# 2016 Juvenile Rockfish Respirometry Protocol

(updated 11/2/16 Melissa)

## PM/Resting Procedures: (~2 hours setup, start by 3pm)

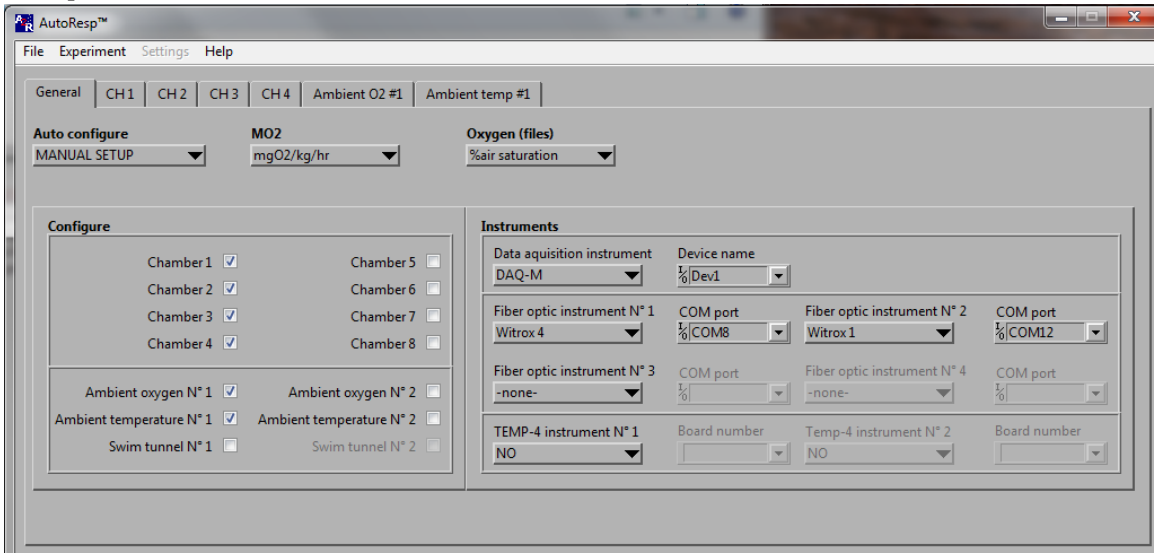
1. **Cooler set-up:**
   a. Rinse system with DI water.
   b. Remove chambers and rinse with hot water.
   c. Fill the chiller with ice and about two liters of fresh water.
   d. Flush saltwater hose for 15 seconds. Fill the cooler with salt water *(use filtered saltwater if there is an algal bloom).*
   e. Ensure all probes are in their proper locations:
      i. If you are running a 7.5 or X treatment, Remove the cap on the Neptune Apex and rinse the probe tip in DI water. Wrap the probe's cable around the chiller hose and clip it in place with a white clip so the tip of the probe is in the water (don't submerge the black portion of the probe!)
      ii. Tie the temperature probe to the right end of the PVC pipe
      iii. Place the ambient DO probe in the small hole on the right side of the PVC pipe
      iv. Insert plastic chamber DO probe holders to connect each chambers' recirculation tubes. Insert DO probes so that the tip falls just above the flow through tube.
      v. Chamber numbers are written on the wall of the cooler. Chamber 1 is the chamber that is physically closest to the number 1, regardless of where its pumps/hoses are located, etc.
      vi. Insert chamber DO probes as follows:

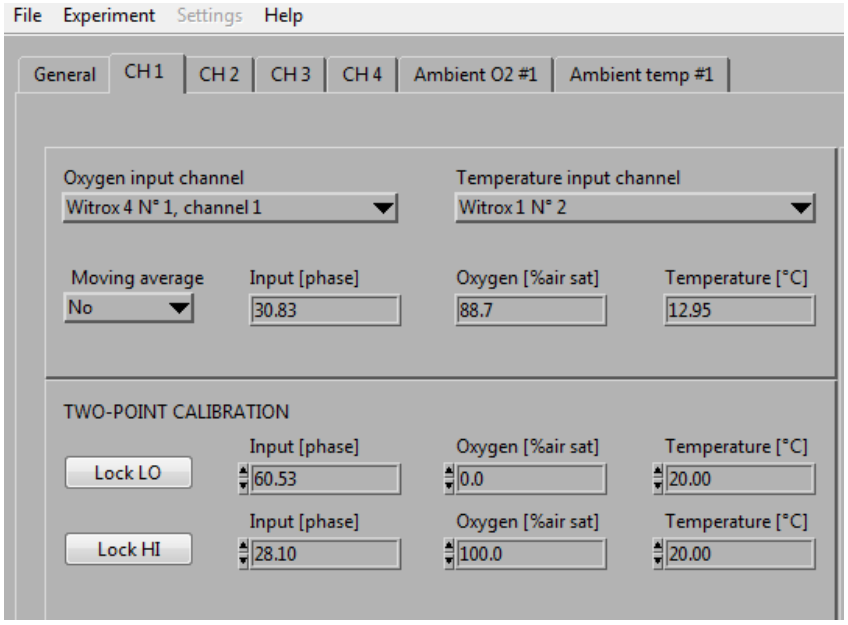
| Probe Label Color | Witrox Channel | Chamber |
|---|---|---|
| White | Channel 1 | Chamber 1 |
| Red | Channel 2 | Chamber 2 |
| Green | Channel 3 | Chamber 3 |
| Blue | Channel 4 | Chamber 4 |

