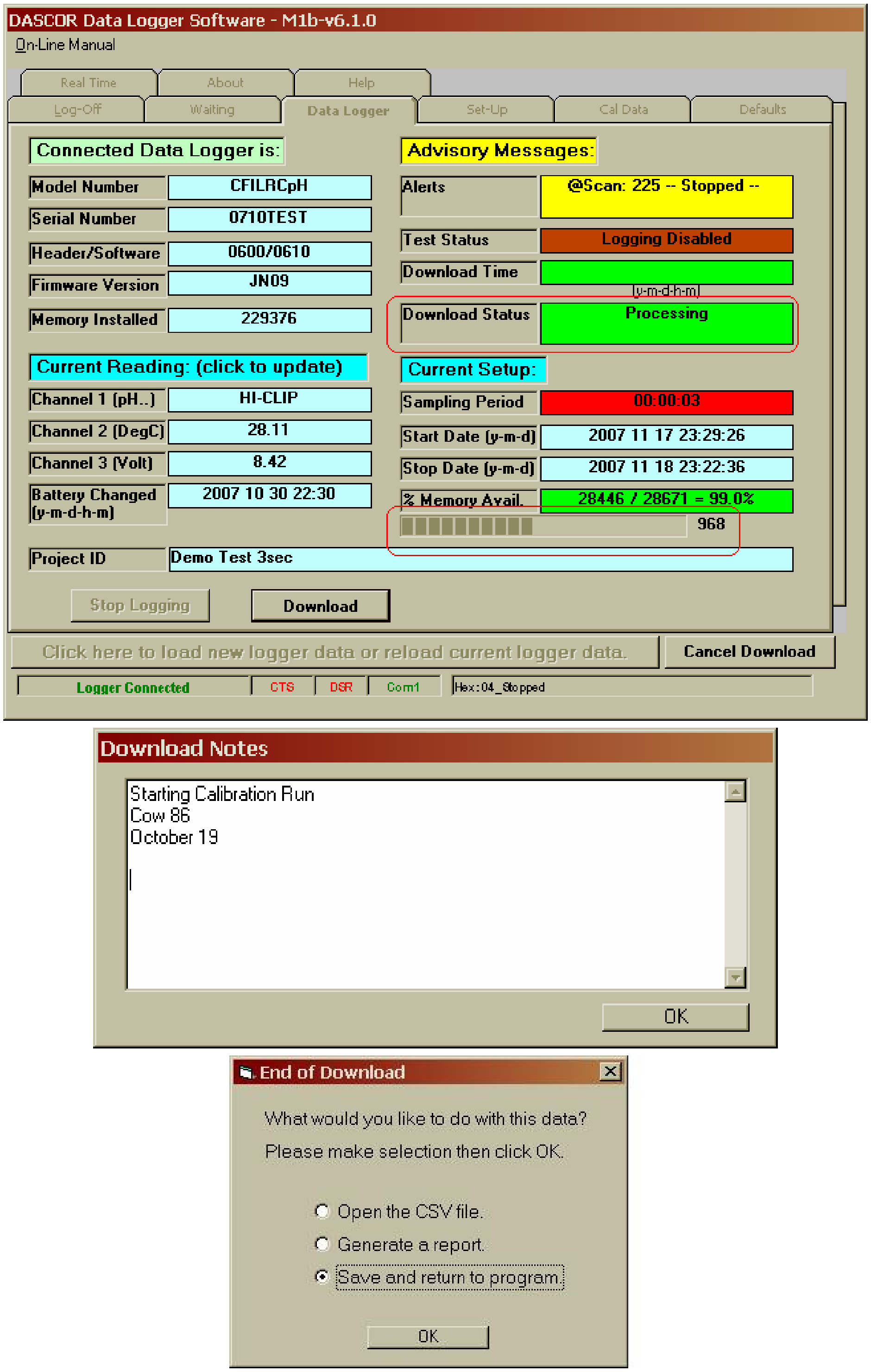
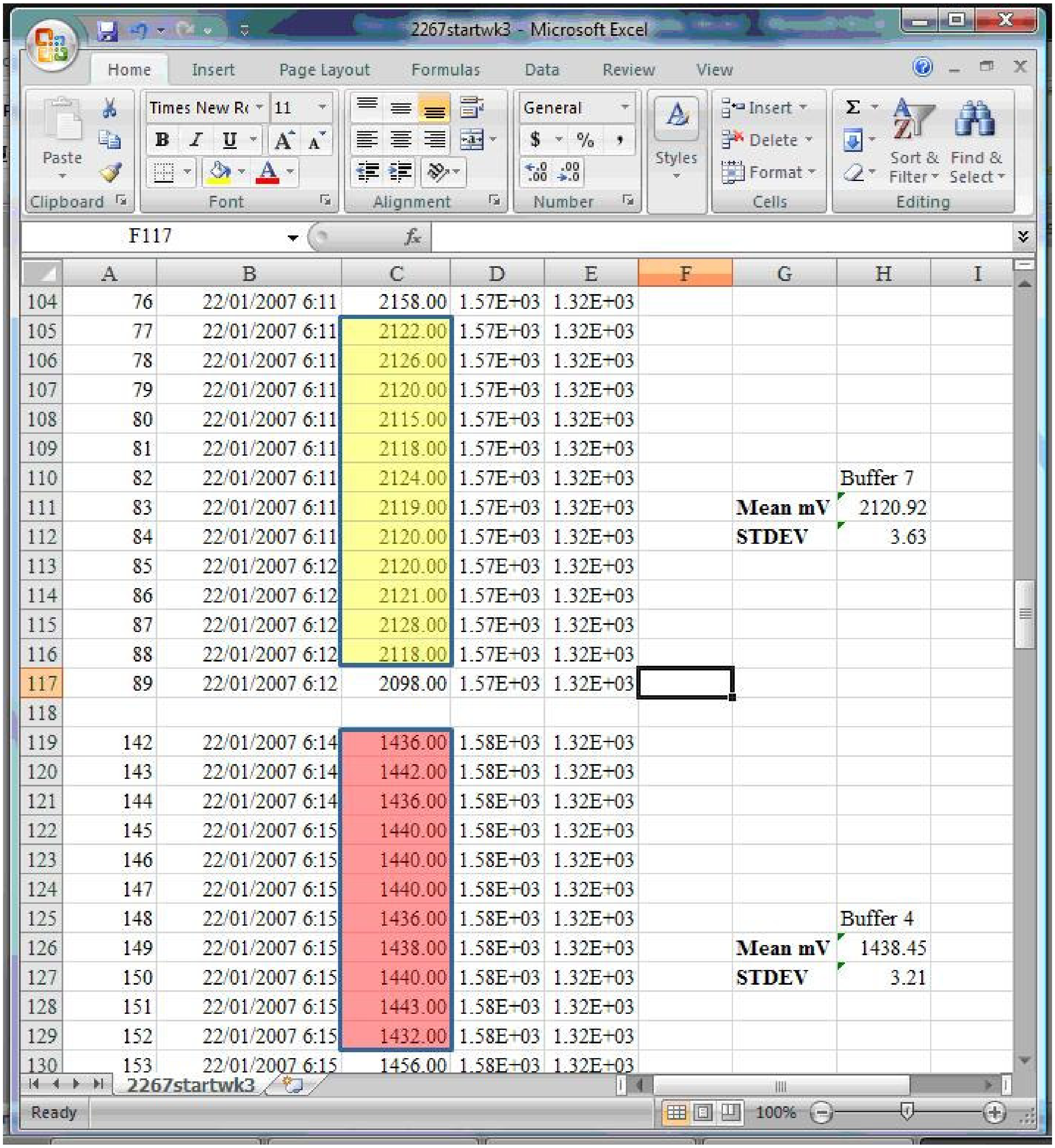


Figure 27, Download Progress Bar and Counter

Open the file to view the mV readings (channel 1) to ensure the sensor is functional (see Figure 28). If the mV readings while the sensor was in buffer 7 and 4 are similar, replace the sensor and repeat the standardization. In the example in figure 28, the mean mV values in buffers 7 and 4 are 2120.92 and 1438.45 mV, respectively. It is often a good idea to plot a time-series of the data to look for significant changes from the expected, and long settling times for the pH sensor. NOTE that only a portion of the complete data file is shown below!



