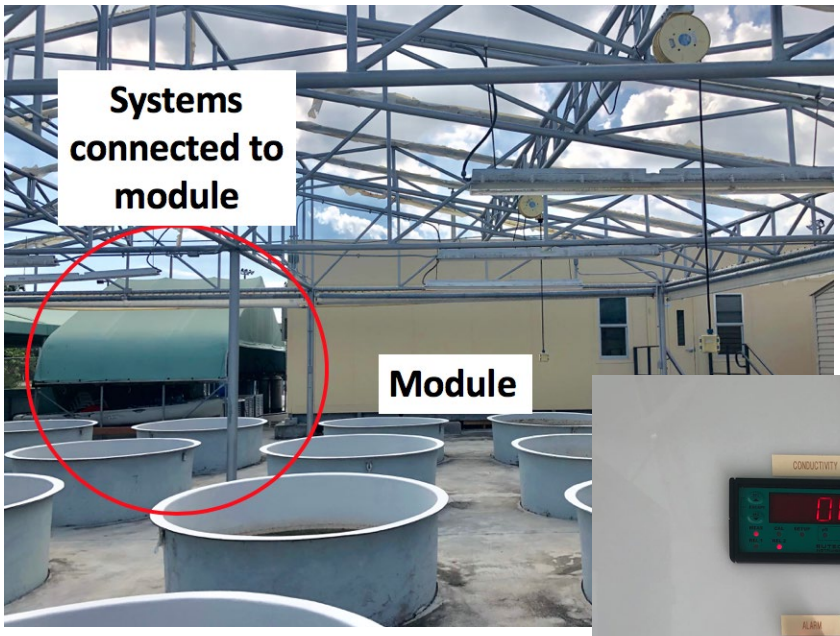
Swimming Performance Protocol
12/04/18

Prior to running a true study, you need to set up the swimming performance tunnel (1.0) and perform velocity and DO calibrations (2.0 & 3.0). This procedure takes about 2 hours but if it is your first time it may take longer. Preferably, do this the day prior to your experimental study (4.0).

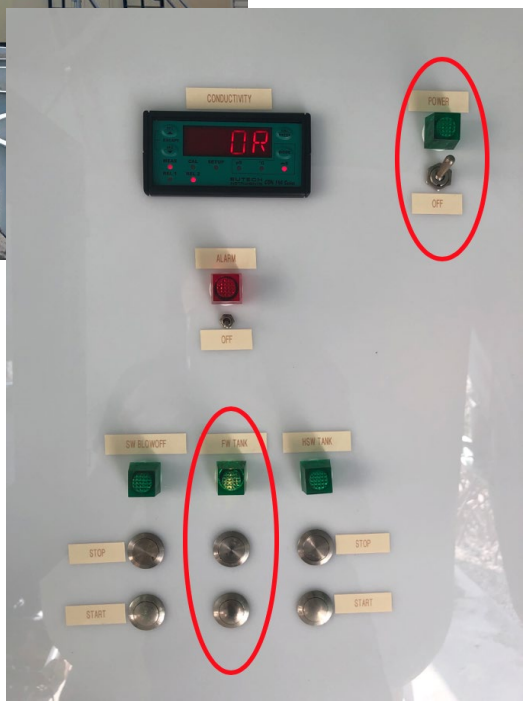
1.0 Experimental Set-Up

1.1 In order to set up the experiment, you first need to fill up with water the blue sump located in the swimming performance room (there is a blue sump under each of the tunnels).
To do this, look at the picture below and go to the systems under the green canopy, to the left of the module building.

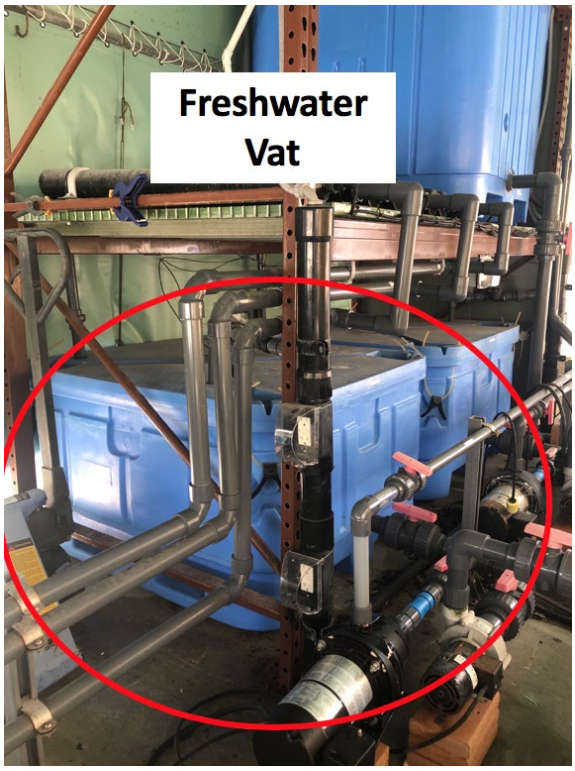


1.2 Direct towards the control box and turn the switch up towards where it says power.

1.3 Once it is on, press start under the label that says FW Tank.

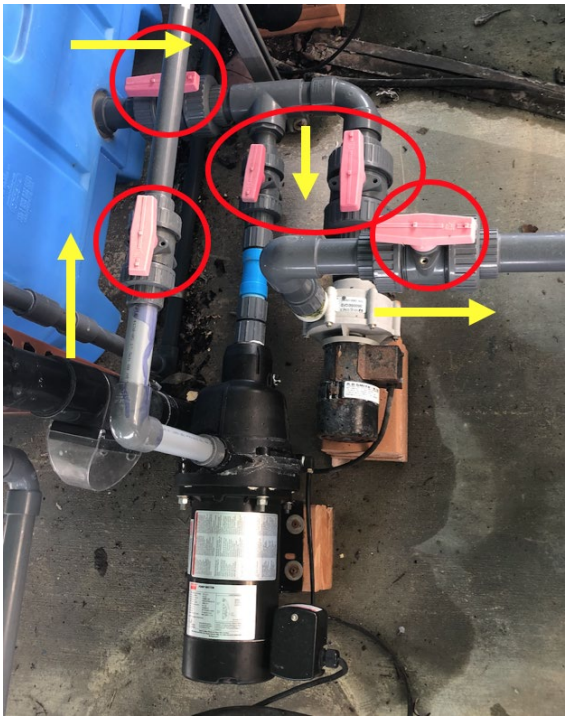


1.4 Head towards the freshwater vat, see picture below, check if it is filled with water all the way to the top. If it is, skip to step #6. If it is not, read step #5.

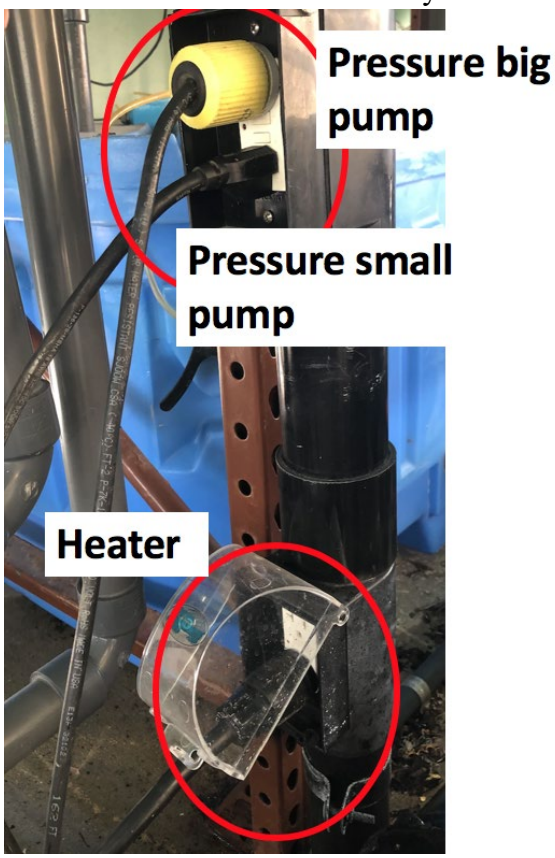


1.5 Turn these valves vertically, so they start pumping freshwater into the freshwater vat. Wait until the vat is completely full.

1.6 Make sure all these valves are open. If one of these is not open, it could cause the pump to run dry (this will damage the pump; it will be poor handling and if the pump breaks, your PI will be responsible for replacing the pump).



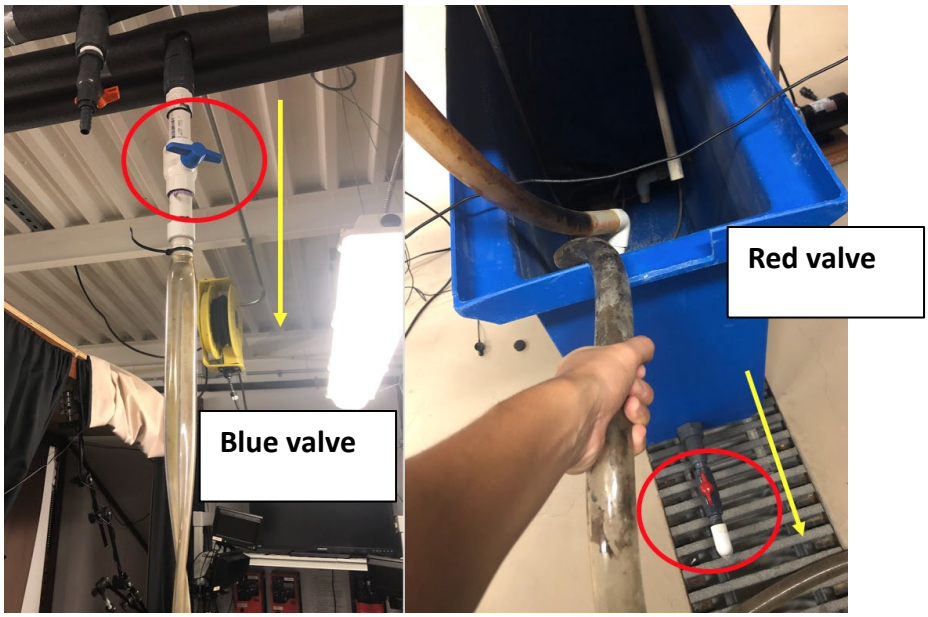
1.7 Once all valves are open, connect the 2 pumps and the heater cords to the electricity outlets. The yellow cord belongs to the big pressure pump that will push the water inside of the module building.
The thin black cord belongs to the small pressure pump that collects the water from the vat and pushes it to the heater.
The thick black cord belongs to the heater.



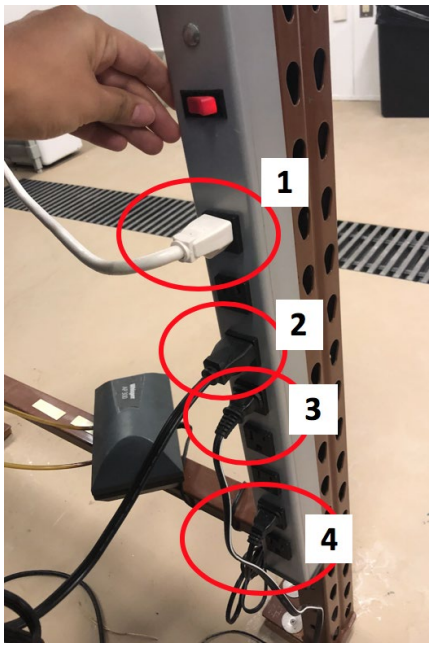
** IMPORTANT **
Once you are done filling the blue sump inside of the module. Immediately:

a) Disconnect the electrical cords,
b) Flip the switch from the control box towards off,
c) And turned valves from step 1.5, horizontally.

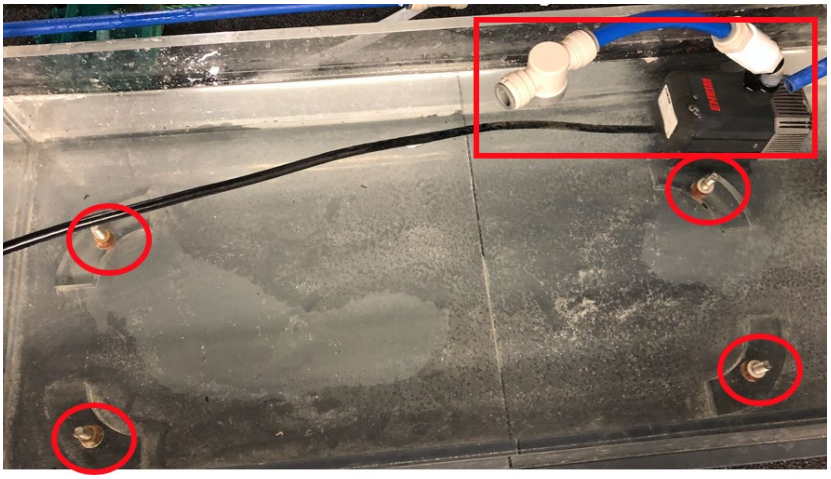
1.8 Inside of the module building, turn the blue valve underneath the ceiling vertically (ON) to fill up the blue sump. Once you do this, it is very important that you hold the hose with two hands because the water comes in with a lot of pressure. It is recommend to leave the red outgoing valve open for about 2 minutes, to get rid of the water that has been sitting in the PVC lines. Once the odor goes away, close the red valve so no more water keeps coming out of the blue sump. Once you are done filling the blue sump, turn the blue valve underneath the ceiling horizontally (OFF).



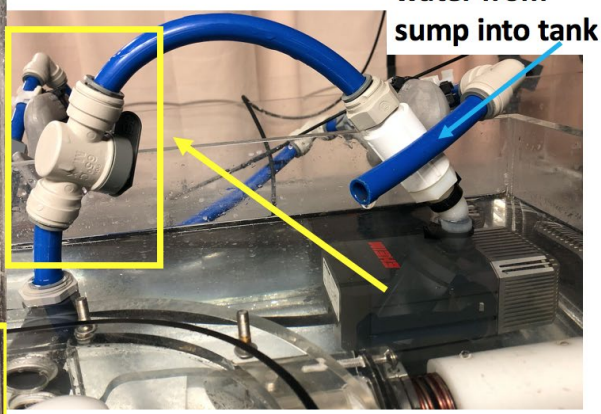
1.9 Once the blue sump is filled, make sure these four electrical cords are connected. The top one connects to another set of extension outlets that turns on the pump in the swimming performance tunnel, see picture (do not turn on the other outlet until further instructions; step #2.1). The second outlet connects the pressure pump that will pump the water from the blue sump into the swimming performance tank. Important **do NOT turn on the pump if the blue sump is not completely filled with water**; sometimes this pump takes a few tries to turn on and push the water inside the swimming tunnel. The third outlet connects to a heater. The bottom outlet connects to the air stones that can be found in the blue sump.



