conductR: an R program to measure stem, leaf or root hydraulic conductance with an electronic balance or pipette

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(this document is best viewed in full screen / presentation mode)

program available from https://uwmadison.app.box.com/v/conductR

for troubleshooting help, feedback, or bug reports, contact ddsmith3@wisc.edu

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You need

- Balance
 - conductR is coded for Sartorius, Mettler, Ohaus or Scientech balances
 - •we use a Sartorius Entris 224-1S with 4-point precision
 - •in theory, any balance with a serial port is capable with a few code modifications
- •USB cable for balance (note: Sartorius cables (part: YCC01-USBM2) are wired differently from others)
- Driver for cable may not be necessary for newer operating systems
 - •e.g. http://www.ftdichip.com/Drivers/VCP.htm for a Sartorius cable
 - •e.g. http://www.prolific.tw for some other cables
- •R (https://cran.r-project.org/)
- tcltk (a package that comes with the standard R installation)
- •X11 or XQuartz (for Macs only this runs tcltk; http://www.xquartz.org/)
- •R Studio (https://www.rstudio.com/; optional but provides a better interface on *Windows* computers than standard R. Standard R is still needed. RStudio may not work well on a Mac)

Communication settings

- Check that your balance will allow manual printing in unstable conditions.
 - For a Sartorius, e.g. Menu>SETUP>PRNT.OUT>PRINT>MAN.W/O
- Determine port settings. Your balance manual should specify how to find these and change them. Here, I will assume:
 - · baud=9600
 - parity=odd
 - data bits=7
 - stop bits=1
 - handshake=software/xonxoff
- Determine port name. This will be COM# in Windows or /dev/cu.usbserial-# on a Mac, where # will vary between ports and computers
 - To find #, turn on the balance and connect it to the computer. For Windows, find # in Device Manager —> Ports. For a Mac, open Terminal (in the Utilities folder) and input: ls -l /dev/cu.* For either system, there may be multiple ports so disconnect and reconnect the balance to determine the correct one. If you see no ports, you may need to install driver for cable. On my Mac, #=00001004, which I'll use in the following examples

Try basic communication

- · Open R
- Load the tcltk package by typing require(tcltk) in the R console.
- Input the following into the R console, replacing the red text with your own port ID (in the first line) and port settings (in the second line):

```
.Tcl('set serial [open /dev/cu.usbserial-00001004 r+]') .Tcl('fconfigure -mode \ \ 0000,0,7,1\ -blocking 0 -handshake xonxoff')
```

Now press the 'print' button on the balance and then in R input:

```
as.character(.Tcl('return [read $serial]'))
.Tcl('close $serial')
```

The weight on the balance should appear in R like:

```
"+" "62.4317" "g" (typical for Sartorius)
"S" "D" "62.4317" "g" (typical for Mettler)
```

- If nothing appears or garbage appears, check port settings (see also Appendix F)
- If sensible numbers appear with a different format see Appendix B

Let the program tell the balance to print

- We need a print command which is Esc-P-CR-LF for Sartorius balances (CR=carriage return and LF=line feed). In the proper format, this command is \x1B\x50\xD\xA (see http://www.asciitable.com/). Other commands are:
 - Mettler: S-I-CR-LF (\x53\x49\xD\xA)
 - Ohaus: I-P $(\x49\x50)^*$
 - Scientech: S-E-N-D-CR-LF (\x53\x45\x4E\x44\xD\xA)**
- Now, run the following lines in R replacing the red text with your port ID (line 1), port settings (line 2), and print code (line 3):

```
.Tcl('set serial [open /dev/cu.usbserial-00001004 r+]' )
.Tcl('fconfigure $serial -mode \"9600,o,7,1\" -blocking 0 -handshake xonxoff')
.Tcl('puts $serial \x1B\x50\xD\xA')
.Tcl('flush $serial')
Sys.sleep(0.1)
.Tcl('return [read $serial]')
.Tcl('close $serial')
```

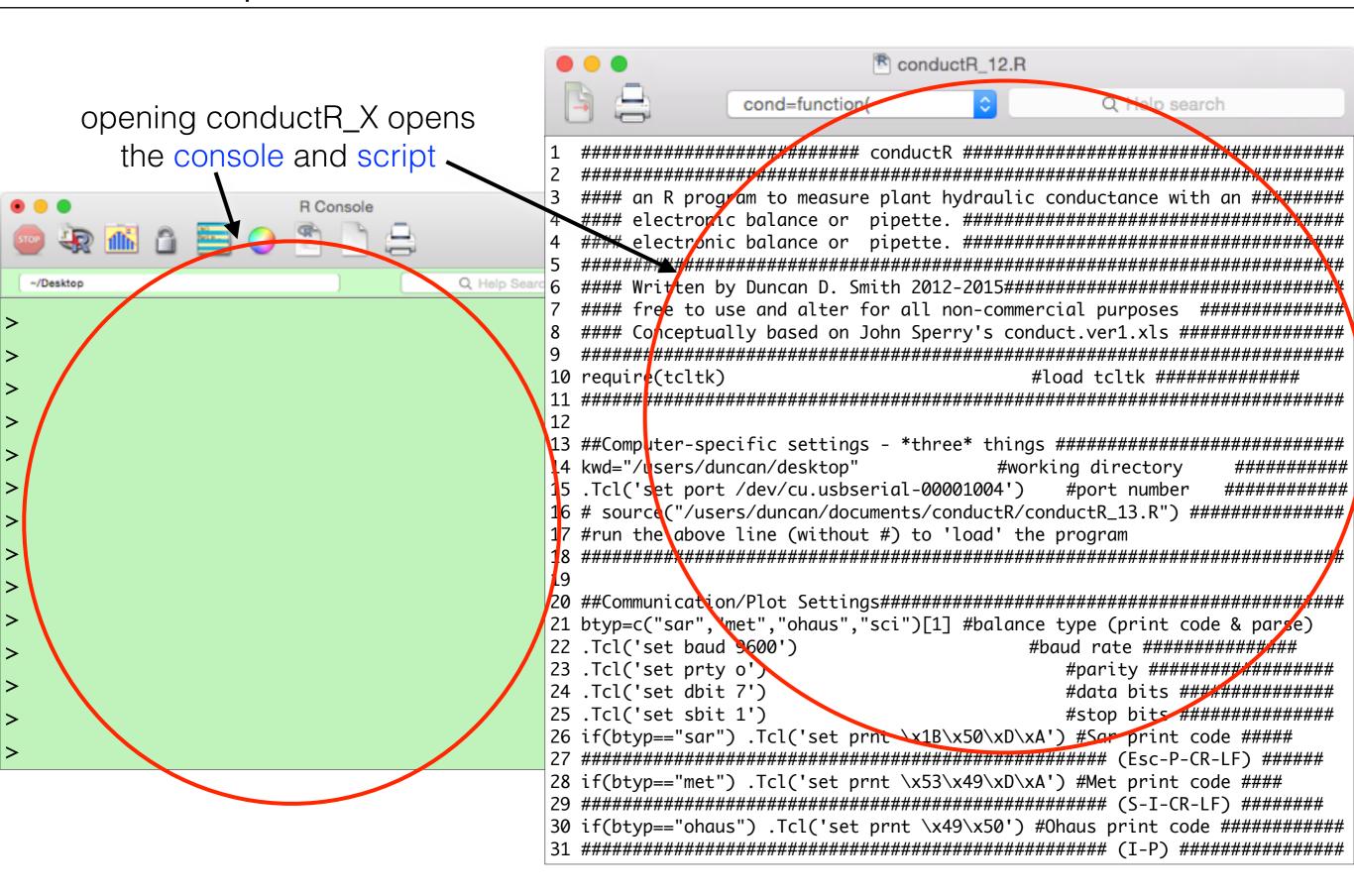
^{*}thanks Stephanie Schmiege

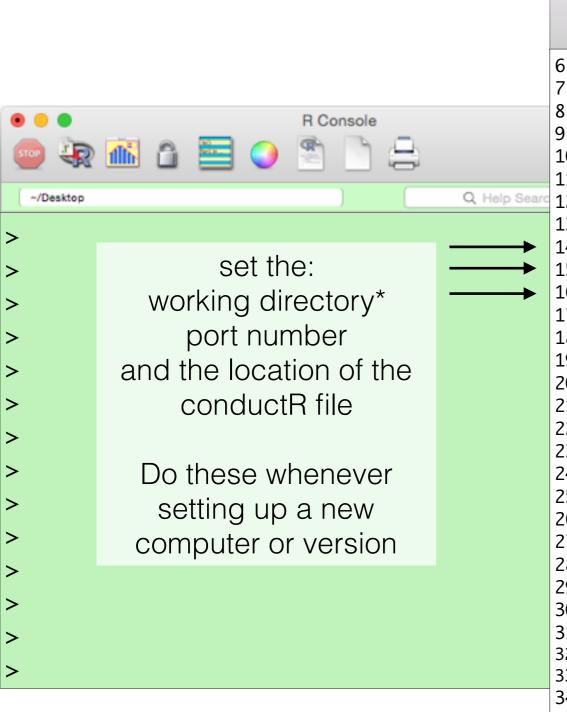
^{**}thanks Greg Vose

Let the program tell the balance to print (continued)

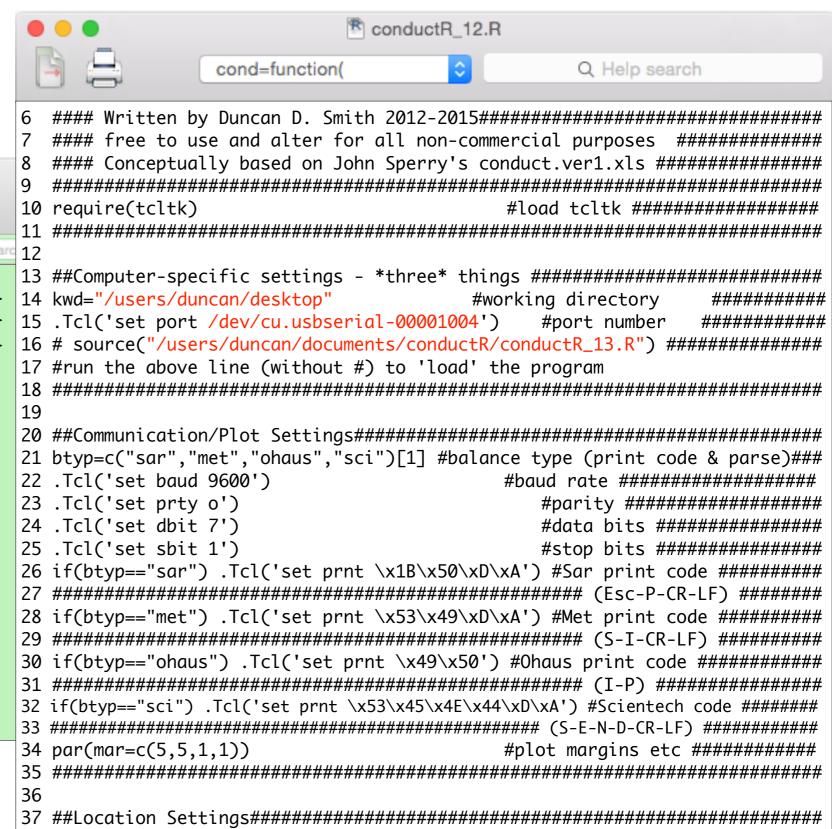
• R should return the weight on the balance. Note that the flush command forces the print command to send and Sys.sleep(0.1) waits 0.1 s, which should be long enough for a response. If it does not work, I would try a simpler command like 'tare' (Esc-T-CR-LF = \x1B\x54\xD\xA for Sartorius) because it will be clear whether the balance received the command or not (i.e. the balance should tare). You might also increase the Sys.sleep() time.

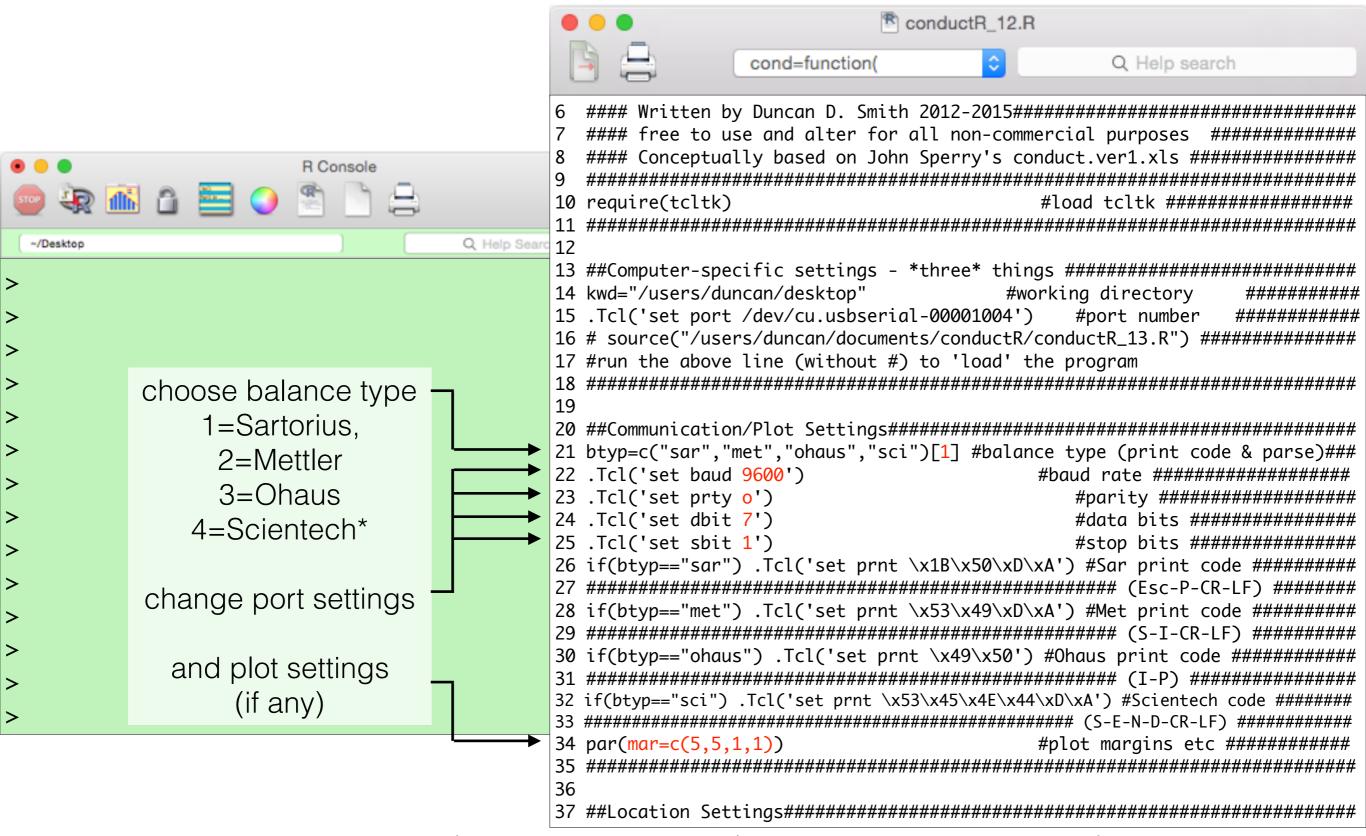
Now to use the program



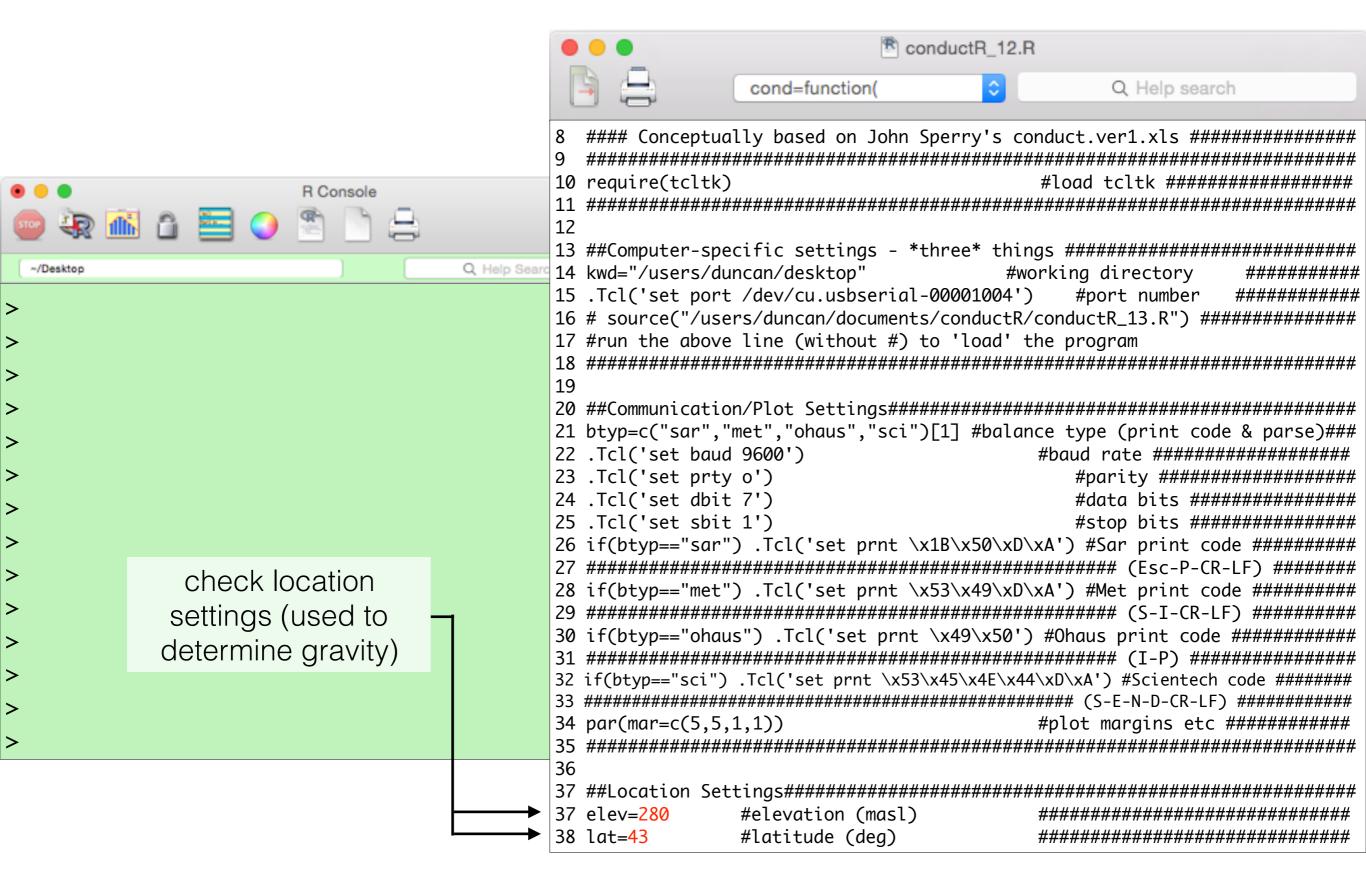


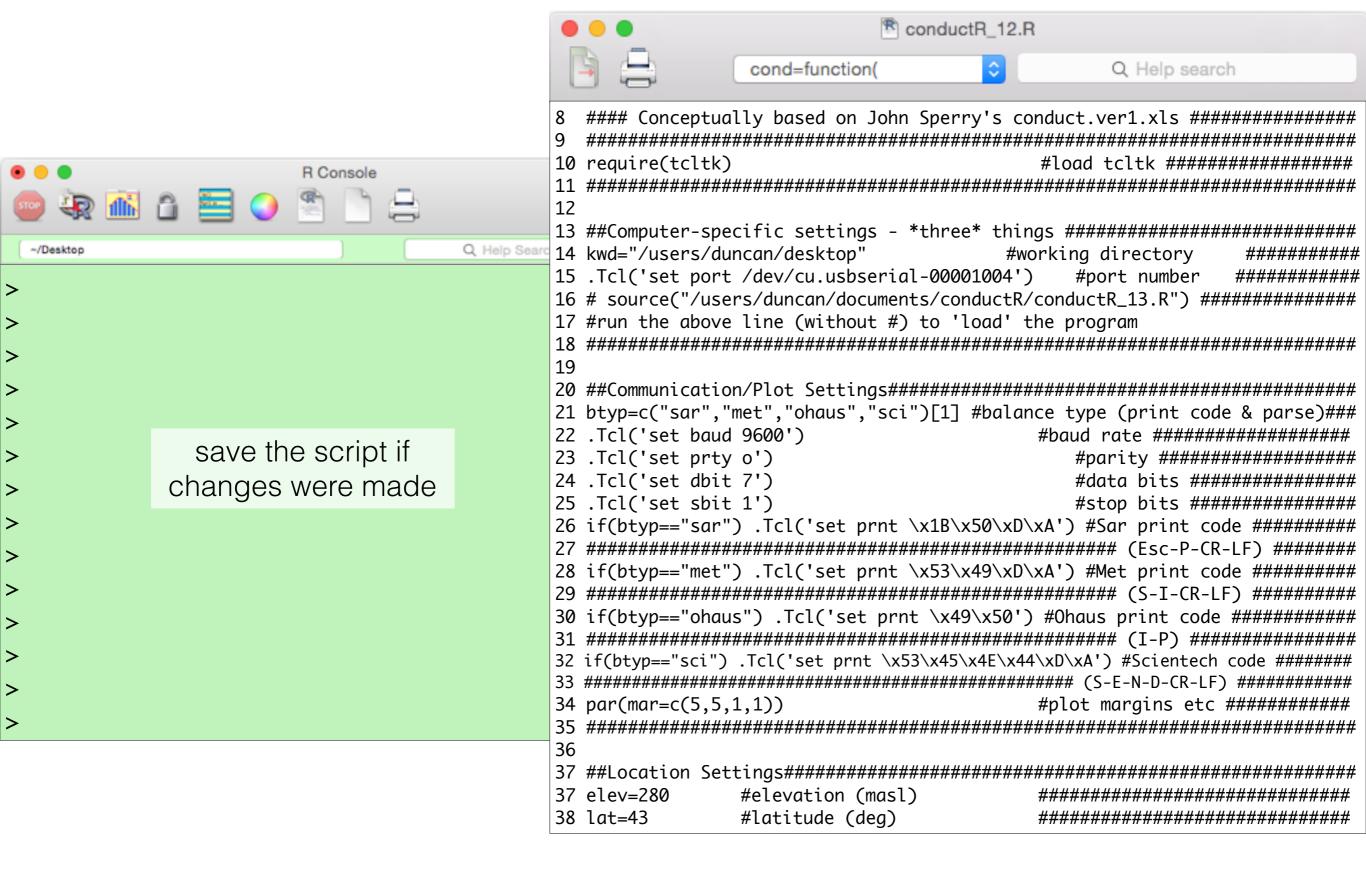
*if you are new to R, type **getwd()** into the console to see your current working directory. Then work out what directory you want files to be saved in.



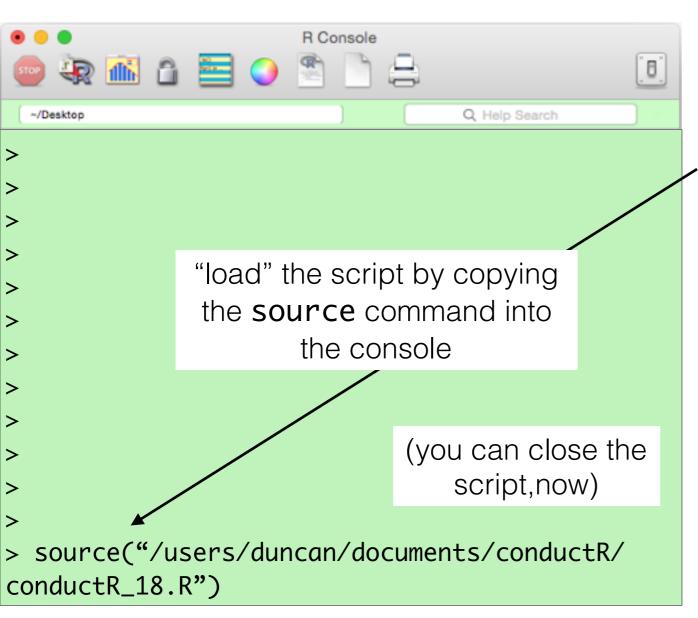


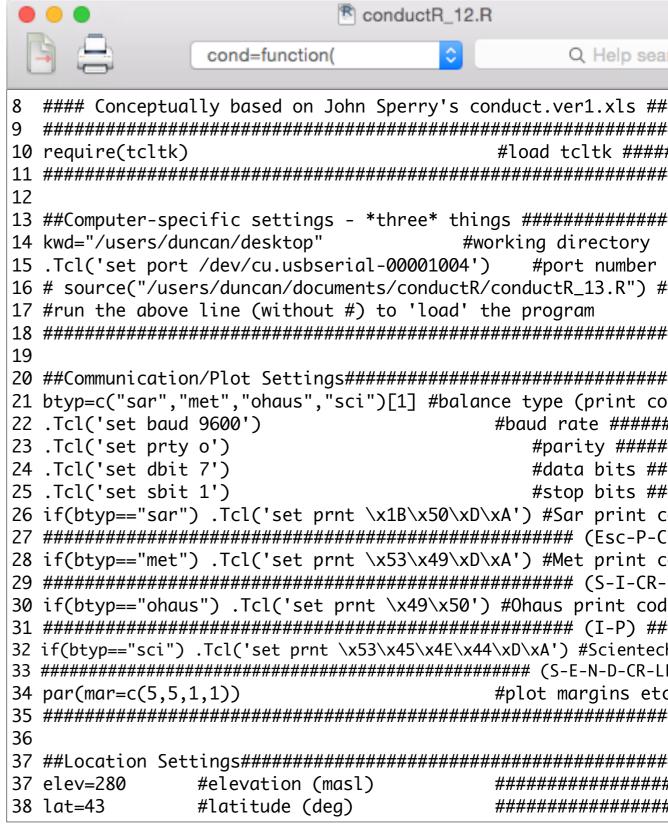
*Balance type determines print code (see lines 26, 28, 30, 32) and how to extract numbers from what balance prints (see decod() function in Appendices A & B) 10