	A	B	C	D	E	F	G	H	I
104	76	22/01/2007 6:11	2158.00	1.57E+03	1.32E+03				
105	77	22/01/2007 6:11	2122.00	1.57E+03	1.32E+03				
106	78	22/01/2007 6:11	2126.00	1.57E+03	1.32E+03				
107	79	22/01/2007 6:11	2120.00	1.57E+03	1.32E+03				
108	80	22/01/2007 6:11	2115.00	1.57E+03	1.32E+03				
109	81	22/01/2007 6:11	2118.00	1.57E+03	1.32E+03				
110	82	22/01/2007 6:11	2124.00	1.57E+03	1.32E+03			Buffer 7	
111	83	22/01/2007 6:11	2119.00	1.57E+03	1.32E+03		**Mean mV**	2120.92	
112	84	22/01/2007 6:11	2120.00	1.57E+03	1.32E+03		**STDEV**	3.63	
113	85	22/01/2007 6:12	2120.00	1.57E+03	1.32E+03				
114	86	22/01/2007 6:12	2121.00	1.57E+03	1.32E+03				
115	87	22/01/2007 6:12	2128.00	1.57E+03	1.32E+03				
116	88	22/01/2007 6:12	2118.00	1.57E+03	1.32E+03				
117	89	22/01/2007 6:12	2098.00	1.57E+03	1.32E+03				
118									
119	142	22/01/2007 6:14	1436.00	1.58E+03	1.32E+03				
120	143	22/01/2007 6:14	1442.00	1.58E+03	1.32E+03				
121	144	22/01/2007 6:14	1436.00	1.58E+03	1.32E+03				
122	145	22/01/2007 6:15	1440.00	1.58E+03	1.32E+03				
123	146	22/01/2007 6:15	1440.00	1.58E+03	1.32E+03				
124	147	22/01/2007 6:15	1440.00	1.58E+03	1.32E+03				
125	148	22/01/2007 6:15	1436.00	1.58E+03	1.32E+03			Buffer 4	
126	149	22/01/2007 6:15	1438.00	1.58E+03	1.32E+03		**Mean mV**	1438.45	
127	150	22/01/2007 6:15	1440.00	1.58E+03	1.32E+03		**STDEV**	3.21	
128	151	22/01/2007 6:15	1443.00	1.58E+03	1.32E+03				
129	152	22/01/2007 6:15	1432.00	1.58E+03	1.32E+03				
130	153	22/01/2007 6:15	1456.00	1.58E+03	1.32E+03				

Figure 28, Checking mV readings & calculating mean mV readings in pH buffers 7 and 4

Once standardization is successful, precede to set-up the logger for measurement.

4.2.3 Method 3: Standardizing the LRCpH system while Logging

A third method is similar to the one above, but is modified as follows: Set up the logger for the full test using a scan period that will be long enough to fill no more than 90% of the memory by the expected end of the test. This allows for limited unforeseen delays in starting or stopping the test. Also allow for about 30 minutes extra logging at the beginning and end. See Section 4.4 below.

Initialize the logger (see Section 4.4), disconnect from the PC, and replace the cover. Next, place the pH sensor in the two different calibration solutions as described above, allowing several minutes in each for the readings to stabilize. When finished with the second solution, place the logger directly into the rumen for the required test period. Note the times. After the test, remove the logger, and repeat the calibration in each solution. Then download and analyze the data. With this method, the calibration information will be at the beginning and end of the test data and an integral part of the data file. However, with this method there is no opportunity to check proper functioning of the systems until the test is complete.

4.3 Setting Date and Time

The date and time that appears on the data retrieved from the data logger originates from the set date and time of the computer used to initialize the logger. Therefore, ensure the date and time is correct on the computer prior to initialization. http://tf.nist.gov/service/its.htm provides downloadable software that will automatically synchronize the PC's internal clock with one of the NIST time servers. If using more than one computer to initialize and download a set of LRCpH systems on a given trial, synchronize the date and time between the computers.

4.4 Initializing the Logger for Measurement

To initialize the logger the user must indicate the number of channels to be recorded and the desired recording interval. Note: Initialization will over-write any data currently recorded on the logger. Ensure you have downloaded data prior to initialization. Begin to prepare the logger for initialization by clicking on the ***Set-Up*** tab (Figure 29). Click on ***2 Ch*** (for pH and temperature), ***3 Ch*** (to add battery voltage) or ***4 Ch*** (if also measuring ORP). ***1 Ch*** is not a valid selection. Choose the scan period and enter (in the above example it is 30 seconds). Please note that the start and stop times that are displayed will change according to the scan period that is entered, the number of channels selected, and the amount of installed memory.

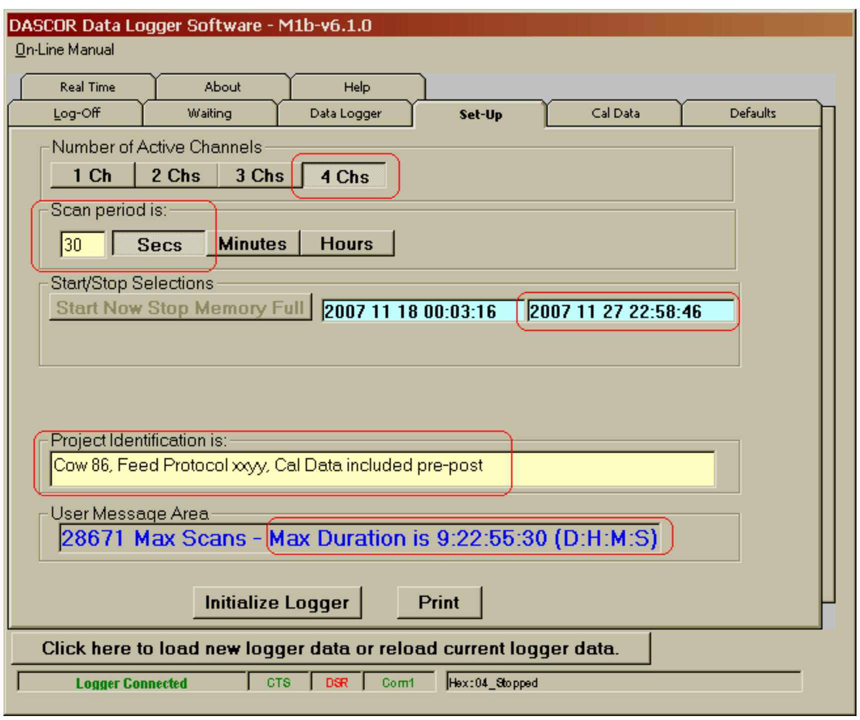


Figure 29, Initializing the logger for ruminal pH measurement

Upon clicking on ***Initialize Logger***, the following confirmation message will appear (Figure 30).

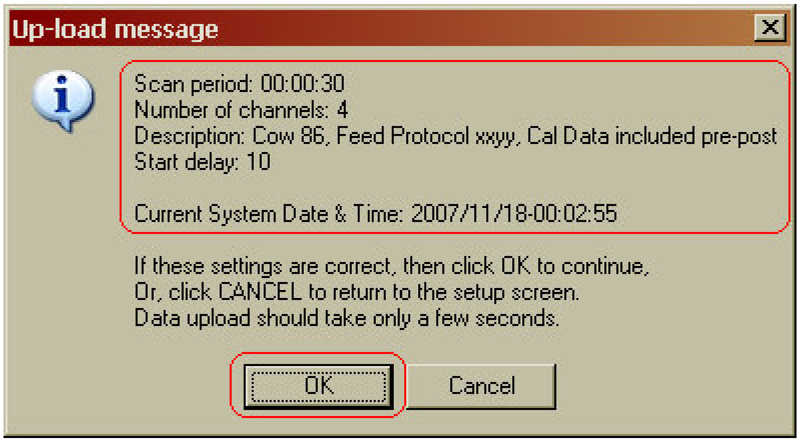


Figure 30, Verification warning when initializing the LRCpH

Check that the scan period, number of channels, description, date, and time are correct, then click ***OK*** (Figure 30) or ***Cancel*** to return and correct the setup.

Another message (Figure 31) will appear to check that it is still OK to upload the logger program. Note: once a new program is uploaded to the logger, all data that was contained in the logger will be lost (i.e. the memory is over-written to collect a new set of data).

Figure 31, Second warning message during LRCpH initialization

The following screen will appear (Figure 32).