1.10 Once the swimming performance tank is half full, ensure that the four small **plastic** o-rings are placed on the bottom screws of the tank. Also, place the little pressure pump inside of the tank in the top right corner (see pump in the picture below).



1.11 Place the tunnel inside the tank, on top of the plastic O-rings by aligning with white cylindrical connecting piece (see photo). Connect the pump hose to the blue hose coming out of the tunnel. The pump inside of the tank will push the water from inside the tank into the tunnel; open the gray valve (vertical). The hose that hangs on top of the pump brings water from the sump into the tank. Screw all corners of the tunnel. Do not press too tight or you will break the



tunnel! (This is very expensive equipment; you and your PI will be responsible for any damages.).

1.12 Next, place these two items in the tunnel. Item #1 is a thin plastic base that goes in the space closest to you. Item #2 needs to be placed under the two screws that pop out of the fan (see Figure 1 circled screws in photo).

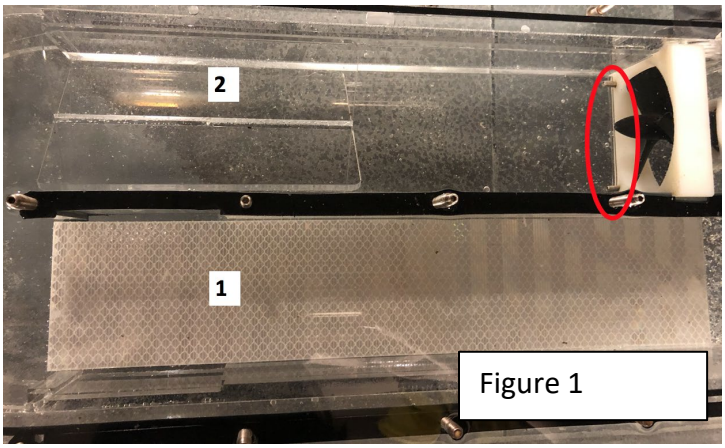
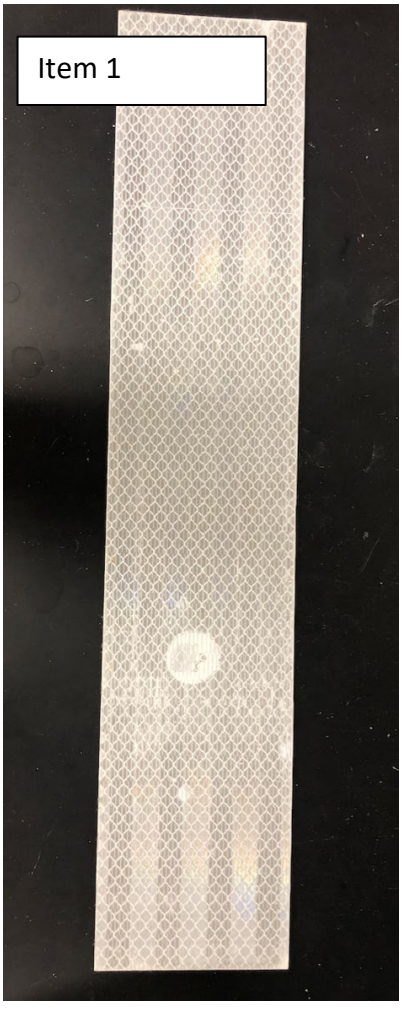


Figure 1

White cylindrical connecting piece



1.13 Then, insert Items 3 and 4 as displayed in the pictures below (Figures 3b and 4b). The top of Item 3 needs to be at the same level as the frame of the tunnel. If the top of this item is not at the same level of the frame of the tunnel, the main tunnel cover will not close properly.

Item number 4: It is hard to see, but in the picture on the right (Item 4b), there is a clear small rectangular divider that has been enclosed in this picture with two yellow lines. Item 4 has to fit between the rectangular piece of plastic and Item 1. Once it is placed correctly, that piece should not move at all. Item 4 has to fit securely between Item 1 and the small rectangular divider in the tunnel. You may need to push a little bit so it fits securely. If it wiggles, it is not in place or secured properly.

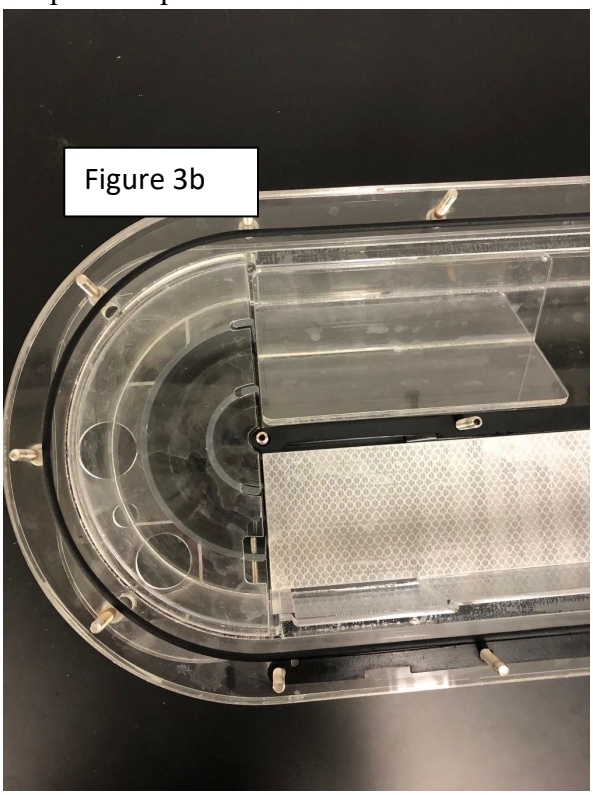
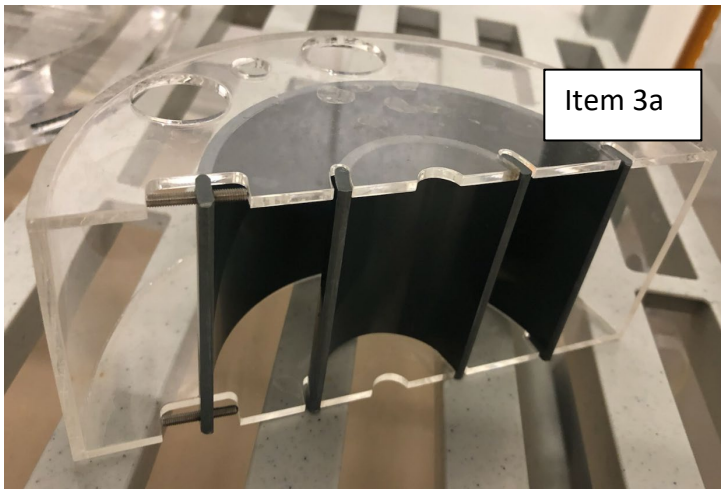


Figure 3b


Item 3a


Item 4a

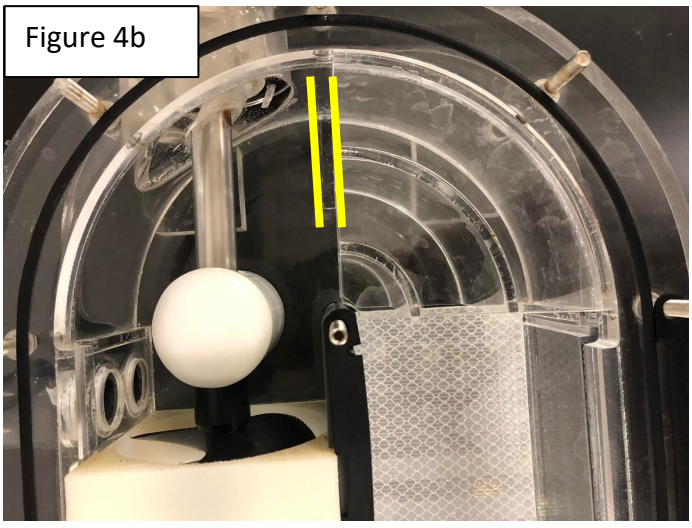
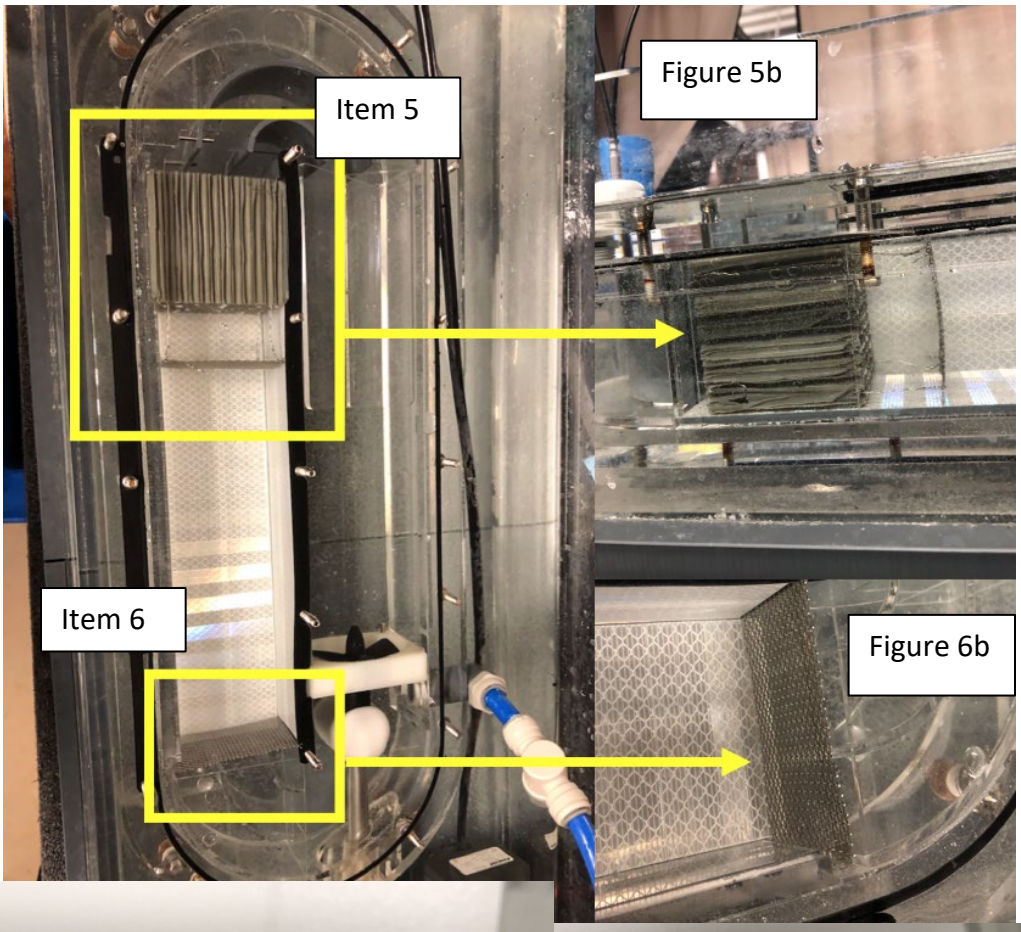
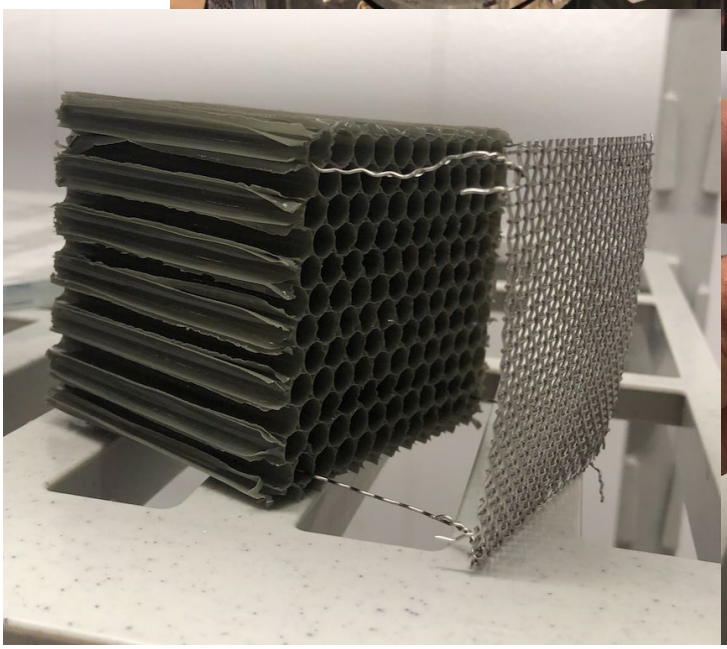
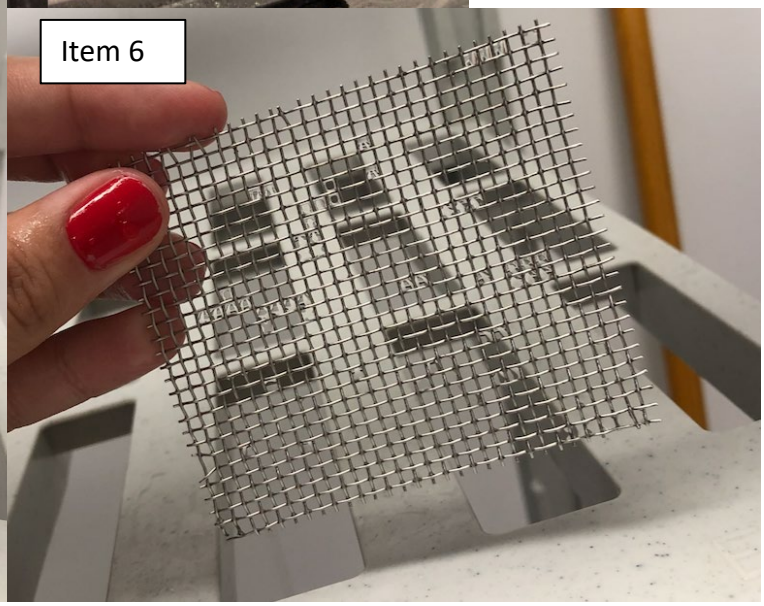


Figure 4b

1.14 Place the last two items as shown in Figures 5b and 6b. Item 5 helps the water to disperse equally across the entire tunnel. Item 5 has a thin metal mesh attached to the front of it, it is there for the same purpose.
Item 6, individual metal mesh, needs to be placed as shown in Figure 6b. It is attached to the right side of the tunnel is also to disperse water evenly.