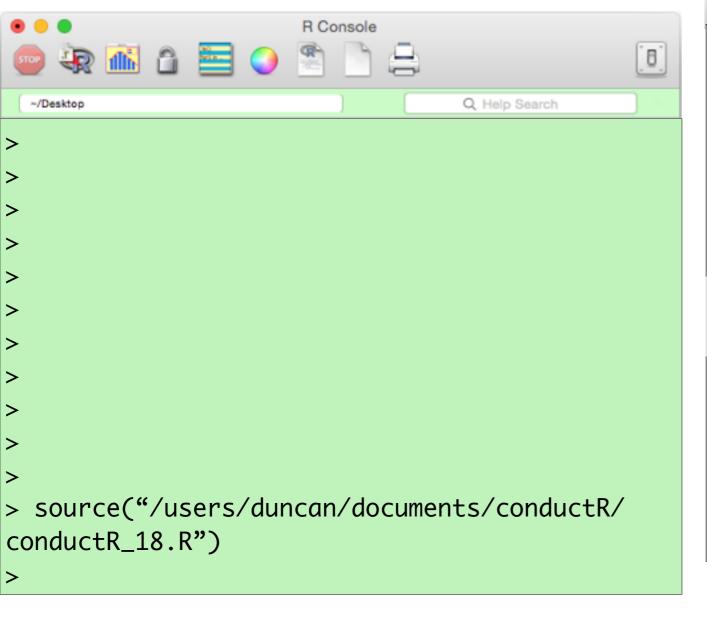


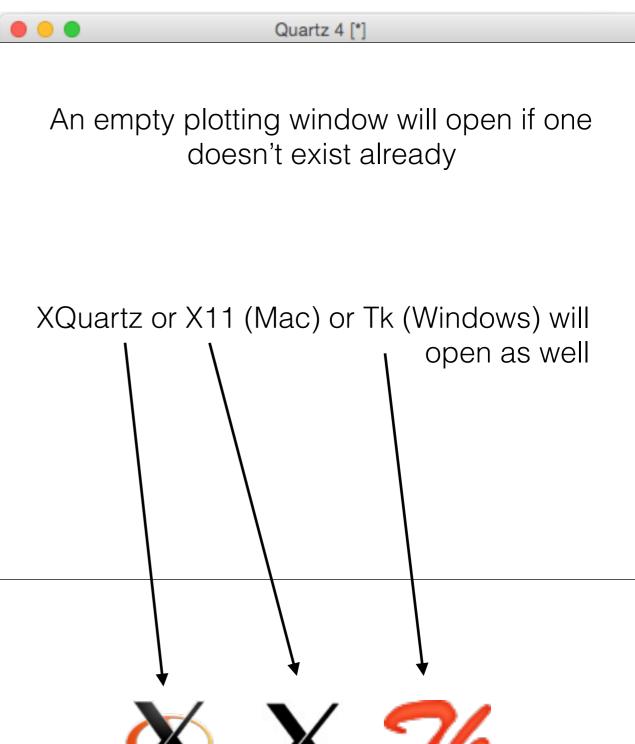
Initiating the program

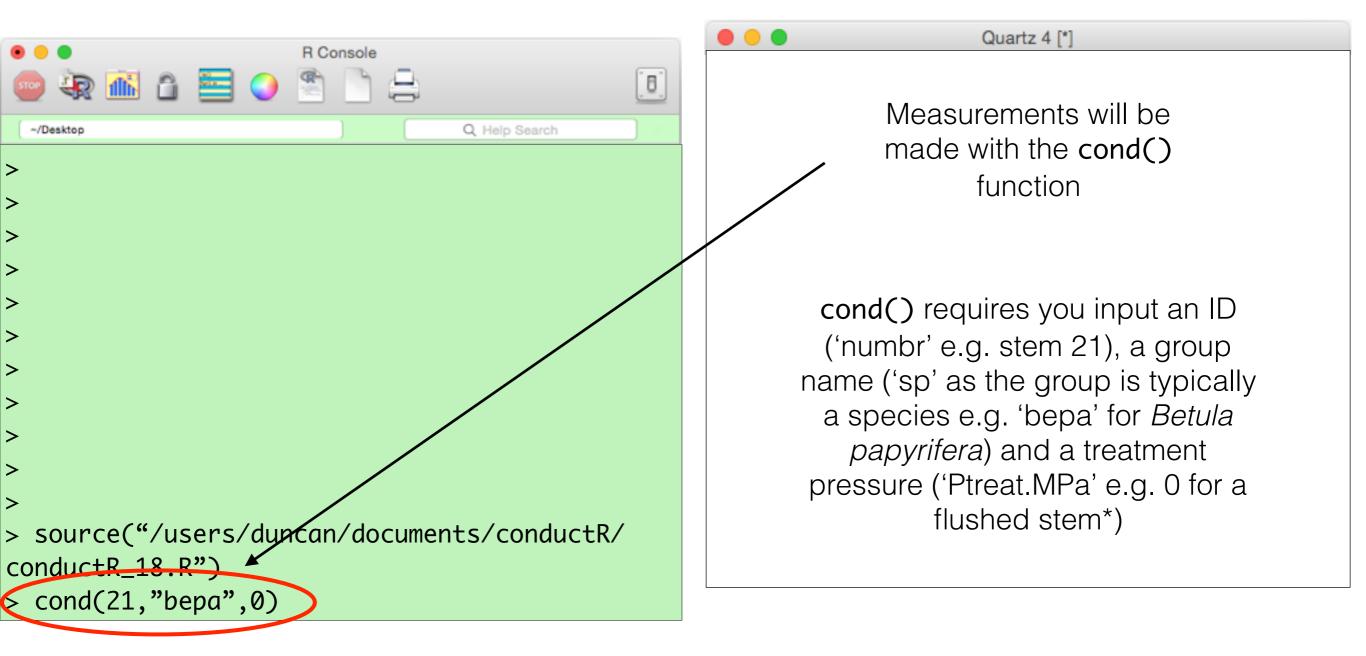




Initiating the program

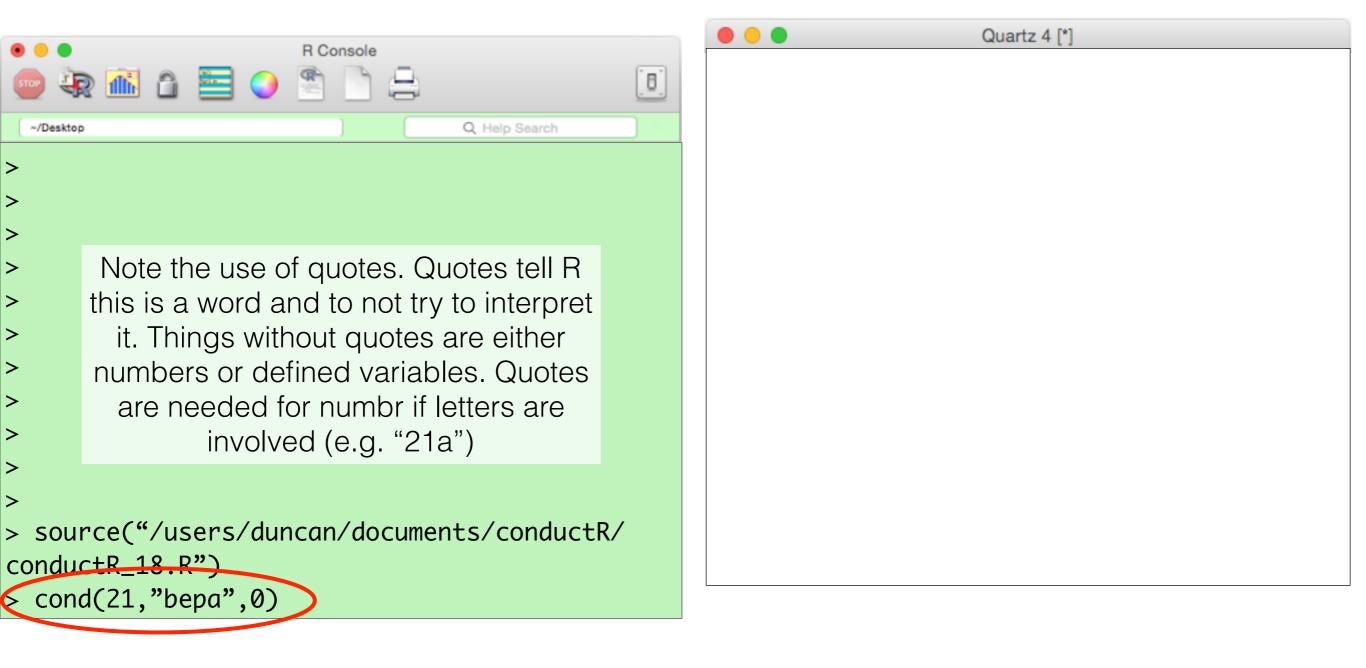


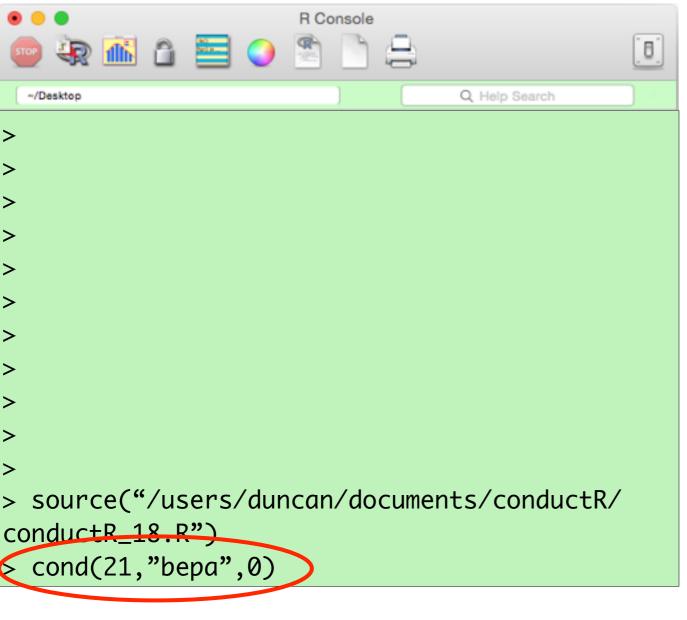




*If measuring native conductance and you didn't measure native xylem pressure, choose some identifying number for Ptreat.MPa (e.g. 999). The program needs a number because it will always try to calculate PLC relative to the most favorable pressure.

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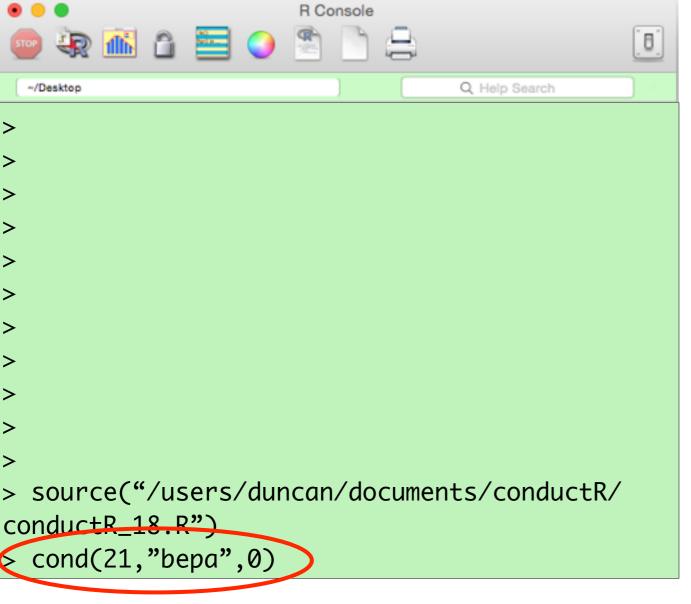


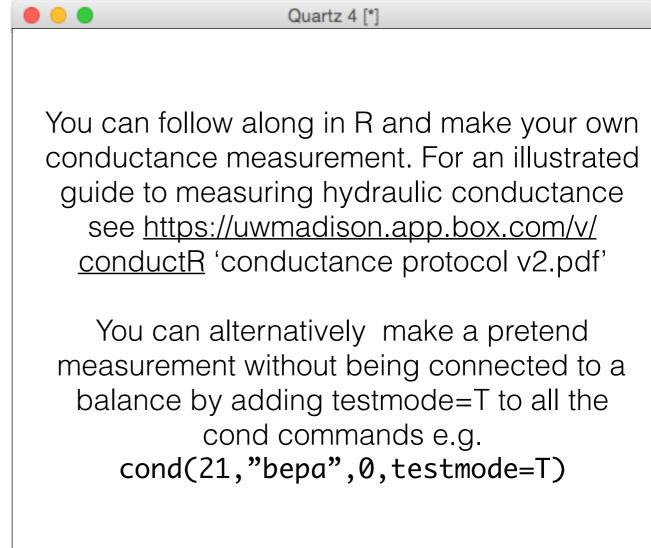
conductR requires numbr, sp and Ptreat.MPa for grouping measurements together. There are other options for cond (see Appendix A) but their defaults are fine for now

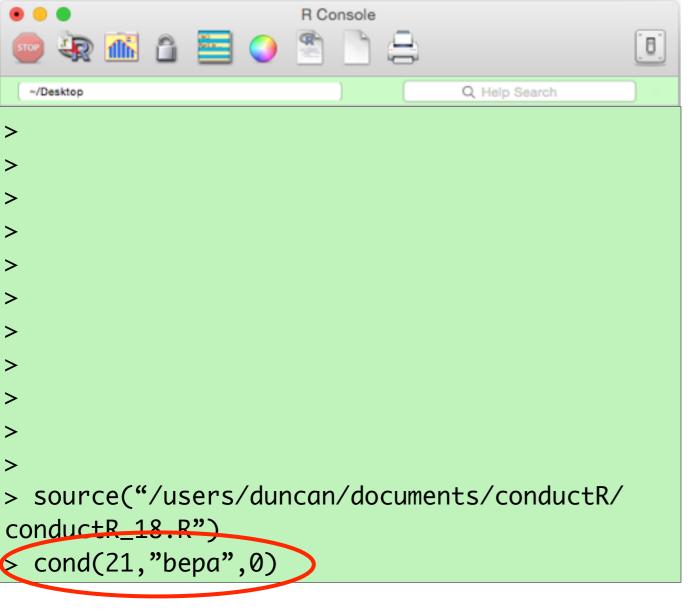
Quartz 4 [*]

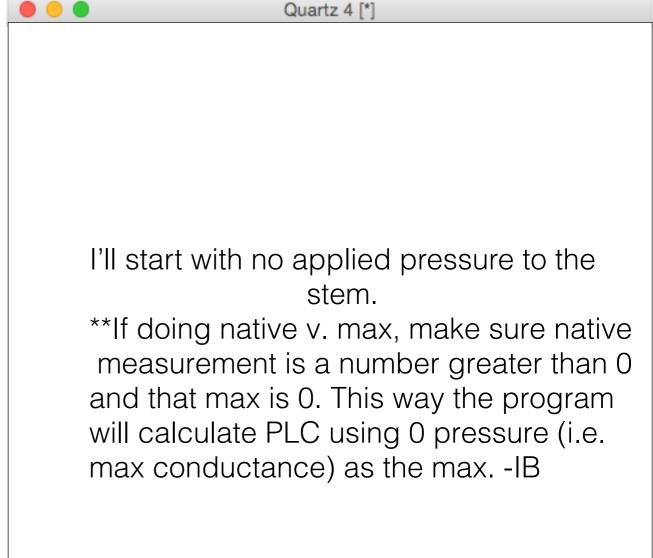
- All measurements with the same sp will be pooled in the same .csv files.
- All measurements with the same sp, numbr and P.MPa (I call this nomID) will be treated as part of the same conductance measurement.*
- Measurements with the same sp and numbr (I call this stemID) will be grouped for calculating PLC

*To the advanced user: a caveat is if you measure a stem more than once at the same treatment pressure, the program will try to group everything into a single conductance measurement. As a solution, you could: 1) exclude points from earlier measurements when prompted 2) use different names (e.g. 21.1, 21.2, 21.3) or 3) input slightly different pressures (e.g. 2.0, 2.00001, 2.00002). I favor option 3.

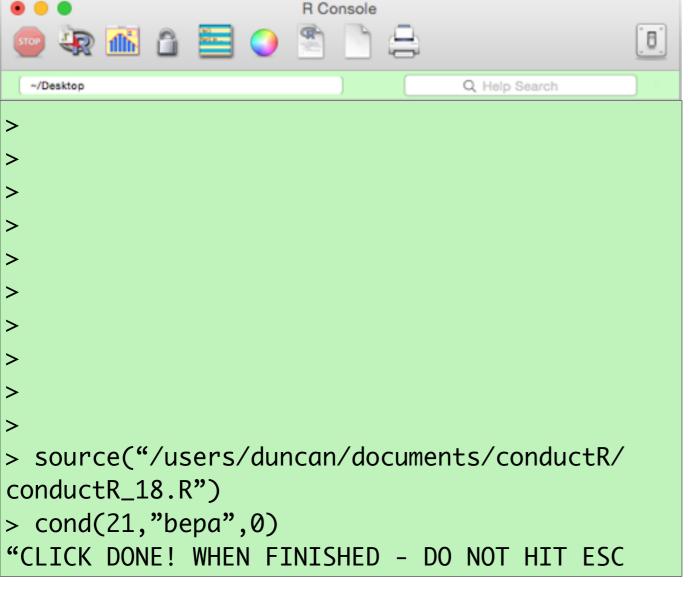


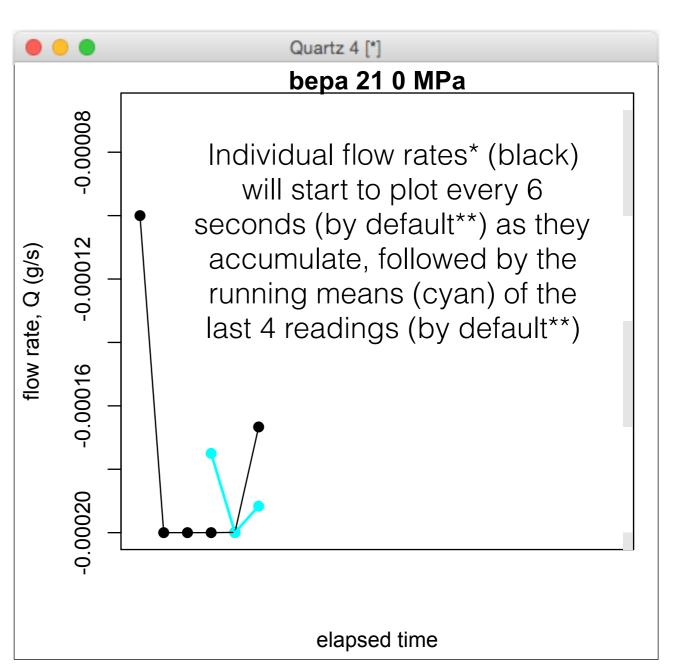






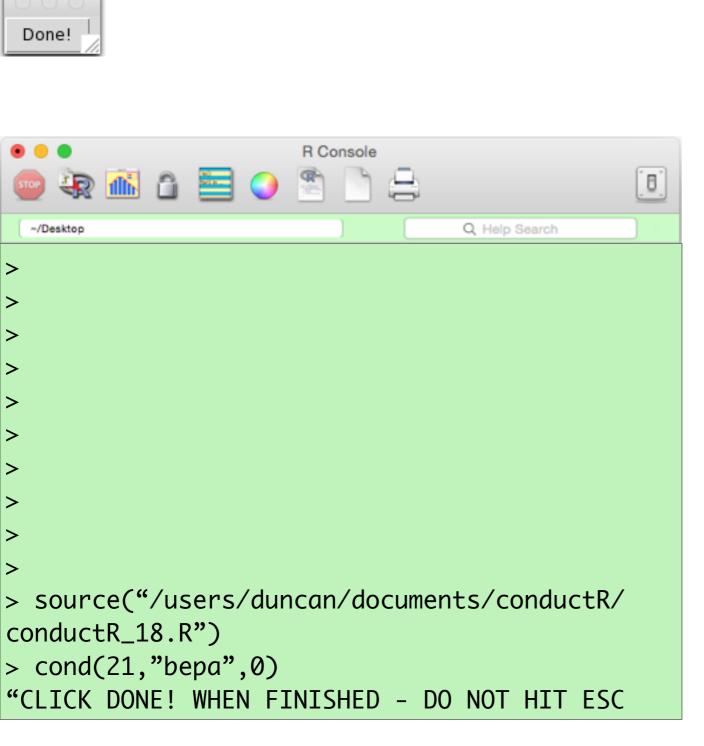


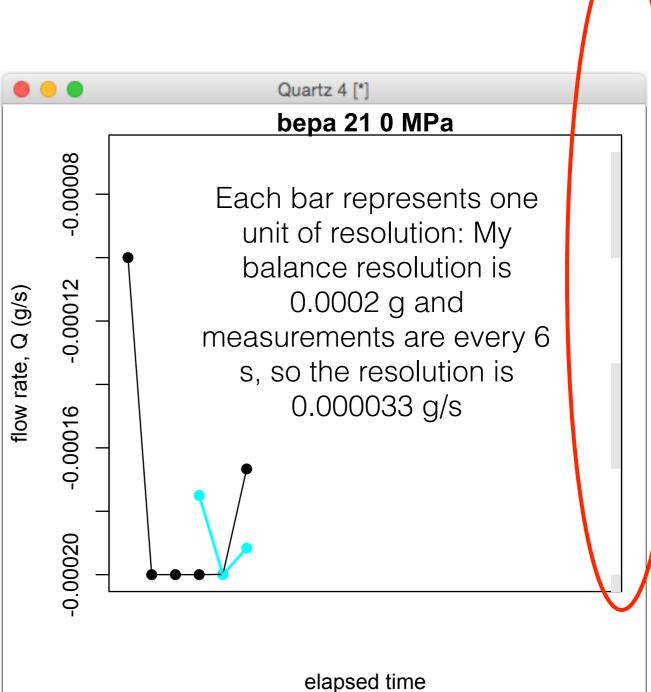


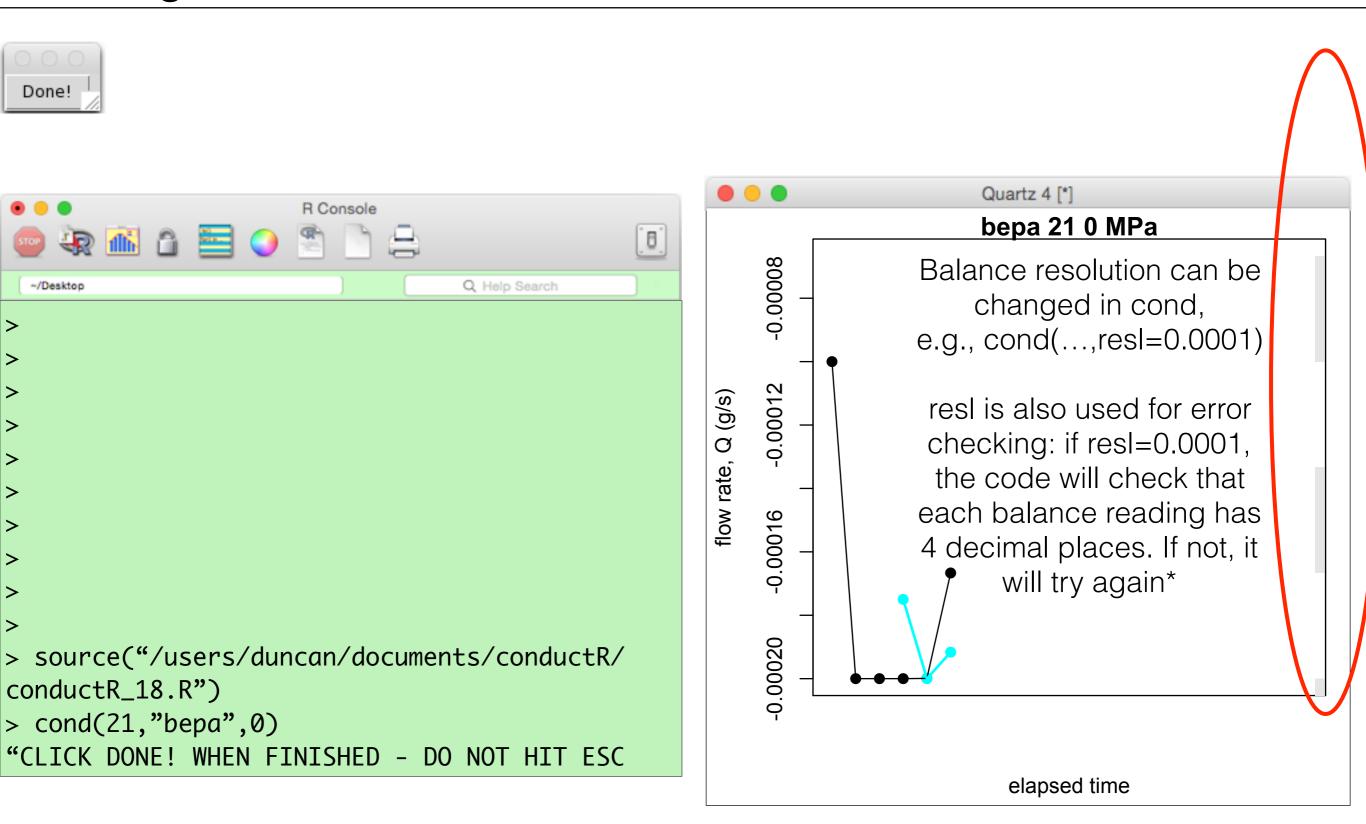


^{*}here, rates are shown as g/s. In the newer version, mg/s is shown instead for ease of interpretation. Data are still recorded in g though.