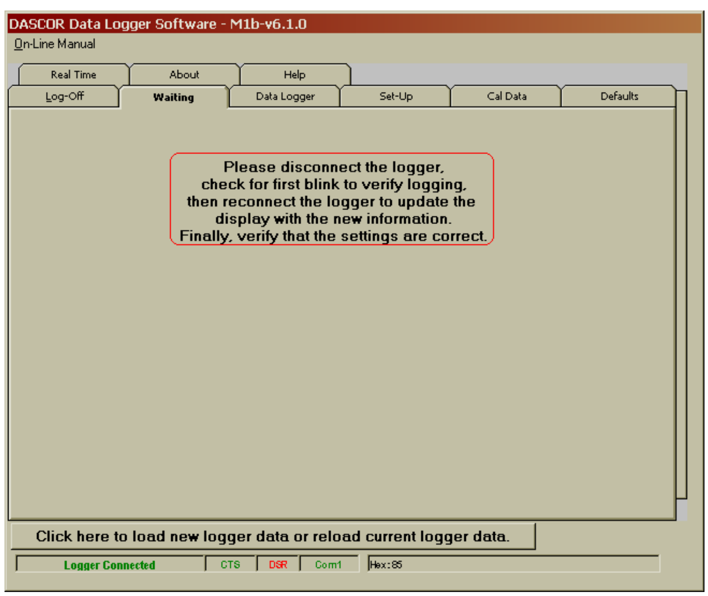


Figure 32, Screen indicating that initialization is complete

Disconnect the communication cable from the logger and watch for the light at the top of the logger to flash red. The first flash should appear 15 seconds after initialization is complete (depending on the ***Default*** tab setting), then continue at the set scan interval that was specified in the ***Set-Up*** (ie. every 30 seconds).

If desired, you may reconnect the logger to the computer to verify your setup. The ***Waiting*** screen with the progress bar will appear and the logger header data will be loaded (Figure 12, right). This logger should load automatically upon connection of the cable to the logger; however, if it does not, disconnect the logger and wait for the ***Waiting*** screen to appear, then try again. Once loaded, the following screen should appear (Figure 33).

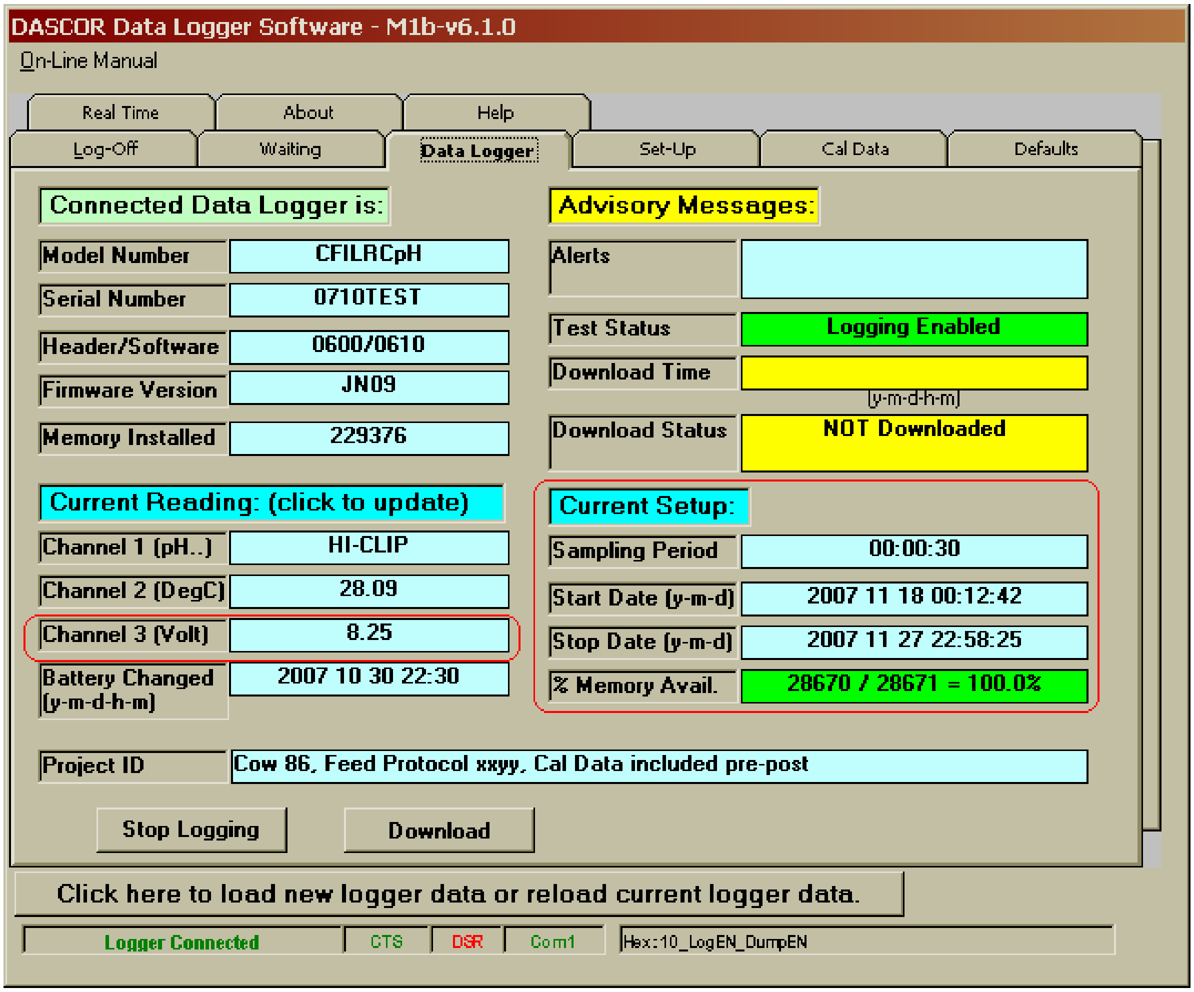


Figure 33, Verifying initialization settings on the Data logger status screen

Check that the logger is logging as intended (indicated by ***Logging Enabled*** in the Advisory Messages) and the sampling period is correct. ***% Memory Available*** should be 100%. Record on the data sheet (see Figure 14) that the logger has been initialized-it is important that you know the system is logging. Note: the battery should be changed prior to use in the rumen to avoid failure when the battery voltage (circled above in Figure 33) is less than 8.0 volts. Available power is unpredictable below 8 volts, and test data can be lost by premature battery failure.

The system can now be removed from the communication cable and the cover replaced (see Figure 4). Screw on the electrode shroud with attached weights (lightly hand tighten as this shroud can be difficult to remove after it comes out of the rumen). See Section 3.1.6 and 6.3 regarding attaching and handling the weights to avoid sensor breakage! Breakage caused by impact of the steel weights on the sensor glass is NOT covered by warranty!

Place the LRCpH system with attached electrode shroud and weights in water at approximate rumen temperature during transport from the computer to the cow.

4.5 Insertion of the LRCpH system into the Rumen

The goal of LRCpH system insertion is to submerse the LRCpH system in the ventral sac of the rumen without causing impaction of ruminal contents in the electrode shroud (Nocek et al., 2002).

Remove the ruminal Cannula plug and, using your arm, create a pathway through the ruminal contents to the ventral sac.

Gently place the LRCpH system into the rumen, weights first, following the path created with your arm.

Once the LRCpH system body is completely in the rumen, reach into the rumen, grab the electrode shroud, and pull the LRCpH system into the ventral sac. By pulling the LRCpH system into the ventral sac you are less likely to cause impaction and potential damage to the rumen epithelium than by pushing it down. You are also trying to keep the weights from impacting the glass part of the pH sensor and prevent breakage.

Once in the ventral sac, ensure there is no particulate build-up in the electrode shroud.

Replace the Cannula plug and record the time on a data sheet such as is shown in Figure 14. pH sensor failure seems to occur during handling, and the data will pinpoint the time of sudden failure—provided the times of calibration, insertion, and removal are known.

4.6 Removing the LRCpH after Measurement of Ruminal pH

Upon removal of the LRCpH system from the rumen, it should be rinsed in warm water to remove adhering rumen material.

Remove the electrode shroud and weights from the end of the system and transfer the system to another bucket of clean water (35-40°C) to thoroughly clean the LRCpH system.

Remove the LRCpH system from the warm water and rinse off the sensor end with deionized (or distilled) water and thoroughly dry the whole system. A small pressurized can used to dust and clean computers can be useful here. High pressure compressed air is not recommended as it may blow contaminants into the connector area past the seals.