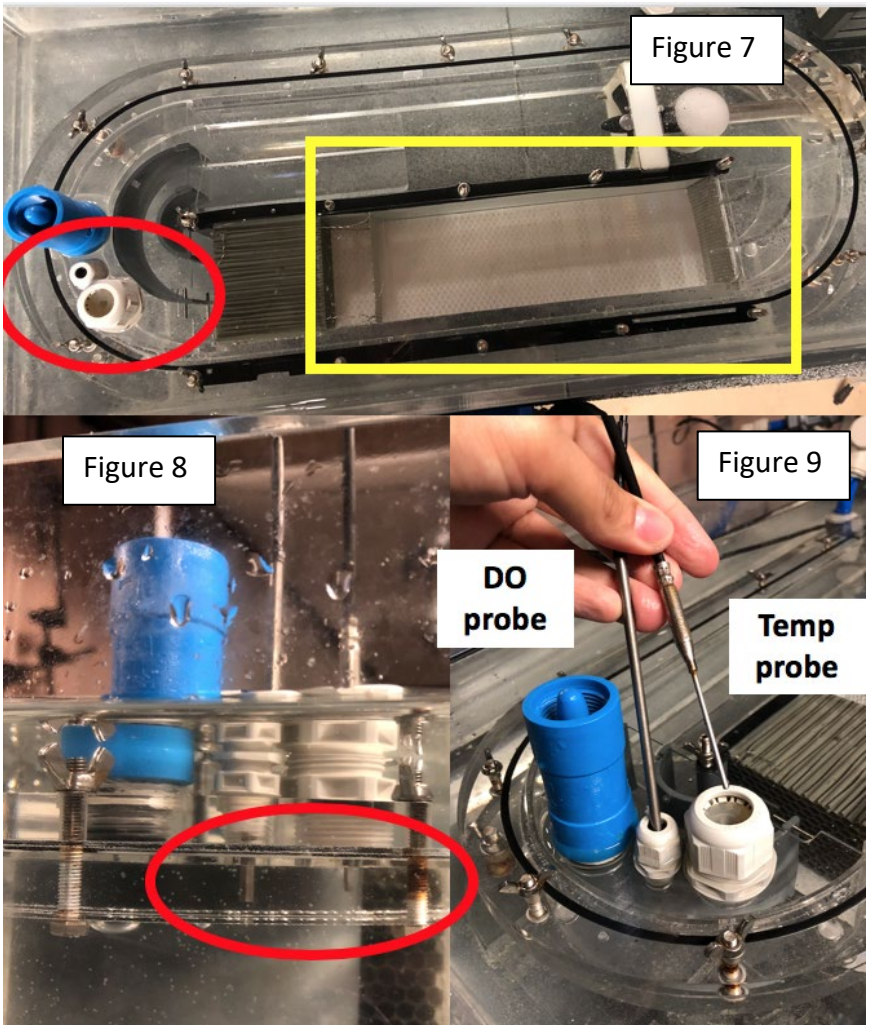






1.15 Screw all nuts in place, do not screw too tight, just with your hand. Do not screw the nuts inside of the yellow rectangle (testing section) (Figure 7).

Insert the DO and temperature probe in their respective holes (Figure 8). Do not push the DO probe all the way in, just the tip, same with the temperature probe (Figure 9).



2.0 Velocity Calibration

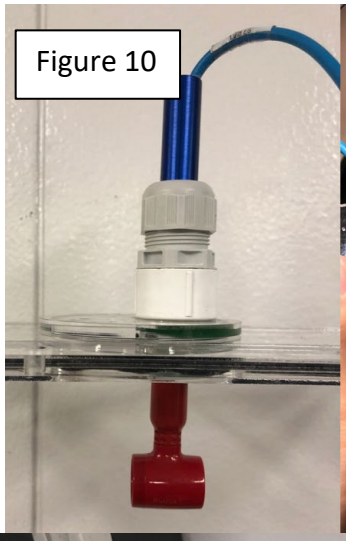
If the system has not been used in more than 2 weeks, you will need to run calibration tests. First start with the velocity calibration procedure (and then proceed with DO calibration, Section 3.0).

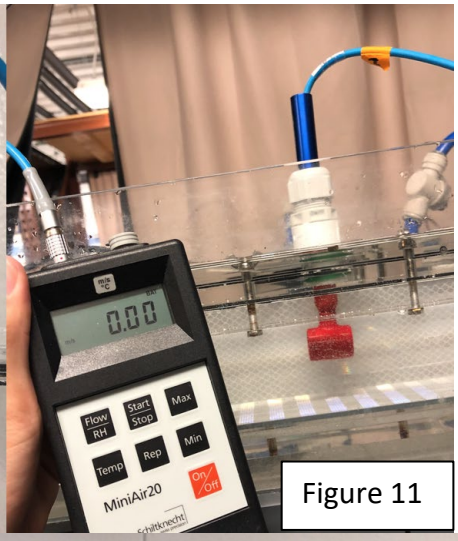
2.1 This is the step in which to turn on the electrical outlet that was mentioned in step #1.9. This will turn on the pump located in the top corner of inside of the tank and will direct the water from inside the tank into inside the tunnel.

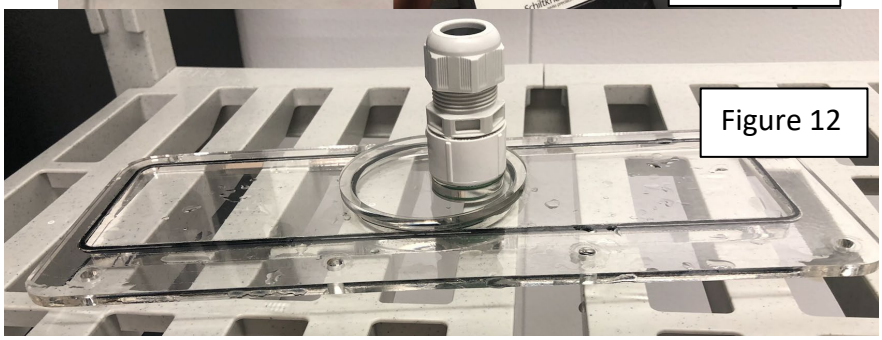
2.2 Flush all the bubbles in the tunnel by following step 2.6 (place the knob on "knob" and increase the velocity until all the bubbles in the system are out) before covering the tunnel.

2.3 Introduce the velocity meter into the small plastic lid (Figure 12) designed to calibrate the swim tunnel (Figure 10). The cover can be found on the drying rack where all tunnel pieces are kept. The velocity meter can be found in the black box labelled "Mini Air Flow Meter" next to system #3.

2.4 Place the velocity probe in the middle of the tunnel, and connect the other end of the probe to the MiniAir20 Box (Figure 11). Make sure that the probe is perpendicular to the lid. Tie the gray plastic screw as much as you can, then screw the top to the tunnel with wingnuts.

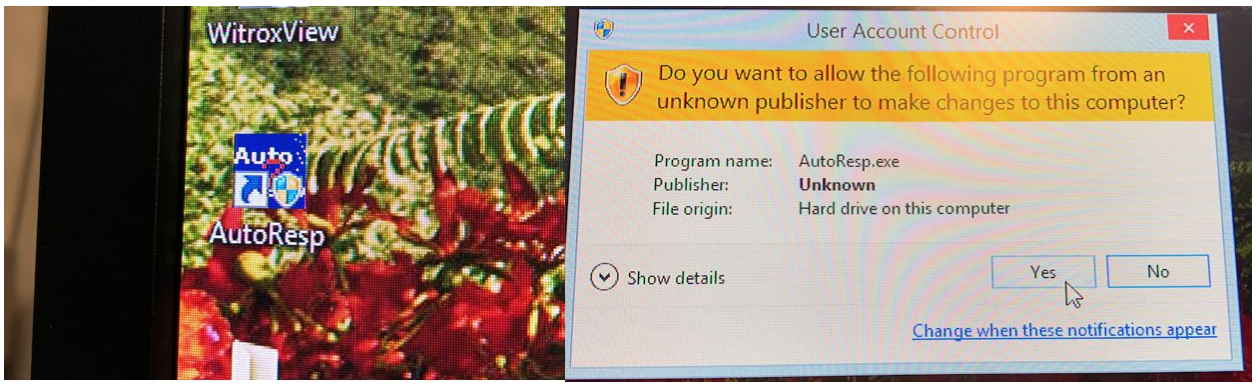






Note: To change MiniAir20 batteries, which are located in the main building (batteries 9V), remove the back lid of the MiniAir20 and change batteries as displayed in the picture below. MiniAir20 will show the sign “BAT” on the screen when battery is running low.

2.5 After you turn on the computer and ***log into to ecotox with password: ecotoxlab*** , click and open AutoResp. Then, click YES tab that pops up.



2.6 Turn on the two loligo Witrox boxes by pressing on the power button (turn on both boxes even if you are just using one tunnel) (Figure 13). Turn on the red box by switching the knob to power. Then, place the second knob on forward, and the last knob on EXT [the knob named "knob" means that if selected, you can increase/decrease the velocity yourself by moving the top first knob. "EXT" means that the swimming tunnel will be controlled by the computer]. The screen that shows the number 0, will show the velocity in RPMs. Once you calibrate the velocity you will be able to convert the RPMs to cm/s (Figure 14).

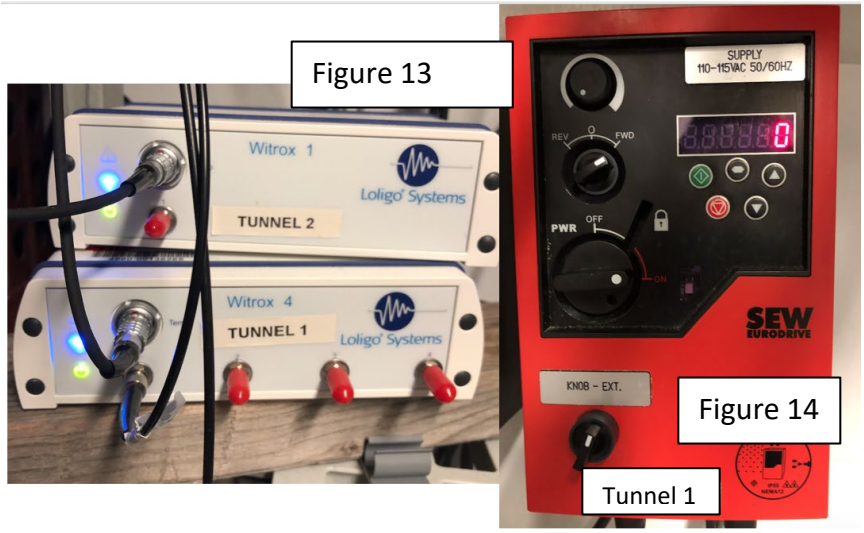
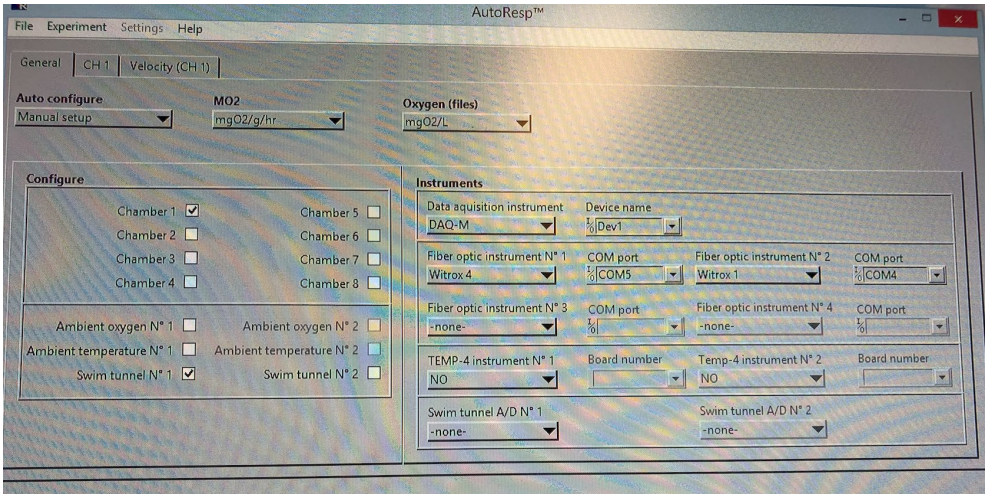


Figure 13

Figure 14

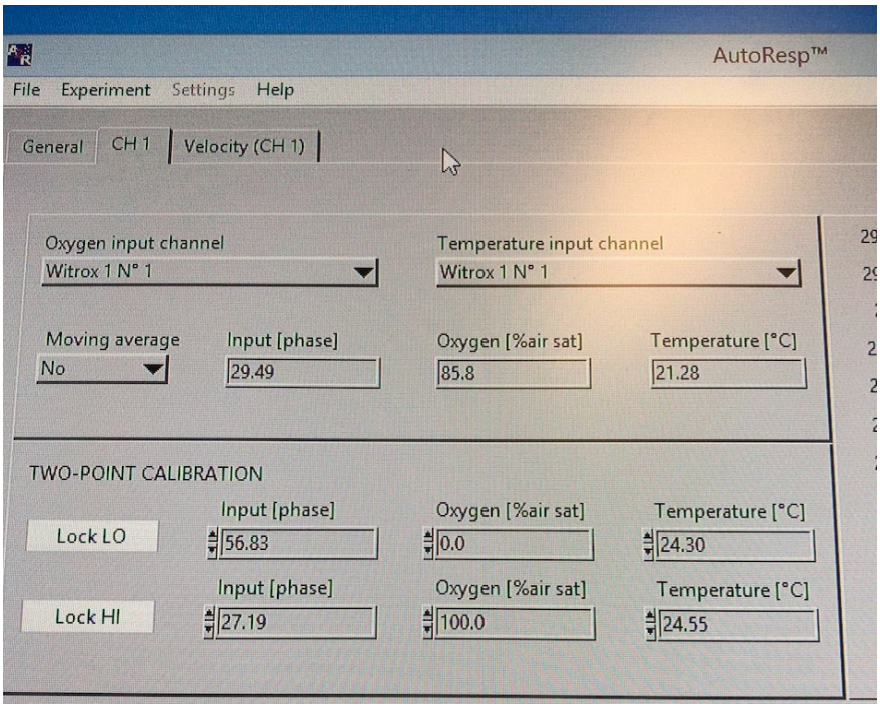
Tunnel 1

2.7 Once you open the AutoResp program, you should select COM5 next to Witrox4; and COM4 next to Witrox 1. This will connect the Witrox boxes to the computer and the red box, you will know the Witrox boxes have connected because the wifi sign on the boxes will not be flickering anymore, they will stay bright blue. Steps 2.6 and 2.7 need to be done in a short time interval so that the wifi signal is not lost; if the wifi signal is lost, turn off and on the Witrox boxes and try step 2.7 again. Everything else should be kept the way it is shown in the picture below.

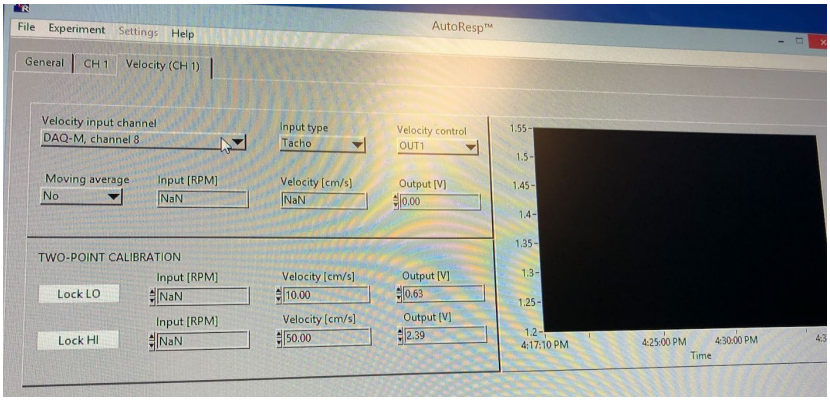


2.8 Before starting the calibration of the equipment. Turn off the pump that pushes the water from the tank into the tunnel. (This is the same pump as in step 2.1).