^{**}see Appendices A & E for details

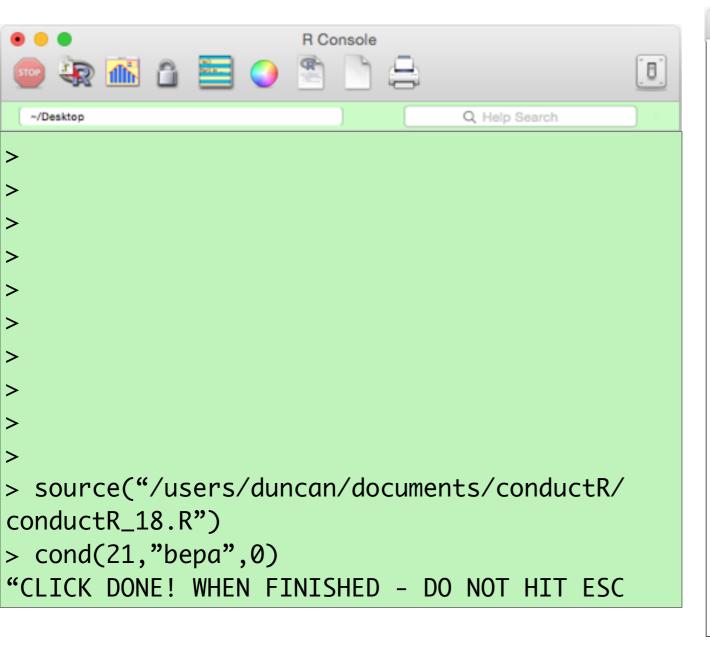


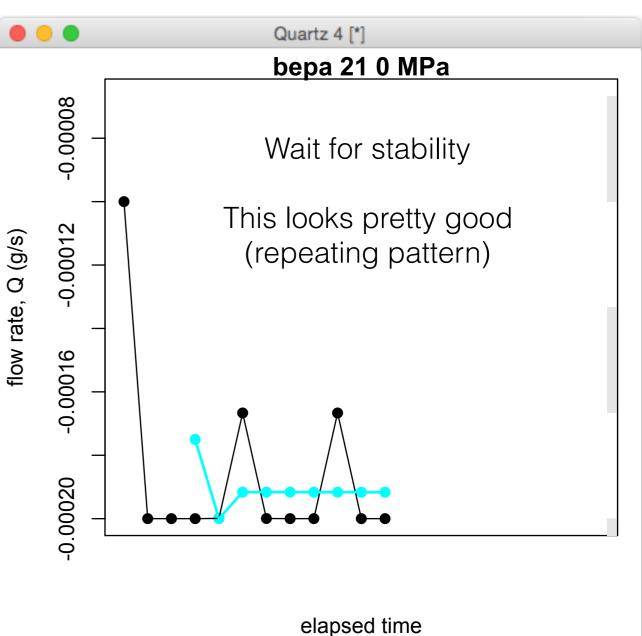


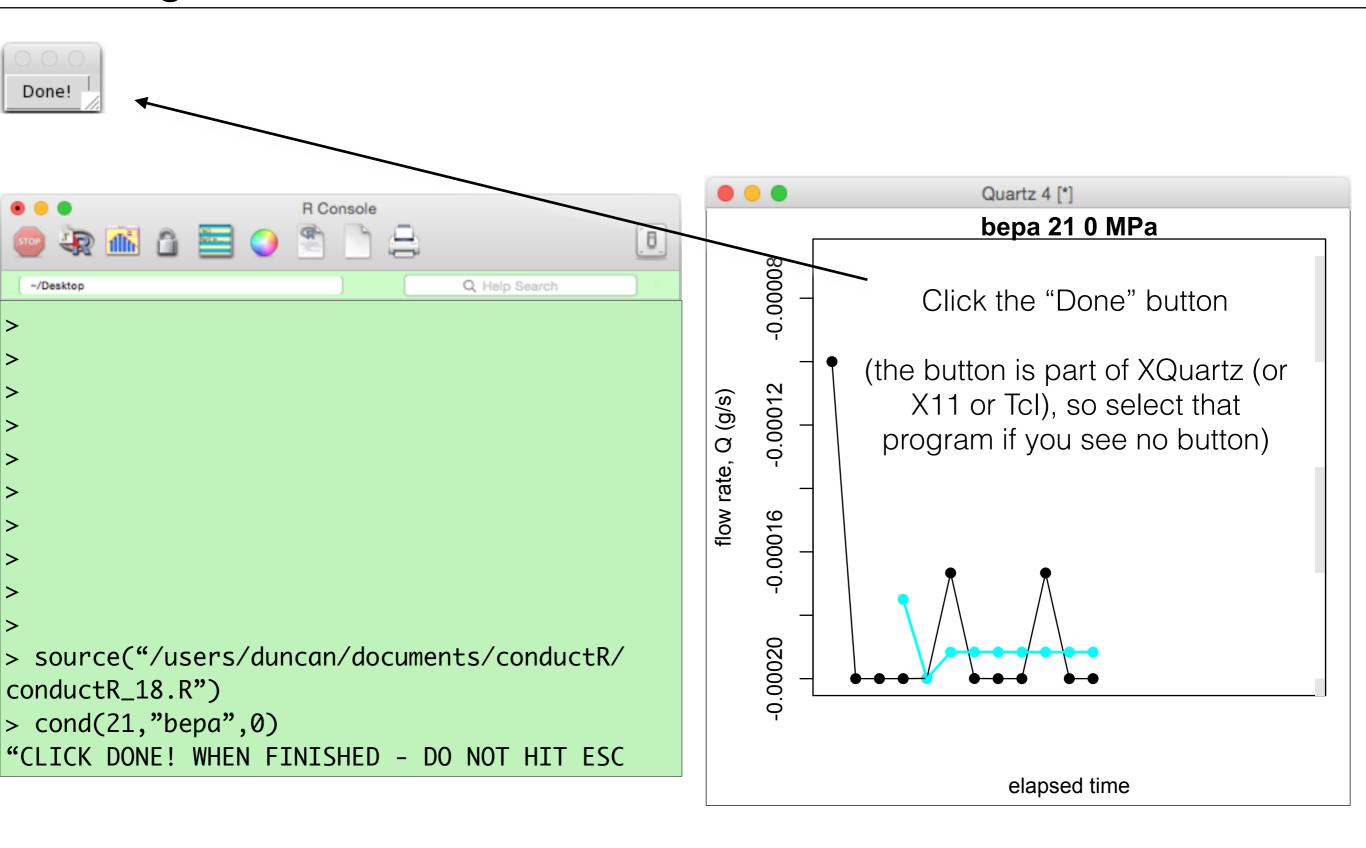


^{*}In my experience, a Sartorius will occasionally drop the last digit, which if undetected, leads to a spike in the calculated flow rate. If too few decimals were received, the program will tell you this in the console.

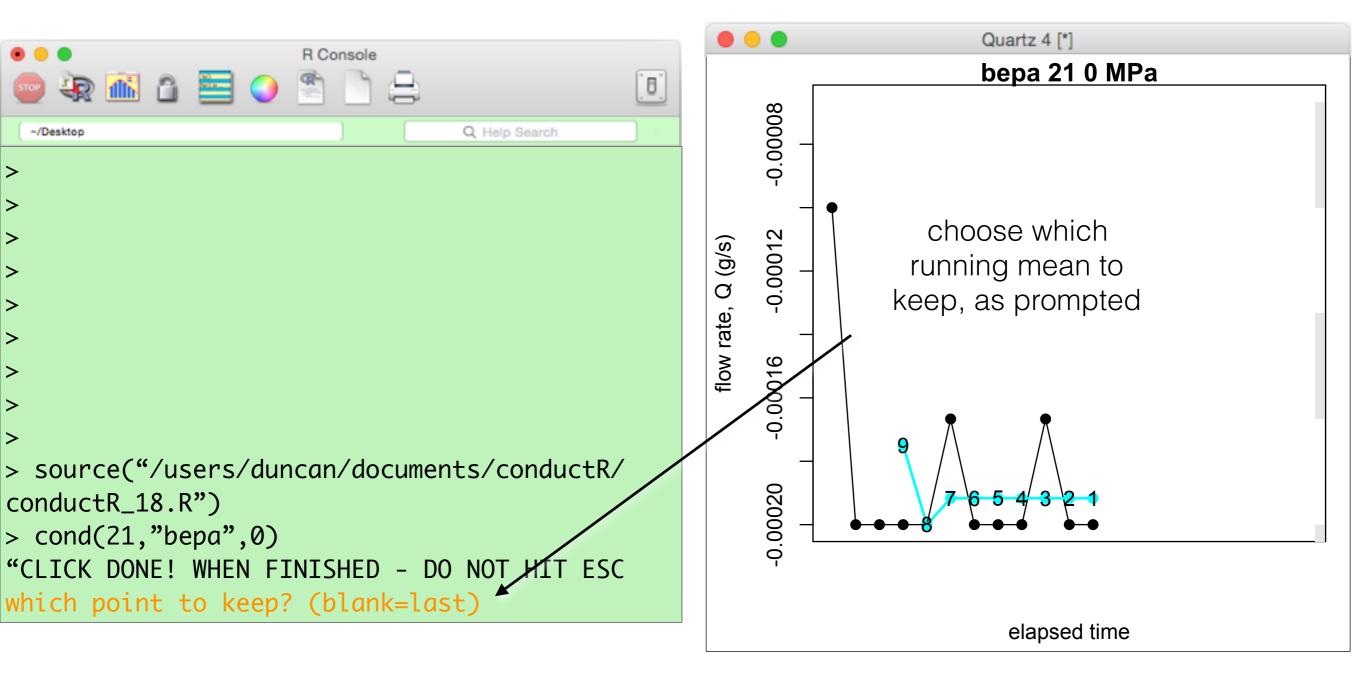


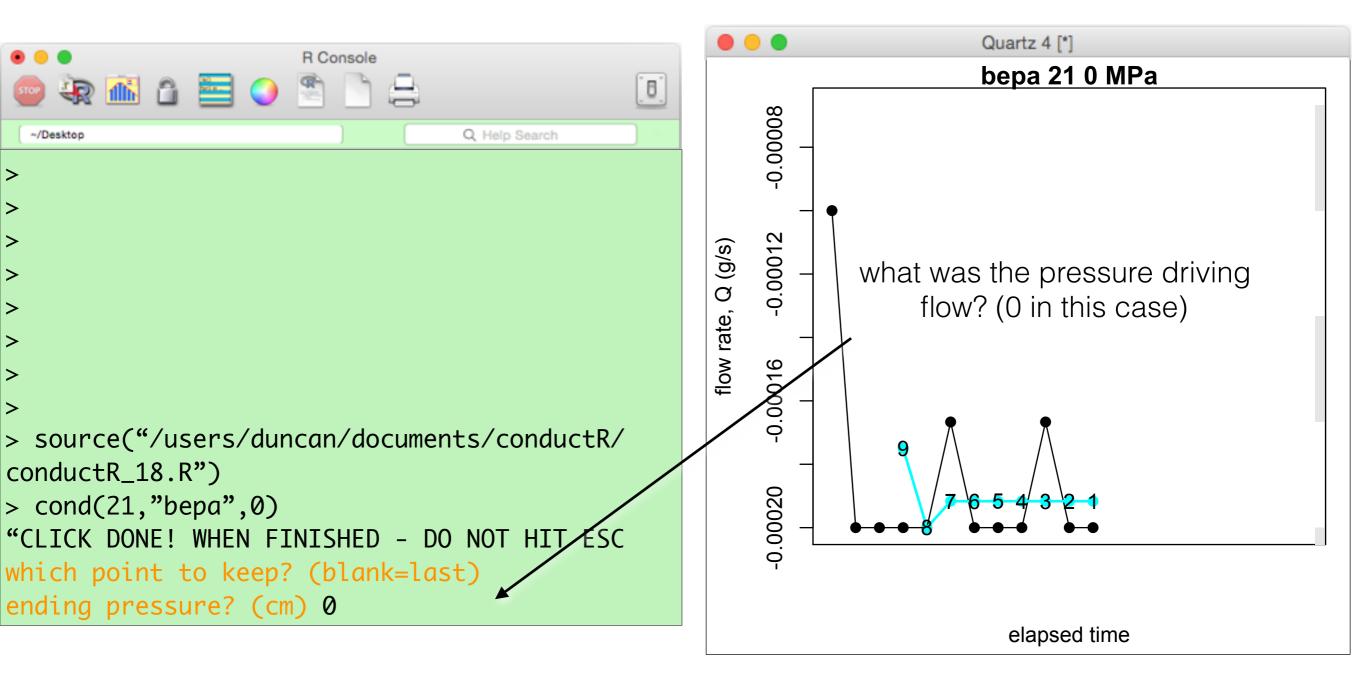


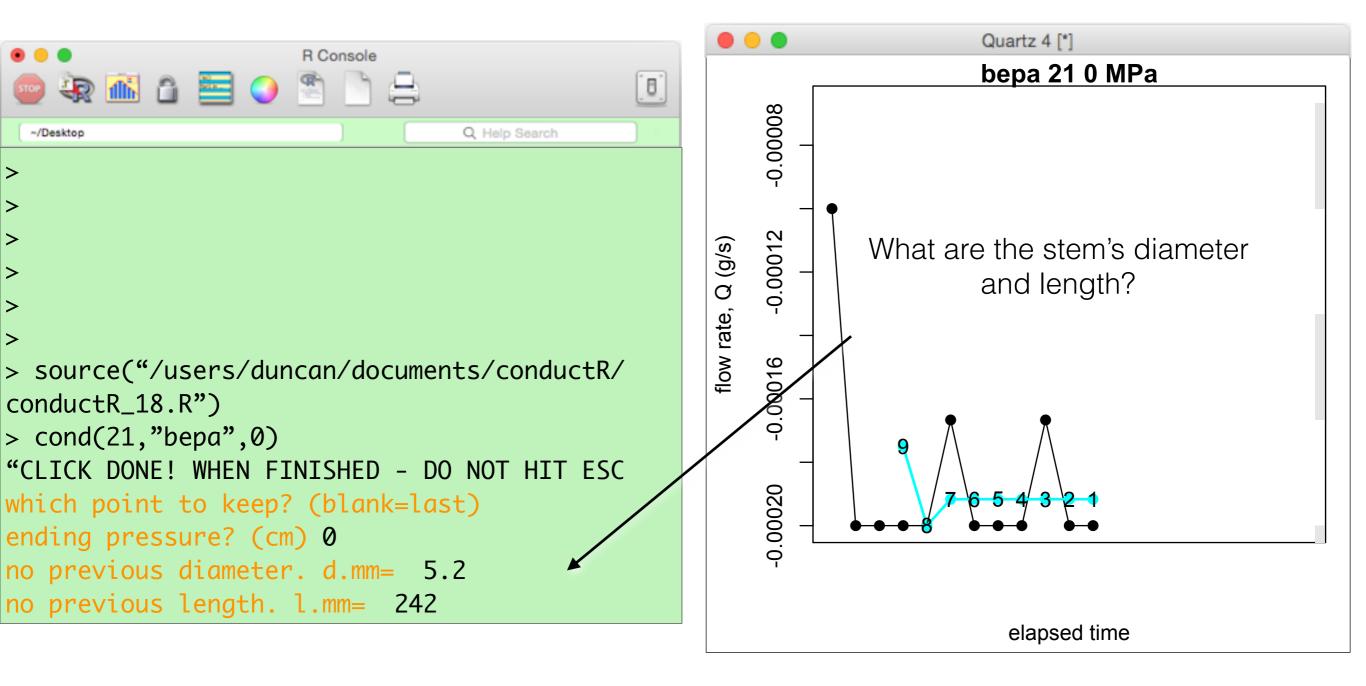


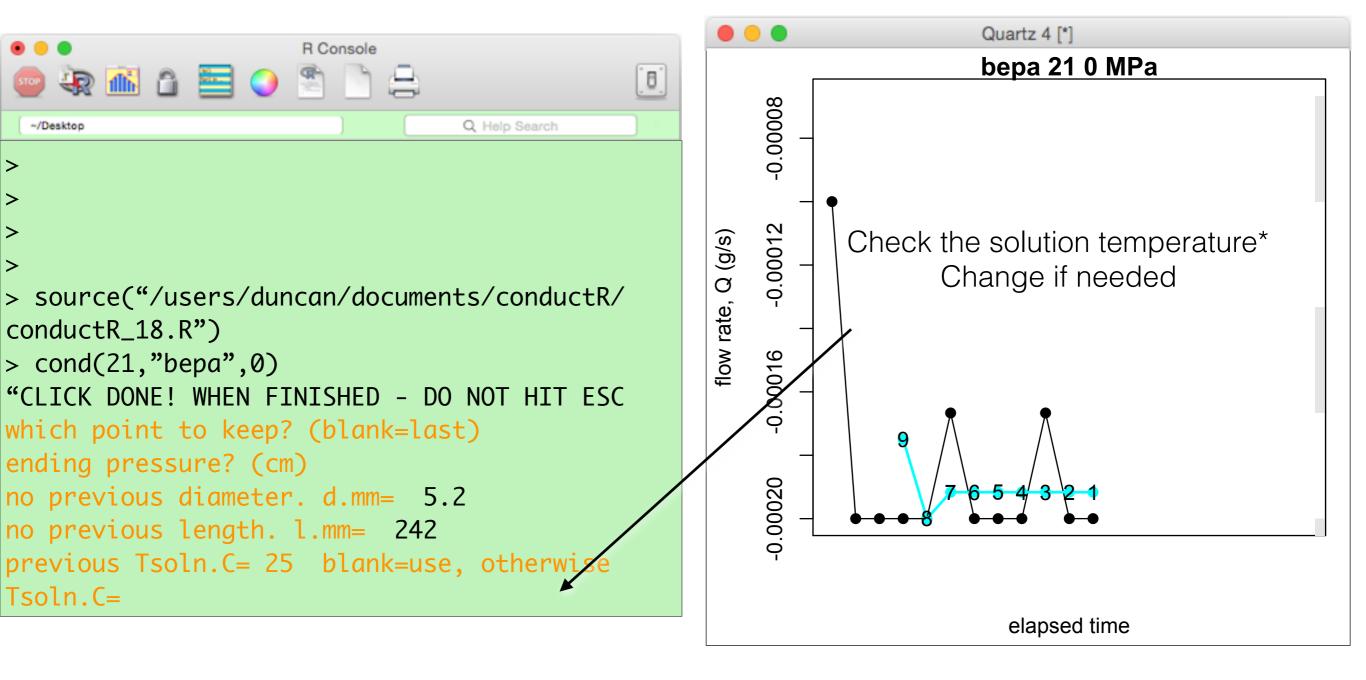




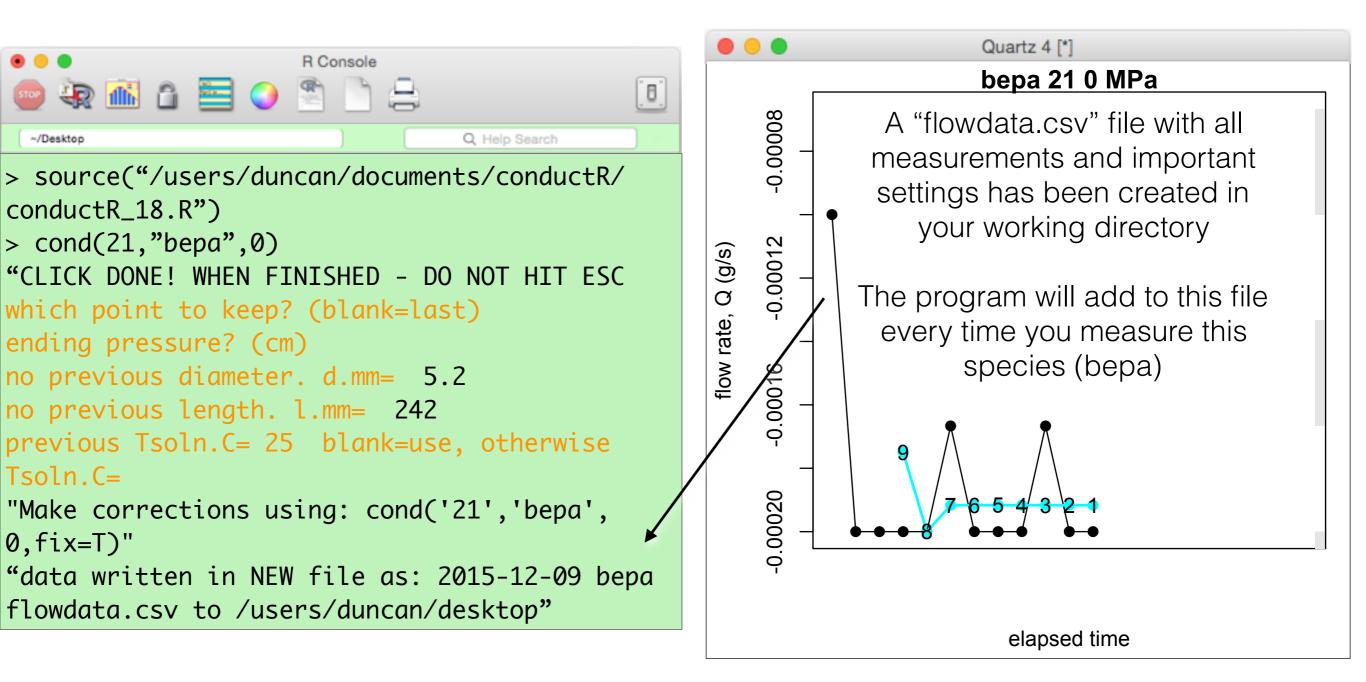




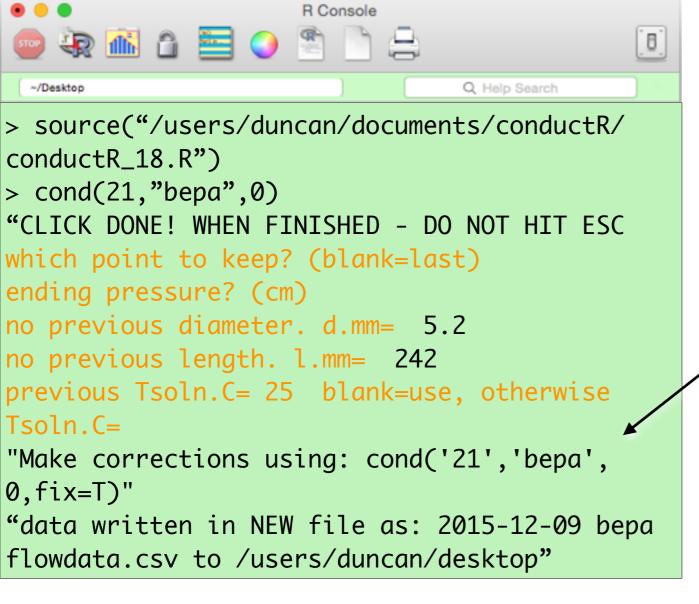


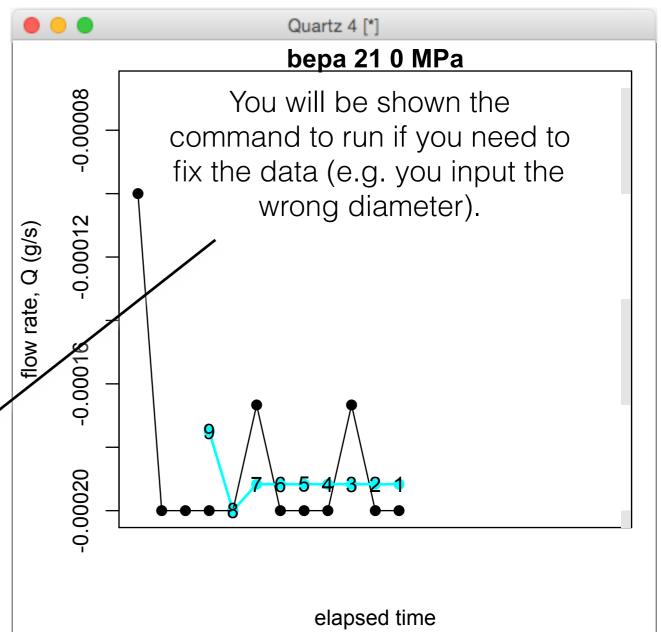


^{*25} C is the default. Conductance will be standardized to 20 C later.

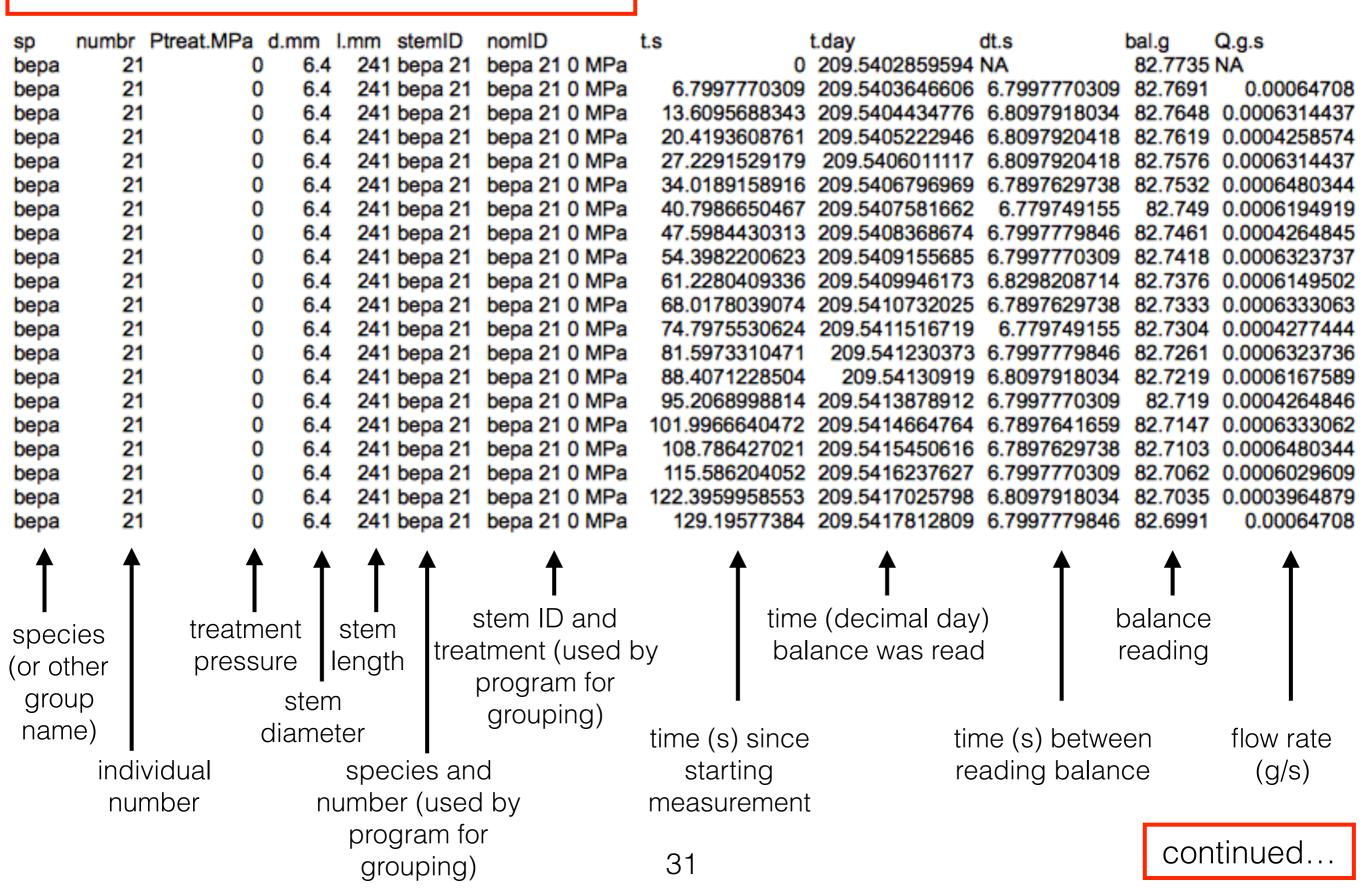


Note that date is added to the file name just to have some unique identifier for your convenience. The program ignores this, so change it if desired.