Remove the outer cover (body type 3) using a pair of screw-drivers or T-wrenches in the cross holes, (a rubber strap oil filter wrench can also be used in this process--see Figure 34). For body type 4, the LRCpH system cap should unscrew by hand. On both old and new body types, there are cross holes on both the body and cap/cover for the insertion of screw drivers or T-wrenches. This is the preferred method of accessing the SIA connector. If a strap wrench is used, it should be used at one end or the other of the cover, not in the middle as this can collapse and damage the cover.

Figure 34, Removing the cover from the original design LRCpH system

Once the cover is removed, use care to ensure that no moisture or rumen contents come into contact with the data logger and electronics board (i.e. keep hands clean and dry while handling the system). Connect the communication cable to the serial connection on the data logger board (see Figure 35).

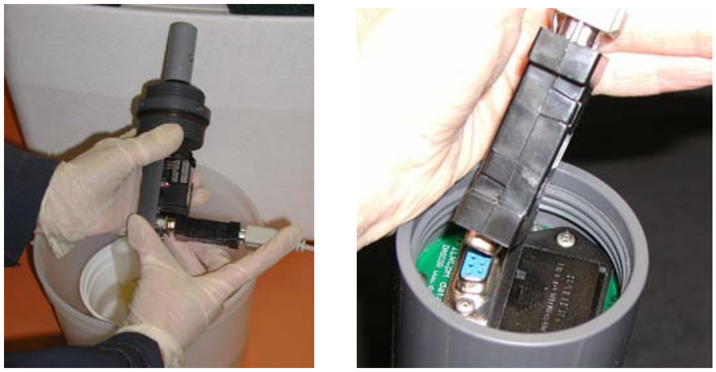


Figure 35, Connecting the serial cable to the data logger (Type 3)

After starting the DASCOR software and connecting the logger, a screen similar to the one below should appear.

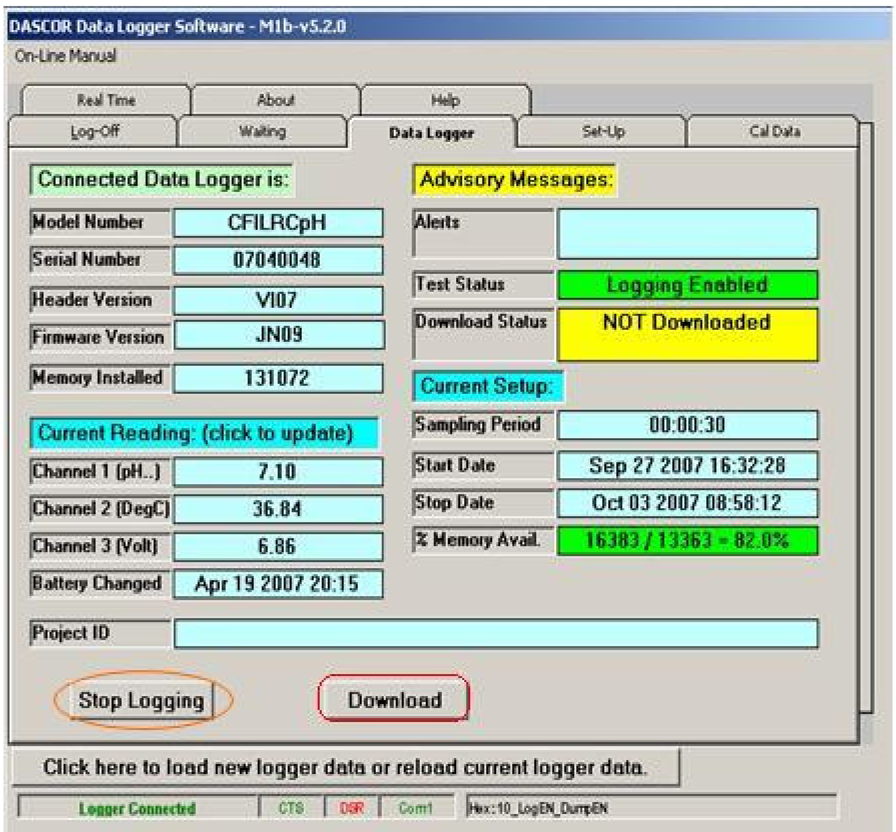


Figure 36, Stopping the logger after ruminal measurement

Note that the Test Status is ***Logging Enabled*** and that less than 100% of memory is available indicating that the logger has been recording data. In this case, the scan interval is greater than the recommended minimum, so clicking the ***Download*** button will initiate the download sequence, which includes stopping the logger. The ***Stop Logging*** button is still available if there are communications problems, but this should only occur at sampling periods less than ten seconds.

Click the ***Download*** button and the following screens will appear to confirm that you really do want to stop the logger (Figure 37). If logging has already been stopped, you will only see the first screen. You do NOT want to abort the download and initialize the logger at this point as all data that has not already been downloaded will be lost.

Figure 37, Confirmation messages for stopping the logger

Again, you will be asked to specify the file path and name. In this case, the filename should be changed to indicate the calibration at the end of the test run, rather than the start.

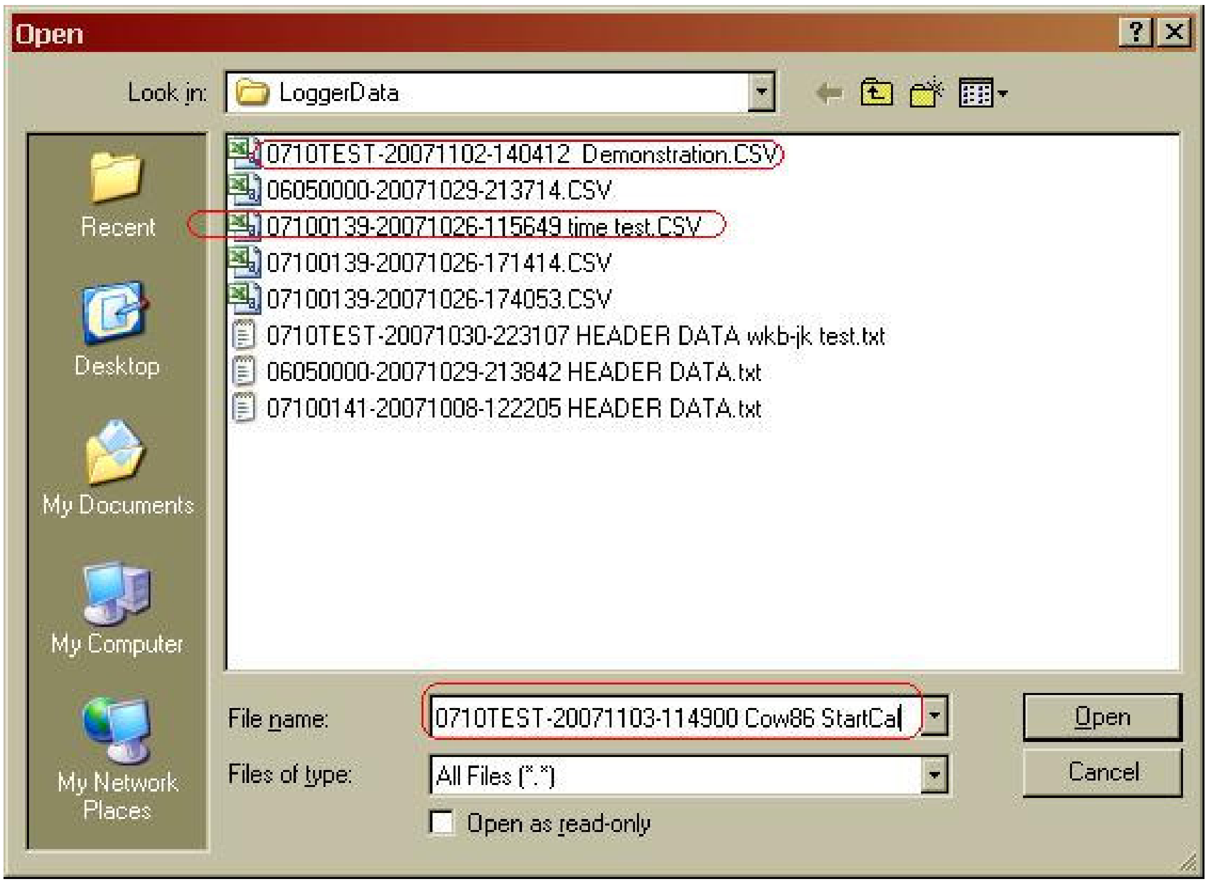


Figure 38, Select the Filename

The following screen will appear when downloading is initiated (Figure 39):