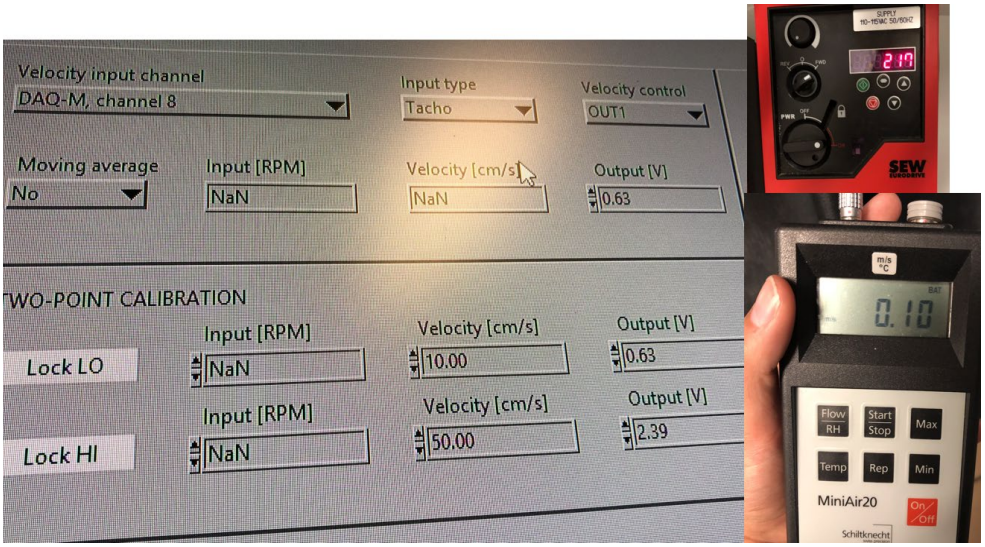
2.9 Click on CH 1, make sure the oxygen input channel is set up to Witrox 1 N1 as well as the temperature input channel. If you do not have these set up correctly, you will not be able to start the experiment. You will come back to this tab when you calibrate the Oxygen Probe.



2.10 **<u>Time to calibrate the velocity:</u>** As soon as you click on the Velocity (CH1), the screen should look similar to this, the top part above the line should look exactly the same. The values under Output [V] are the values that the last person that used this equipment used to calibrate the water velocity. This screen shows that the last person calibrated 0.63 V to be 10cm/s, and 2.39 V to be 50 cm/s.

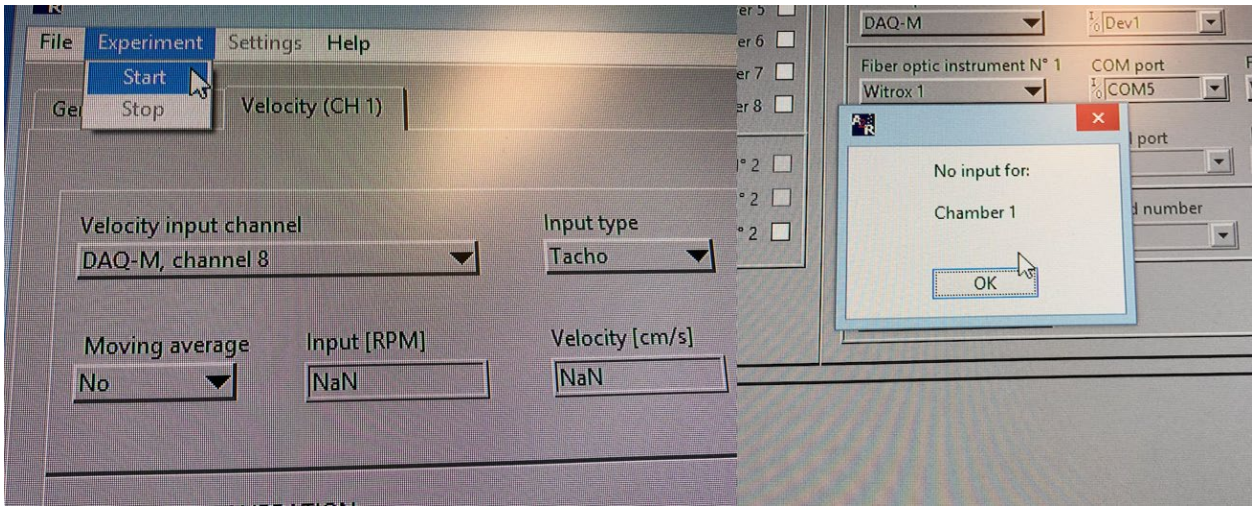


2.11 To start, it is helpful to look at the last calibrations. In this case, 0.63 V = 10cm/s & 2.39 V = 50 cm/s. You can always change these extremes velocities if your experiment needs it (10 and 50 cm/s settings were used with Sailfin molly, *Poecilia latipinna*; 10 and 75 cm/s settings were used with blue gill, *Lepomis macrochirus*, and cobia, *Rachycentron canadum*; ultimately, it is dependent of organism type and their physiology). As an example, 2.39 was entered under the "Output [V]" above the line and corroborated with the MiniAir20 apparatus that 2.39 V was indeed 50 cm/s. If the MiniAir20 indeed indicates that 2.39 V is 50cm/s, you need to press "Lock HI" to make the Output [V] next to "Lock HI" that same number. In case, MiniAir20 showed a value different from 50cm/s, you would have to play around with the voltage in the computer until the MiniAir20 showed 50cm/s in the MiniAir20 display/screen and then press the "Lock HI" button. You have to write all these values in Form #1 (see page 20). The red box will give you the velocity in RPM, also write down this value. Then repeat with the lowest velocity, and press "Lock LO" (see picture below).

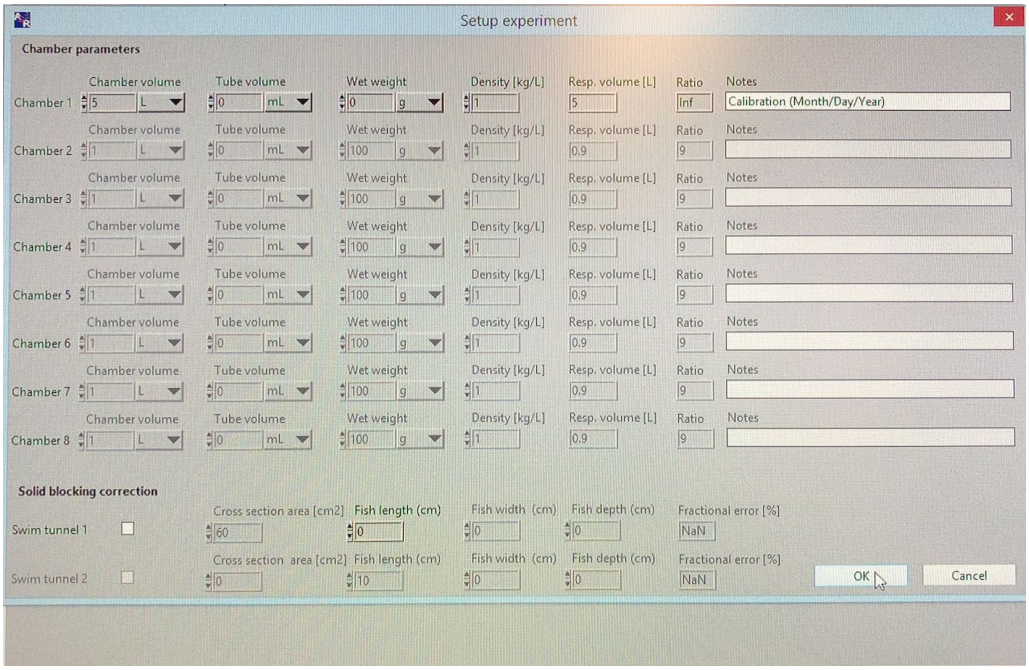


NOTE: If the current calibration values are very different from the last calibration (±1.5 (V), the tunnel system has not been assembled optimally/properly. See if all the pieces are fitting properly (Step 1.10-1.14). If there are too many bubbles inside the tunnel, remove the lid and push out the bubbles.

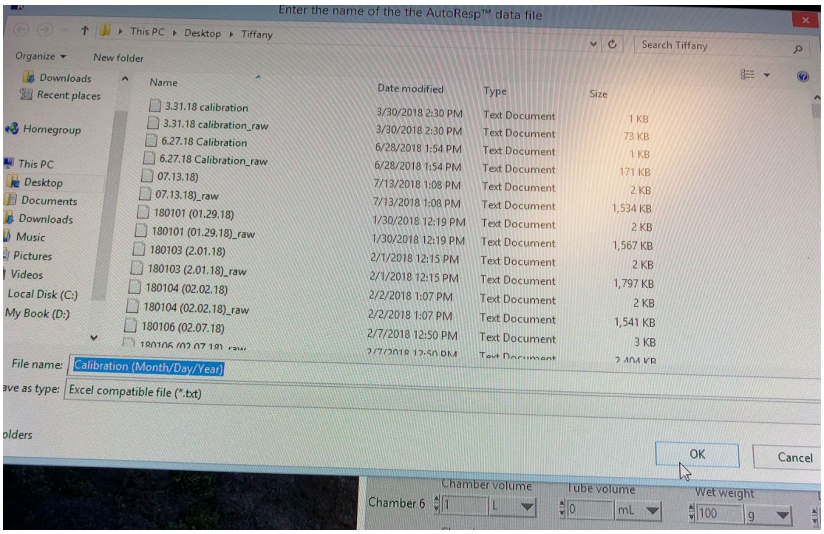
2.12 Click on “Experiment” and then on “Start”. If an error message pops up like the one down below and right, then you must be missing something, re read all steps above.



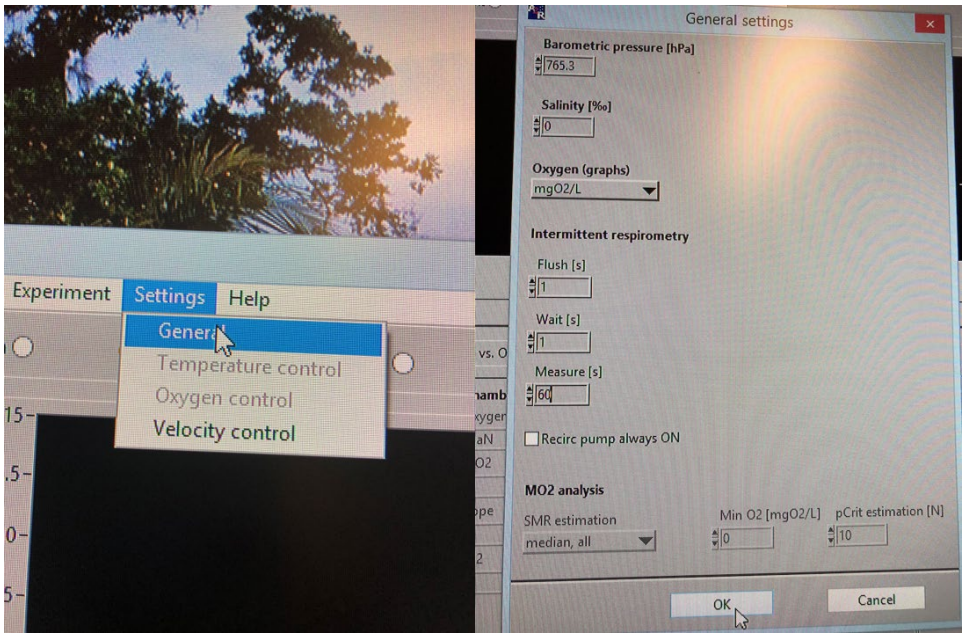
2.13 Once you start the experiment, the following screen will pop up. The only active numbers should be in row chamber 1. All digits should be zero except: Chamber 1, Chamber volume, resp volume. The chamber volume and the resp. volume should always be the same. Unclick swim tunnel 1 under solid blocking correction. You will only use the first row of this screen, and you will write Calibration (Month/Day/Year) under Notes, and then press OK.



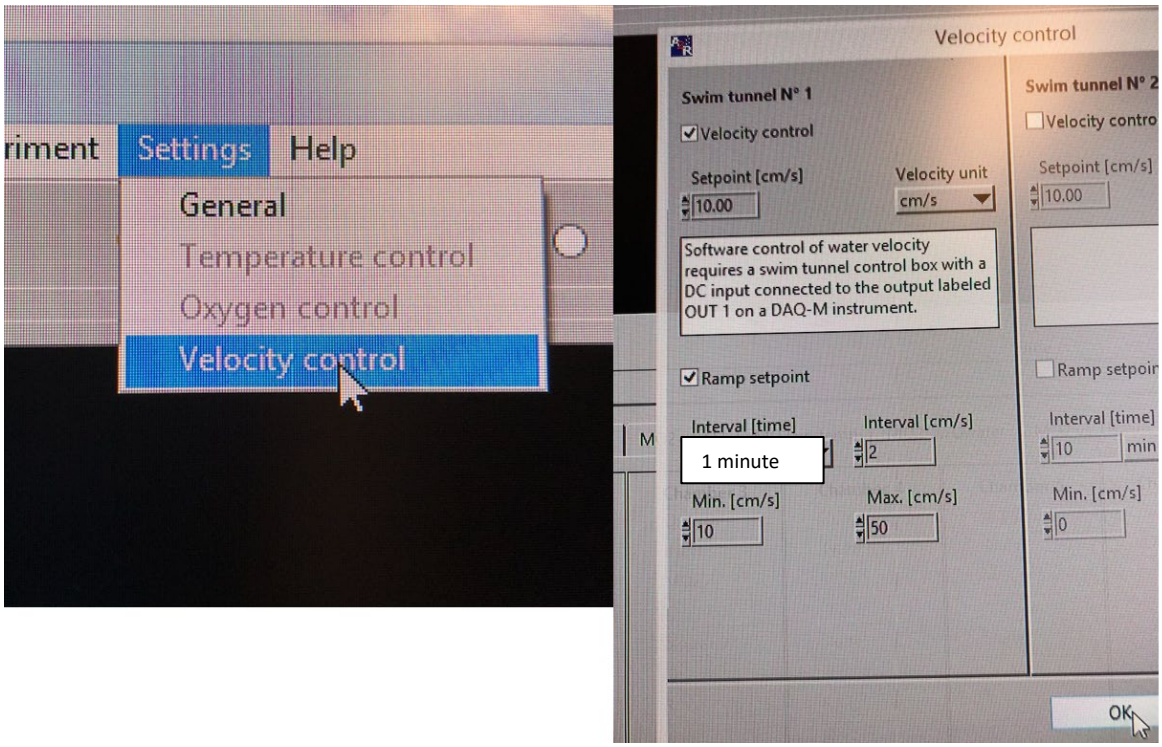
2.14 After pressing okay in the previous step, this screen will pop up. You can decide where to save your file, but make sure you also save it with Calibration (Month/Day/Year), and then press okay. This is the same procedure that you will follow to save a real experiment.