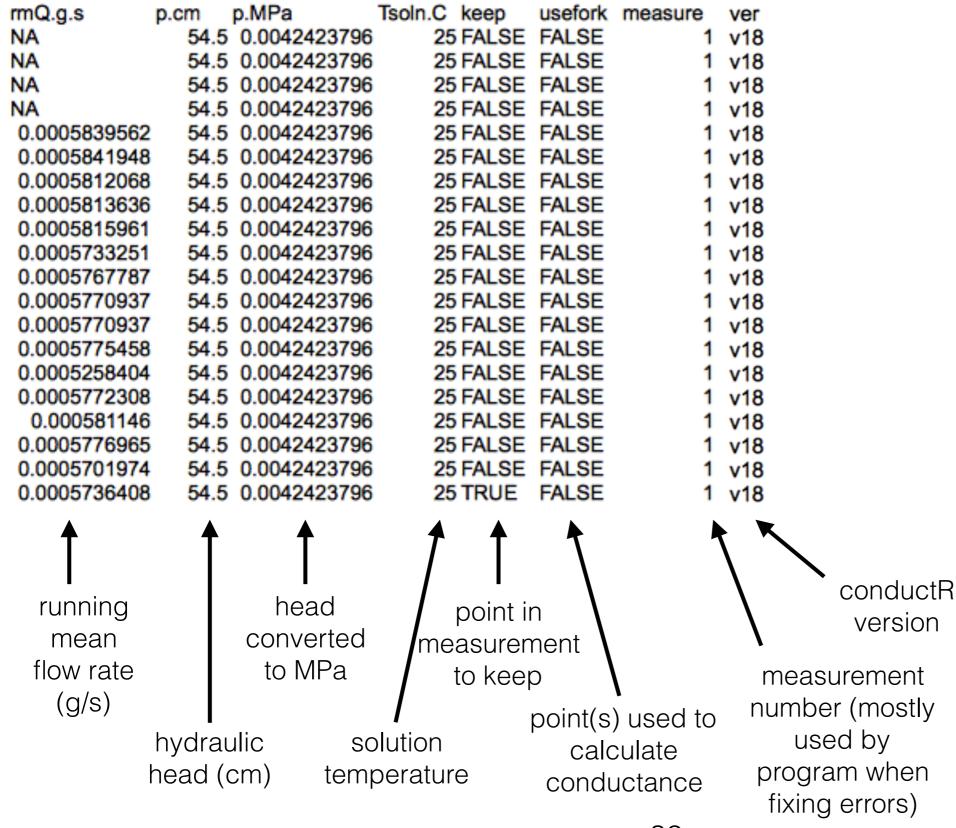


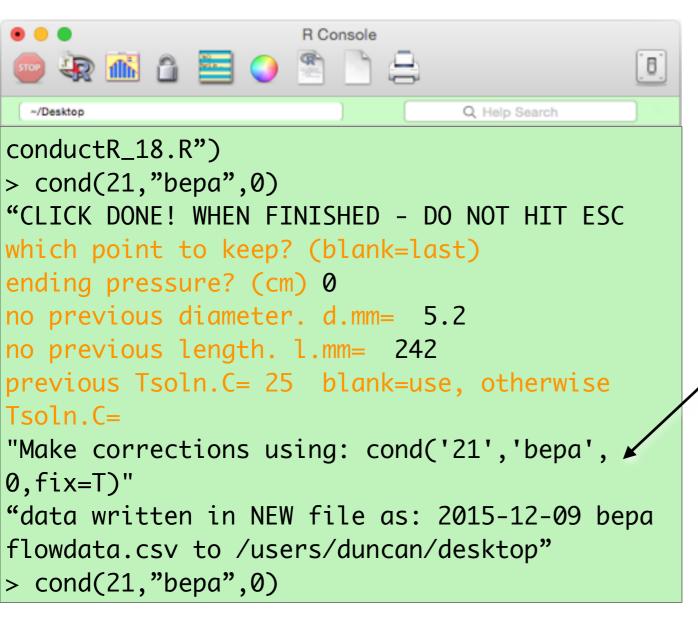


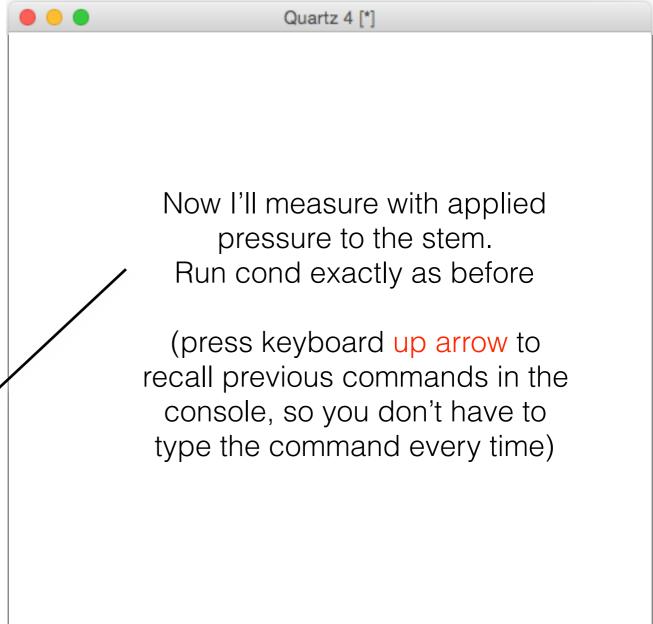
The saved flowdata.csv file looks like this



The saved flowdata.csv file looks like this

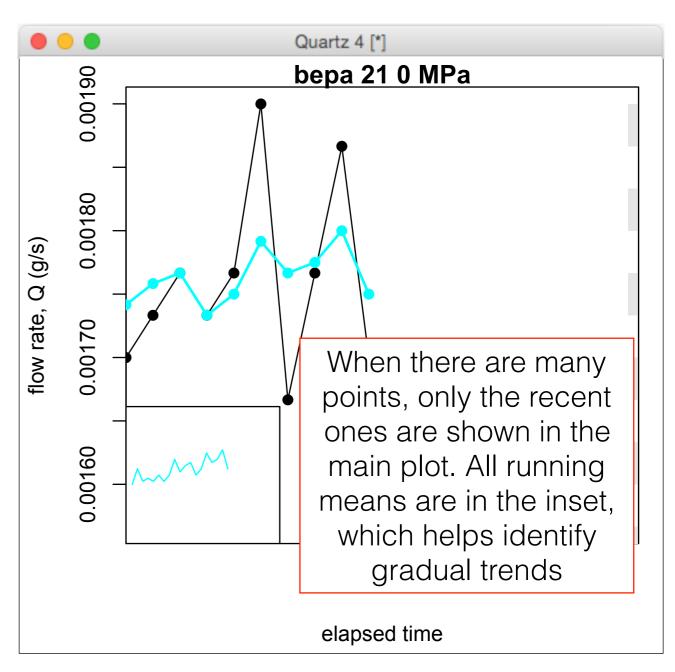




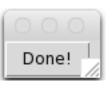




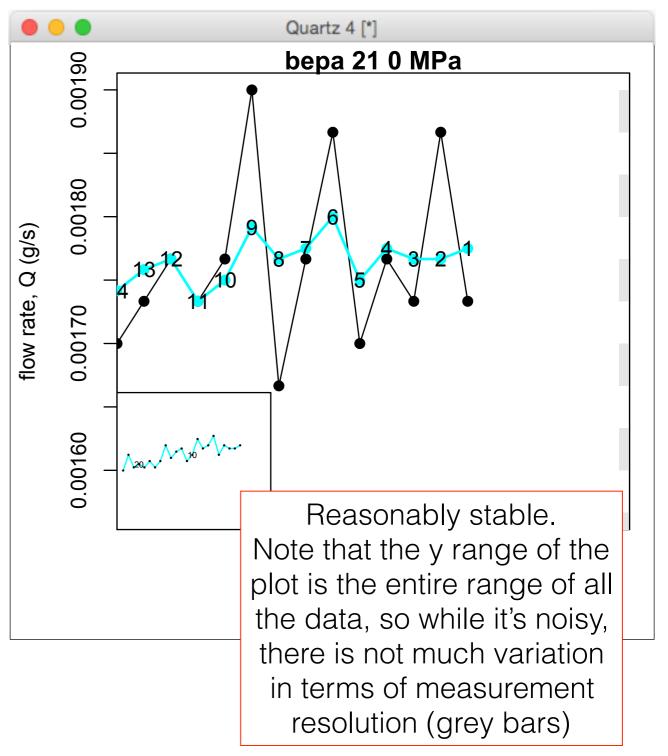
```
R Console
                                              8
                                 Q Help Search
> cond(21,"bepa",0)
"CLICK DONE! WHEN FINISHED - DO NOT HIT ESC
which point to keep? (blank=last)
ending pressure? (cm) 0
no previous diameter. d.mm= 5.2
no previous length. l.mm= 242
previous Tsoln.C= 25 blank=use, otherwise
Tsoln.C=
"Make corrections using: cond('21','bepa',
0, fix=T)"
"data written in NEW file as: 2015-12-09 bepa
flowdata.csv to /users/duncan/desktop"
> cond(21,"bepa",0)
"CLICK DONE! WHEN FINISHED - DO NOT HIT ESC!"
```

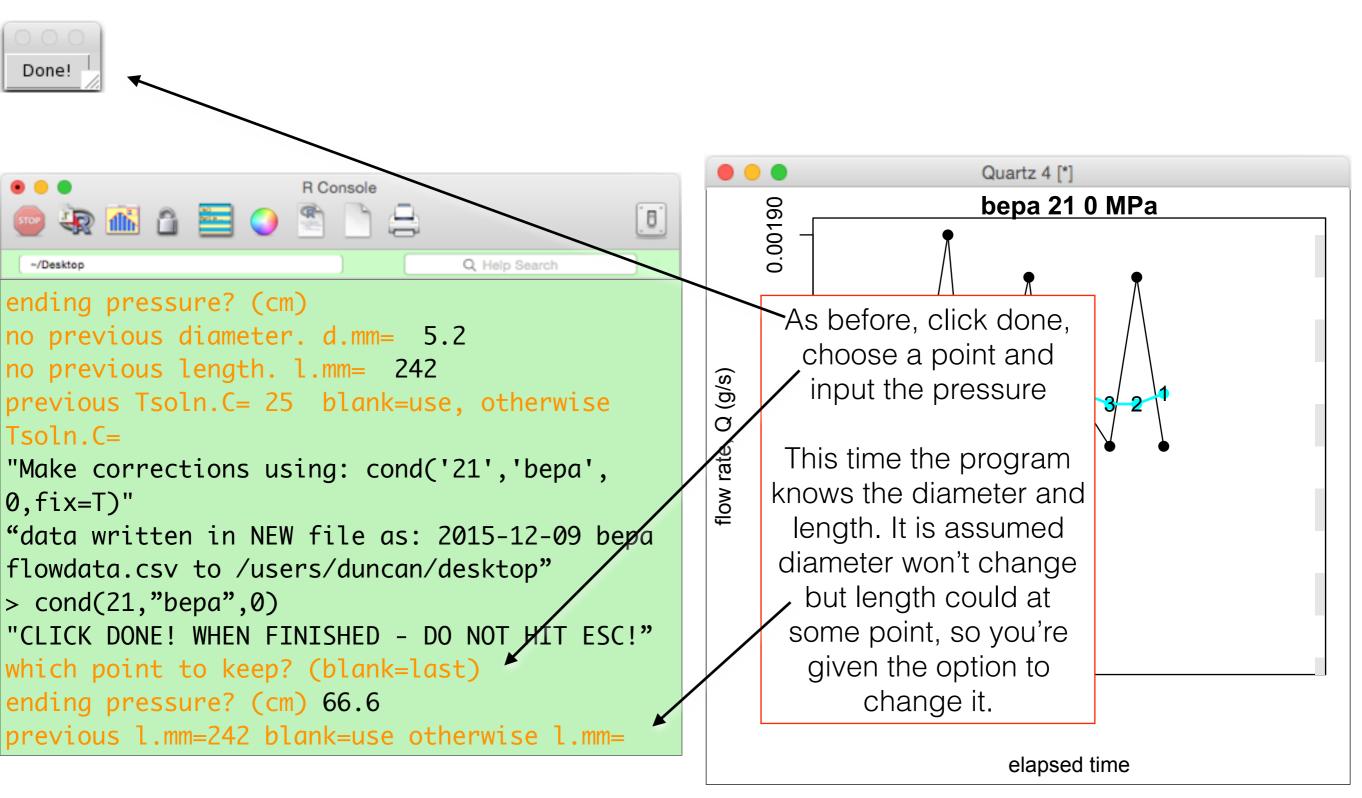


Inset size (as a fraction of total plot size) is controlled by ovrl in cond. See Appendix A



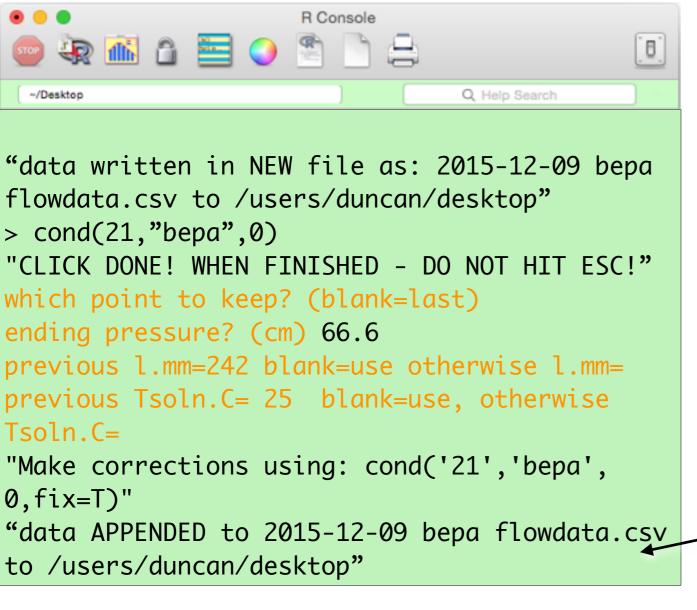
```
R Console
                                              8
                                 Q Help Search
> cond(21,"bepa",0)
"CLICK DONE! WHEN FINISHED - DO NOT HIT ESC
which point to keep? (blank=last)
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previous Tsoln.C= 25 blank=use, otherwise
Tsoln.C=
"Make corrections using: cond('21','bepa',
0, fix=T)"
"data written in NEW file as: 2015-12-09 bepa
flowdata.csv to /users/duncan/desktop"
> cond(21,"bepa",0)
"CLICK DONE! WHEN FINISHED - DO NOT HIT ESC!"
```

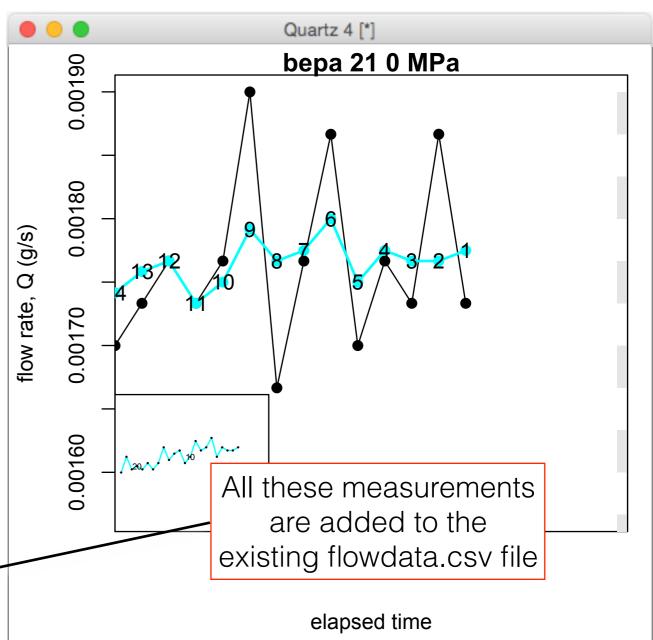


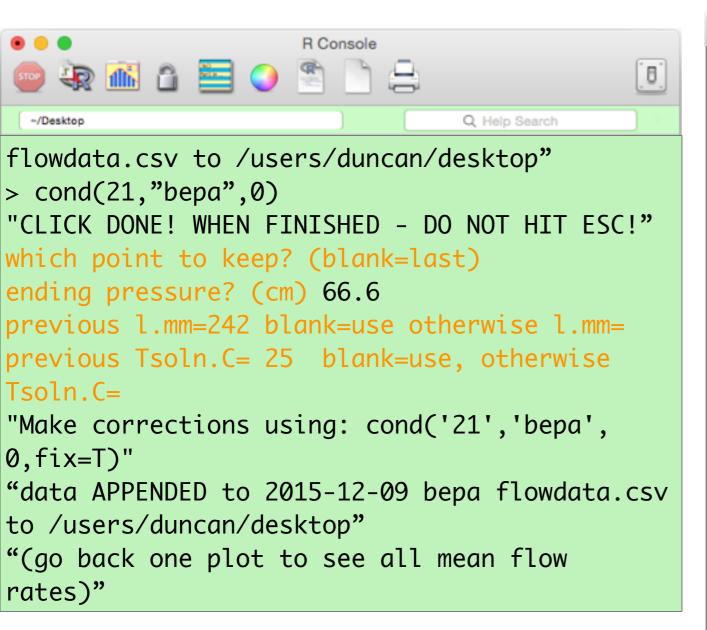


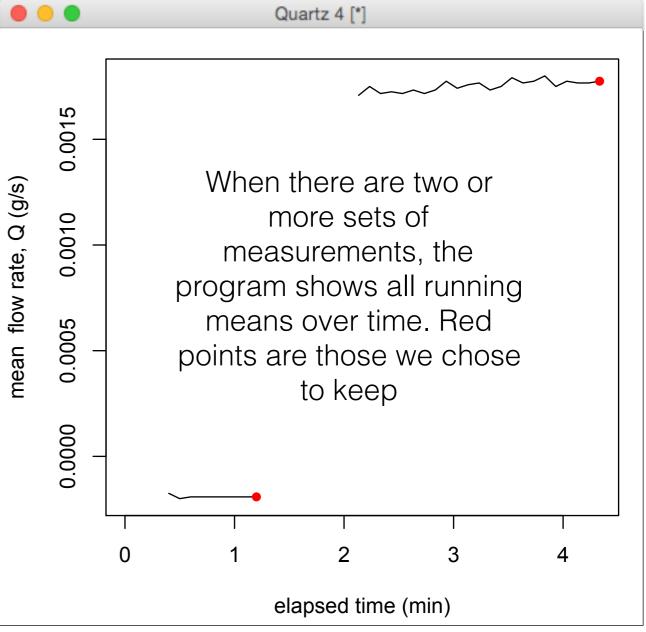
^{*}if you change length or diameter partway through a conductivity measurement, the program will use the most recent values to calculate conductivity. It is NOT assumed the physical dimensions changed during measurement.

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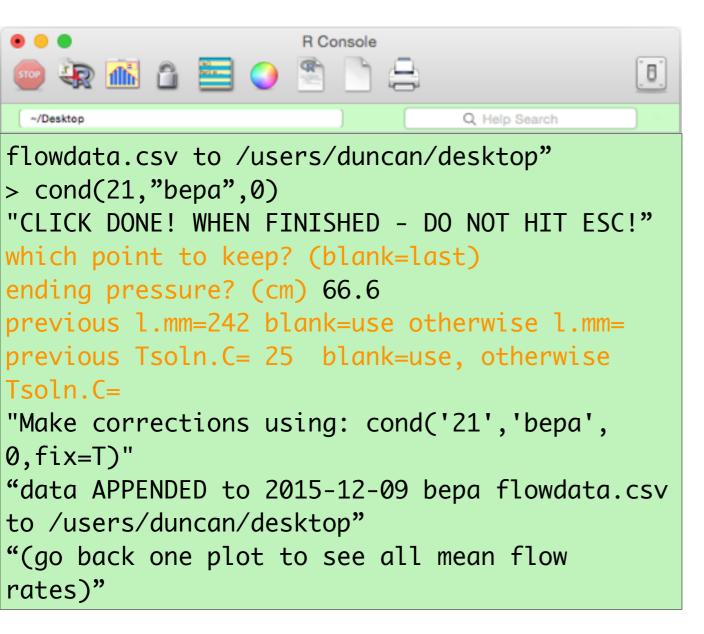


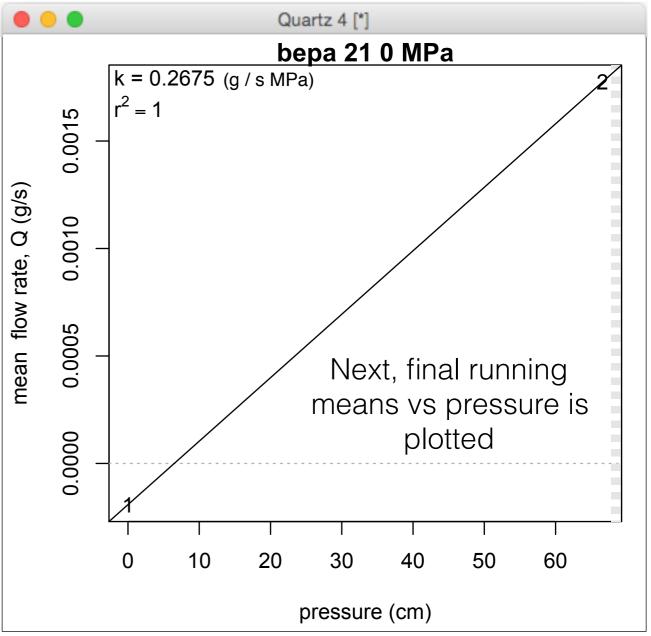


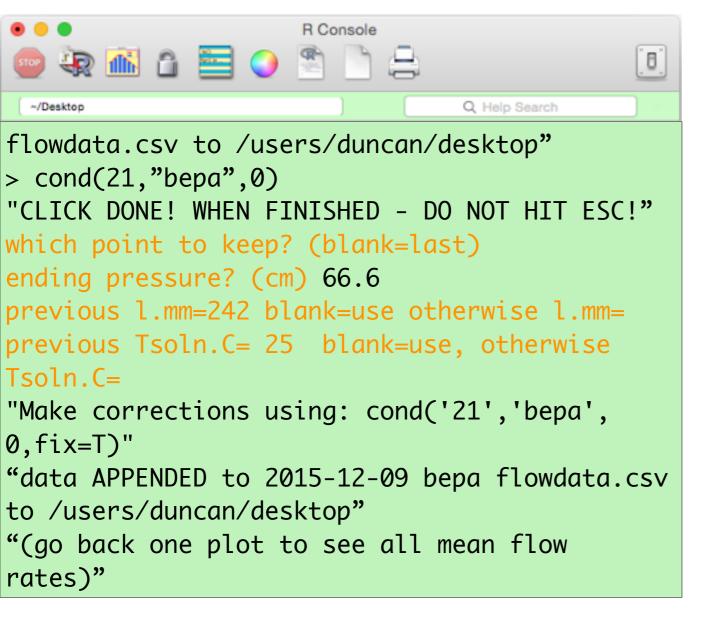


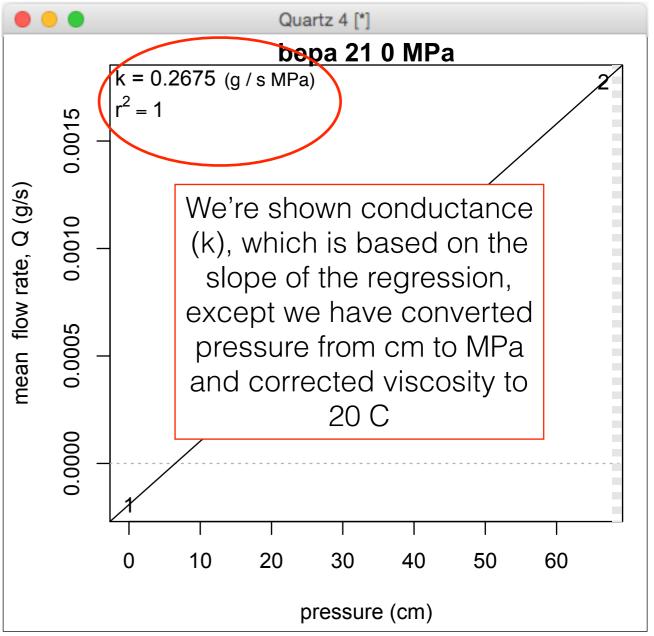


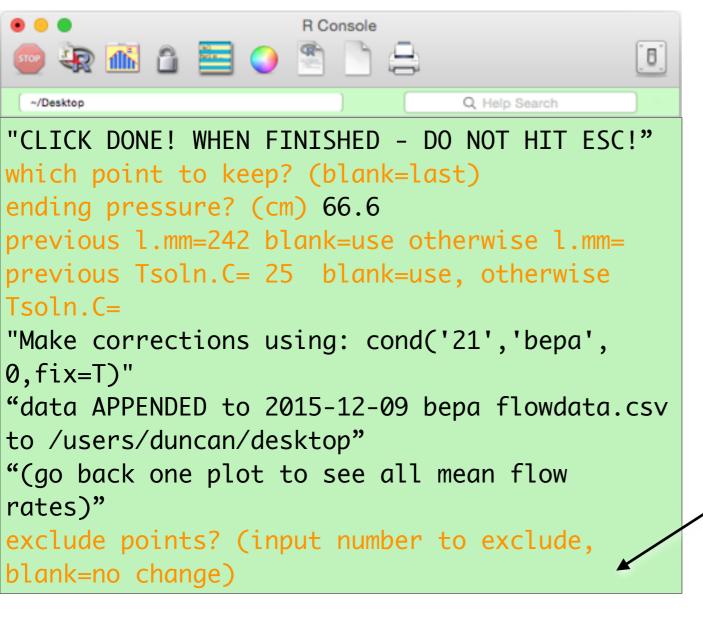
The program shows this plot for only 1 second before making the next plot. To toggle through plots, select Quartz window and use left and right arrow keys while holding **%**