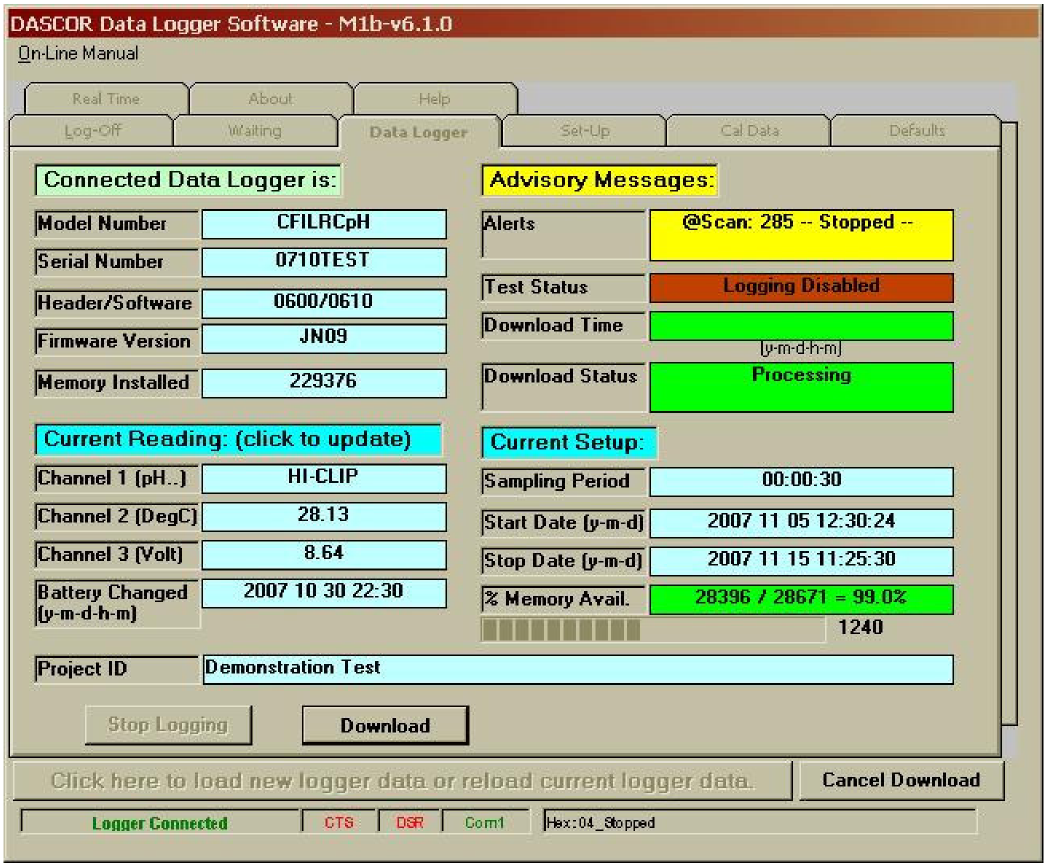


Figure 39, Data logger status screen indicating that data is being downloaded

As the downloading progresses, the progress bar will continue to move to the right (Figure 39) until downloading is complete (full line). It will take approximately 5 minutes to download four channels of data that have been logged for 24 hours at 30-second intervals. This time varies <u>widely</u> depending on the total data points taken, and the computer and operating system used, and may be as long as 45 minutes if the maximum number of data points are taken, and a slow computer is used.

When downloading is complete (the number to the right of the progress bar reaches "0"), the ***Download Notes*** screen will appear (Figure 40), allowing the insertion of test notes at the end of the ".csv" file. You must click ***OK*** to complete this operation. ***ENTER*** will move you to the next text line.

Figure 40, Screen to imput downloaded notes

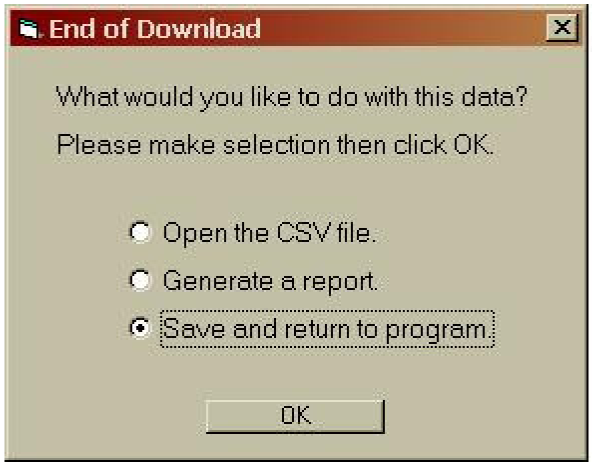


Figure 41, Saving the file and returning to the DASCOR program

Click on ***Save and return to program*** then ***OK*** (Figure 41). This is the point when the data is saved to the file that you specified earlier (see Figure 38).

4.7 Post-Measurement Standardization of the LRCpH system

Post-measurement standardization is required to determine how mV readings have changed over time. This process is identical to the pre-measurement standardization.

Repeat the procedure in Section 4.2 to record the mV corresponding to the pH 7 and 4 buffers at the end of measurement and record on a record sheet (see Figure 14). This is done to account for any drift that may occur in the sensor system after being in the rumen.

Put the sensor in the pH 7 buffer solution first, as the pH 4 buffer solution will act as a mild cleaner of the sensor end and may change how the sensor was reading in the rumen prior to removal.

A study by Penner et al. (2006) concluded that daily standardization of new sensors is not required and the duration between consecutive standardizations could be extended to 72 hours. The risk that is associated with leaving the system in place for this period of time is the possibility of sensor failure. With the LRCpH system, sensor failure can only be detected upon removal from the rumen. If there is a limited amount of time in the experiment in which to record ruminal pH measurements, a shorter interval between sensor checks is recommended. More frequent inspection will result in fewer days of lost data in the event of sensor failure. Under most circumstances, sensors have performed normally for an average of 25 days (in the rumen). These sensors also have a limited shelf life. See Figure 6 to determine age of the sensor.

Remember that it is possible to remove the logger from the rumen and check the integrity of the sensor using the ***Current Reading*** command to display several sequential readings on the ***Data Logger*** tab, or use the ***Real Time*** tab for extended monitoring. Use of the ***Current Reading*** function does not require that the logging be stopped, and the logger can be returned to the rumen after a brief check. To use the ***Real Time*** tab, select the option that does NOT stop the logger. This will allow the logger to send data to the screen every time it logs at the programmed rate. Be sure to check the physical integrity of the sensor glass!

4.8 Checking the data

After downloading the data and conducting the post-measurement standardization, open the downloaded ".csv" file in Excel and check the data to ensure that the system was working properly. Verify that the date and time stamps are correct, and that the mV values (and resultant pH values) recorded look reasonable (see Figure 42). If there was a problem with a sensor, the data may stay the same for a long period of time or there may be extreme changes in mV in a short period of time. Generating a time-series plot is always a good idea to visualize settling times during calibration. Figure 43 shows a plot of the sample test data above. Note the times when the sensor was in calibration buffer solutions at the beginning and end of the "test data." The need for several minutes of settling time following a step-change in pH should be obvious. The settling time will increase with sensor age and use, but may be partially restored by cleaning. Also note that the temperature is fairly stable and the battery voltage is good. The ORP sensor was exposed to air and handling and bounced wildly, including a short period below the allowable full scale range ("Low Clip" in the data). Portions of the sample below have been slightly reformatted for legibility.

If the data appear normal, then the LRCpH system may be initialized to start logging for more days in the rumen, or if the trial is complete (including a post-test calibration), the sensors should be removed and cleaned by following the instructions in Section 4.9, **Cleaning and Storage of Sensors**. In the sample (Figure 42), note the left set of columns (lite-blue) is raw data in mV, while the right set columns (lite-green) have been converted to Engineering Units (pH, DegC, Volts, and mV) using the slope, offset, and TARE values directly above each column. The slope and offset values are from the factory calibration and are stored in the logger. The TARE defaults to zero, but you may change this number to introduce an offset which will be applied to all of the converted cells in the same column. You may also change the slope and offset values to match your own calibration data, but be sure to record the original values in case you need to restore them.