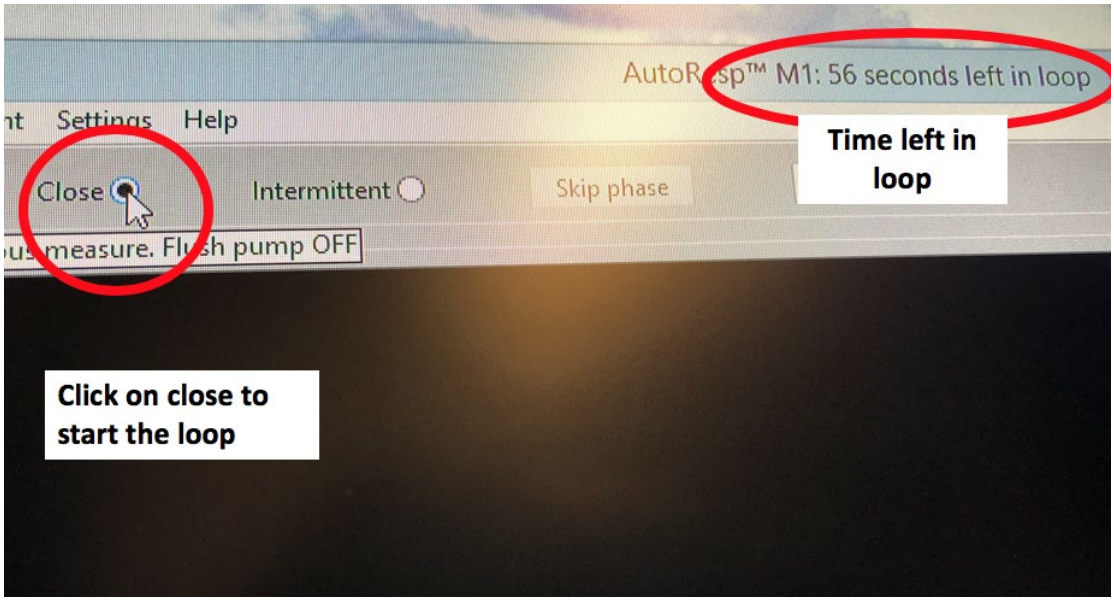
2.15 Click on “Settings” and then click on “General”, the image below will pop up. You can change the Barometric pressure depending on the barometric pressure of the day (note: having to change it is very unusual). The only thing that you have to change on this window is the number of seconds under Measure [s]. The number 60 in this case indicates that every 60 seconds, the velocity will automatically increase by 2 cm/s. You can modify it: you will enter a number < 60 seconds to calibrate faster or a number > 60 to have more time to write the values down on the Form #1 (see page 20). Press okay.



2.16 Press “settings” and then “velocity control”. Then, the “velocity control tab” will pop up and you will first click “velocity control”, there, you will put the lowest velocity that you chose in step 2.11. Then, you will click on “ramp setpoint” and you will type the seconds that you chose in the previous steps under “interval [time]”, and next to it, under “interval [cm/s], you will choose by how much you want to increase your velocity in cm/s (this will depend on your experimental protocol); that is, this is where you indicate at which rate you want to increase your experimental velocity from your Min to your Max. For example, if the experiment Min velocity is 10cm/s and Max is 50cm/s and the interval is 2cm/s, this means that every interval time the velocity will increase by 2 (so 10, 12, 14, 16… 48, 50). Under Min [cm/s] you will put the lowest velocity that you chose on step 2.11 and as well as the Max [cm/s] velocity that you chose on step 2.11. Press okay.



2.17 Once you pressed okay, the water flow in tunnel will start, but it will not change from 10cm/s (number that I put on step 12 under setpoint [cm/s]) unless you press CLOSE. Once you press close, you will see a timer on the top of the screen. Every 60 seconds, the computer will increase the velocity by 2cm/s.



Form #1.

FLORIDA INTERNATIONAL UNIVERSITY — PAGE: _____ OF _____
ECOTOXICOLOGY & RISK ASSESSMENT LABORATORY — FORM No: 373
NORTH MIAMI, FL — EFFECTIVE DATE: 05/14/14

LOLIGO SWIMMING TUNNEL FLOW SPEED CALIBRATION LOG

CALIBRATION DATE		TIME		ANALYST	

TUNNEL ID		PHASE	SPEED (cm/s)	VOLTAGE (v)
1	HIGH		50	2.39
	LOW		10	0.63
	HIGH			
	LOW			

TUNNEL 1				TUNNEL 2			
INPUT SPEED (cm/s)	RPM	V (cm/s)	Voltage	INPUT SPEED (cm/s)	RPM	V (cm/s)	Voltage
10	Values that the red box gives you	Values that the MiniAir20 gives you	0.63	40	Values that the red box gives you	Values that the MiniAir20 gives you	
12				42			
14				44			
16				46			
18				48			
20				50			2.39
22							
24							
26							
28							
30							
32							
34							
36							
38							

FLOW METER ID:		SERIAL NUMBER:	

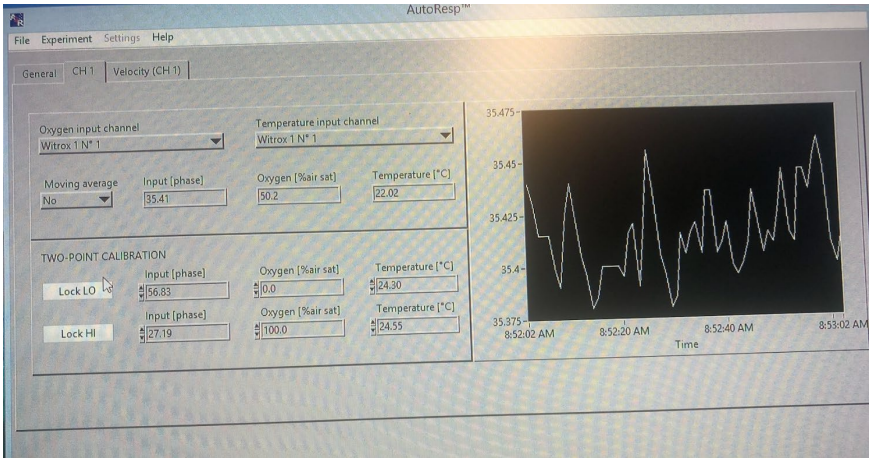
Form #1 will help you to keep track of your calibrations, and also to inform the next person that will use the equipment of your previous calibration (Form #1 is kept in the binder next to the computer monitor called “Loligo System Calibration Log”; this binder must never leave the room). You can modify the input speed as you desire, but the protocol recommends that you start with 10cm/s, the highest velocity will depend on the species that you will be using. The increase in velocity will also depend on your protocol. In step 2.16, you instructed the software to increase the velocity by 2cm/s every 60 seconds (1 loop). Therefore, every 60 seconds you will have to write down on Form #1, the input speed that you are aiming for (column 1), the rpm values that you see on the red box (column 2), and the velocity that you read on the MiniAir20 (column 3). You will only be able to write the voltage of the first and last speed, you get these from the voltage used in step 2.11.

If you realize that the MiniAir20 is not giving you correct values, you have to go back to step 2.11 and adjust the extremes, and set up a new experiment and try again, until the values of the MiniAir20 match your input speed. You will never obtain exact values, but they should not vary too much (± 2) from the desired speeds. Calibrations should be done every 10-14 days, or when you replace a piece/item of the tunnel. Always keep the pieces from tunnel 1 and tunnel 2 separate.

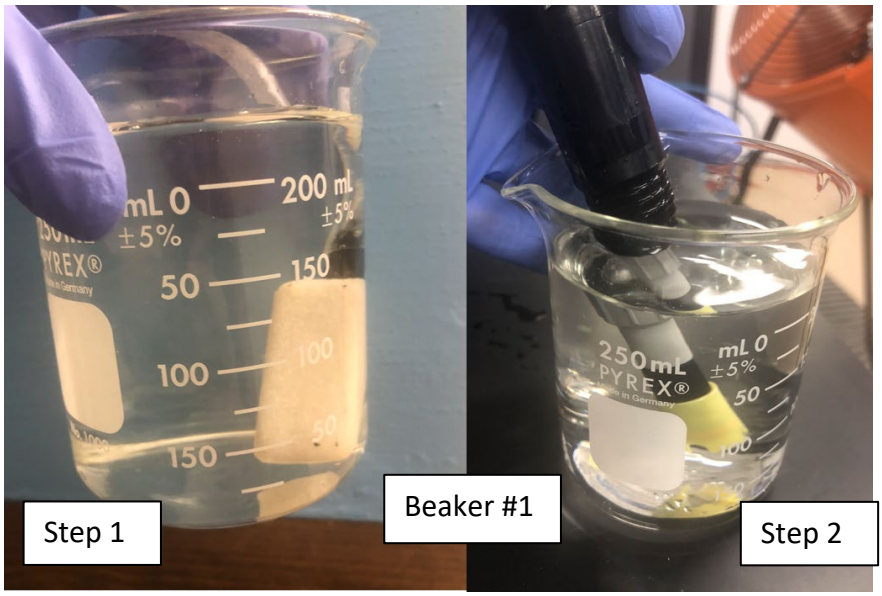
Do not forget to write your name, calibration date and time, the tunnel ID, flow meter ID, Serial number of flow meter ID. No need to enter or record phase space. Once you are done with the calibration, press on the “Experiment” tab on the top, and stop.

3.0 DO Calibration

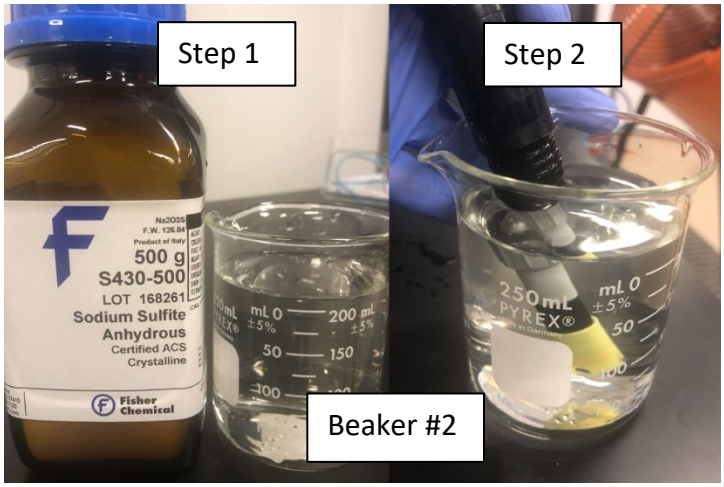
3.1 The one tab that you will only need for this calibration is CH1, if using swim tunnel #1. Other tabs will be available if you are using other tunnels.



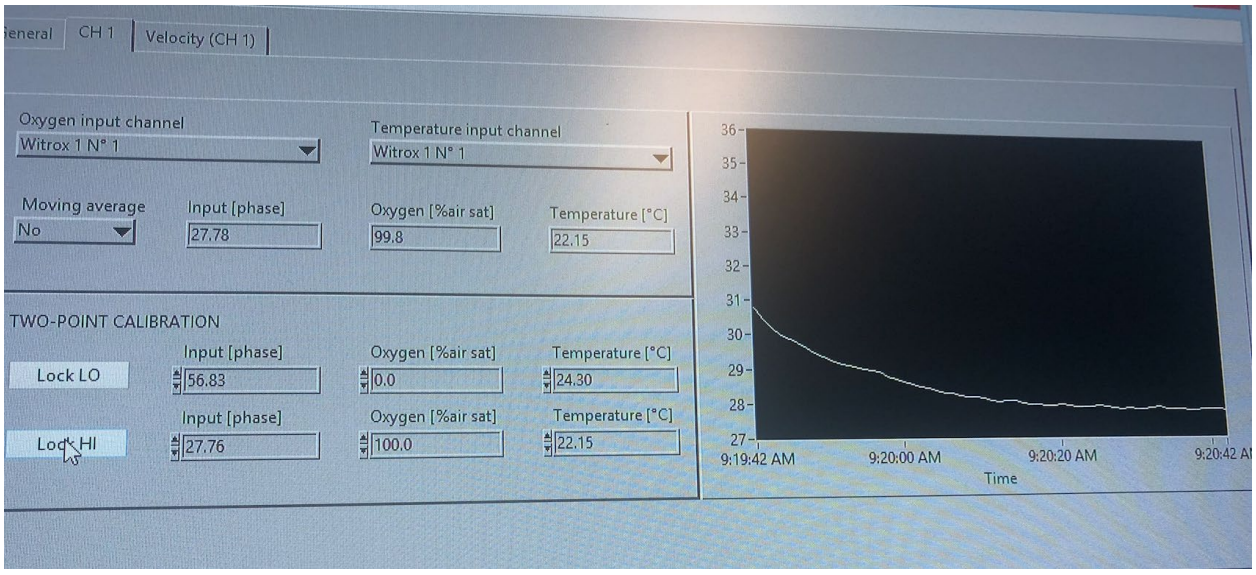
3.2 Before starting the calibration, you need to get two 250 ml beakers filled up to 200ml with freshwater. You will saturate the water in the first beaker to 100 % by introducing an air stone (Step 1). You will confirm that the water is saturated by using a DO probe such as YSI Pro2030 (Step 2).



