Experiment FB-1: Osmoregulation

Equipment Required

PC or Mac Computer

IXTA, USB cable, Power supply

FT-302 Force Transducer

Ring stand and clamp

Basket and pennies (each weighs about 3 g)

5 x 250 ml beakers

2 x 100 ml graduated cylinders.

Forceps

Marine Worms or Garden slugs *** note if using slugs, adjust the type of water used

Artificial Seawater (see Appendix)

FT-302 Setup

1. Locate the FT-302 force transducer in the IXTA kit and plug the DIN8 connector into the Channel A5 input of the IXTA.

Figure FB-1-S1: The FT-302 force transducer.

2. Attach the transducer to a ring stand using a 90^{o} clamp, so that the transducer is horizontal.
3. Attach a weight pan to end of the transducer arm.

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Figure FB-1-S2: The FT-302 force transducer connected to the IXTA.

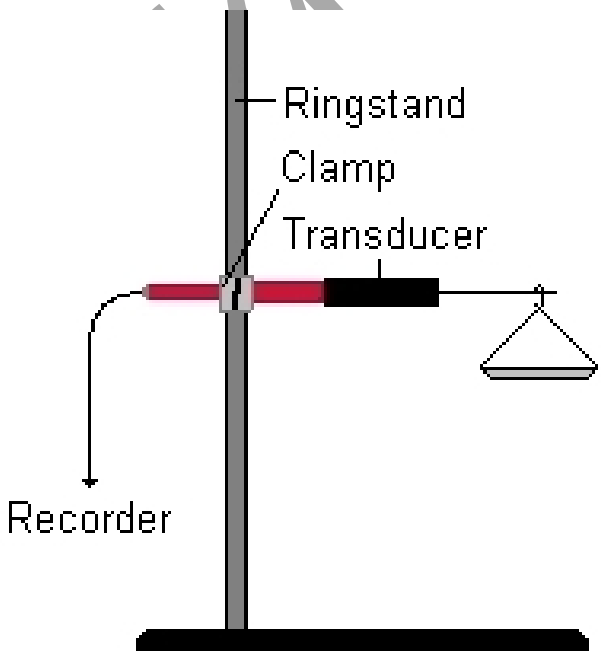

Figure FB-1-S3: Equipment setup to record weight placed in a pan.

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Preparation of Solutions

1. Place or write a label on each 250 ml beaker: 100%, 90%, 80%, 70% and 50%.

Table FB-1-S1:Composition of Solutions for Experiment

Labeled Beaker	Artificial Seawater (ml)	Deionized Water (ml)
100%	200	0
90%	180	20
80%	160	40
70%	140	60
60%	120	80

2. Make up the seawater solutions and place in the appropriately labeled beakers.

Calibration of the FT-302 Force Transducer

1. Type “No Weight” in the Mark box. Click Record, and press the mark button to attach the comment to the recording. Record for ten seconds with no weight hanging from the arm or hook of the transducer.
2. Type “5 grams” in the Mark box. Hang a 5 gram weight on the arm or hook of the transducer. Press the mark button. Record for ten more seconds.
3. Click Stop to halt the recording.
4. Select Save As in the File menu, and name the file. Choose a destination on the computer in which to save the file. Click on the Save button to save the data file.

Unit Conversion

1. Scroll to the beginning of data when no weight was attached to the force transducer.
2. Use the Display Time icons on the LabScribe toolbar to adjust the display time of the Main window to show the complete calibration data on the Main window.
3. Click the Double Cursor icon so that two vertical cursors appear on the Main window. Place one cursor on the flat section of data collected when no weight was attached to the force transducer, and the second cursor on the flat section of data collected when the 5 gram weight was attached to the transducer.

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4. Click on the arrow next to the title of the force transducer channel to open the channel menu.
5. Select Units from the channel menu and Simple from the Units submenu.
6. In the Simple Units Calibration window, select 2 point calibration from the pull-down menu in the upper-left corner of the window.
    - Put a check mark in the box next to Apply units to all blocks. Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
    - Enter “Zero” in the corresponding box to the right of the voltage recorded when no weight was attached to the transducer. Enter “5” in the box to the right of the corresponding voltage recorded when the 5 gram weight was hung on the hook of the transducer.
    - Enter the name of the units, grams, in the box below the weights. Click on the OK button in the lower right corner of the window to activate the units conversion.