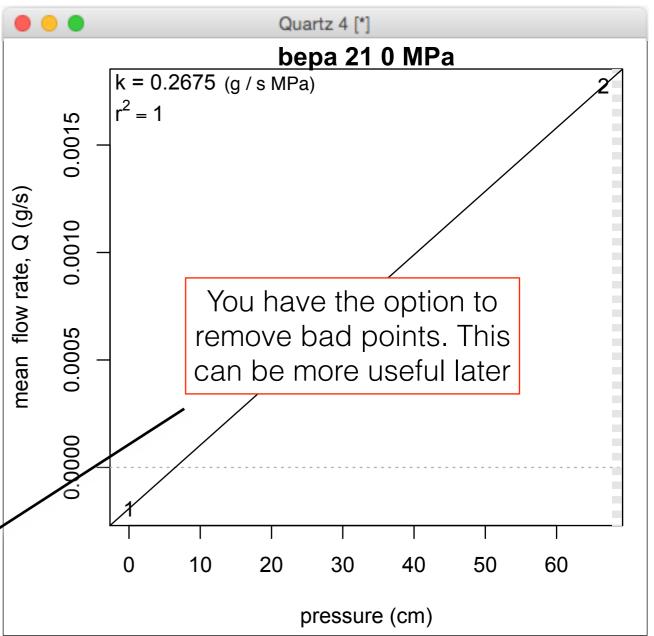


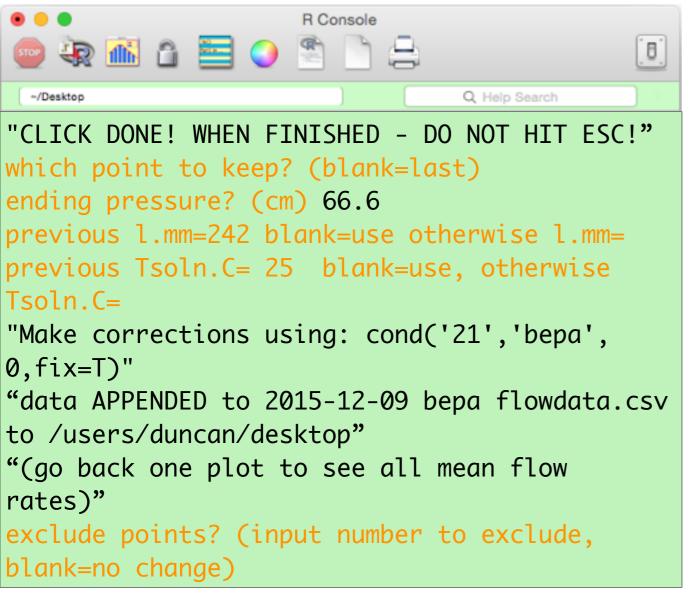


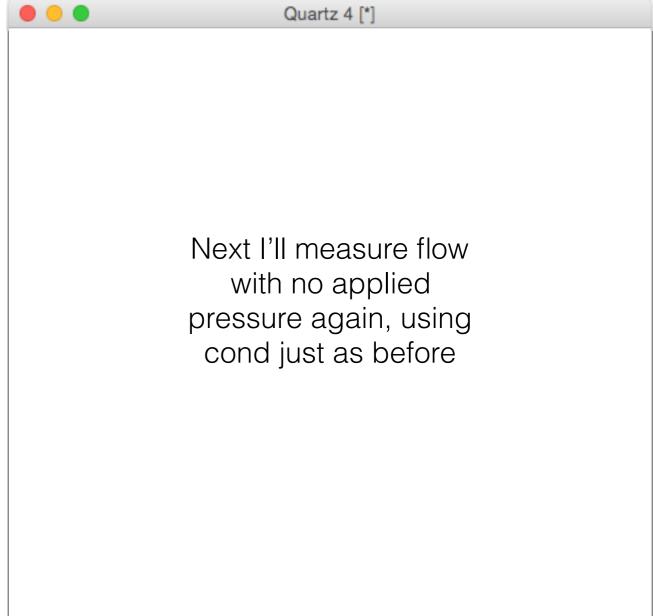


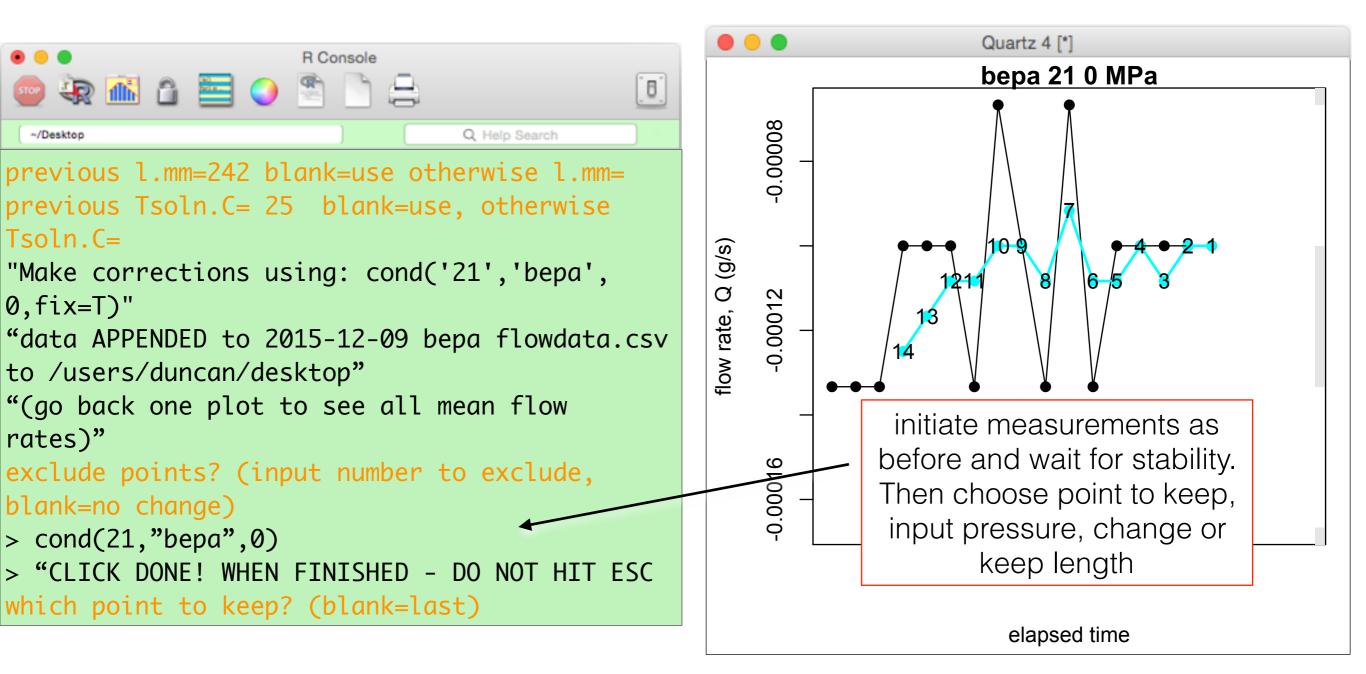


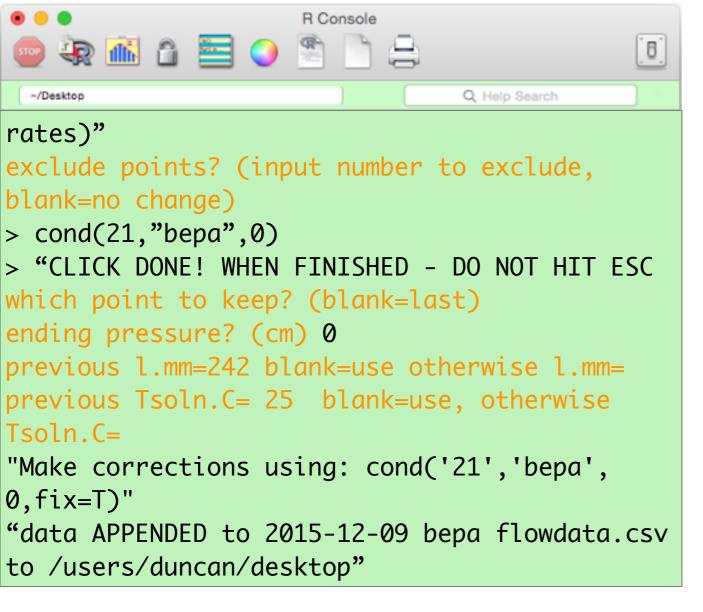


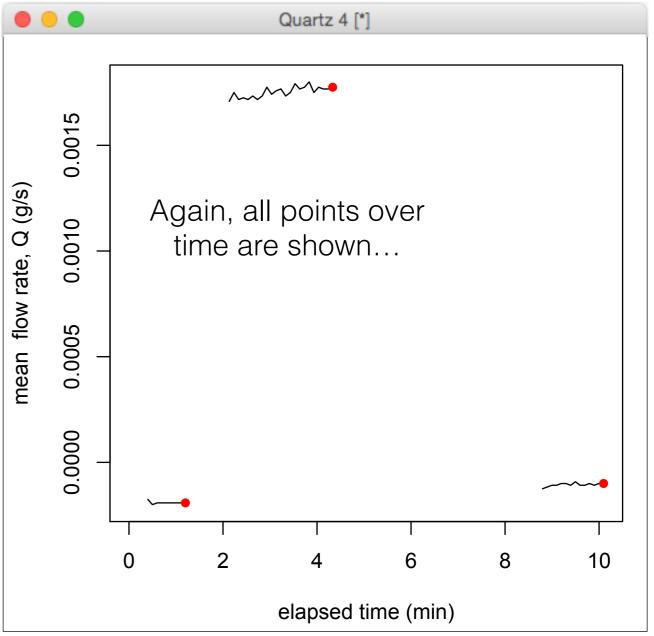


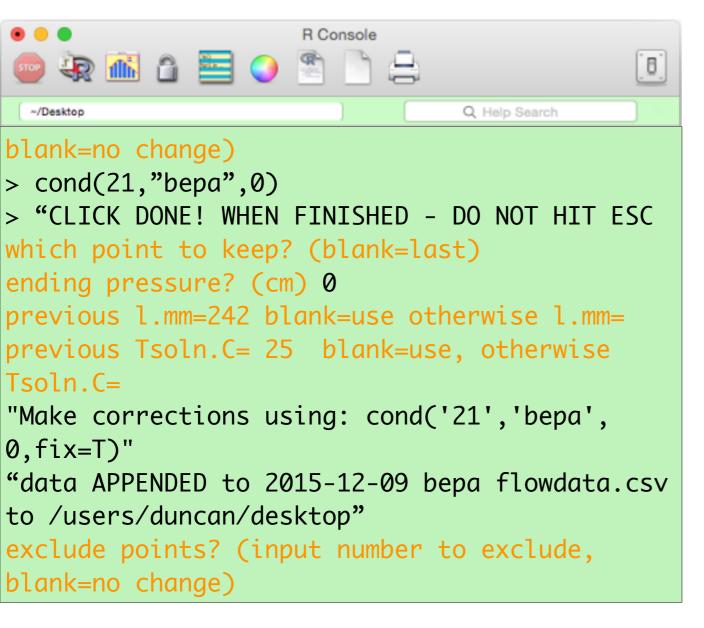


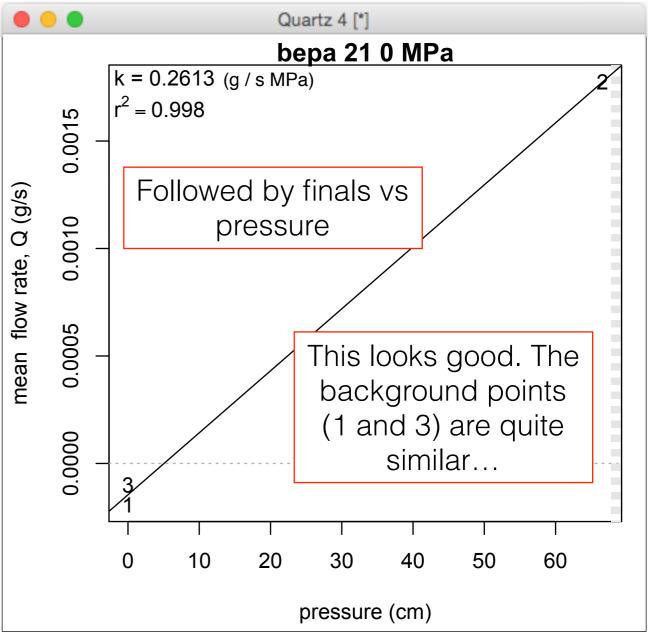


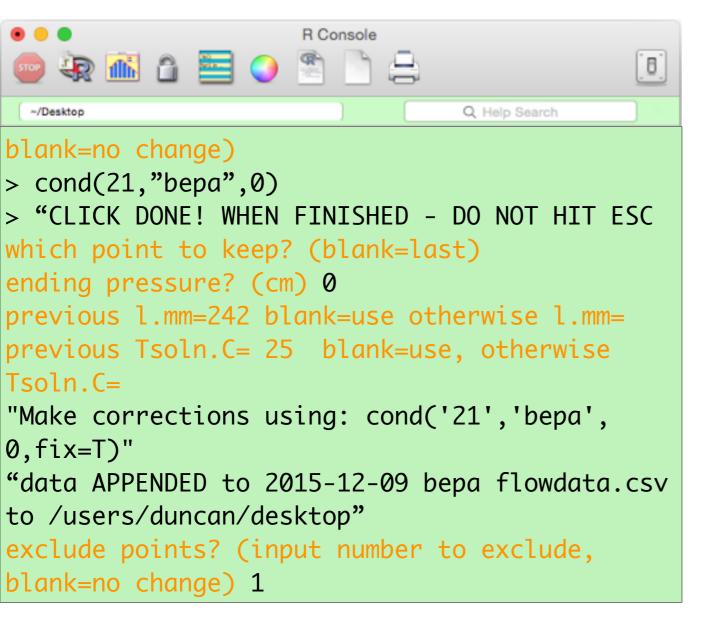


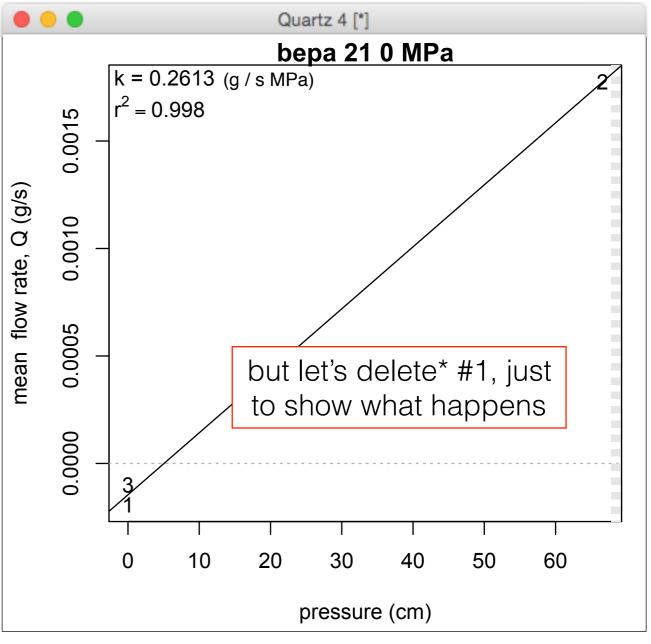




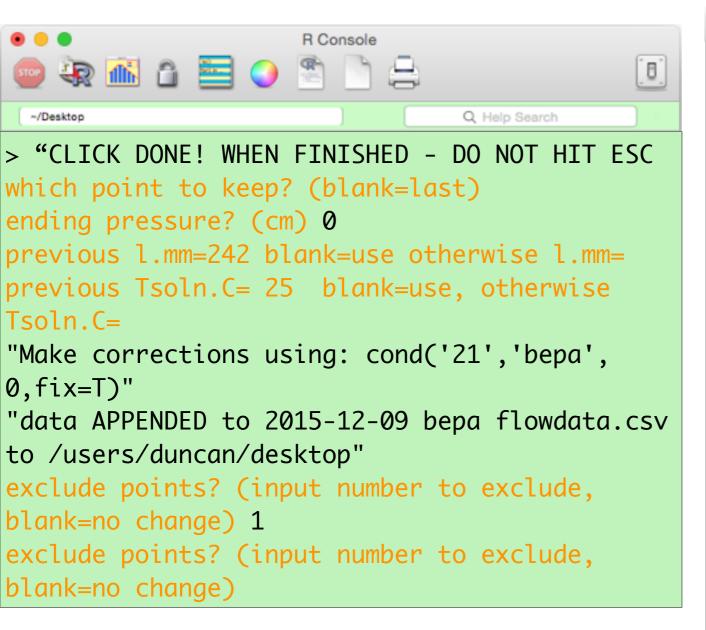


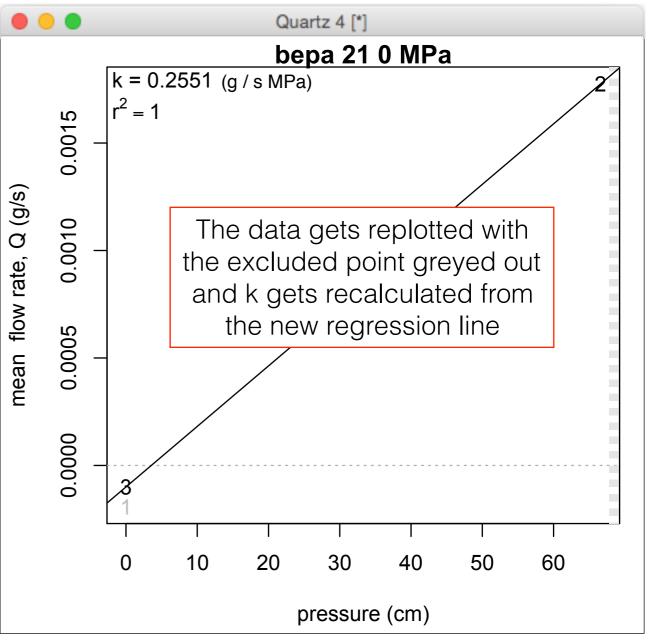


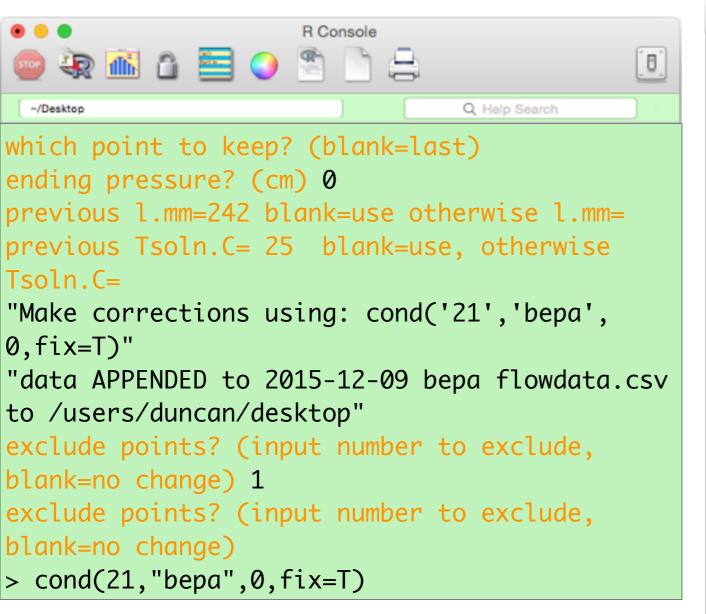


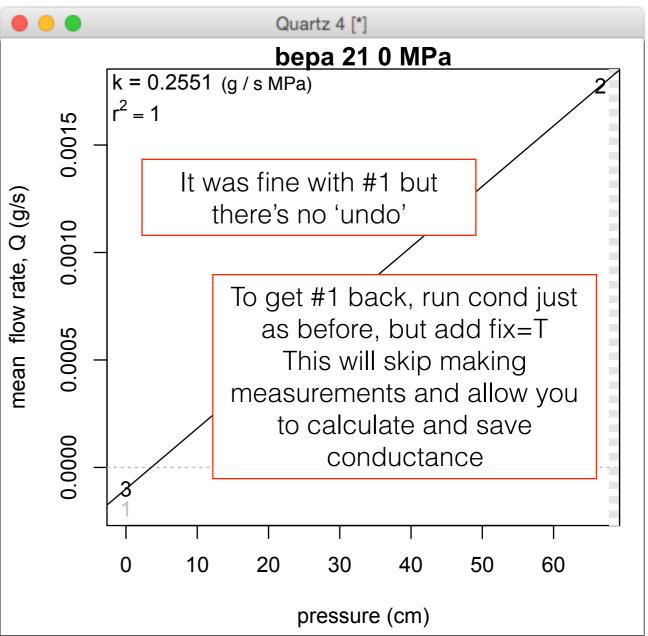


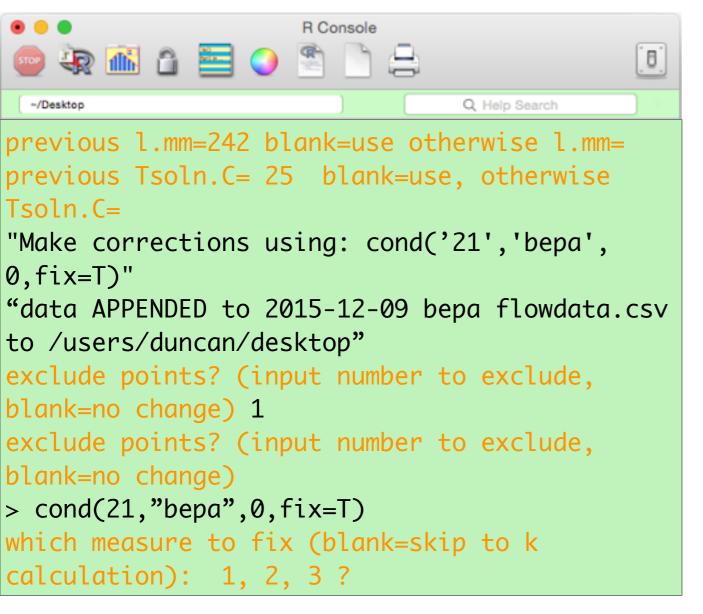
*don't worry, those data aren't really gone