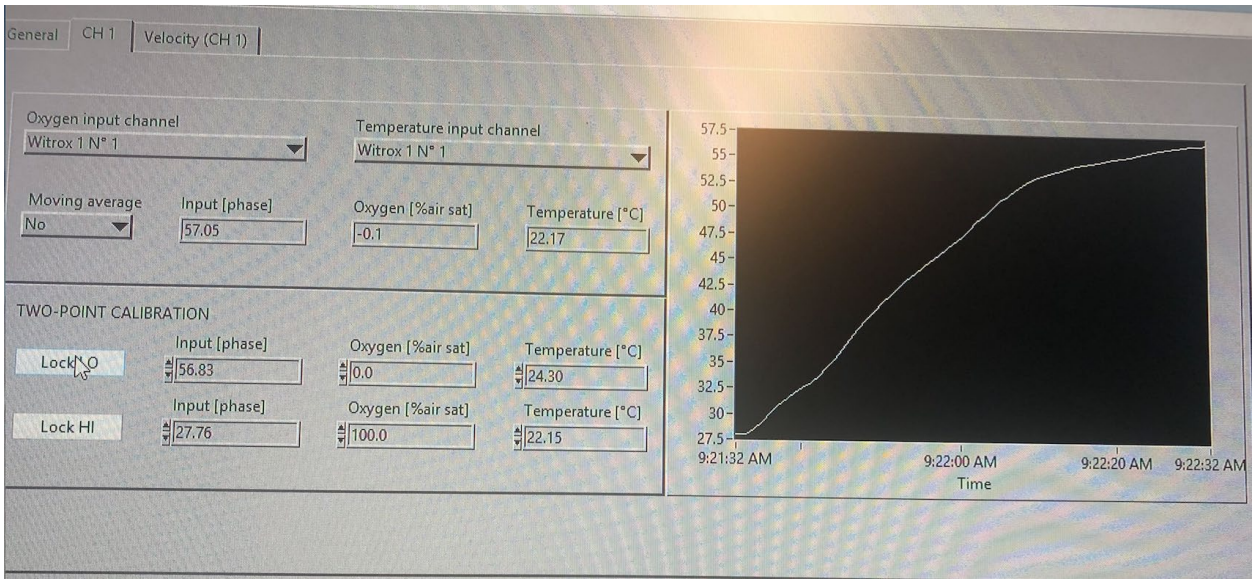
3.3 In the second beaker, you will add ~3 g sodium sulfite anhydrous. Mix for about a minute until all the grains dissolve. BE SURE TO BE WEARING PROTECTIVE GEAR, INCLUDING A FACE MASK AND GLOVES, AND TO DO THIS IN THE FUME HOOD!



3.4 Once you have the two beakers ready, you will first insert the swimming performance DO probe from the swimming tunnel into the **saturated beaker**. When you insert the probe, do not forget to gently move it side to side. While you move the probe, the numbers on the computer under "Oxygen [air sat%]" will start increasing and the numbers under "Input [Phase]" will start decreasing. You will also see the line in the graph going down, this shows the same as the values under "Input [phase]". Once the values under "Oxygen [%air sat]" reach close to 100, click on "Lock HI", and write these values on Form #2.

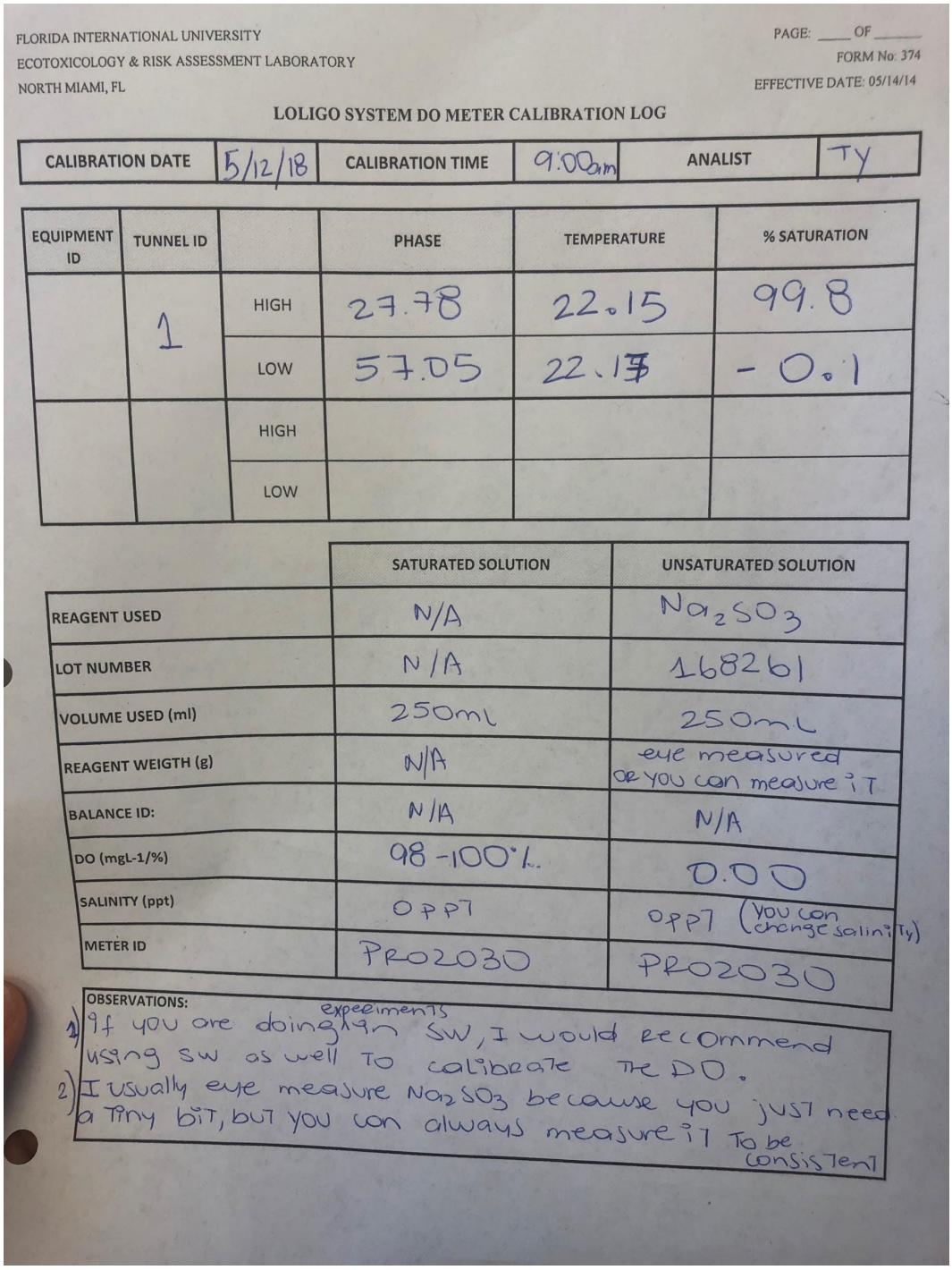


3.5 Then, insert the probe in the beaker with very low oxygen. The values under “Input [phase]” will start increasing, and the ones under “Oxygen [%air sat]” will start decreasing. The line in the graph will start increasing as the values under “Input [phase]” start increasing. Once the values under Oxygen [%air sat] get close to zero, press “Lock LO” and write these numbers down on Form #2.



You will be able to find the oxygen consumption information in the folder where you will be saving your actual experiment (not the calibration, but your project experiment). As you collect data from your experiment, you will be able to see the raw data: changes in oxygen every few seconds, and in another file summarized data which only addresses the change of oxygen per time that you put in step 2.15.

FORM #2



FLORIDA INTERNATIONAL UNIVERSITY
ECOTOXICOLOGY & RISK ASSESSMENT LABORATORY
NORTH MIAMI, FL

PAGE: ____ OF _____
FORM No: 374
EFFECTIVE DATE: 05/14/14

LOLIGO SYSTEM DO METER CALIBRATION LOG

CALIBRATION DATE	5/12/18	CALIBRATION TIME	9:00am	ANALIST	TY

EQUIPMENT ID	TUNNEL ID		PHASE	TEMPERATURE	% SATURATION
	1	HIGH	27.78	22.15	99.8
		LOW	57.05	22.17	-0.1
		HIGH			
		LOW			

	SATURATED SOLUTION	UNSATURATED SOLUTION
REAGENT USED	N/A	Na_2SO_3
LOT NUMBER	N/A	168261
VOLUME USED (ml)	250mL	250mL
REAGENT WEIGTH (g)	N/A	eye measured or you can measure it
BALANCE ID:	N/A	N/A
DO (mgL-1/%)	98-100%	0.00
SALINITY (ppt)	0 PPT	0 PPT (you can change salinity TY)
METER ID	PRO2030	PRO2030

OBSERVATIONS:
1) If you are doing/an experiments SW, I would recommend using SW as well to calibrate the DO.
2) I usually eye measure Na_2SO_3 because you just need a tiny bit, but you can always measure it to be consistent

You have completed calibration steps required to start an experiment if you have not run one in more than two weeks. Also, remember that you should perform the calibration the day prior to when you intend to run the actual swimming performance experiment/trial.

4.0 Swimming Performance Protocol

Objective

To assess fishes swimming performance through the evaluation of two activities: "prolonged swimming" speed (Ucrit) performance and exercise metabolism. The biological endpoints (or effects criteria) measured in each of the latter activities can be used to compare between chemical exposed (or other environmental insult) treatment groups and untreated controls to determine potential sub-lethal effects of chemical exposure on swimming physiology.

Background Information

In the case of Aquatic Toxicity studies, a swimming performance trial begins with an exposure to a test material. Once the water is prepared (mod-hard FW or synthetic SW), the proper dilution is prepared and a 13.3L holding container was filled with 8L of test material. With the exposure set-up ready, a fish is captured, weighed, measured, and then placed into the exposure container. The fish is then individually exposed in the test material media for 96 h (this time changes depending on your experiment objectives). After the exposure is complete, the fish is removed and placed into the swim tunnel/respirometer. If this is not an aquatic toxicology study, follow your protocol.

The tunnel is sealed, monitoring devices are setup and the tunnel is flushed with oxygen saturated water to maintain the DO and temperature (see steps 1.0 Experimental Set-Up). The area around the tank is surrounded by black curtains to eliminate any outside extraneous influences. The fish is allowed time to acclimate to the swim tunnel without any flow in the tunnel. Acclimation time is typically a minimum of 30 minutes, but sometimes it may need to be longer based on how the fish is responding to being in the swim tunnel. Some fish take longer to adjust and calm down. This cannot be a set time period, as each fish reacts differently, as it is to be expected in a behavioral study. Once the fish has calmed down (i.e., determined by observation via camera connected to a remote monitor, see step 5.8 a & b), a slow flow (10 cm/s, this is your "Lock LO" that was entered during velocity calibration) is initiated in the tunnel, and should last a period of 10 minutes; this is considered the second acclimation process. At the beginning of the second acclimation is when you should turn off the pump explained in step 2.8 & 2.1 to start accounting for the fish oxygen consumption.

Autoresp is the name of the software that controls the RPM of the propeller in the swim tunnel. It also records all of the collected data such as temperature and DO concentrations. When a swim trial begins, the water velocity is increased step by step in about 2 to 5 cm/s increments (it will depend on your range-finding studies; in the velocity calibration example, 2 cm/s was used). The velocity increase is dependent on the species being used. Range-finding and control studies are conducted to get an idea of what a fish species U_{crit} may be. This information is used to modify the design of the steps of future swim studies that are relevant to you. It would be expected that to make the study design appropriate for the species being used, an investigator would need to modify the design of the study based on (1) the organism used and (2) after several range-finding studies have been conducted. The estimated U_{crit} (critical swimming speed) of a species would need to be separated into about ten steps. That way, there will be a high enough resolution to get data around the estimated U_{crit}. For example, if a U_{crit} estimate was 55 cm/s, a step velocity increase of 4 or 5 cm/s would be appropriate. A step increase of 10 cm/s would be inappropriate as you would reach the U_{crit} value by the fifth step of the trial.

The duration of a step may also need to be modified based on the test organism. The desired endpoint partially dictates the step duration. U_{crit} values for burst, sustained, or prolonged swimming (see more detail in protocol BP-ERAL218, document can be found in Ecotox drive) may be determined in swimming performance studies. Burst swimming is typically observed with extremely rapid increases in speed to determine a maximum U_{crit} value called U_{max}. Sustained swimming studies are conducted with longer steps from 5 to 15 minutes and sometimes longer. The early step durations may be shortened to be able to prevent exceedingly long study duration times. This is called ramped U_{crit}.

A swimming performance study can generate a variety of quantitative endpoint values (e.g., critical swimming speed, aerobic scope), which can be used as a level of fitness for an individual organism (Tierney and Farrell 2004, Tierney 2011). Swimming performance studies monitor critical swimming speed and oxygen consumption over time while increasing the velocity of water over several steps that the fish is confronted with. It is similar to a stress test in humans. The obtained data can provide MO_{2max} (maximum rate of oxygen consumption), MO_{2min} (minimum rate of oxygen consumption), total O_2 consumption, aerobic scope (absolute or fractional difference between minimum and maximum oxygen consumption), and U_{crit} (critical sustained or endurance swimming speed).

Organism size is a variable that needs to be matched with the swim tunnel being used. There needs to be enough room in the swim tunnel for the fish to swim freely and not erratically due to stress from cramped space. This makes it more practical to use a size class of fish rather than an age to select the fish used in a study. The 5L swim tunnel has a swim chamber having dimensions of 30 cm in length, by 7.5 cm in width and height. It was determined that fish within 3-10 cm of length were the most appropriate size for the 5L swim tunnel.

The termination of a study is determined by the behavior of the fish during the trial. When a fish is exhausted, it may exhibit several behaviors that may include pressing against the back gate, resting its tail on the gate while maintaining their orientation in the tunnel, or other strategic positioning of their bodies that allow them to rest. These behaviors must be discouraged to have a successful test and an accurate endpoint. To avoid such undesirable behaviors, use light stimuli and/or reversal of the flow in the tunnel. If these stimuli do not encourage the fish to continue swimming, the trial is terminated (i.e., remove fish from tunnel, you cannot use the data recorded from this individual fish; use another fish to continue). The amount of stimuli and the decision to terminate a study is up to the study conductor. Experience on behavioral observations of a specific fish species is instrumental in being able to determine when a fish is exhausted.

In swimming performance testing or other behavioral studies, it is necessary to be able to modify certain parameters of the experiment to make it appropriate for the organism being used. In aquatic toxicology, this is different than the strict aquatic toxicity test guidelines of an US EPA or American Society for Testing and Materials (ASTM) protocol. As more studies are conducted and the investigators learn more about the organisms used, parameters of these studies may change.

Additional Background Information

Swimming performance in fish refers to any quantifiable component of swimming ability that has to do with endurance, metabolic rate, maximum acceleration, turning radius, etc. Performance thus mediates fitness. Beamish (1978) provides an extensive review of methodologies used in studying swimming capacity in fish.

Swimming performance can be classified into three categories (Beamish, 1978): sustained, prolonged, and burst (or sprint). Definitions are included below. We will use "prolonged swimming" (Ucrit) and aerobic scope for activity to assess the effects of chemicals or other environmental stressors. Such stressors (e.g., pollutants and changes in temperature) can adversely affect energy metabolism by reducing the maximum (active) rate of oxygen consumption or aerobic scope which is related to swimming performance. The latter may be affected by muscle contractility and/or the rate of gas transfer by the gills and the circulatory system and/or adverse effects on the nervous system.

"**Sustained swimming**" includes all levels of activity from spontaneous movements to cruising speeds which can be maintained in excess of 200 min without resulting in muscular fatigue. In effect, sustained swimming can be maintained for long periods without resulting in muscular fatigue. Sustained performance includes individual as well as schooling fish and routine activity which represents daily movements-foraging and station holding (steady and unsteady swimming); theoretically, sustained performance can be maintained indefinitely.