In the 10 gram range, the FT-302 will deliver approximately 75 mV/gram at x1 gain and approximately one tenth of that in the 100 gram range. The FT-302 is now ready for use.

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Experiment FB-1: Osmoregulation

Calibration of FT-104 or FT-302 Force Transducer

Aim: To calibrate the force transducer used to measure weight gain or loss of the animal.

Approximate Time: 15 minutes

Procedure

1. Make sure that the iWorx box is turned on and the FT-104 or the FT-302 force transducer is connected.
2. Type **No Weight** in the Mark box to the right of the Mark button. Click the Record button, and press the Mark button to the left of the mark box. Record for ten seconds with no weight hanging from the arm or hook of the transducer.
3. Count the number of pennies you have and multiply their number by three (the weight of a penny in grams).
4. Type the **Pennies** in the Mark box. Place the pennies on the weight pan and press the Mark button. Click the AutoScale button next to the channel title area. Record for ten more seconds.

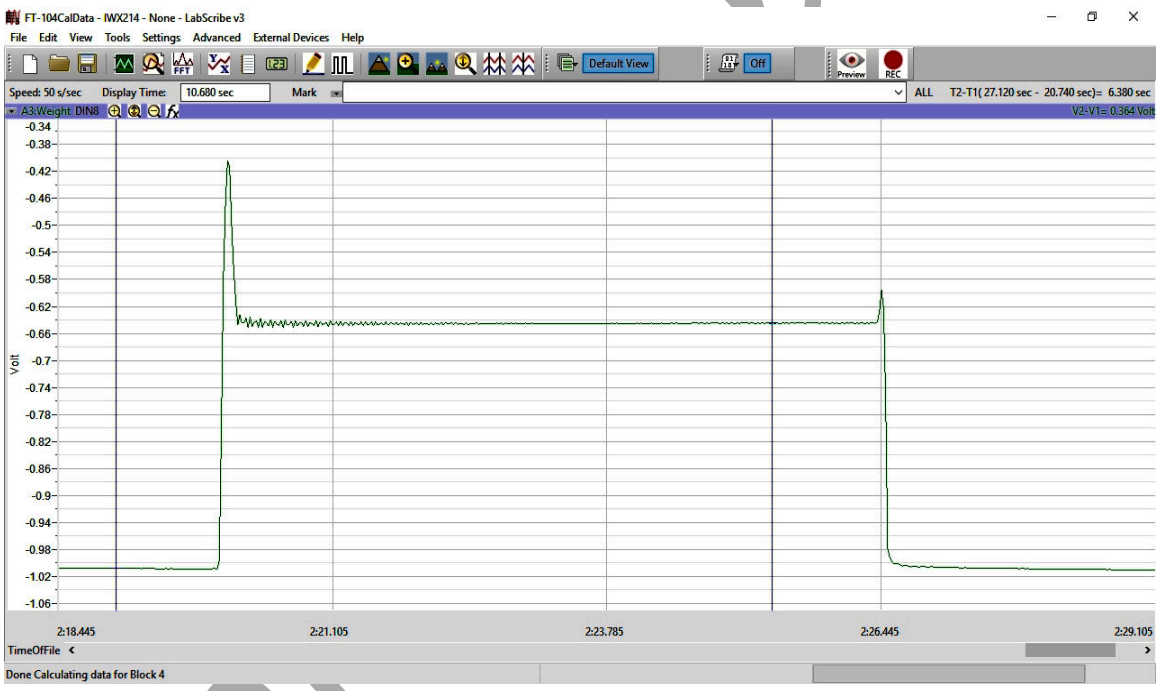

Figure FB-1-L1: Recording of different known weights used to calibrate FT-104/FT302 force transducer.

5. Click Stop to halt the recording.
6. Select Save As in the File menu, type a name for the file. Click on the Save button to save the data file.

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7. Remove the pennies from the weight pan.