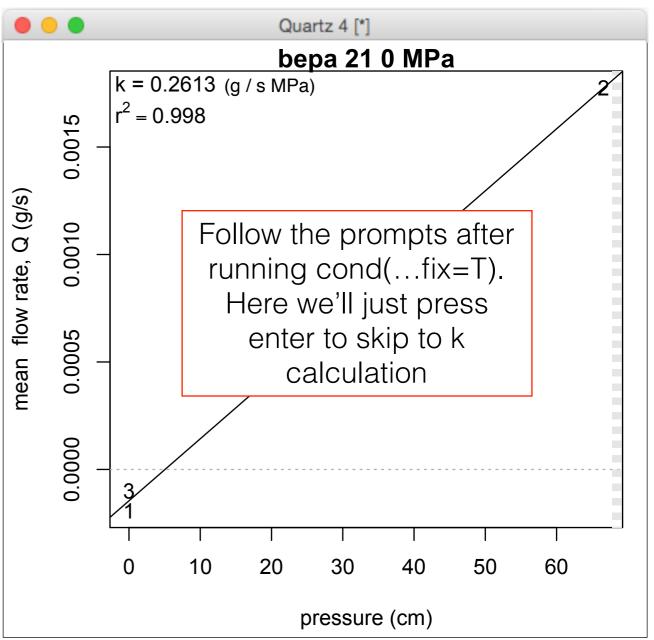


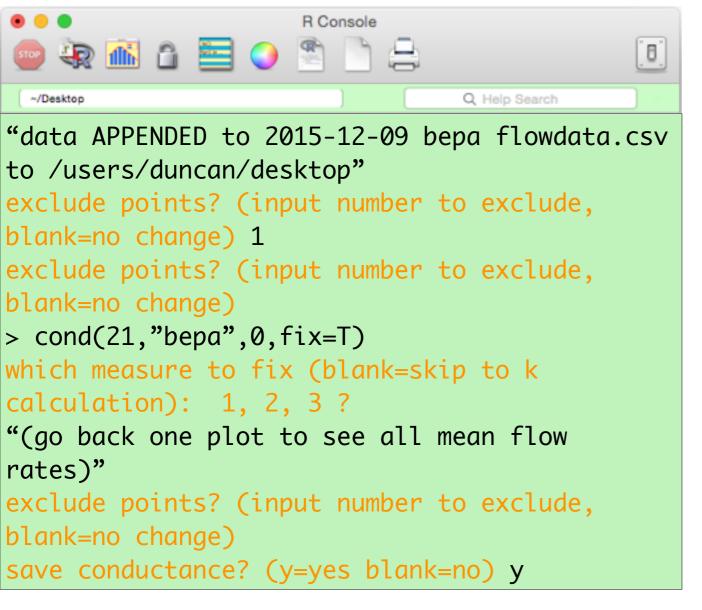


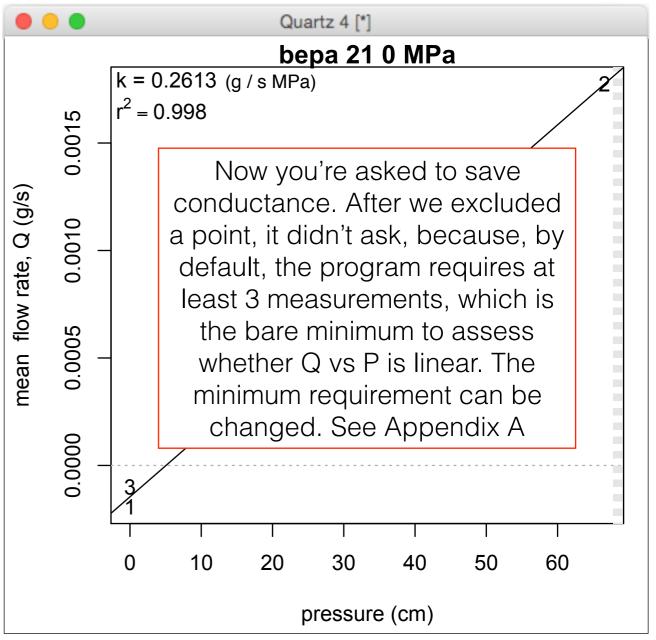


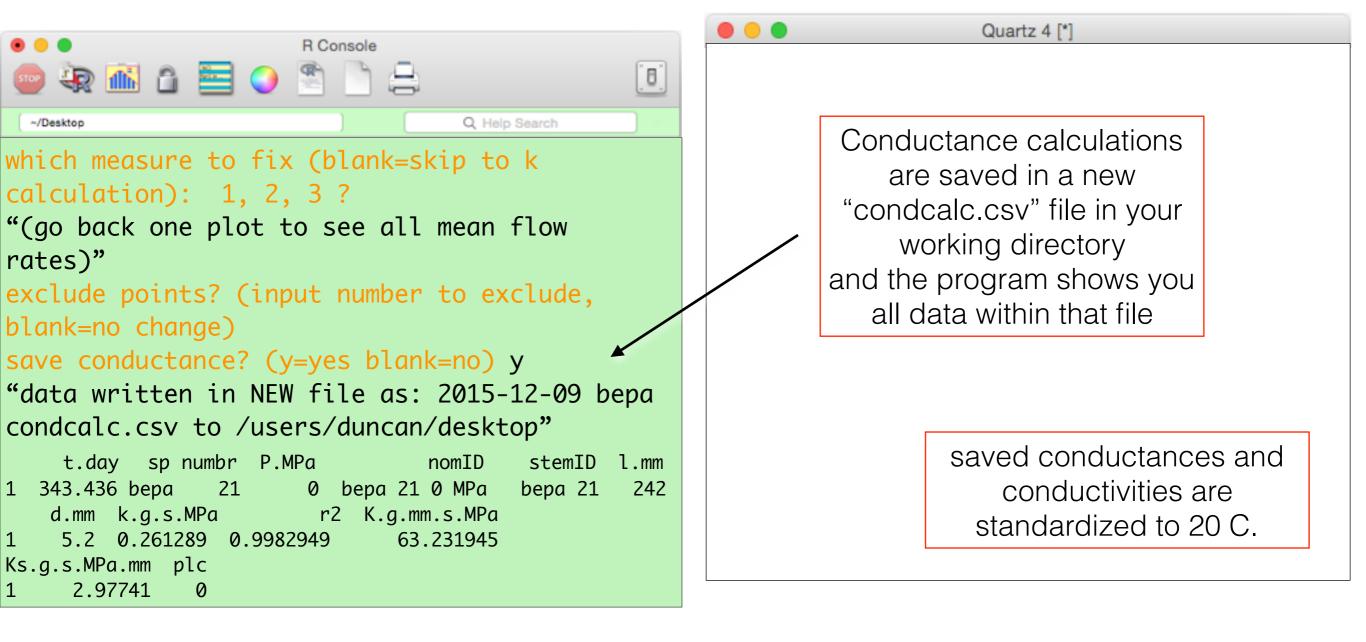






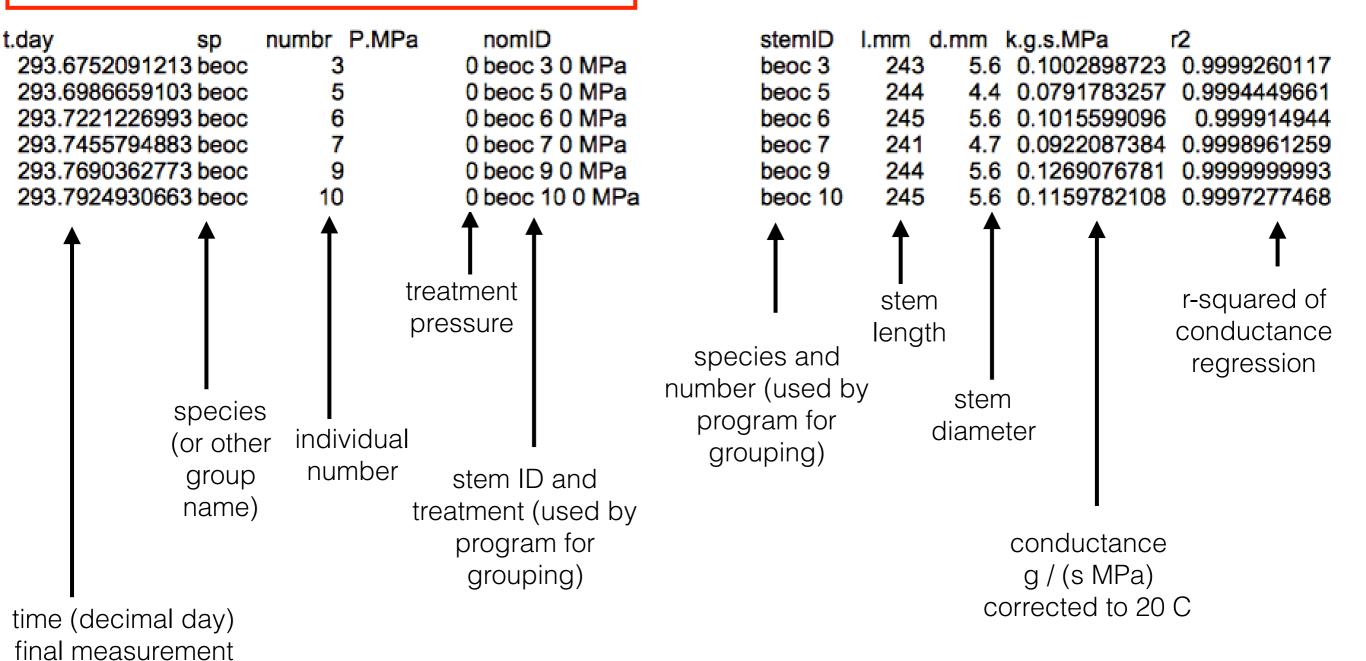




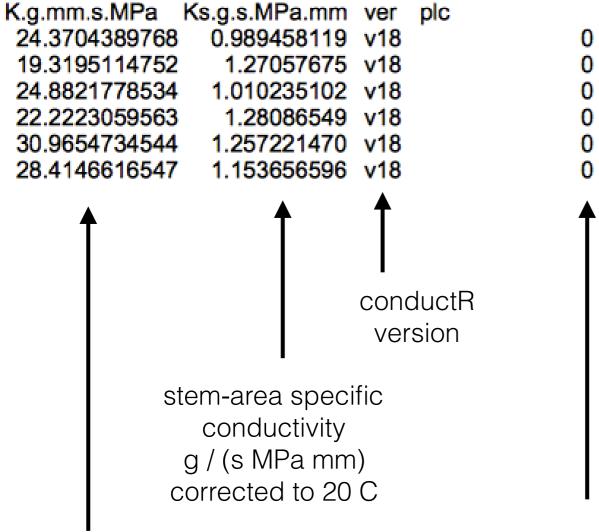


The saved condcalc.csv file looks like this

was completed

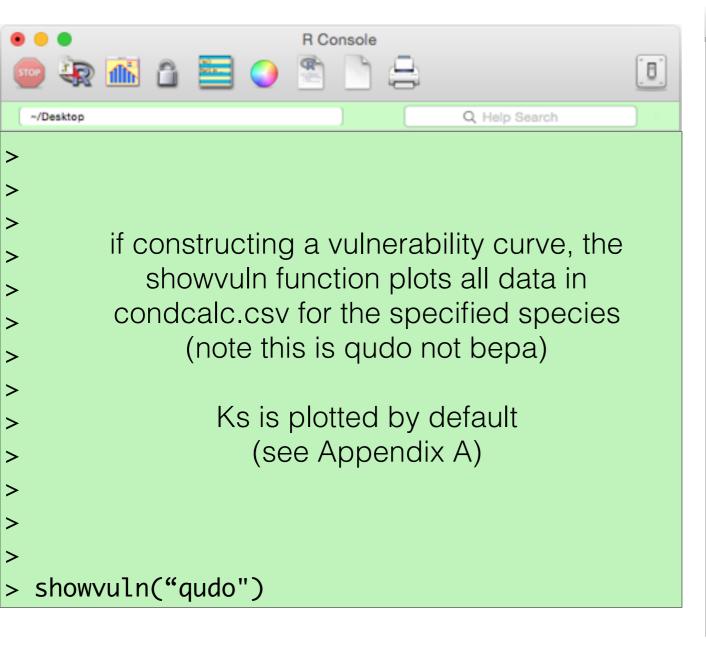


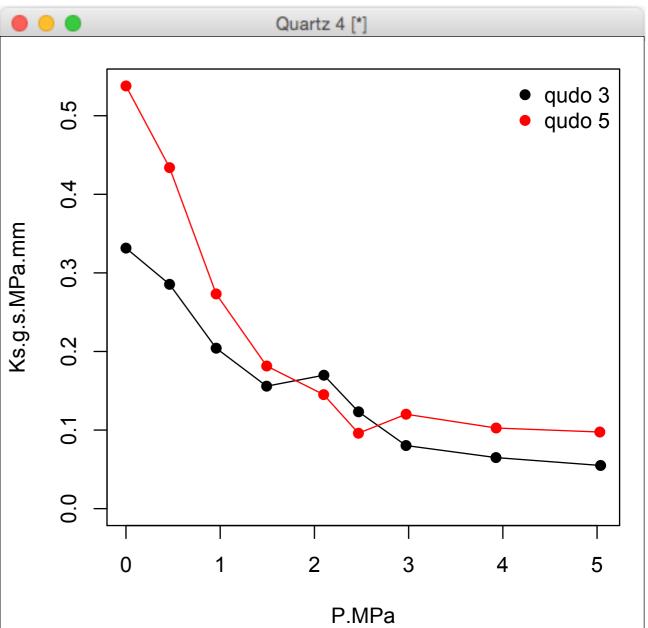
The saved condcalc.csv file looks like this



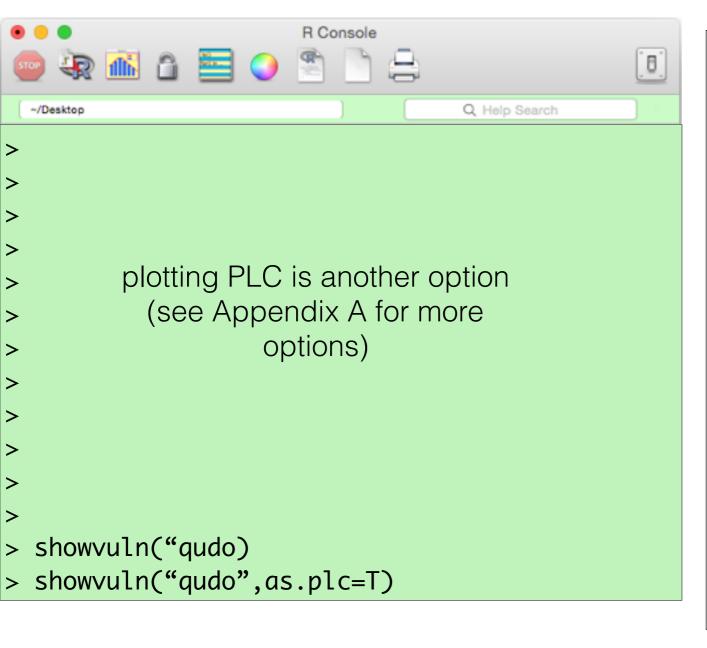
conductivity (g mm) / (s MPa) corrected to 20 C percent loss conductivity, using K at most favorable treatment pressure as maximum K.

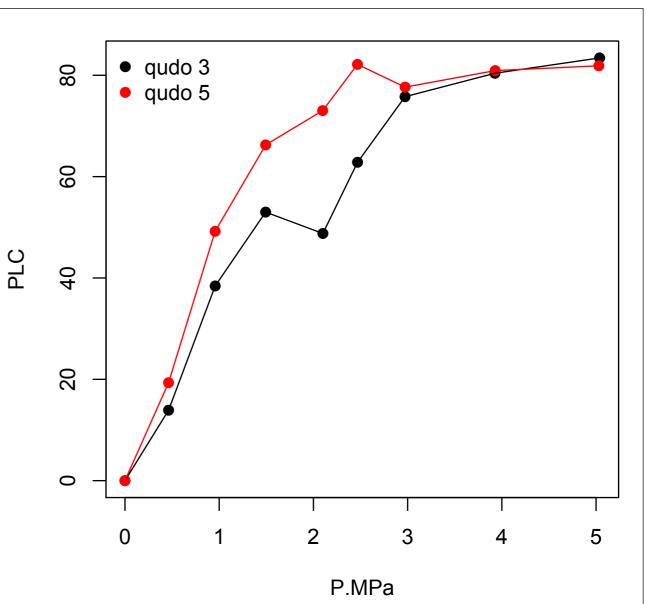
Viewing vulnerability





Viewing vulnerability

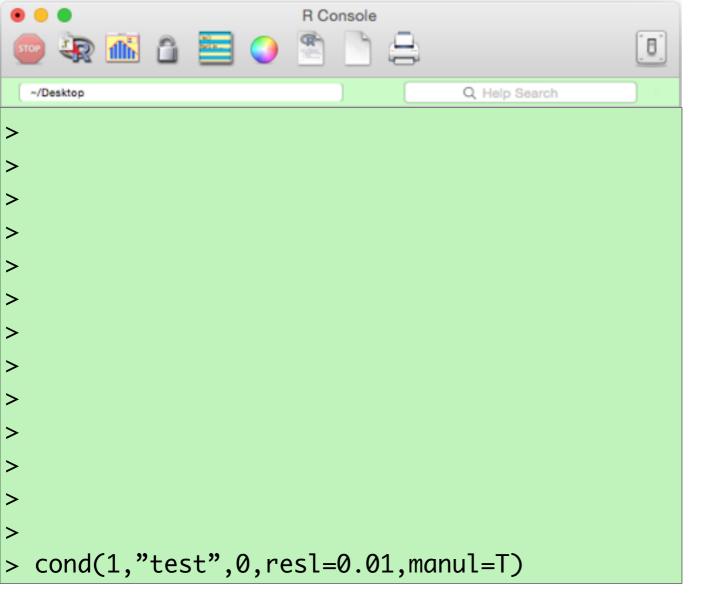


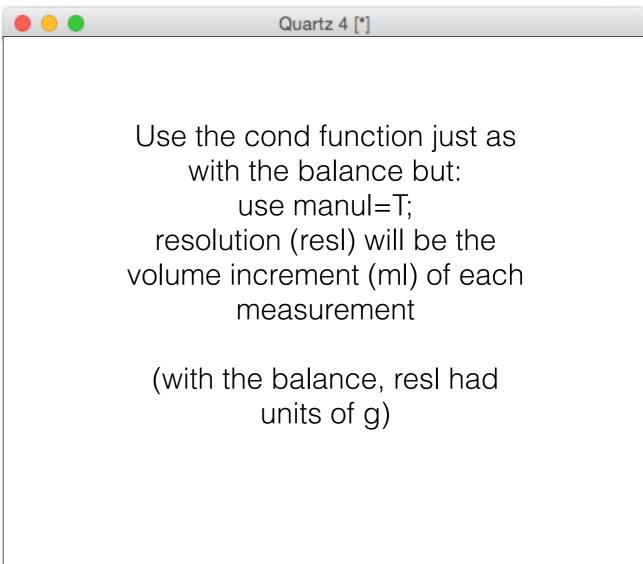


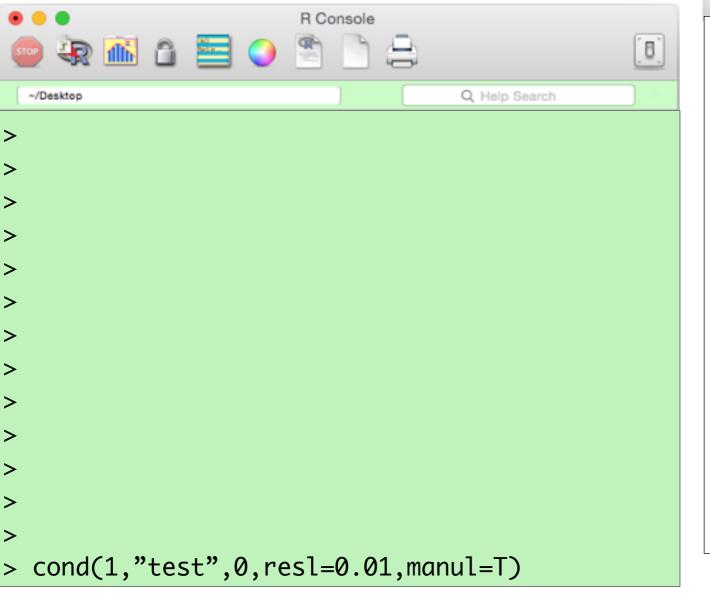
Measurements with a pipette: manual mode

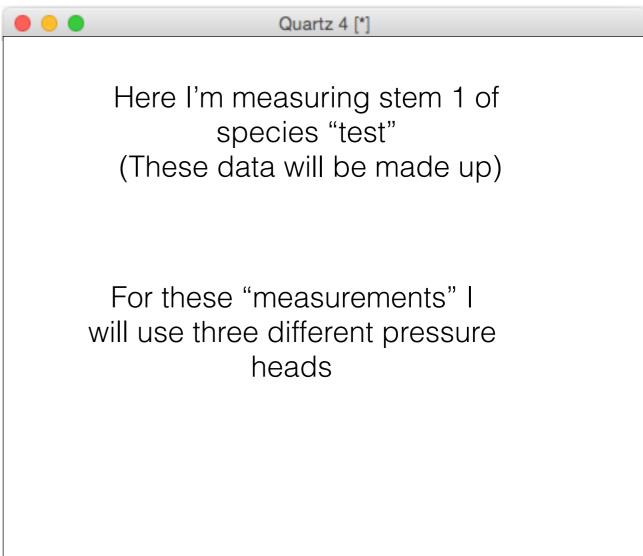
Most details of the program are the same except you control the measurements:

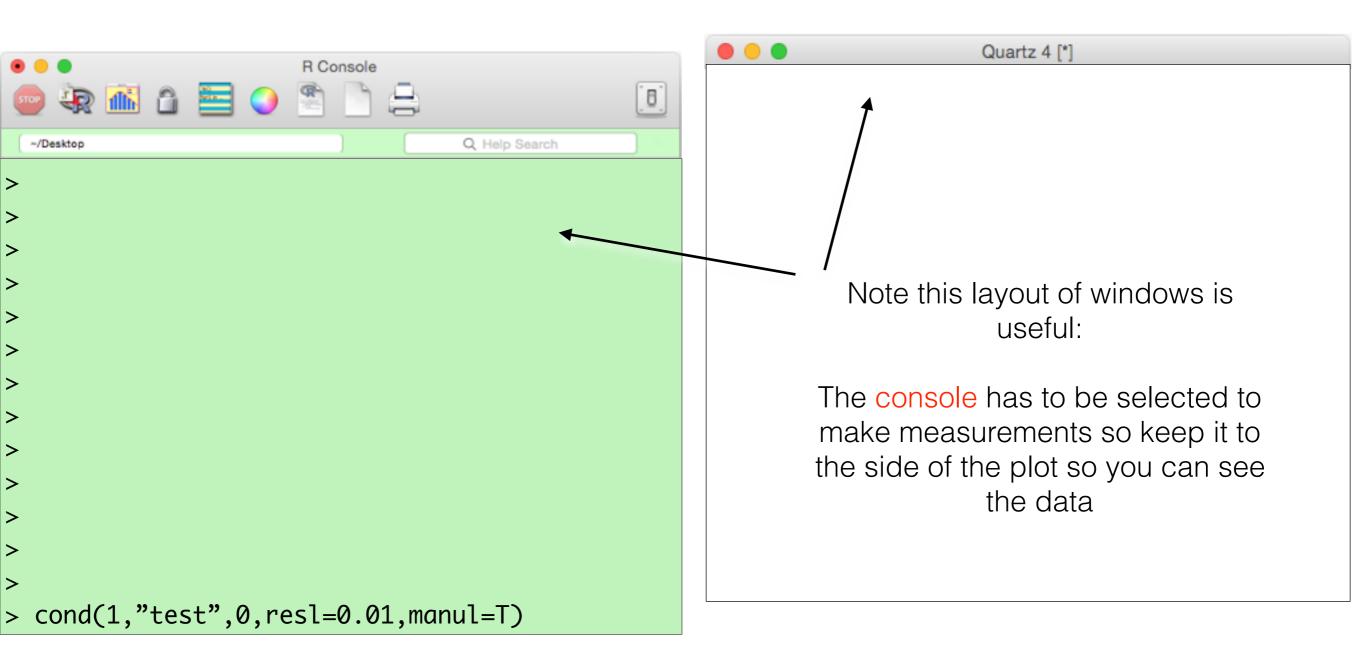
Instead of the program measuring change at fixed times, you tell the program when a fixed unit of liquid has moved into (or out of) the xylem