"**Prolonged swimming**" speed is of shorter duration (20 sec-200 min) and results in fatigue. In the field, it is difficult to separate sustained and prolonged swimming and fatigue; fish seldom maintain a given speed as long as 200 min. To measure the maximum prolonged swimming speed of fishes, the measure of "critical swimming" speed (Ucrit) was developed. This measure has been frequently used in numerous species and experimental conditions (e.g. water quality changes such as contaminant eposures). Ucrit is measured for those fish that swim through ten or more 'steps' before reaching fatigue. The steps consist of fixed increases in speed and duration. The steps can vary from 10 min to 2 h, and the test ends when the fish fails to maintain position for the full interval at a given step. Swim performance tests of Ucrit have validity especially for those fish species that are migratory or normally swim for long periods. Beitinger and McCauley (1990)

emphasized that swimming performance stamina is probably not as important in many inactive fish species, which rely more on burst swimming for the capture of food and evasion of predation. Brett (1964) created a precise method of calculating Ucrit as the speed of the last fully completed step plus the temporal fraction of the final uncompleted step. Most swimming performance studies involving contaminants have measured Ucrit.

The highest speed that a fish can attain is called "**burst speed**". These high speeds can be maintained in most fish for less than 1 min and are characterized by an initial acceleration phase of unsteady swimming followed by a steady phase termed "sprint". The need for short-term high swimming performance is often essential for survival of many species as it facilitates the capture of prey/food, avoidance of predators, or the negotiation of rapid currents. Additionally, many larval fish do not have the ability to swim in a steady manner, but rather use small bursts. These bursts may be restricted to specific periods of the day (Shamchuk and Tierney, 2012).

Note that there are two unique swimming speeds of importance to pelagic fish: (1) the optimal cruising speed, which maximizes the distance traveled per unit energy expenditure and (2) the optimal foraging speed, which maximizes the rate of flow of surplus energy, or production in its broadest sense.

Methods

A 5-L Brett-type swimming tunnel (Loligo Systems) is used for the swimming performance trials where the fish is contained within a portion of the chamber that maintains a laminar flow of water at a desired speed. The swimming tunnel should be thermo-regulated at 25±1 °C using a recirculating water bath connected to sump with a submersible heater. Oxygen consumption is measured with an optical dissolved oxygen (DO) meter (Witrox®, Loligo System). Flow speeds are calibrated using a flow meter (Miniair®20) as recommended by the manufacturer. All the test parameters including the swimming speeds are controlled with an attached computer through the software Autoresp™. Oxygen levels should be mantained maintained at ≥4 mg/L. For experimental set-up and calibration steps, see steps 1.0 to 3.0. For experimental studies (once calibration has been completed), see step-by-step instructions in step 5.0 on how to use the software and all equipment.

Fish will be designated as either a treatment group or a control group. Fish should fasted 24 h prior the swimming trials. Individual fish should be netted at random from the holding tank, measured and weighted and placed into the swimming chamber which is surrounded by black curtains to limit any external influence. Fish should be allowed to acclimate to the tunnel without flow until no signs of stress are observed and then for a minimum of 30 minutes at a speed of 10 cm/s (Peake et al., 1997 proposed 30 min). The acclimation process should be monitored with cameras mounted in front of the tunnels (see step 5.8) and more time should be added if any sign of stress is observed. Each critical swimming speed test should be at 10-15 cm/s and performed as a ramp-U_{crit} test which allows for changes in step duration (Jain et al., 1997). Step duration and speed intervals should be adjusted specific to each species as are illustrated in Figure 1 and should be based on range-finding swim trials. The speed should be increased to approximately 75% of the expected U_{crit} for the particular fish species in small steps of 5 min duration intervals, after which step length should be increased to 15 min, for a goal of a total of approximately 10-13 steps (Jain et al., 1997; Plaut, 2001; Tierney, 2011; the actual total number of steps will be determined as per range-finding studies). Step speed and duration should be designed based on the swimming capabilities of the species obtained in previous range-finding studies with the respective species and control group.

There are two important scenarios to bear in mind as you assess if experiment has ended; that is, if U_{crit} has been reached:

1. "Cheating fish": Some fish will position their tail at the back of the tunnel to help themselves propel against the water flow; others will be resting at the bottom corner of the tunnel and not swimming at all. You can motivate them not to rest by using a reversal of water flow and/or light stimuli. If the fish does not stop "cheating" or is unwilling to swim, the test is considered invalid. You must end the test and use another fish.

2. "Fatigued fish" (determining the U_{crit}): Fish will be pushed by the water flow against the back of the tunnel; fish will appear fatigued and breathing heavily (you will see operculum moving fast). This means that they are closed to reaching the U_{crit}. To find the U_{crit}, you will use the reversal of water flow, and wait ~5 s to let the fish swim to the front of the tunnel; then, you turn the water flow back to forward, and observed if the fish is pushed to the back of the tunnel or if it

continues to swim. If the fish is again pushed to the back of the tunnel, reverse the flow and let it swim forward. You will repeat this exercise up to three times before determining U_{crit}. When the U_{crit} is reached, you can end the test. Record the time to reach U_{crit} to the second (see formula below to calculate U_{crit}).

The swimming performance endpoints generated in this protocol are: U_{crit}, $MO_{2max,}$ and MO_{2min}. These three endpoints are described below.

U_{crit} : Critical, sustained, or endurance swimming speed is determined using steps that start at 5 min and end at 15 min (step length, see Figure 1 below). Speed increments (step height) need to be adjusted so that the total number of steps is ~10 (see Figure 1 below). Now, calculate the U_{crit} using the next formula:

$$U_{crit} = U_f + U_s \times (t_f / t_s)$$

U_f : Speed of the last fully completed step (cm/s) (speed prior to fatigue step)

U_s : Speed increment for each step (cm/s)

t_f : Amount of time spent on final step (fatigue step) (s)

t_s : Duration of the last fully completed step (s)

MO_{2max}: Maximum recorded oxygen consumption rate over the course of the swimming performance trial. **MO_{2max}** usually occurs in the final 2 or 3 steps of the swim test. It may not occur in the final step as fish tend to use anaerobic metabolism more extensively as they approach $U_{crit/max}$.

MO_{2min} : Lowest recorded oxygen consumption rate over the course of the swimming performance trial. **MO_{2min}** usually occurs in the Acclimation period or in the first 1-3 steps of the swim test. Based on the design of the test, this should be termed routine MO_2 as it is likely not representative of the true minimum MO_2 of the animal due to stress of handling and being placed in a novel environment.

Aerobic Scope: The absolute or fractional difference between resting and maximal oxygen consumption (MO_{2max} - MO_{2min} or MO_{2max} /MO_{2min}) provides an estimate of the aerobic scope for activity beyond routine activity.

Dissolved oxygen recorded during the test is used to calculate oxygen consumption per time unit per weight unit (MO_2). MO_2 maximum and minimum are calculated as well as the aerobic scope. Aerobic scope is obtained both as absolute and fractional difference between minimum and maximum oxygen consumption (MO_2max - MO_2min or MO_2max /MO_2min).

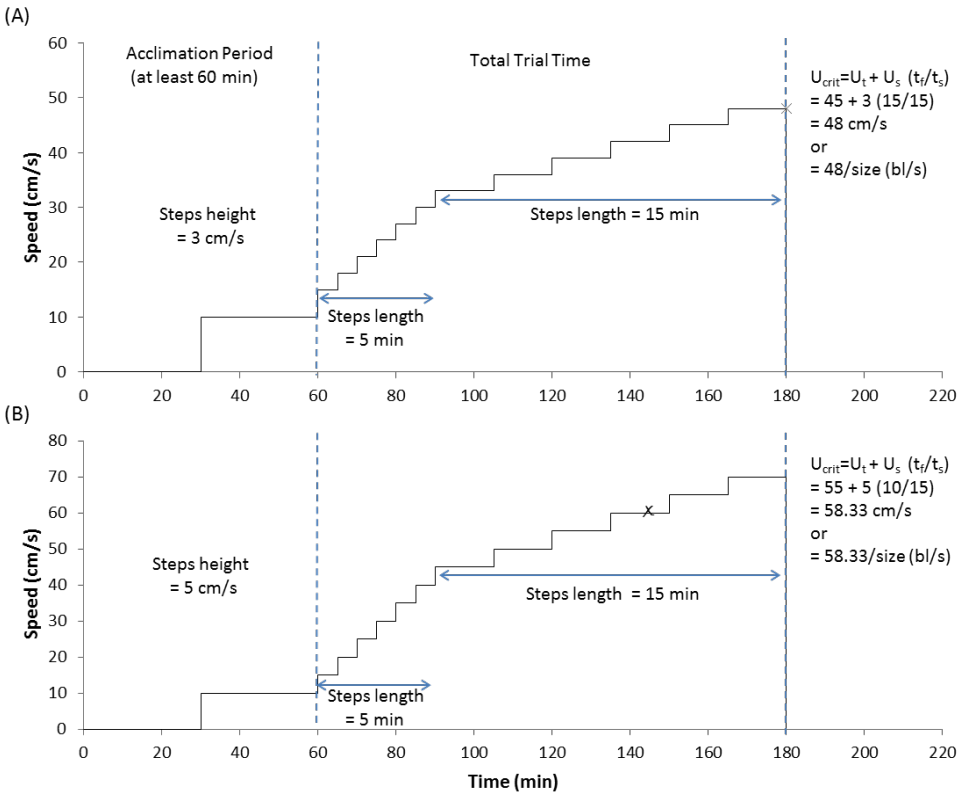


Figure 1. Description of the ramp-U_{crit} methodology used for the swimming performance studies conducted from April 2014 through October 2014 with **(A)** Sheepshead minnow (*Cyprinodon variegatus*) and **(B)** Florida pompano (*Trachinotus carolinus*). A hypothetic example of U_{crit} calculation was presented for each case.

It is important to remember that you can modify this protocol as it best fits your organism. Every fish species is different and have different Ucrits.

5.0 Swim Tunnel Step by Step Procedure

Note: The form (Form #3) that you will need for running the experiments is at the end of this document. (Important: Steps 5.1 to 5.7 are also explained in detail with photos in Step 1.0 'Experimental Set-up').

5.1 Assemble swim tunnel interior components (do not install either lid) and fill with water.

5.2 Remove any bubbles from the straitening vanes and the straitening block (honeycomb material) using the water flow from the filling line.

5.3 Place main top cover on as if it were a slide cover to limit the amount of air trapped under the lid and turn on the swim tunnel using manual control (dial set to 'KNOB' on controller) to force out any remaining bubbles. Avoid high speeds as this will break up bubbles and make them more difficult to remove.

5.4 Insert temperature and oxygen probes, install the test section cover, and seal all remaining ports (do not use wingnuts yet). Run the swim tunnel again at an intermediate speed to ensure all air is removed. Remove and replace the covers as needed to let air escape.

5.5 Once all bubbles are removed, loosely screw on the wingnuts around the main cover (do not use wingnuts around the test section) and then hand tighten in a crisscross pattern.

5.6 Set the controller to Ext. and start the Autoresp software. Start a new experiment (under the experiment menu) and input swim tunnel and estimated fish information (Note: This info is used to generate estimates only and will not influence final results).

5.7 Oxygen meter (SOP#3.7.9.3) and motor speeds should be calibrated before use (every 10-14 days).

5.8 Set up the camera before introducing the fish, to avoid unnecessary stress to the fish.

a) You will be using gopros (Hero 3) to observe the experiment (Figure 15, see photos below), you could also film the experiment if you want. The gopros can be found in the grey cabinet located in the swimming performance room. Make sure to add the external battery to the gopro so it lasts longer and you do not have to change the camera in the middle of the experiment (Figure 16, see photos below).

NOTE: All gopros and external batteries should be charged a day prior to the experiments. The batteries run out very fast.

Figure 15

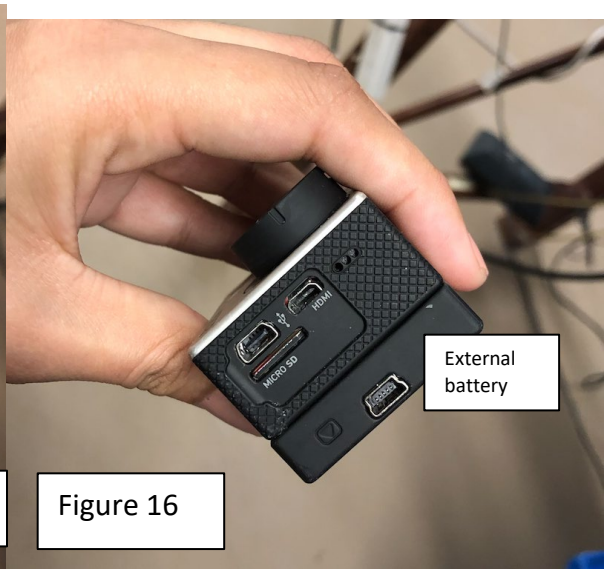


Figure 16

b) Place the gopro upside down so that the hdmi connections are pointing towards the computer (Figure 17, see photos below). Then, connect the hdmi cord that comes out from the screen where you want the video project on (Figure 18, see photos below), and turn on the screen (Figure 19, see photos below). You can also use the gopro clicker to turn on and off the gopro without manually turning on the camera. Make sure to charge the clickers as well (Figure 20, see photos below).