The yellow sections show data inserted into the ".csv" file during download which includes the date and time of the download, file name and path, and the notes you entered at the end of the download. You will also notice that the test was ended by stopping the logger. The red circles highlight other areas that might be of particular interest, such as the date the battery was replaced, and the number of times the logger has been set up for a test using that battery, as well as the total number of times the logger has been initialized. (The table is continued on the next page.)

der Downloaded:	2007/09/15 19:36	WARNING: A Status Event occurred. First event noted occurred at scan number 954.								
Status Flags set at Download:	@Scan: 954 -- Stopped Logging --									
Description:										
Download Time Zone:	0	Initialization Time Zone:	0							
	NOTE: Time Zones are the same.									
Model Version:	CFILRCpH	Serial Number:	070901xx	Cal-Interval:	0	A/D Resolution:	12			
Period:	0:00:10	Waits:	0:00:15	scans:	28671	Active Chans:	4			
Init Date:	2007/09/15 16:37	Start Date:	2007/09/15 16:37	Stop Date:	2007/09/19 0:16	Installed Chans:	4			
FactoryID:	UNlock:39339.870	FactoryID:	UNlock:39339.870							
Engine ID:	M1b03-pH	Engine SN:	070901xx	SigCon ID:	pH-T-Vb	SigCon ID:	+ORP			
Header Ver:	600	Firmware Ver:	JN09	Memory Size:	229376					
Battery Installed:	2007/09/14 20:52	Battery Inits:	2	Total Inits:	2					
						Chans:	chan. 1	chan. 2	chan. 3	chan. 4
						Location:	pH..	Temp	Vbat	ORP.
						Model:	S655CD-H	1K392RTD	M1b03-pH	971716..
						Serial:	....	....	070901xx	....
						Cal Due:	2008/09/30	2008/09/30	2008/09/30	2008/09/30
						TARE:	0	0	0	0

LRCpH Users Guide Release 2007-Dec-26.doc, Printed 2007/Dec/29

						Slope:	1.77E-03	0.0193695	5.99E-03	0.249444
						Offset:	2.56722	-15.0308	0.0231335	-622.633
						EU's:	pH..	DegC	Volt	mV..
Downloaded on:	2007/09/15 19:36	at LocCode:	T	by:	FactoryInt	as file:	C:\LoggerData\9-15-07\unit113 100Kbias			
Scan#	Date_Time	Chan 1	Chan 2	Chan 3	Chan 4		pH..	Temp	Vbat	ORP.
Start of Downloaded Data		mV	mV	mV	mV		pH..	DegC	Volt	mV..
1	2007/09/15 16:37	2513	2051	1314	0		7.0123903	24.696045	7.8985005	Low Clip
2	2007/09/15 16:38	2513	2050	1314	0		7.0123903	24.676675	7.8985005	Low Clip
3	2007/09/15 16:38	2514	2050	1315	0		7.0141592	24.676675	7.904494	Low Clip
4	2007/09/15 16:38	2514	2050	1315	0		7.0141592	24.676675	7.904494	Low Clip
5	2007/09/15 16:38	2514	2050	1314	0		7.0141592	24.676675	7.8985005	Low Clip
6	2007/09/15 16:38	2514	2050	1315	0		7.0141592	24.676675	7.904494	Low Clip
7	2007/09/15 16:38	2514	2050	1315	0		7.0141592	24.676675	7.904494	Low Clip
DATA REMOVED FOR EXAMPLE										
943	2007/09/15 19:14	824	1937	1325	113		4.0247689	22.487922	7.9644283	-594.44583
944	2007/09/15 19:15	824	1938	1325	92		4.0247689	22.507291	7.9644283	-599.68415
945	2007/09/15 19:15	824	1938	1325	70		4.0247689	22.507291	7.9644283	-605.17192
946	2007/09/15 19:15	824	1937	1325	48		4.0247689	22.487922	7.9644283	-610.65969
947	2007/09/15 19:15	824	1937	1325	28		4.0247689	22.487922	7.9644283	-615.64857
948	2007/09/15 19:15	824	1937	1325	4		4.0247689	22.487922	7.9644283	-621.63522
949	2007/09/15 19:15	824	1937	1325	0		4.0247689	22.487922	7.9644283	Low Clip
950	2007/09/15 19:16	824	1938	1325	0		4.0247689	22.507291	7.9644283	Low Clip
951	2007/09/15 19:16	824	1938	1325	0		4.0247689	22.507291	7.9644283	Low Clip
952	2007/09/15 19:16	824	1938	1325	0		4.0247689	22.507291	7.9644283	Low Clip
953	2007/09/15 19:16	824	1938	1325	0		4.0247689	22.507291	7.9644283	Low Clip
954	2007/09/15 19:16	824	1939	1317	0		4.0247689	22.526661	7.9164808	Low Clip
End of Downloaded Data										
Start of Download Notes										
WARNING: Status Event.										
First Status Event occurred at scan number 954.										
Alerts: @Scan: 954 -- Stopped --										
End of Download Notes										

Figure 42, Example of data retrieved through Excel after downloading

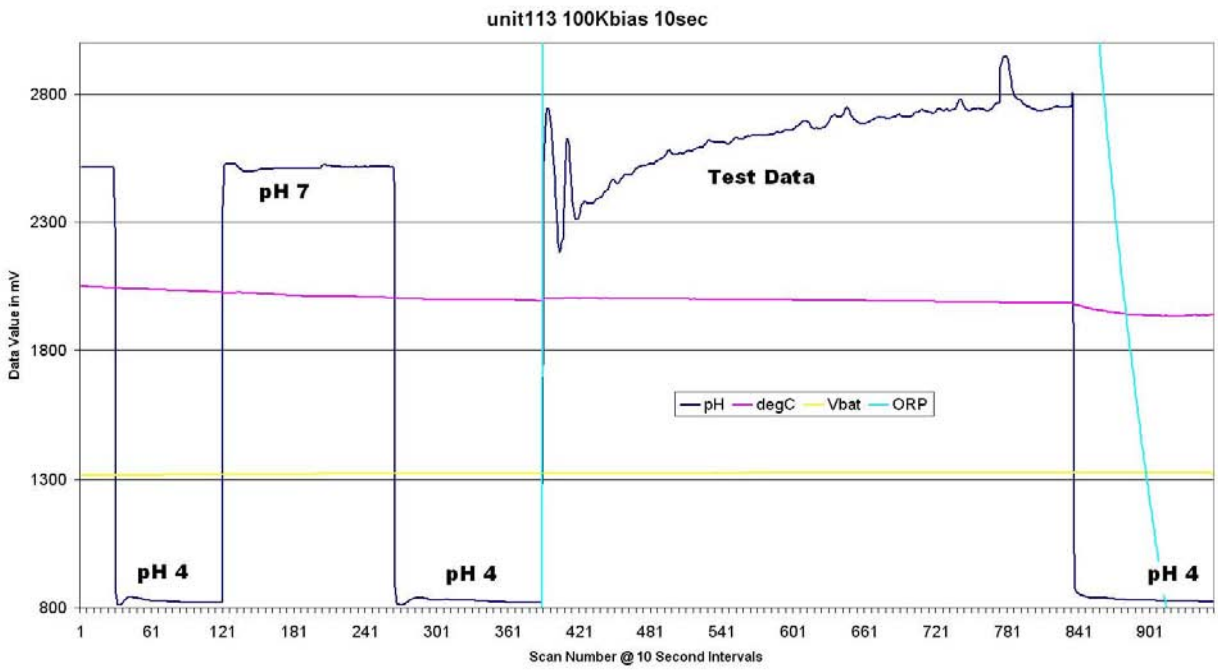


Figure 43, Sample Test Data Time Series Chart

4.9 Cleaning and Storage of Sensors

Why clean? The rumen tends to leave a protein film over the glass surface of the sensor and on the white frit forming the contact with the reference electrode chemistry. In time, this will build up in the pores in the glass and frit, cause shifts in readings and increasingly slow settling times. Eventually the sensor will fail.