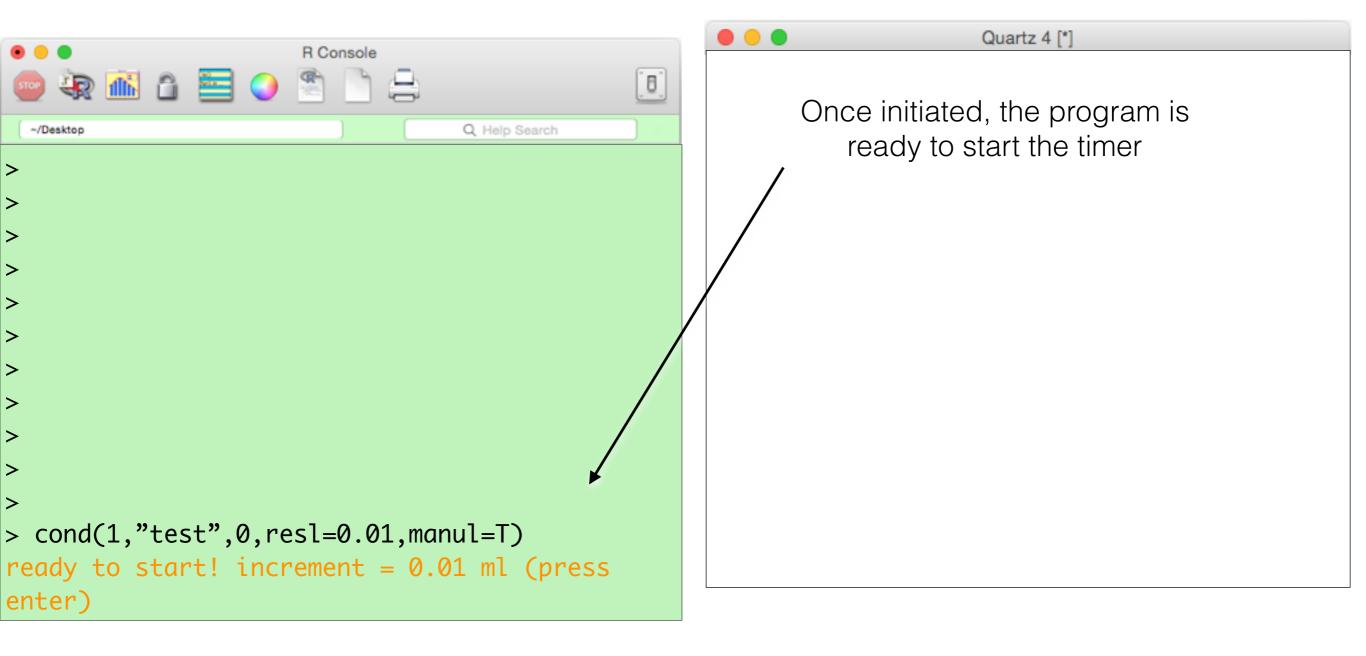


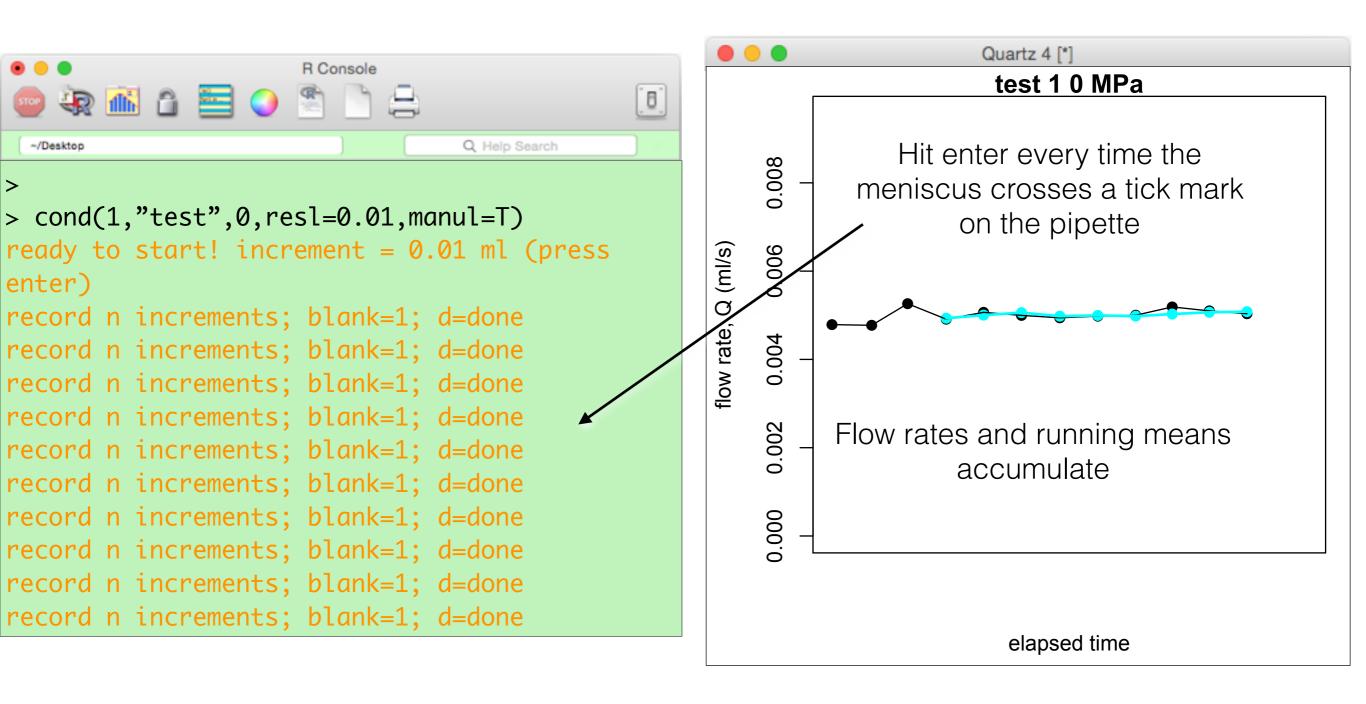


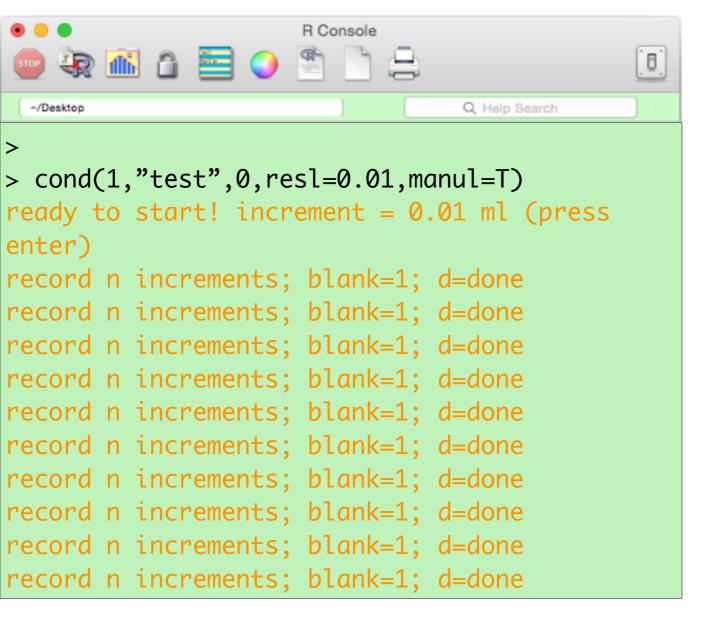


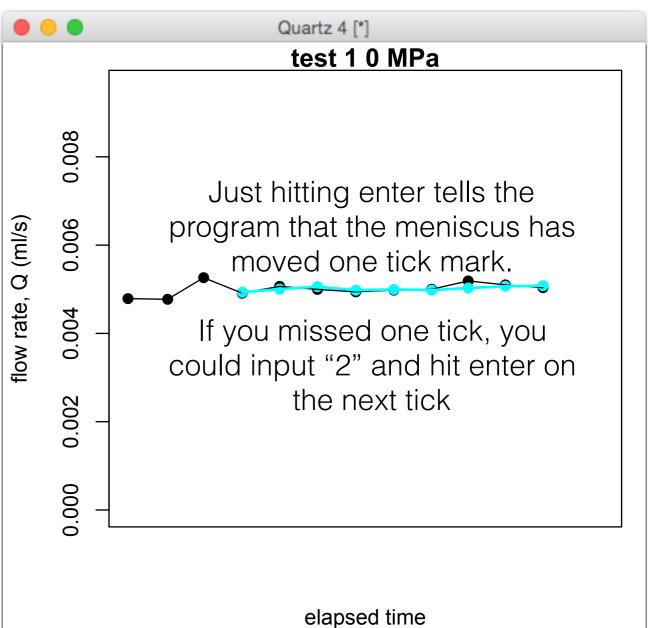


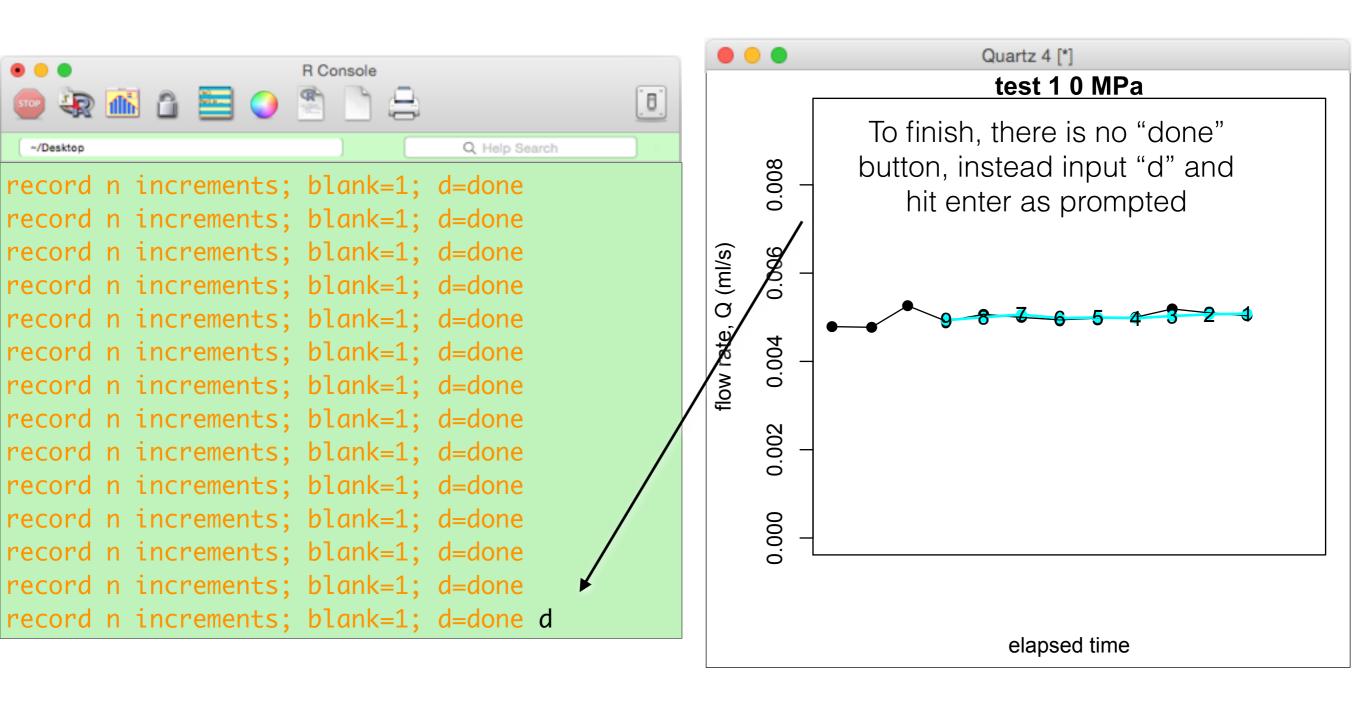


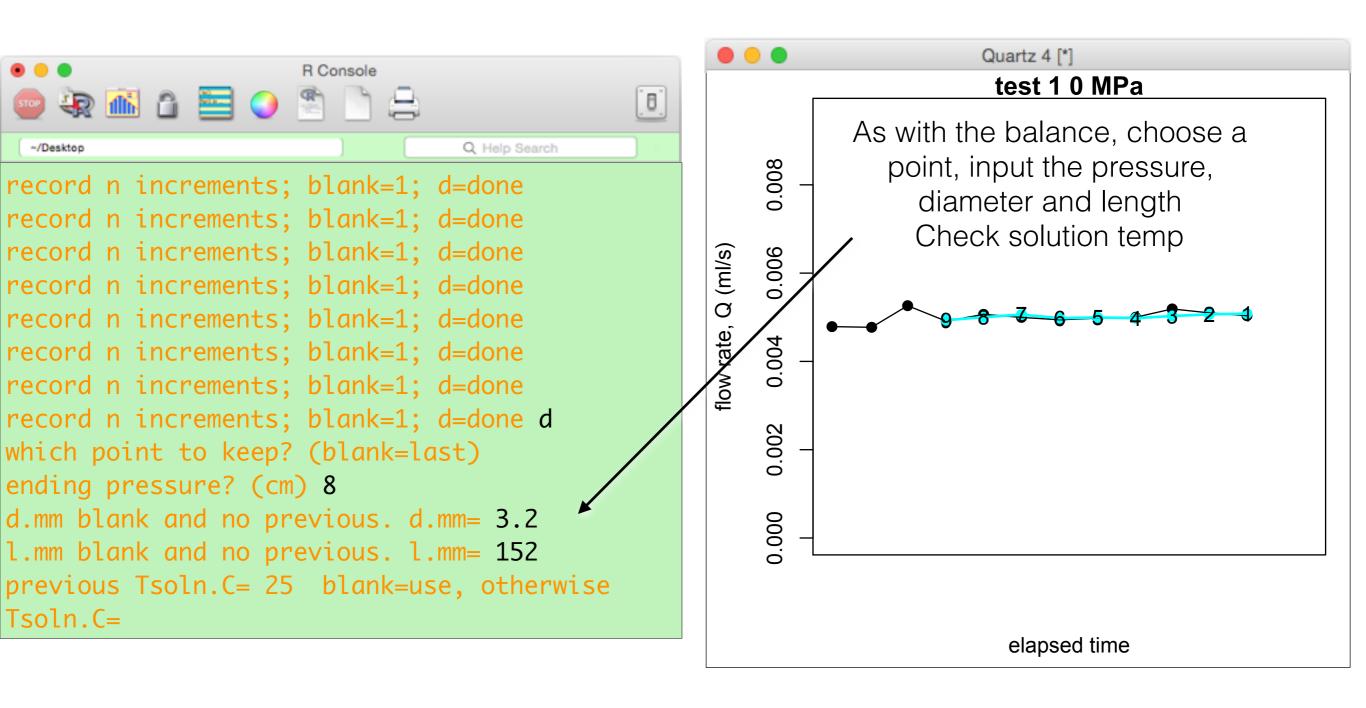


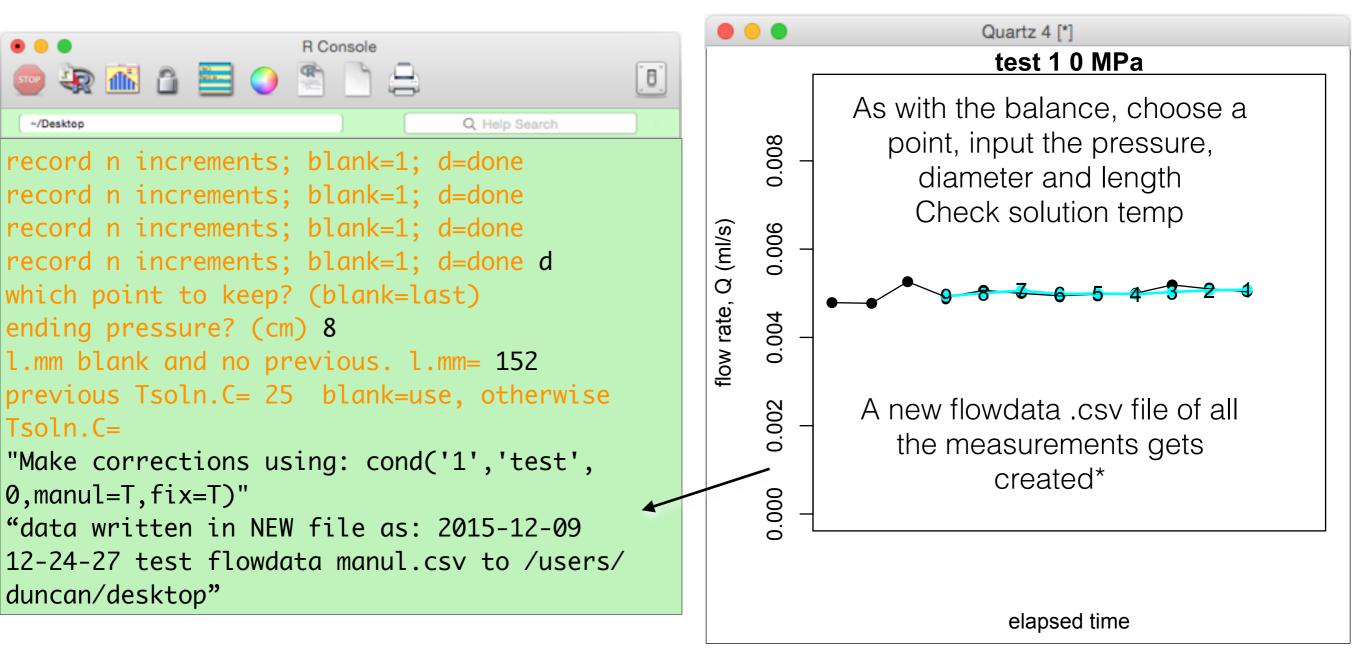




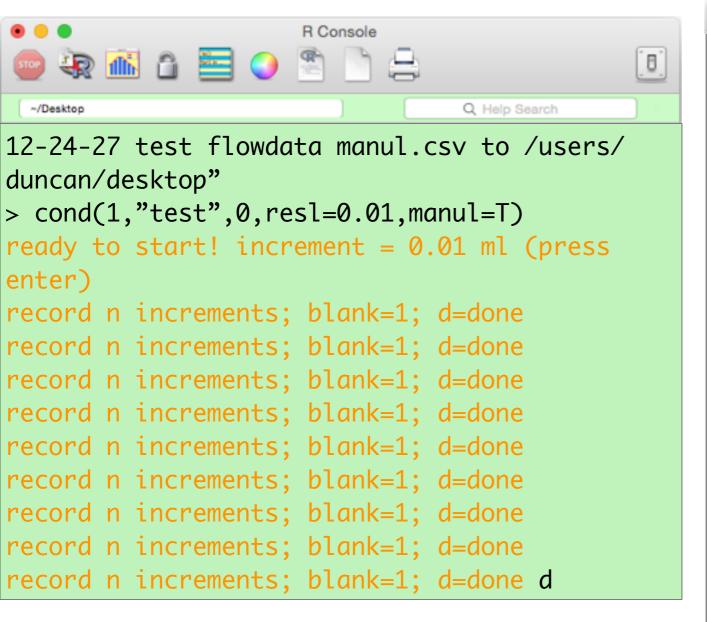


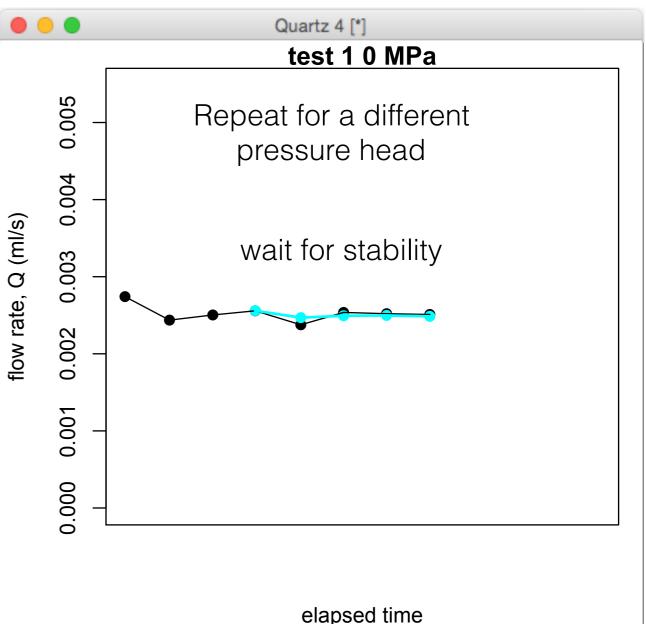


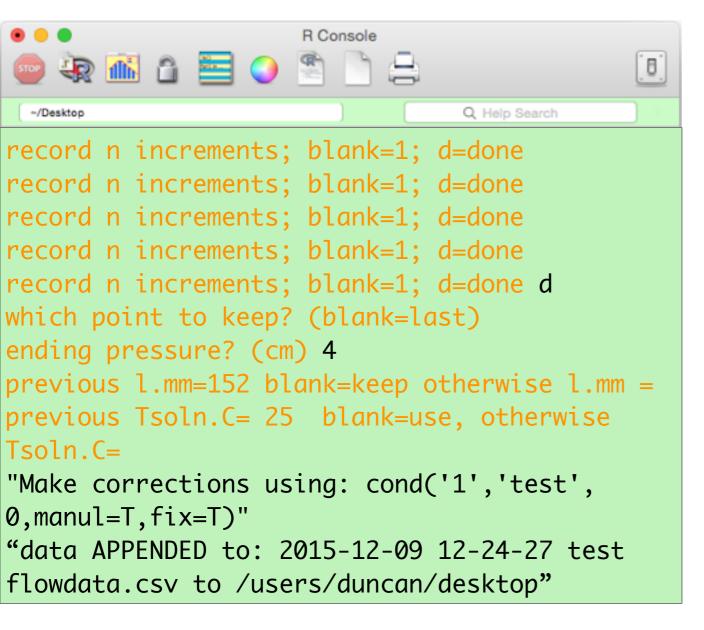


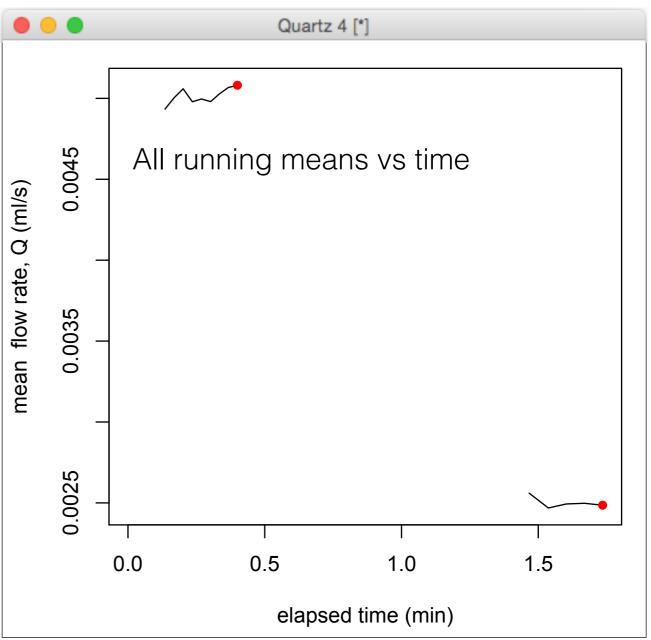


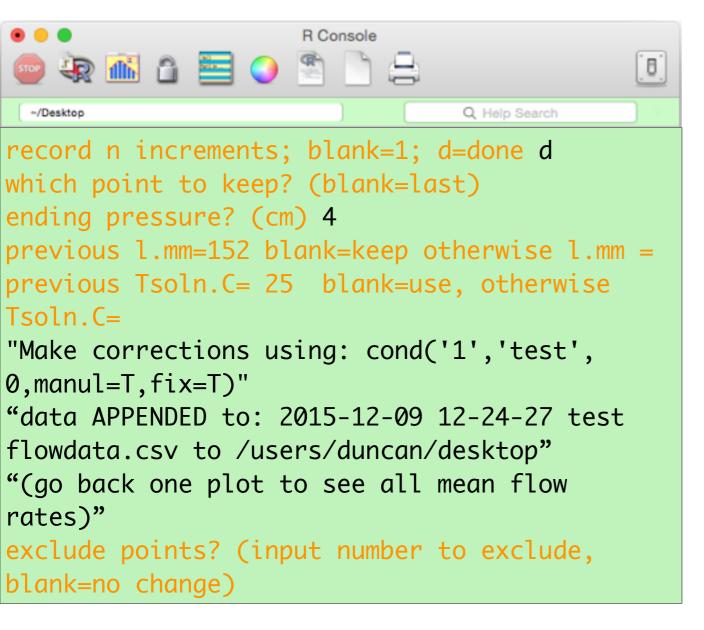
*this .csv will have nearly the same layout as one created when using the balance. The key difference is units will be in ml instead of g. Note that 'manul' appears in the file name. This prevents an error if you happen to use the balance and pipette on the same species