Figure 17

Figure 18

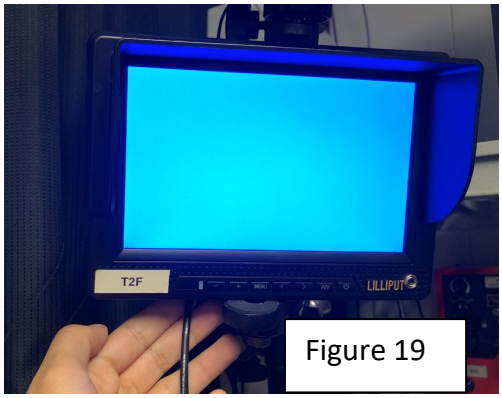
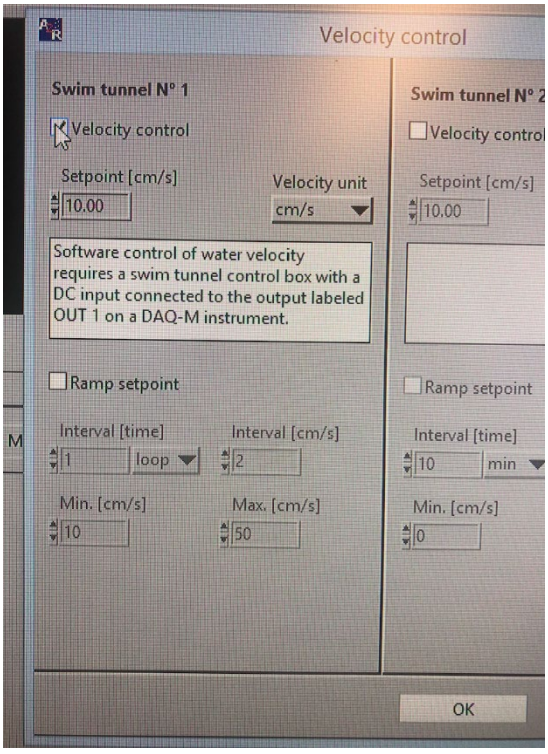


Figure 19

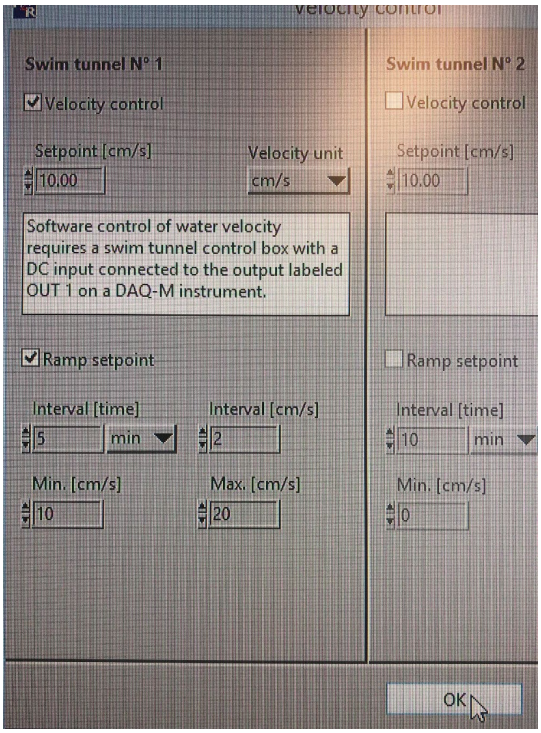
Figure 20

5.9 Transfer the fish into the test chamber section and lock down the test section cover as described above (Step 5.5). Let the fish acclimate until it has calmed down; close curtains. The circulation setting should be set at ***flush*** by clicking the white circle at the top of the Autoresp window. Flush means that there is water going in and out of the tunnel.

5.10 Once the first acclimation has completed, turn switch off (see more details in section 2.8 & 2.1) to avoid any more water from flowing into the tunnel from the tank. Then, start the second acclimation where you will have the fish swimming at a low velocity (e.g. 10cm/s) for 10 minutes. To start the second acclimation, turn on the swim tunnel (Velocity Control under settings menu) by setting it to run at the acclimation speed (e.g., Sailfin molly 10cm/s; see photo to the right) and select ok. Note the exact start time (to the second) from the time on the right-hand side of the live Oxygen graph when the 'Data' tab is selected.



5.11 Then, to start the first step to calculate ramp Ucrit, open the velocity control window and turn on the ramp option by checking the ramp setpoint box (see photo to the right).

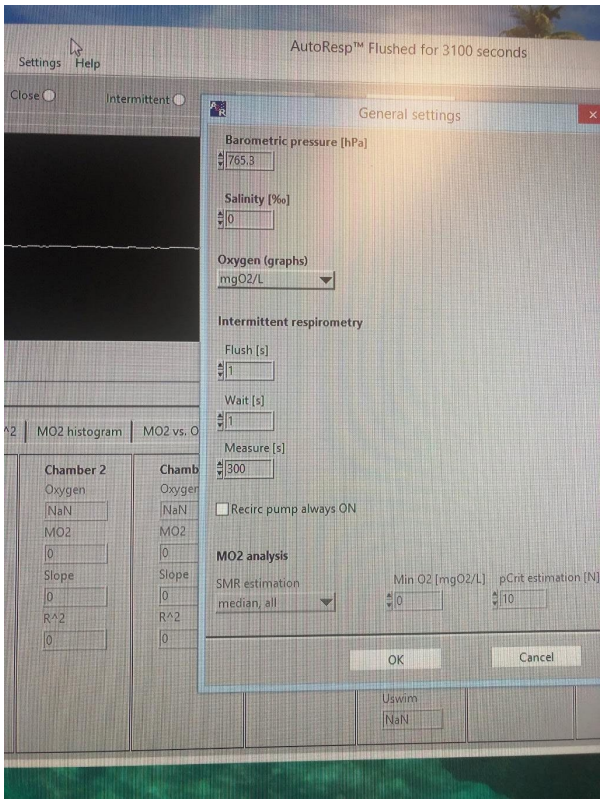


5.12 For the first few steps where you increase your step duration by 5 minutes, you write 5 min under "Interval [time]".

5.13 The value under "Interval [cm/s]" tells the software by how many cm/s you want to increase your velocity every 5 minutes.

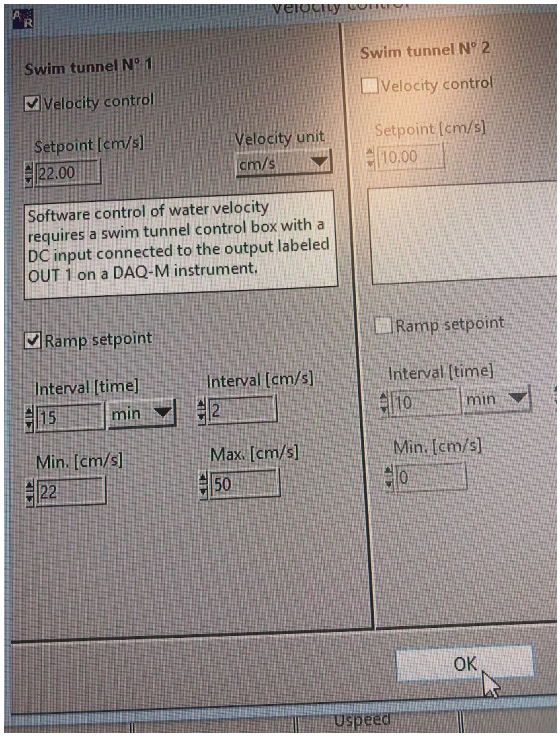
5.14 "Min. [cm/s]" is the minimum velocity at which you will start your step 1, and "Max. [cm/s]" is the last step that you want at 5 minutes. Press okay.

5.15 As soon as you press okay, you will click on settings and then on general. Because the first steps lasted a period of 5 minutes = 300 seconds, enter 300 number under "Measure [s]". This indicates that the software will measure the oxygen consumption every 300 s (see photo to the right).



** Make sure that as soon as you press okay, you CLOSE the system by setting the circulation to ***close*** by clicking the white circle at the top of the Autoresp window. By the time you select close, the pump that pushes water from the tank into the tunnel should have already been turned off at the beginning of the second acclimation (e.g. 10 cm/s for 10 min). If you do not select close at the start of step #1, the velocity in your experiment will not automatically change steps, and the oxygen changes will not be recorded. **

5.16 Once you decide to increase the step length to 15 or 20 minutes (you choose this based on your study objectives), you will again open the velocity control window and change the values. On step 5.14, 20cm/s was determined to be the "Max. [cm/s]", the last step at Interval [time] of 5 minutes. Now, the velocity that follows 20cm/s, in this example 22cm/s, will now go under "Setpoint [cm/s]" and "Min. [cm/s]". See photo to the right.



5.17 You will change the "Interval [cm/s]" to 15 minutes, and change the value under "Max. [cm/s]" to the max velocity that you think your fish can obtain; then press okay. This was the same number that you set for "Max [cm/s] on step #2.11 during calibration.

*** Once you hit OK, the ramp will start automatically since you already closed the system before ****

5.18 If the fish ever cheats (e.g. tries not to swim by laying his tail in the back of the tunnel, or finds a spot in the tunnel where water does not go as fast), you should reverse the flow of the water by switching the knob of the red control box towards reverse. Do this a max of 3 times, if the fish does not co-operate, then terminate the experiment and mark it invalid. If the fish starts swimming again, continue with the experiment. Opening the black curtain sometimes also helps fish to resume its swimming. (Also explained in the Methods section.)

5.19 Once the fish finishes the experiment, write the exact time down and fill out **Form #3**. Form #3 will help you keep track of your entire experiment. Click on the "Experiment" tab and press "stop", and then "yes". See below for Form #3.

5.20 Turn on the pump that flushes the water from inside of the tank into the tunnel. Take out the fish from the tunnel and euthanize the fish if it has been exposed to chemicals.

5.21 If using freshwater:

a) Water should not be used for more than a week.

b) Every **week**, remove/disassemble every tunnel piece and rinse well with DI water.

- Be sure not to switch/mix tunnel pieces, if you do this, you must calibrate the velocity again.

If using saltwater:

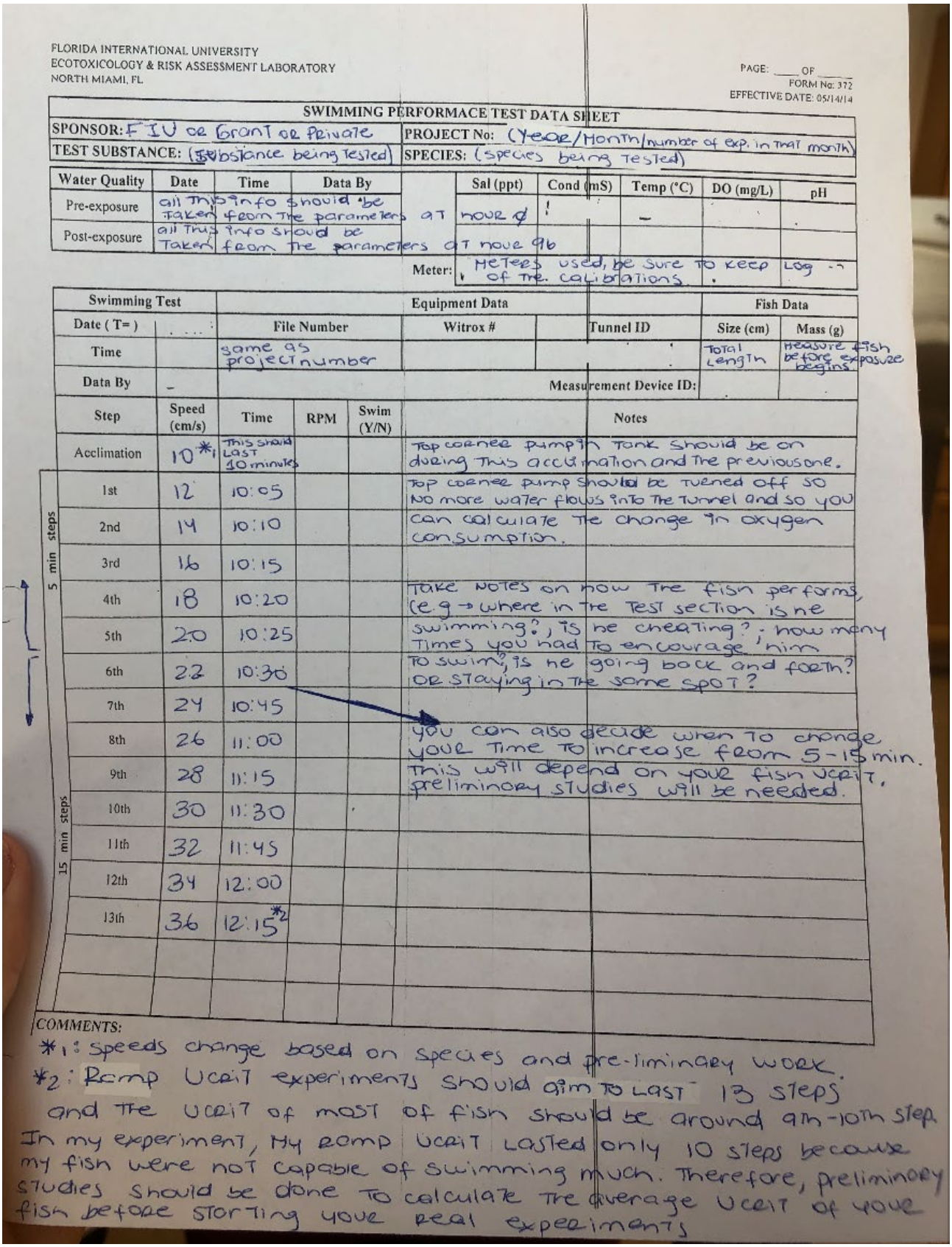
a) Water should not be used for more than a week.

b) Every **day,** remove/disassemble every tunnel piece and rinse well with DI water.

- Be sure to not switch the tunnel pieces, if you do this, you must calibrate the velocity again.

5.22 Look for the folder where you saved the experiment/trial and import data into excel sheet so the columns are rearranged.

Form # 3 (Note the instructions written on the form.)



FLORIDA INTERNATIONAL UNIVERSITY
ECOTOXICOLOGY & RISK ASSESSMENT LABORATORY
NORTH MIAMI, FL

PAGE: ____ OF ____
FORM No: 372
EFFECTIVE DATE: 05/14/14

SWIMMING PERFORMACE TEST DATA SHEET

SPONSOR: FIU or Grant or Private | PROJECT No: (Year/Month/number of exp. in that month)
TEST SUBSTANCE: (substance being tested) | SPECIES: (species being tested)

Water Quality	Date	Time	Data By	Sal (ppt)	Cond (mS)	Temp (°C)	DO (mg/L)	pH
Pre-exposure	all This info should be Taken from the parameters at hour 0							
Post-exposure	all This info should be Taken from the parameters at hour 96							
Meter:				METERS used, be sure to keep of the calibrations			Log	

Swimming Test		Equipment Data			Fish Data	
Date (T=)		File Number	Witrox #	Tunnel ID	Size (cm)	Mass (g)
Time		same as project number			Total Length	measure fish before exposure begins
Data By		Measurement Device ID:				

Step	Speed (cm/s)	Time	RPM	Swim (Y/N)	Notes
Acclimation	10 *1	This should LAST 10 minutes			Top corner pump in Tank should be on during This acclimation and the previous one.
1st	12	10:05			Top corner pump should be turned off so No more water flows into the Tunnel and so you
2nd	14	10:10			can calculate the change in oxygen consumption.
3rd	16	10:15			
4th	18	10:20			Take NOTES on how the fish performs, (e.g → where in the Test section is he
5th	20	10:25			swimming?, Is he cheating?, how many Times you had to encourage him
6th	22	10:30			to swim?, is he going back and forth? OR staying in the same spot?
7th	24	10:45			
8th	26	11:00			you can also decide when to change your Time to increase from 5-15 min.
9th	28	11:15			This will depend on your fish Ucrit, preliminary studies will be needed.
10th	30	11:30			
11th	32	11:45			
12th	34	12:00			
13th	36	12:15 *2			

5 min steps

15 min steps

COMMENTS:
*1: Speeds change based on species and pre-liminary work.
*2: Ramp Ucrit experiments should aim to last 13 steps and the Ucrit of most of fish should be around 9th-10th step. In my experiment, My ramp Ucrit lasted only 10 steps because my fish were not capable of swimming much. Therefore, preliminary studies should be done to calculate the average Ucrit of your fish before starting your real experiments