Units Conversion

1. Scroll to the beginning of data when no weight was attached to the force transducer.
2. Use the Display Time icons on the LabScribe toolbar to show the complete calibration data on the Main window. The required data can also be selected by:
   - Placing the cursors on either side of data required.
   - Clicking the Zoom between Cursors button on the LabScribe toolbar to expand the complete calibration data to the width of the Main window.
3. Click the Double Cursor icon. Place one cursor on the flat section of data collected when no weight was attached to the FT-104 or FT-302, and the second cursor on the flat section of data collected when the pennies were attached to the transducer.

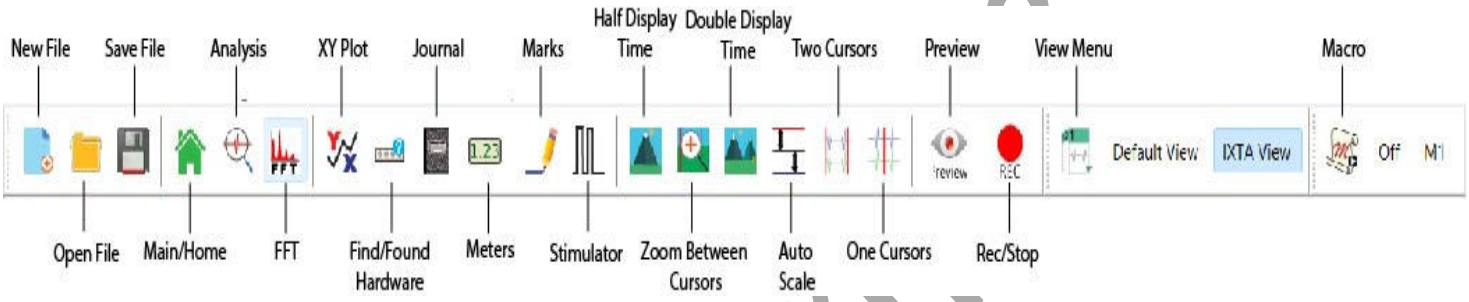

Figure FB-1-L2: The LabScribe toolbar.

4. To convert the output of the force transducer from a voltage to the grams of force:
   - Click on the arrow next to the title of the Weight channel to open the channel menu.
   - Select Units from the channel menu and Simple from the Units submenu.

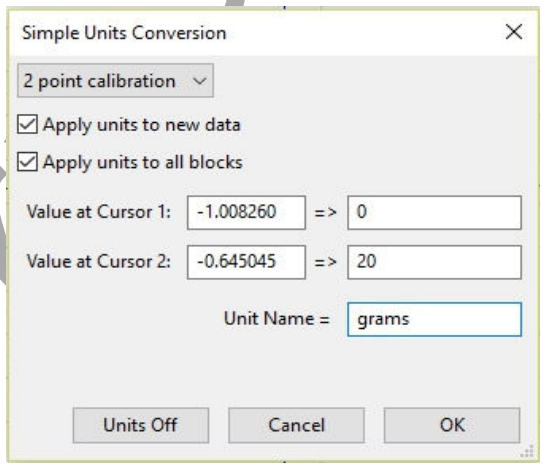

Figure FB-1-L3: The Simple Units Conversion dialogue window with the voltages at the cursors set to equal the weight used in calibration.

Work Sample - Lab

5. The Simple Units Calibration window will appear. On this window:
    - Select 2 point calibration from the pull-down menu in the upper-left corner of the window.
    - Put a check mark in the box next to Apply units to all blocks.
    - Notice that the voltages from the positions of the cursors are automatically entered into the value equations.
    - Enter zero in the corresponding box to the right of the voltage recorded when no weight was attached to the transducer. Enter the weight of the pennies in the box to the right of the corresponding voltage recorded when the weight of the pennies was hung on the hook of the transducer.
    - Enter the name of the units, grams, in box below the weights. Click on the OK button in the lower right corner of the window to activate the units conversion.

Practice Weighing Technique

Aim: To develop a consistent technique of weighing a worm.