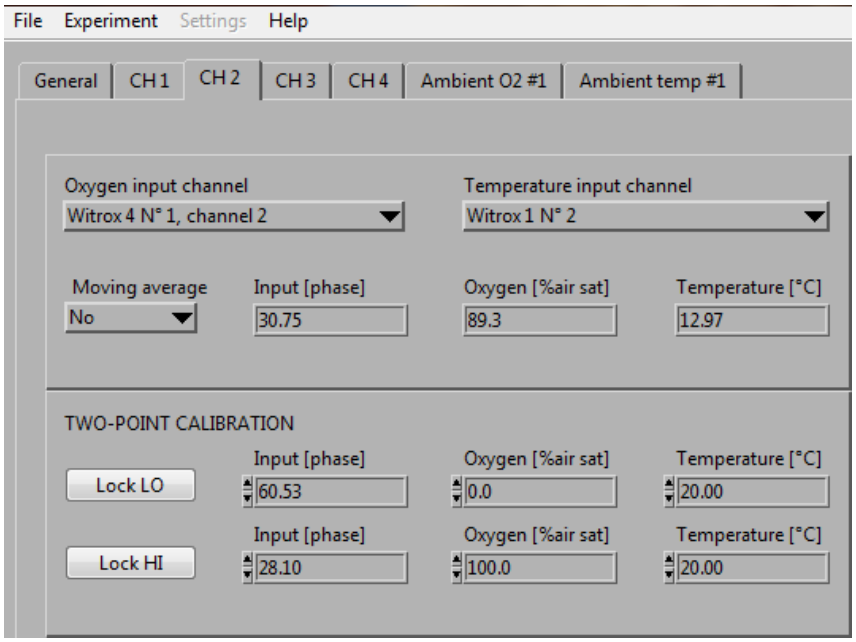
   f. **Plug the water bath recirculation pump into the power strip on the desk.** The recirculation pump will run continuously throughout the trial to ensure proper mixing of water in the cooler.
   g. Turn on the DAQ power switch to start the pumps.

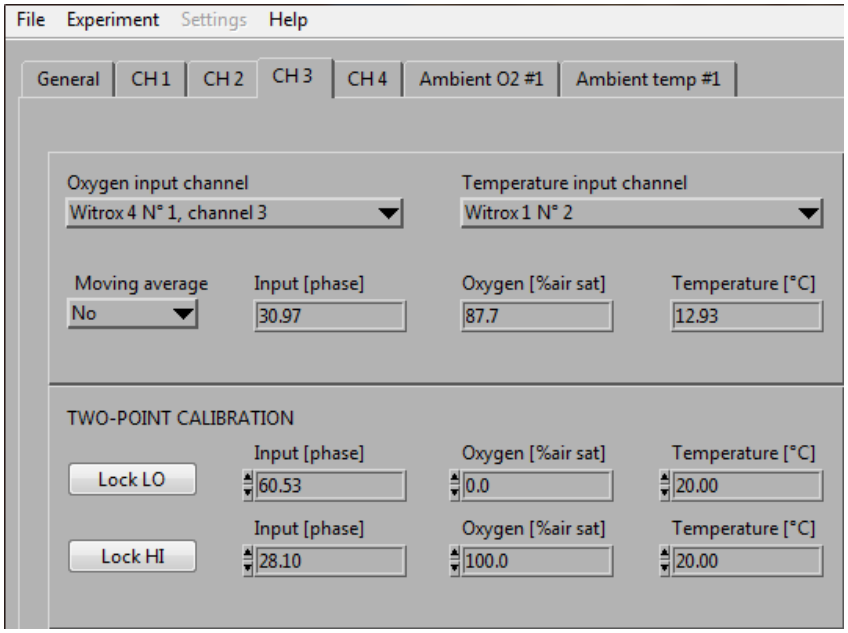
2. **AutoResp Setup:**
   a. Right click on the AutoResp program and choose "run as administrator"