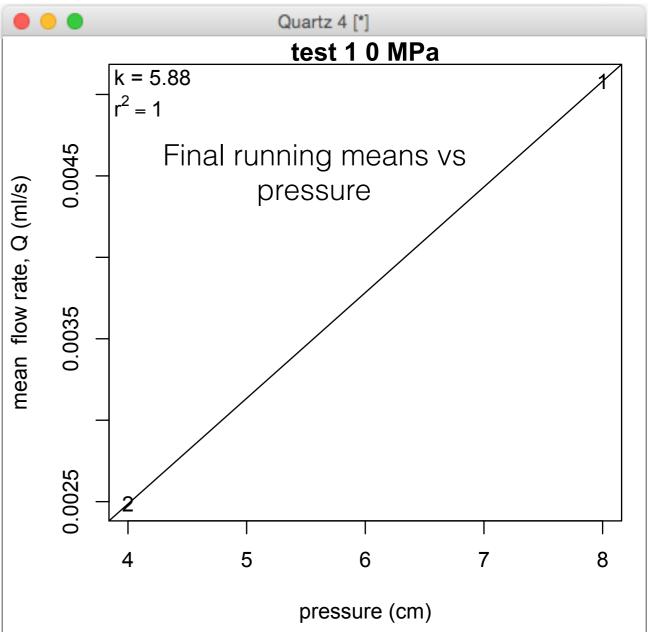


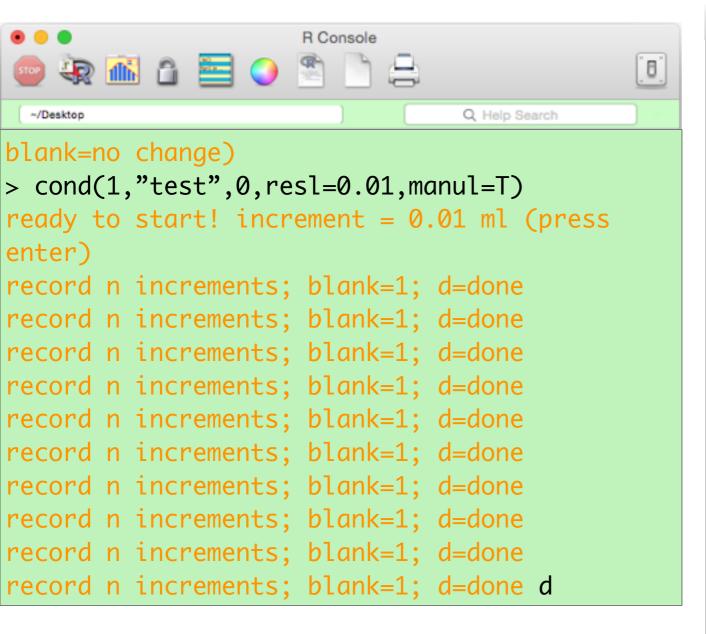


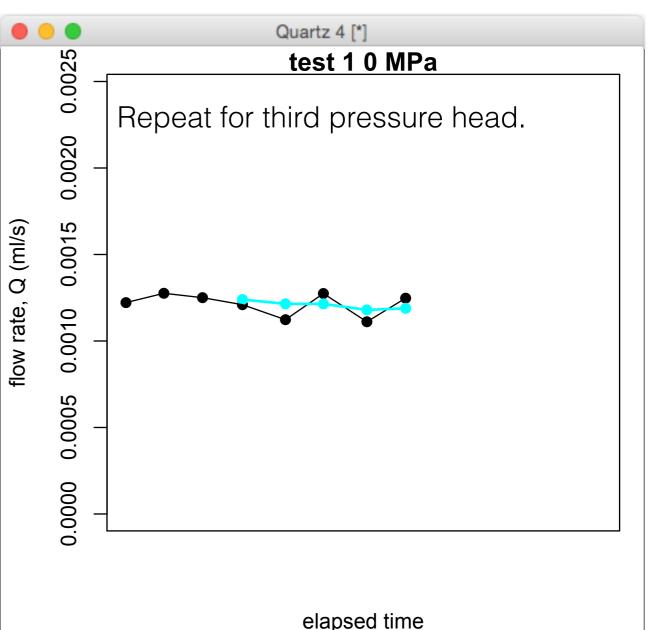


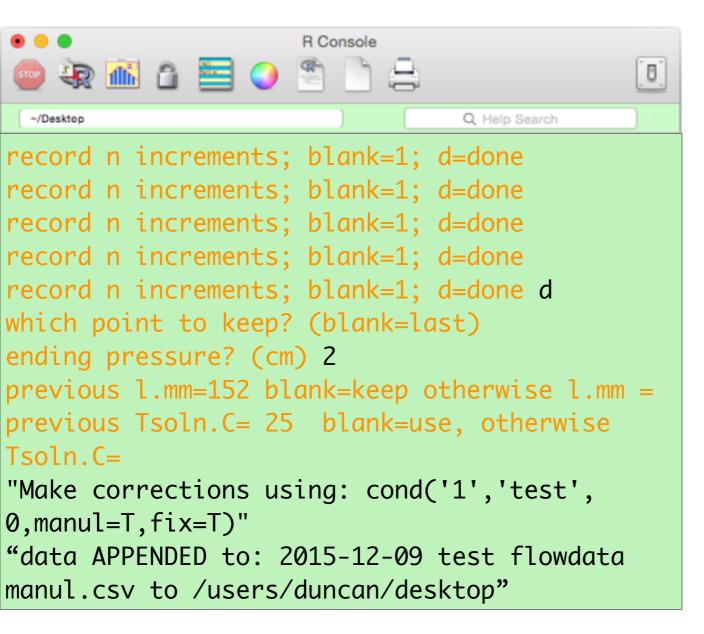


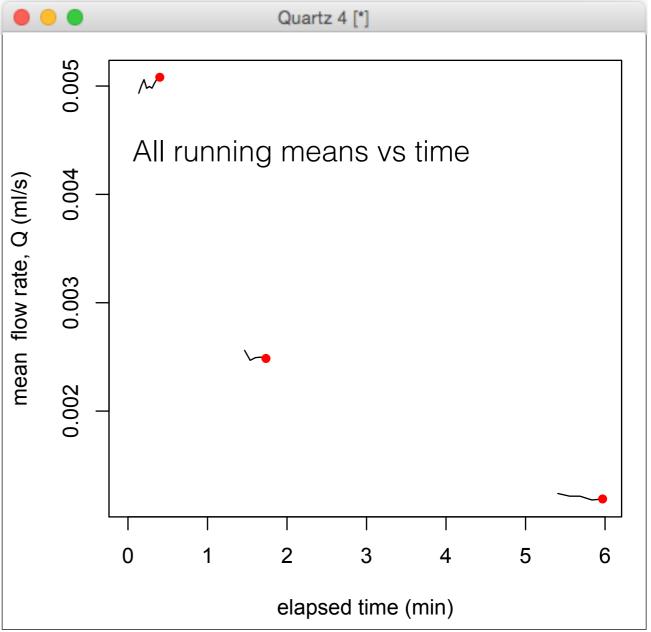


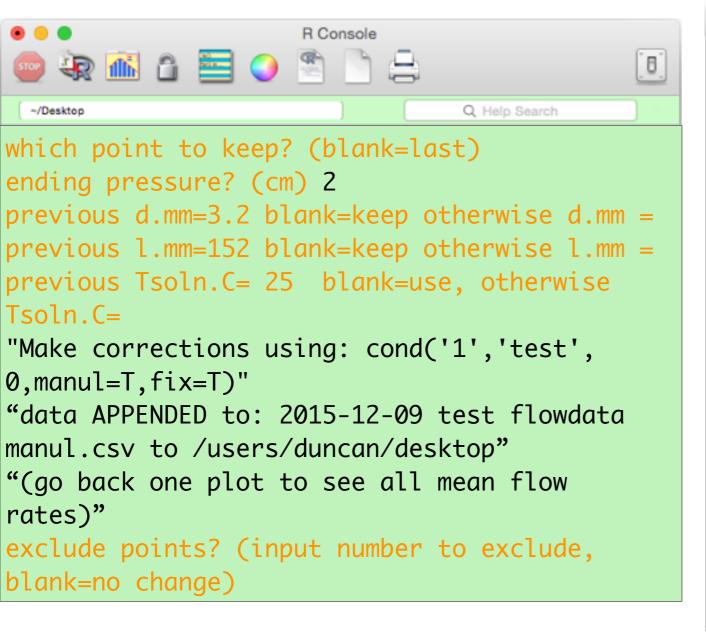


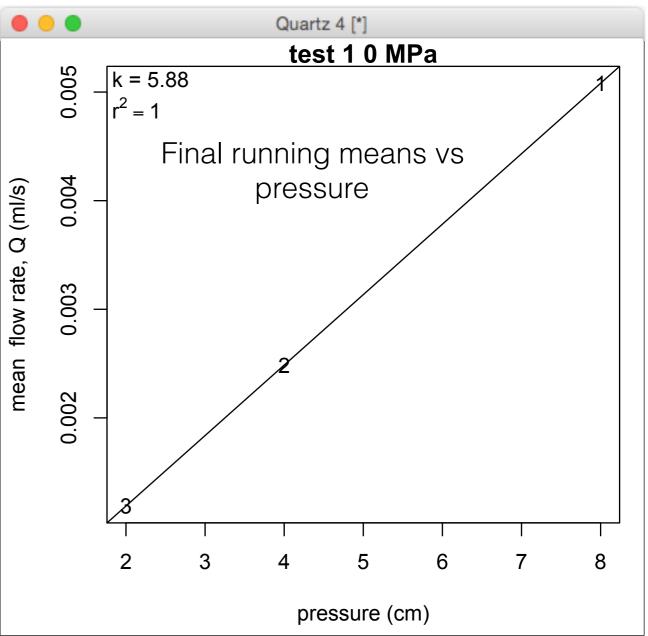


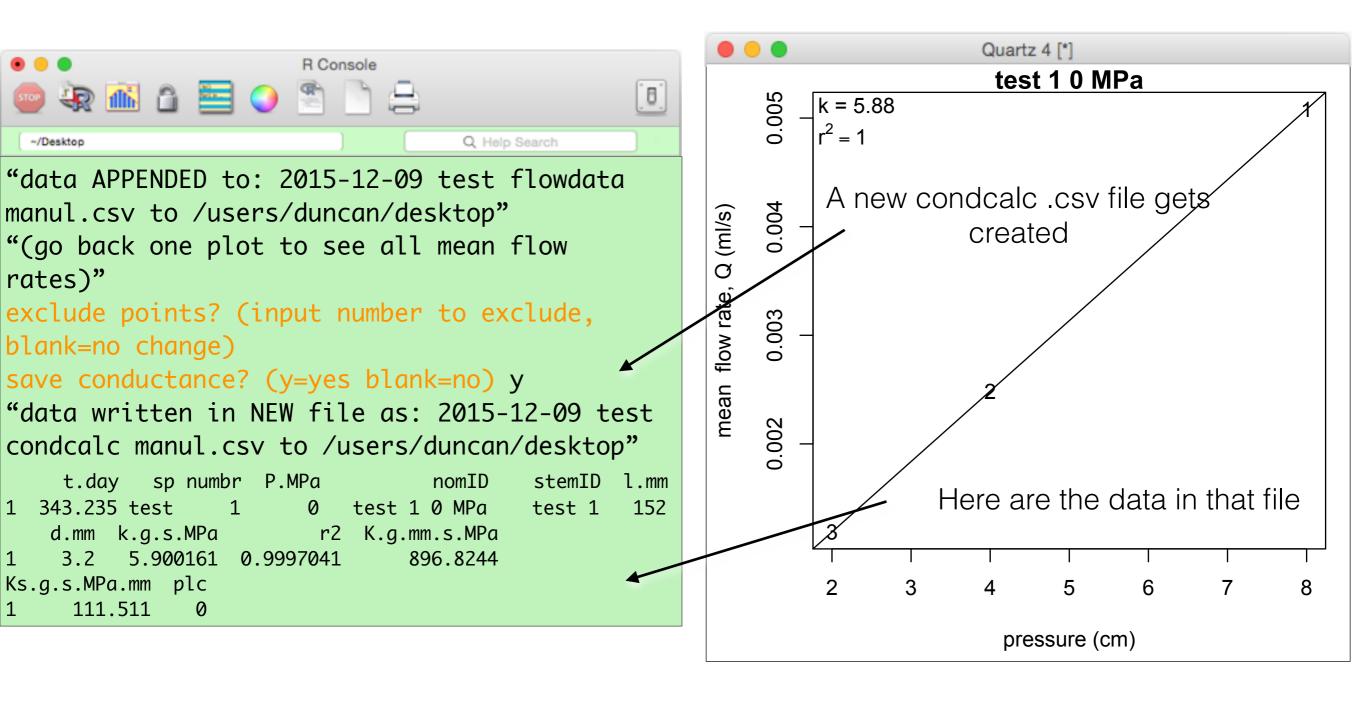










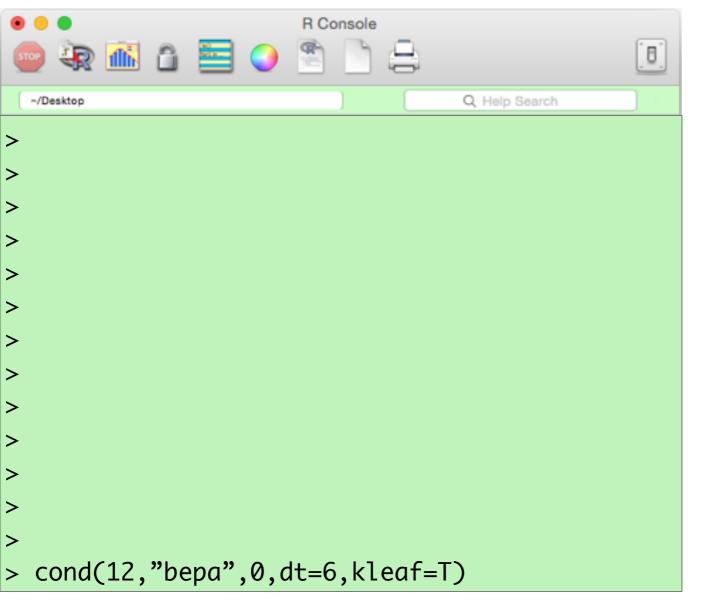


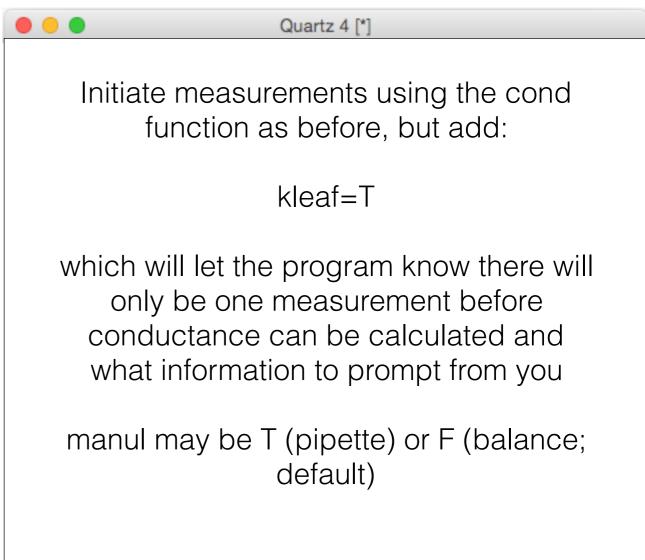
Measuring leaves (balance or pipette)

The program may be configured to measure hydraulic conductance of leaves with the evaporative flux method

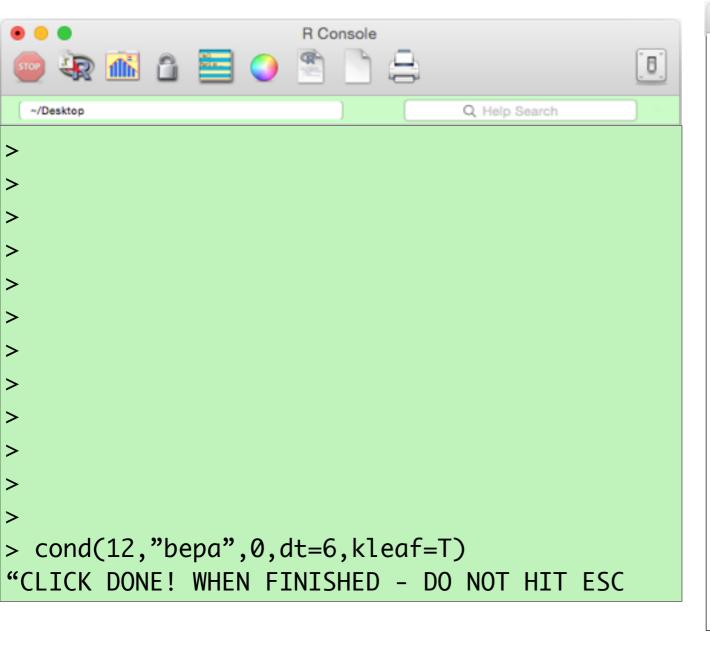
The protocol of measuring background flow rates doesn't lend itself to leaf hydraulics very well. The flow through a transpiring leaf depends on leaf water potential, which cannot be measured without disrupting hydraulic conductance (e.g. pressure bomb or chamber psychrometry).

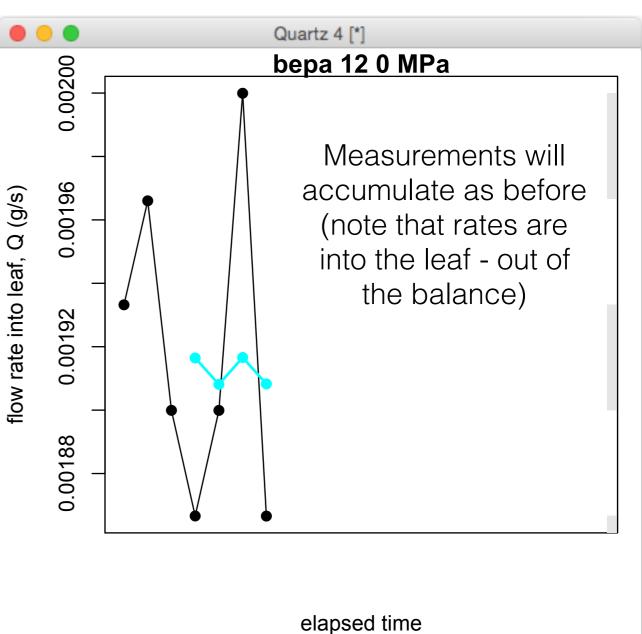
Given this limitation, the program calculates leaf hydraulic conductance from a single flow rate and water potential



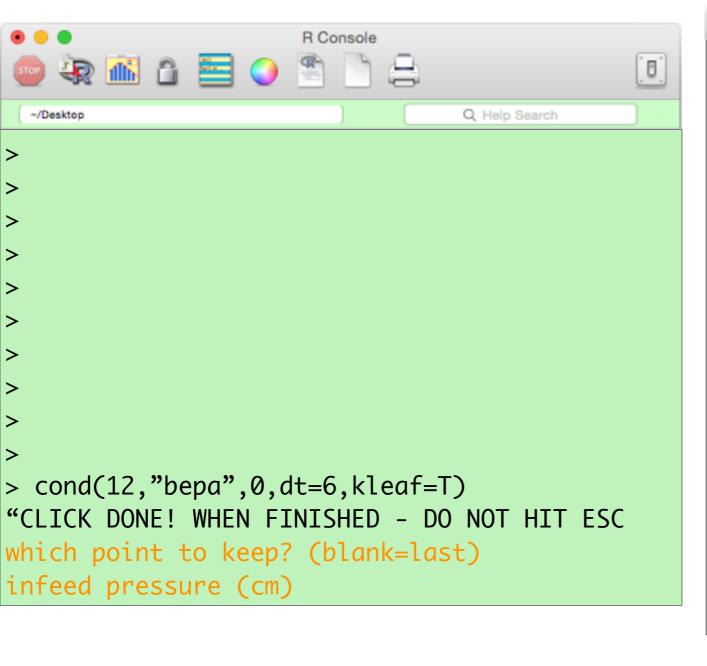


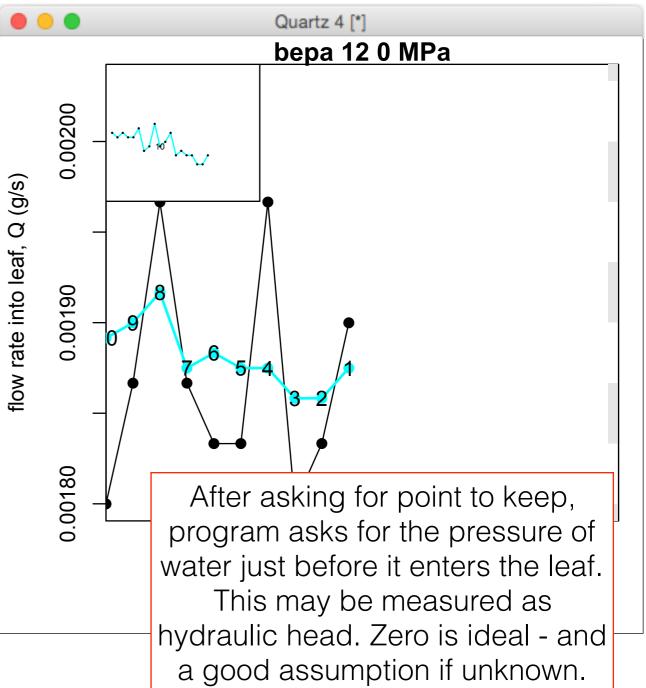


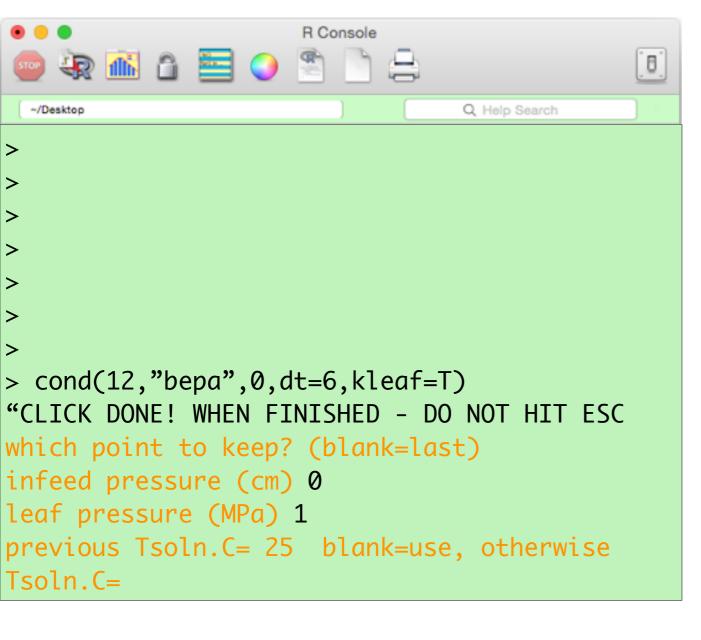


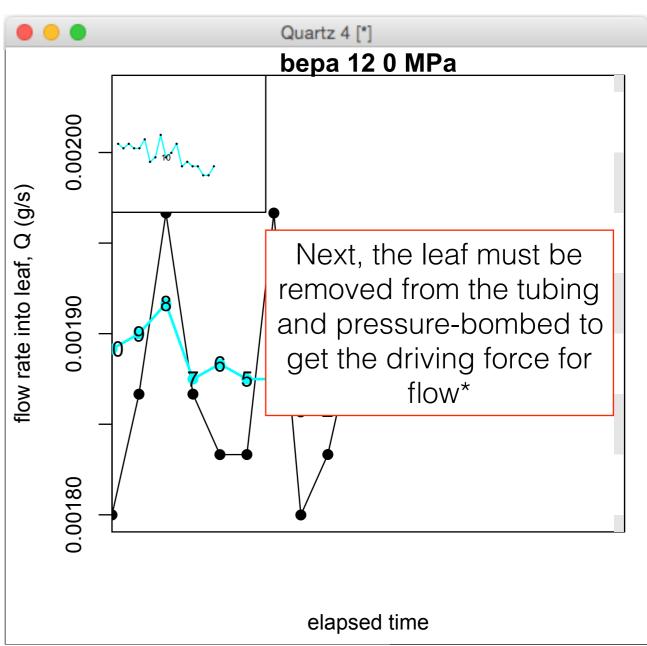




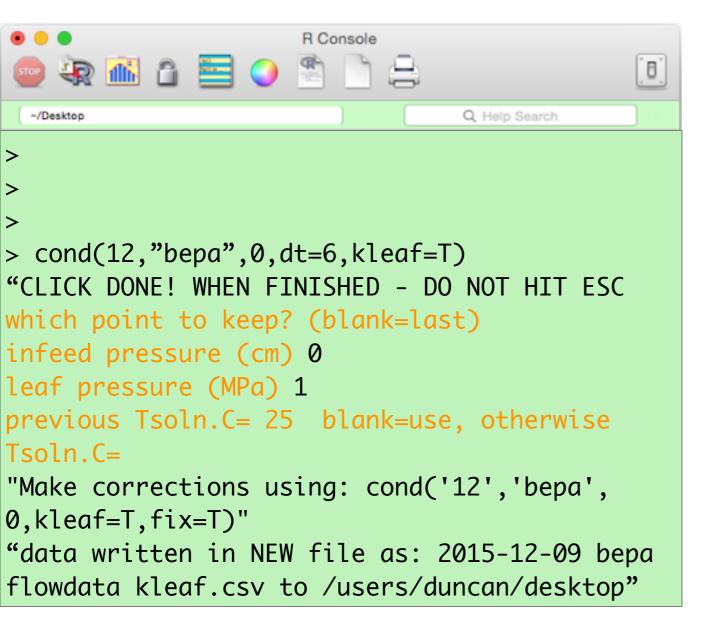


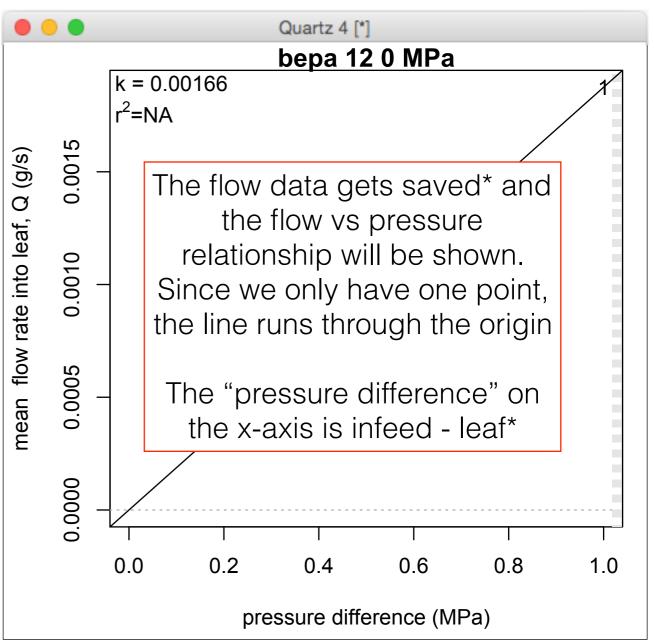




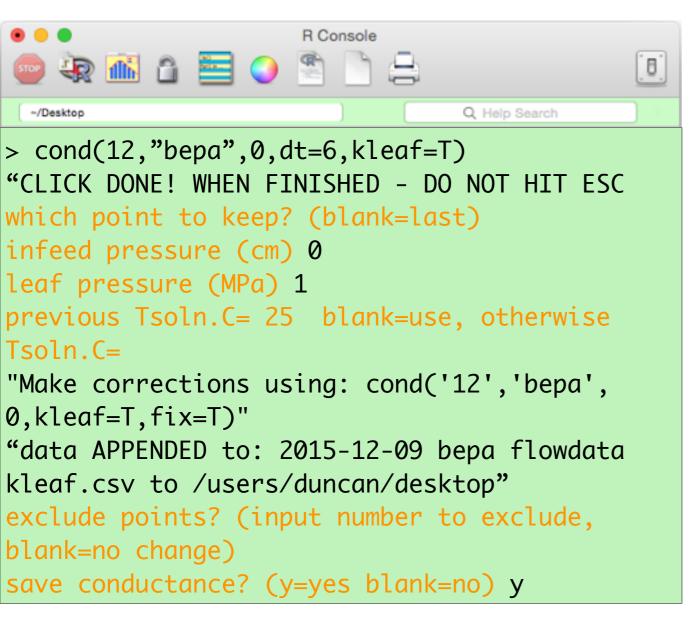


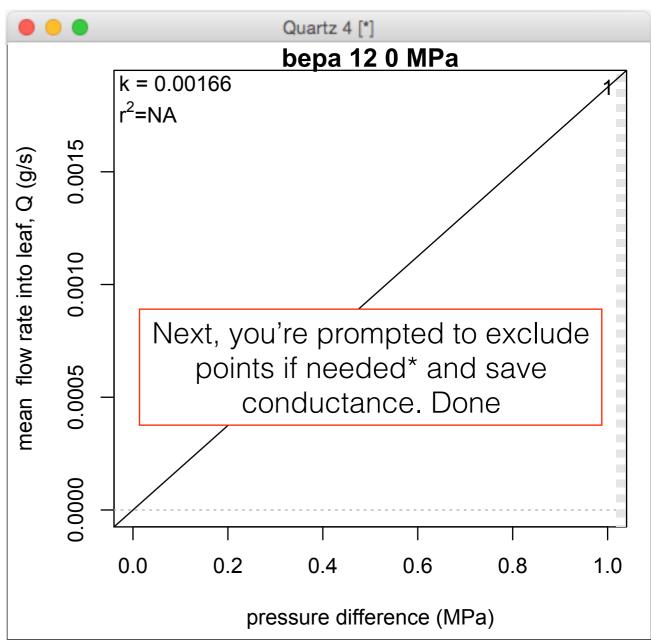
^{*}The assumption is that leaf pressure is a negative number. If your input is positive, the program will make it negative.





*this .csv will have a different layout from one created when measuring stems (or roots). Note that 'kleaf' appears in the file name. This prevents an error if you happen to measure leaves and stems or roots on the same species





^{*}admittedly, removing points may not make sense as you can't create a point unless you measure xylem pressure and therefore prevent further hydraulic measurement

Parameters of the cond function (and their defaults)

```
cond(
                        # stem number (or letter, use quotes with letters)
      numbr,
                        # species (must be in quotes)
      sp,
      Ptreat.MPa=0,
                        # treatment pressure in MPa [must be a number; can be
                         c(rpm,rmax.mm) if using a centrifuge - see pcent() function]
      runmean=4,
                        # number of values in running mean
      dt=6,
                        # target time (s) between balance readings
      resl=0.0001,
                        # resolution of balance (g) or pipette (ml)
                        # leave space for this many measurements when making a new
      mxplot=10,
                         plot after existing one is full
                        # manual mode (T for use with pipette)
      manul=F,
                        # create random numbers instead of reading the balance
      testmode=F,
                         (useful for demonstration or when altering code)
                        # size of inset showing overall running mean flow data - as
      ovrl=0.3,
                         a fraction of plot size (0=no inset)
                        # is measurement on a leaf? If T, will ask for leaf pressure
      kleaf=F,
                         and not diam or length. Also nmin=1
                        # the minimum number of points required to calculate k from
      nmin=3,
                         Q vs P
      fix=F)
                        # option to skip measurements and change an input in a
                         previous measurement and/or calculate conductance
```

Parameters of the showvuln function (and their defaults)

```
showvuln=(
                                # species
    sp,
    yax=c("k.","K.","Ks")[3],
                                # what to plot on y axis (conductance,
                                 conductivity or specific conductivity (default),
                                 respectively)
                                # option to plot percent loss conductivity, if T,
    as.plc=F,
                                 yax is ignored
    horiz=90,
                                # add a horizontal line at this height in PLC
                                 plot (e.g. use 90 PLC as a cutoff and see how
                                 close you are)
                                # option to return entire condcalc.csv file
    retrn=F)
```

Note: this function will search the working directory for .csv files that contain "sp condcalc". If there are multiple matches, it will ask which one to use.

Miscellaneous functions and their parameters

```
pcent(
              # returns pressure induced by centrifuge in MPa
                # RPM of centrifuge
    rpm,
    rmax.mm, # radius from center of rotation to meniscus in rotor cup
    r=0
                # point of interest in stem relative to center of rotation
                Note: function uses density of water = 998.2 \text{ kg m}^{-3} (20 C)
rpmcent(
               # returns RPMs needed to induce given pressure
                # desired pressure in MPa (needs to be negative)
    р,
                # radius from center of rotation to meniscus in rotor cup
    rmax.mm
    r=0
                # point of interest in stem relative to center of rotation
                Note: function uses density of water = 998.2 \text{ kg m}^{-3} (20 C)
phead(
               # converts pressure head in cm to MPa
                # head in cm
    p.cm,
                # density of water in kg m<sup>-3</sup>
    rho,
                # gravity in m s<sup>-2</sup>
    grav)
```

Internal functions

cond() uses functions corner() and resbar() to respectively add text and resolution bars to plots

Times are represented in R as "seconds since 1970". julr() helps convert this to the more useful decimal day.

Balance outputs are interpreted by **decod()**, which has options for Sartorius, Metler-Toldeo, Ohaus and Scientech balances. More details in Appendix B.

frbind() forces rbind() to work even when column names/numbers do not match. This prevents erroring out if you have added/removed columns or changed their names. While it prevents an error, it will not rectify changed names with the names it expects. The code just creates the new columns that it expects.

Appendix B: The decod() function

The internal function decod() parses the balance output into a useable number. For example, a Sartorius of Scientech balance might print

```
"+" "62.4317" "a"
 "62.4317" "g"
```

To parse this, the program needs to know it will print multiple components and the first component is either a sign or a number. The decod() is programmed to look at the first component and decide if it's a sign. If it's a sign, then the second component must be a number so we'll take that sign and apply it to the number. In the code, which component is the number is called wch (i.e. wch=1 or wch=2, here). On a Sartorius balance, by default, the above printout may be preceded by "N" which throws off the above logic. To remedy this, change the balance settings e.g.

Menu>SETUP>PRNT.OUT>PRT.INIT>OFF

A Mettler balance is somewhat simpler in that it might print:

where the number is always the third component (i.e. wch=3), which will include the negative sign if needed. For an Ohaus balance, wch=16.

If communication is working but you have a different balance or you're having parsing issues, there is a commented line (~ line 106) that says #print(as.character(tst)). I suggest uncommenting this and running the program for a bit with stable vs unstable and positive vs negative weights to see what the general format is and which component is the number. Then change decod() [if you're comfortable doing so] or ask me for help.

Appendix C: Dealing with input errors

A number of features prevent the user from inputting values that cause the program to error. For example, when asked which point to keep, the input can only be an integer between 1 and the total number of mean flow rates, which often helps if you accidentally enter pressure instead (often not an integer). Pressure, diameter, length and temperature must be numbers. The program will ask for new values if these criteria are not met.

Some accidents are accepted by the program though. If you realize you just entered the wrong value, **proceed through the remaining prompts.** Most common errors are fixed by subsequently running cond(...,fix=T). After finishing the measurement, the program gives you the exact code you need to run. Using fix=T will prompt you for which measurement to fix (i.e. the numbers shown in the Q vs P plot), what to fix (options depend on measurement type - leaf or stem/root), and what the corrected value is. Changes are made to the flowdata csv file then the program proceeds as if a measurement was just completed, allowing you to calculate conductance if needed. If you already calculated conductance with an error, you can manually delete the bad calculation after adding the new calculation.

Appendix D: Known issues / cautions

After measurement, warning(s) stating NAs introduced by coercion are sometimes given. These may be due to bad balance outputs or bad inputs for pressure, diameter or length. This is not known to affect measurements but the warning may cause concern.

Prior to version 14, density of water was always assumed to be 25 C for calculating hydraulic head. User-inputted temperature was only used to calculate viscosity to correct conductivity to 20 C.

You will lose data if you:

- Press ESC instead of clicking Done!
- Press ESC instead following the prompts (actually useful to cancel a measurement)
- Have the flowdata.csv or condcalc.csv file open in another program (e.g. Excel) when ending a measurement (may only matter in Windows)

You will confuse things (and possibly lose data) if you:

- Rename flowdata.csv or condcalc.csv files
- Rename or delete columns in flowdata.csv or condcalc.csv

Appendix E: Anticipated questions:

How do I save my data?

Data are automatically saved to your working directory after every measurement. You need not save anything in R except the conductR code if you alter it

How do I change the defaults in cond()?

In the conductR code where it says cond=function(...) [~line 41] you'll see all the function arguments and their defaults. Change as desired, save and re-source the code into the R console.

What if I input the wrong ____?

Your options are to either 1) start over (good when you just started a measurement and, say, used the wrong ID) 2) use cond(...,fix=T) and follow the prompts or 3) manually edit the saved data file(s). If you calculated conductance with a wrong input, you can recalculate by following the prompts of cond(...,fix=T). But you will need to manually delete the wrong values from condcalc.csv

Why is this not an R package?

It seemed simpler this way to keep everything organized in one file. Plus, this way the user has full access to and control over the code, which allows things like default arguments to be changed. However, there is potential for problems if your variable names overlap with mine. Besides the functions (see Appendix A) and .Tcl variables (i.e. port settings), I use: kwd, elev, lat, btyp

Appendix F: Observations for various balances

With a new Mettler-Toledo balance, I had success changing balance settings to enable Host (instead of Printer), use 9600 baud, 8 bits, no parity, 1 stopbit, handshake Xon/Xoff, end of line <CR><LF>, Ansi/Win character set, continuous mode Off. However, in conductR I used default port settings (including 7 bits, odd parity).

With an old Mettler-Toledo balance, I had success when balance settings matched the default conductR settings (as expected). But this balance would only communicate with a Windows computer.

With a GeneMate balance, port settings were not alterable and were baud 9600, 7 data bits, space parity, 1 stopbit, handshake none or xon/xoff. But default conductR port settings (including odd parity) worked fine, as did the Sartorius print command (Esc-P-CR-LF).

My ability to test different balances is very limited. Please pass along info that might help others.