Approximate Time: 15 minutes

Procedure

1. Use forceps to remove a worm from seawater. Blot the worm with paper towels to remove excess water.
2. Type **No Worm** in the Mark box to the right of the Mark button.
3. Click the Record button to record a baseline of ten seconds while only the weight pan is attached to the transducer. Continue recording.
4. Type **Worm** in the Mark box and mark the recording. Place the worm on the weight pan attached to the transducer.
5. Continue to record for ten seconds after the worm was placed on the weight pan. Click Stop to halt the recording.
6. Replace the worm in the seawater.
7. Repeat Steps 1 through 6 for two other worms.

Data Analysis

1. Scroll through the data file to the weighing of the first worm.
2. Use the Display Time icons to adjust the Display Time of the Main window so that the output of the transducer before and after the worm was placed on the weight pan is displayed on the Main window.
3. Data can be collected from the Main window or the Analysis. If you choose to use the Analysis window, click on the Analysis window icon in the toolbar.

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4. The mathematical functions, V2-V1 and T2-T1, should be showing. The values for these parameters are displayed on screen.
5. Maximize the height of the trace on the Weight Channel by clicking AutoScale All from the toolbar.
6. Place the cursors on the recording before and after the addition of the worm to the weight pan. The value for V2-V1 on the Weight channel is the weight of the worm.
7. The weight of the worms can be recorded in the on-line notebook of LabScribe by typing their names and values directly into the Journal.
8. The functions in the channel pull-down menus of the Analysis window can also be used to enter the name and value for V2-V1 from the recording to the Journal. To use these functions:
    - Place the cursors at the locations used to measure the weight of the worm.
    - Transfer the name of the mathematical function used to determine the weight to the Journal using the Add Title to Journal function in the Weight Channel pull-down menu.
    - Transfer the weight to the Journal using the Add Ch. Data to Journal function in the Weight Channel pull-down menu.
9. Repeat Steps 2 through 8 to find the weights of the other two worms weighed in this exercise. Record the weights in the Journal and on Table FB-1-1.
10. Select Save in the File menu.

Table FB-1-1: Weight of Worms Recorded During Weighing Practice.

	Weights (g)		
	Worm 1	Worm 2	Worm 3
Practice			

Osmoregulation

Aim: To measure changes in the weights of five (5) worms placed in different osmotic environments.

Approximate Time: 30 minutes

Procedure

1. Use forceps to remove a worm from seawater. Blot the worm with paper towels to remove excess water.
2. Type **No Worm** in the Mark box to the right of the Mark button.

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3. Click the Record button to record a baseline of ten seconds while only the weight pan is attached to the transducer. Continue recording.
4. Type **Worm 1** in the Mark box. Place the worm on the weight pan attached to the transducer. Press the mark button to mark the recording.
5. Continue to record for ten seconds after the worm was placed on the weight pan. Click Stop to halt the recording. Note the time when the worm was weighed.
6. Record the weight of the worm in the Journal and on Table FB-1-2.
7. Place the worm in the 100% seawater (or appropriate environment) solution.
8. Repeat Steps 1 through 6 for each of the four other worms used in this exercise. Each worm goes into a different solution: 90, 80, 70, or 60% seawater.
9. Every ten minutes, remove each worm from its solution. Blot the worm, weigh it, and return it to the same solution.
10. Record the weight of the worm in the Journal and on Table FB-1-2.
11. Weigh all the worms until you have seven weight values for each worm.

Data Analysis

Graph the weight of each of the five worms in a different solution as a function of time.

Questions

1. Does the weight of the worm in 100% seawater change? Is the weighing of the worms accurate?
2. Which worm gained weight at the fastest rate? If weight gain indicates water intake, explain the results in terms of concentration gradients?
3. Do any of the worms stop gaining weight towards the end of the experiment? How do you explain this observation?
4. Explain what you think would happen if the animal was placed in a very concentrated sea water solution. How can you support your answer?

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Table FB-1-4: Weights of Worms Placed in Solutions of Different Osmotic Strengths Over Time.

	Weights (g)				
Solutions	100%	90%	80%	70%	60%
Time (min)	Worm 1	Worm 2	Worm 3	Worm 4	Worm 5
0					
10					
20					
30					
40					
50					
60					

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