After each use, and definitely prior to storage, pH sensors should be cleaned. There are several ways to clean them, and several ways NOT to clean them. First, the NOT: Do NOT use chemical solvents used for cleaning paintbrushes, such as acetone, paint thinner, etc. However, dilute isopropyl alcohol is OK, as is soap and water, or dilute acids. Many labs have an enzyme-based cleaner for labware that works very well, and is recommended by the sensor manufacturer: Tergazyme (or Terg-A-Zyme) by Alconox[5].

Upon completing a measurement period in the rumen, sensors should be removed from the data logger unit. To remove the sensors, turn the sensor counterclockwise to release the pins from the BNC connector and pull out firmly past the rubber rings. The sensor end should then be cleaned in a detergent (such as Terg-A-Zyme) solution (warm, not hot) for a **MAXIMUM** of 15 minutes, rinsed in deionized (or distilled water), and left to soak in pH 4 solution overnight prior to storing in electrode storage solution until the next use. Bulk storage solution is available from DASCOR.

Using the Alconox product, clean the sensor as you would any lab glassware—<u>being very careful not to get the connector wet</u>. Cool or warm water is OK. Hot water is not necessary. Follow with a good rinse in clean water, then storage solution. Finally place the cap, partially filled with storage solution[6], over the end of the sensor. The caps originally placed on the loggers during manufacturing should be retained and reused for storage. In the event storage solution is not available, standard pH 4 buffer solution may be used. A toothpick or paperclip may be used to allow air to escape when replacing the cap partially filled with storage solution. The overall quantity of storage solution is not critical, but there must be enough to wet the glass. The sensors may be stored in any position once all air is removed.

Buffer solution that leaks out around the cap quite often crystallizes. Just rinse it off, or wipe it off with a damp towel. It's messy, but not dangerous.

4.10 Storage of LRCpH systems

The data logger units should be stored in a clean, dry place with the covers lightly in place so as not to trap any moisture that may have gotten into the interior of the logger. The battery should always be removed. Some circuitry is always powered, and will needlessly drain the battery. Battery removal and replacement will cause an error message to appear on the ***Data Logger*** tab under test status indicating a ***power-on reset***. This is normal when replacing batteries. If the battery is new, be sure to click the ***battery replaced*** button on the ***Calibration*** tab.

5.....The Alconox website, including links to world-wide distributors: http://www.alconox.com/static/techbull/techbull_tergazyme.asp

6.....The storage solution liquid provided in the cap by Sensorex is standard pH-4 buffer solution, but has some additional KCl added for storage. DASCOR can provide bottles of storage solution. Please let us know if you are interested.

5 Data Analysis

In this section we provide recommendations on how to convert the mV readings to pH units. We do not attempt to demonstrate how to summarize pH data once converted from mV to pH.

5.1 Converting Raw mV Data to pH

The LRCpH system allows users to collect frequent mV readings that are later converted to pH units using the pre-measurement and post-measurement standardizations. The assumption is that the change in mV readings from the pre- to the post-measurement standardizations is linear and constant and fairly represents sensor drift due to aging and use.

Calculate the slope and y-intercept for the pre- and post-measurement standardizations. A spreadsheet with worksheets that demonstrate the following calculations is included with the User's Guide or software installation package.

Using the raw mV file, calculate the number of observations (n). To calculate the offset and the pre- and post-measurement coefficients use the following equations.

Offset = 1 ÷ (n-1)

Pre-measurement coefficient_1 = 1

Pre-measurement coefficient_2 = Pre-measurement coefficient_1 - offset

Pre-measurement coefficient_3 = Pre-measurement coefficient_2 - offset

etc.

Post-measurement coefficient_1 = 1 – pre-measurement coefficient_1

Post-measurement coefficient_2 = 1 – pre-measurement coefficient_2

etc.

Convert the mV readings to pH using the following equation.

pH_1 = mV_1 reading [(pre-measurement slope × pre-measurement coefficient_1 + post-measurement slope × post-measurement coefficient_1)] + (pre-measurement y-intercept × pre-measurement coefficient_1 + post-measurement y-intercept × post-measurement coefficient_1)

5.2 Temperature Correction

Looking at the sample data in the sample spreadsheet (**LRCpH Barn Worksheet_2007.xls**), the pH varies from six to seven, and the temperature stabilizes between 38 and 40 degrees. The pH sensor introduces a shift in its readings that changes with the sensor's temperature and with the pH being measured. Since the procedures discussed previously include calibrating the sensors at rumen temperature, and the pH stays close to seven, the effects of temperature on the sample data is probably less than 0.03 pH units, and might be ignored, depending on the accuracy requirements of the test protocol.

All sensors and electronic systems are subject to temperature-induced variations. The change in value with temperature is generally referred to as the "temperature coefficient" or "tempco" for short. In fact, the ability to measure temperature with a platinum RTD (**R**esistive **T**emperature **D**evice) is due to its highly predictable change in resistance due to temperature. DASCOR's data logger electronics are designed to have a minimal tempco, and any changes in the measurements due to the electronics are usually well below the accuracy of the sensors, and can be ignored for most purposes.

However, pH measurements are subject to temperature effects introduced by the sensor itself, which often cannot be ignored. The following is extracted from a white paper[7] on the Sensorex Website. The Sensorex Website includes some excellent tutorial material on pH sensors in general.[8]

"When measuring pH using a pH electrode the temperature error from the electrode varies based on the Nernst Equation as 0.03pH/10C/unit of pH away from pH7. As shown in the table below [Figure 44], the error due to temperature is a function of both temperature and the pH being measured. Note that there is no error at pH7 and 25 C. Temperature compensation can be achieved manually or automatically. Manual temperature compensation is usually achieved by entering the temperature of the fluid being measured into the instruments menu and then the instrument will display a 'Temperature Compensated' pH reading. This means that the temperature [sic] is corrected to the value expected at 25 C. Automatic temperature compensation requires input from a temperature sensor and constantly sends a compensated pH signal to the display. Automatic temperature compensation is useful for measuring pH in systems with wide variations in temperature."

pH vs. Temperature Error Chart

	pH 2	pH 3	pH 4	pH 5	pH 6	pH 7	pH 8	pH 9	pH 10	pH 11	pH 12
5°	.30	.24	.18	.12	.06	0	.06	.12	.18	.24	.30
15°	.15	.12	.09	.06	.03	0	.03	.06	.09	.12	.15
25°	0	0	0	0	0	0	0	0	0	0	0
35°	.15	.12	.09	.06	.03	0	.03	.06	.09	.12	.15
45°	.30	.24	.18	.12	.06	0	.06	.12	.18	.24	.30
55°	.45	.36	.27	.18	.09	0	.09	.18	.27	.36	.45
65°	.60	.48	.36	.24	.12	0	.12	.24	.36	.48	.60
75°	.75	.60	.45	.30	.15	0	.15	.30	.45	.60	.75
85°	.90	.72	.54	.36	.18	0	.18	.36	.54	.72	.90

Figure 44, pH Errors with Temperature

Note: *Values in light blue are less than 0.1 error and may not require temperature compensation. Values in gray are temperature and pH in which there is no error in pH from temperature.*

Note that at 25 C, or at a pH of 7, there is no effect of temperature. However, as the pH and/or temperature move away from the central point, the "error" can approach 1 pH unit! Calibration solution temperatures should be held as constant as possible at a temperature matching that of the rumen.

When the data logger downloads the collected data to your PC, a ".csv" file is generated. The rightmost columns (temperature and battery voltage) are based on factory calibration, and the values given are correct and in engineering units such as degC and Volts. The pH column, however, is based on a theoretically perfect sensor, and is NOT temperature compensated at the present time. However, the temperature is available and can be applied to the raw data to provide a pH value corrected for temperature based errors in the sensor. If you would be interested in seeing this done automatically in a future release of the software, please drop DASCOR a note.

[7] http://www.sensorex.com/support/education/pH_education.html

[8] The following link takes you to the Sensorex support page, which is well worth exploring: http://www.sensorex.com/support/support.html

6 Troubleshooting

6.1 Troubleshooting Chart

Problem	Possible Cause	Solution
Low battery warning	Battery voltage <6.7	Replace 9 V battery
Won't ***Load Header*** to the computer	Poor connection Scan Interval < 5 Sec. LRCpH system battery may be dead	Check that the cable connections between the computer and logger are securely in place Click ***Stop Logging*** and/or short Pause pins. Replace the 9 V battery in the LRCpH system, and try logging on again
Won't download	Poor connection Program error COMM-port problem	Check that the cable connections between the computer and logger are securely in place Exit Dascor LRCpH program, reload, and try downloading again Check the proper comm. port is selected under the defaults tab
File Not Found error	Default path not set Improper installation of the software	Re-specify the download path on the ***defaults*** tab. Uninstall then re-install the software. Contact DASCOR if problem repeats.
Reads the same mV in both pH 4 & 7 buffers	Sensor failure	Replace sensor
mV values won't stabilize during standardization	Electrical interference Sensor failure	Disconnect computer from electrical source (i.e. unplug and run on computer battery only) Replace sensor

6.2 Sensor Breakage Issues

According to the pH probe manufacturer (Sensorex), more than 98% of pH electrodes will reach you in excellent working condition. Sensors that fail during normal handling or during first use may be returned for possible warranty replacement. Sensorex will examine the glass fracture surfaces, and can easily identify and differentiate failures caused by manufacturing stress crack defects, or by mechanical causes such as impacts. So, if you feel a probe failure was premature, and was not caused by dropping, or impacting the weights, rocks, or tramp metal in the rumen, then return the cleaned sensor to DASCOR (freight prepaid), and we will have it examined. A replacement will be provided by Sensorex IF the failure is determined to be manufacturing related, such as stress fracture, electrical short or open, or other workmanship or material issues.

The most fragile shape for a pH sensor is the spherical lab style. Second in line is the flat face. The most robust shape is the hemisphere. There are minor trade-offs between glass thickness, sensitivity, and surface area that give the hemispherical shape roughly the same performance as the flat glass electrodes, but with improved strength. Sensorex states that the increase in strength is roughly 5:1. HOWEVER, and this is important, any glass shape can be broken if it is struck by a hard object, or has too much pressure applied to it. Also, with the hemispherical sensors, there is more exposure of the glass, and the potential for impact is increased.

In an ongoing effort to increase the life expectancy of the pH sensors, and on the recommendation of the manufacturer, DASCOR is adding a new part number: 971761. The new sensors are identical in function to the S655CD-HT's we have been delivering, and include the gel reference electrolyte. However, the S655's have a flat glass electrode, while the 971761's have a hemispherical glass electrode. The price is also the same. Both electrode types can be ordered directly from DASCOR.

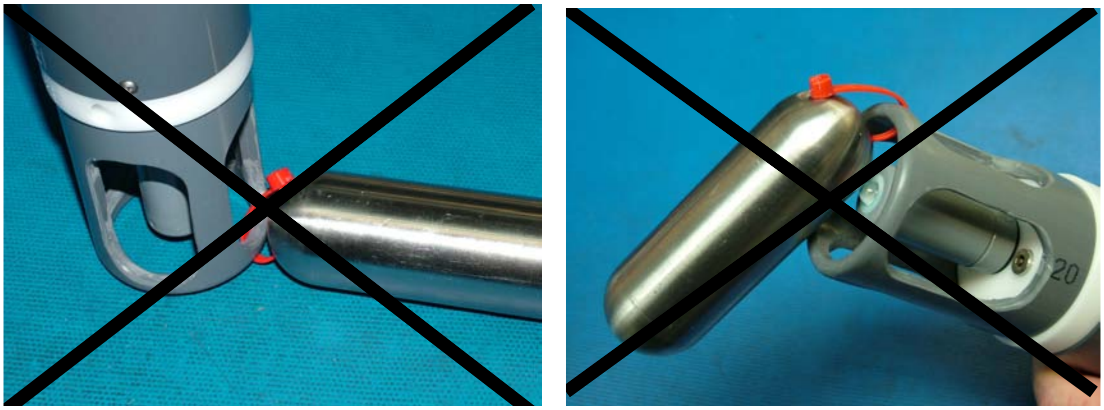
Over the past few years, approximately 15 sensors have been returned to DASCOR, of which one or two were covered by warranty replacements. This is in keeping with a <2% failure rate. The remainder were identified by Sensorex as being damaged in use, and not covered by warranty.

The good news is that a possible cause and mitigation for electrode glass breakage has been identified. Please examine the photographs below. (see Figure 45) Note that if the wire ties connecting the weights to the shroud are around the bottom ring, there is a strong probability that the weights will swing into the sensor area and impact the glass, particularly during insertion thru the Cannula. We believe this has been the major cause of previous glass breakage in the rumen. The cure is simply to move the attachment to the vertical struts, keeping the connecting loop fairly short. The weights will have adequate freedom of motion, and will continue to hold the logger in the proper position—but will not be able to impact the glass.

One-quarter inch extension rings are also available to move the shroud outward and provide additional protection for the glass part of the pH Sensor. The extension rings are recommended for use with the Hemi-glass style sensors to compensate for their longer length. Staggering the weights slightly can allow for easier insertion and removal thru the Cannula opening without pulling excessively on the wire ties or requiring the weights to be in close contact. These rings will be provided with all new loggers, and on request to retrofit older loggers.

Finally, the weights themselves can be coated with a resilient Plastisol material similar to that used to manufacture the cannulas. DASCOR can also provide Plastisol boots to retrofit on to existing stainless steel weights. There is an added cost for the Plastisol coating or boots.

New sensors are also being designed which will include a built-in protective ring.



<u>DO NOT</u> ATTACH THE WEIGHTS THIS WAY!

<u>DO</u> ATTACH THE WEIGHTS THIS WAY!

Figure 45, Mounting the weights

Another possibility for impacting the glass is dropping the logger assembly onto the bench, or hitting the bottom of the beaker holding the calibration solution. The logger is heavy, and enough weight on the sensor will cause breakage—whether it has the flat or hemispherical glass. The shroud, when properly installed, will prevent contact with the glass under normal handling, so it should always be in place (except when changing or cleaning the electrodes). If you encounter problems, and feel that the shroud needs to extend farther out, use a spacer ring.

Figures 46 is a suggested method of handling the calibration buffers—please note that the hemispherical glass does not touch the bottom of the buffer container. This method can be applied to the procedures given earlier with the larger Nalgene bottles. Note that this procedure is easier if the steel weights are removed for calibration, then re-attached prior to placement in the rumen.

Figure 46, Using the Shroud during Calibration

If you follow these procedures, and continue to have glass breakage, please contact DASCOR immediately!

kborsum@dascor.com

or by phone: 1-760-796-7788.

7 Seeking Help

In order to further assist new and current users with minimal impact on valuable research time, DASCOR is starting an email list server or blog page which will be moderated by the staff at DASCOR. All registered users will have access and we encourage users to post questions and answers for all participants. Further information about the list can be obtained by sending a request to listmanager@dascor.com. Please include the word "[info]" including the square brackets in the subject line. Complete information will be sent by return email.

8 Acknowledgements

DASCOR specializes in developing instrumentation, and has relied heavily on input from the end users to guide the development of their loggers for specific applications such as ruminal research. In order to respond to requests from the field for procedures and guidelines for using these loggers, Bev Farr of Lethbridge Research Centre, in conjunction with Karen Beauchemin and Greg Penner, has created this *User's Guide* for use of the Lethbridge Research Centre Ruminal pH measurement system (LRCpH). The intent of this guide is to provide practical suggestions for ruminal pH measurement with the LRCpH. This guide is the result of their efforts, and is intended to answer the majority of the questions that new users may generate.

9 DASCOR Contact Information

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Shipping (FedEx/UPS):	862 Ball Ave., Escondido, CA, 92026
Voice:	1-760-796-7788
Fax:	1-760-796-7785
Panic:	1-619-794-7788 (Kelly's cell)
Website:	www.dascor.com
Email:	websales@dascor.com or kborsum@dascor.com

We have been getting an incredible amount of spam in recent months, and have had to tighten up our email filters at the risk of losing your emails. Please **call** if you don't get an expected response in a reasonable amount of time!

10 References

Nocek, J.E., J.G. Allman, and W.P. Kautz. 2002. Evaluation of an indwelling ruminal probe methodology and effect of grain level on diurnal pH variation in dairy cattle. J. Dairy Sci. 85:422-428.

Penner, G.B., K.A. Beauchemin, and T. Mutsvangwa. 2007. The severity of ruminal acidosis in primiparous Holstein cows during the periparturient period. J. Dairy Sci. 90: 365-375.

Penner, G.B., K.A. Beauchemin, and T. Mutsvangwa. 2006. An evaluation of the accuracy and precision of a stand-alone submersible continuous ruminal pH measurement system. J. Dairy Sci. 89:2132–2140.