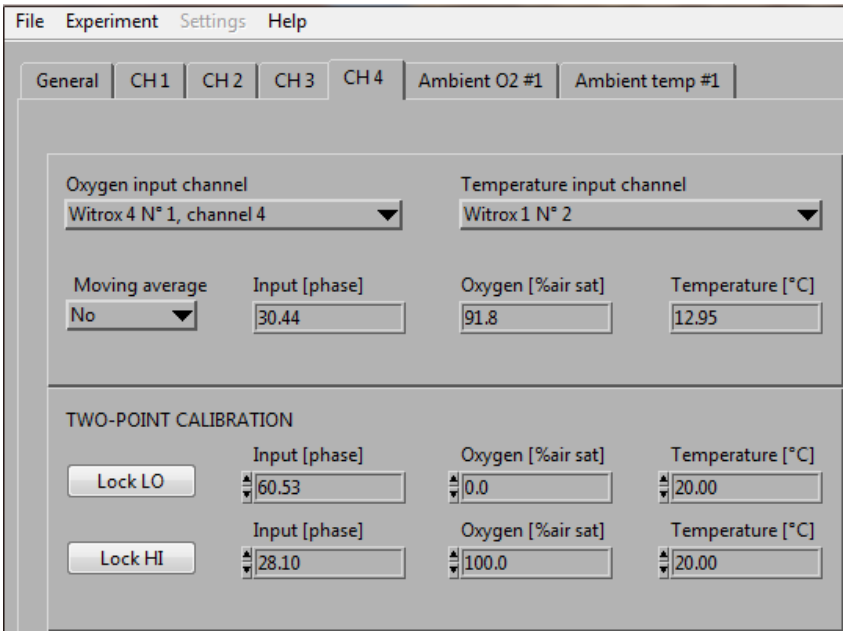
b. Set up the tabs as follows:






c. Once all settings are input correctly, click Experiment -> Start

d. Save the experiment on the desktop with a name like "test" that can easily be found and deleted later.

e. Set the system to "Flush"

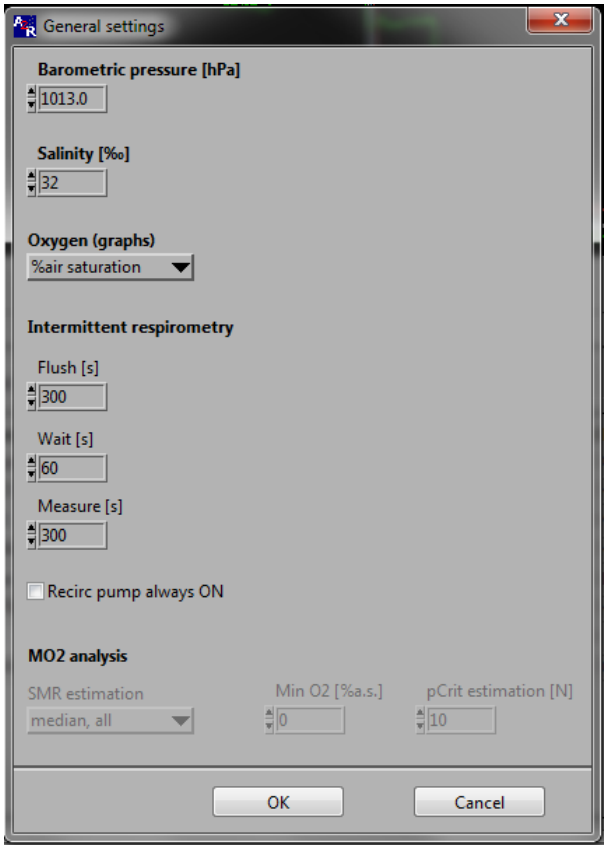
f. Input the following values on the screen so they are saved for when you start the actual experiment:

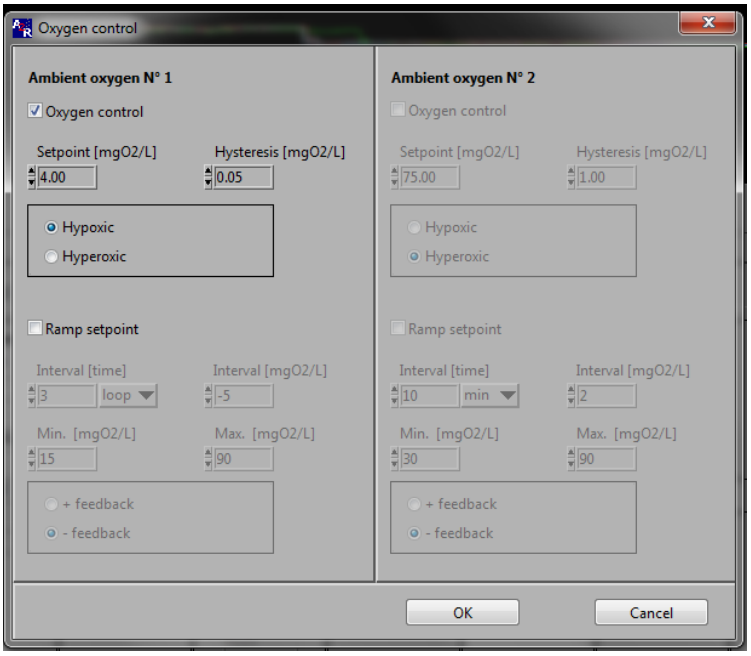
i. General Settings:

- Find the actual barometric pressure at the weather station on the second floor at NOAA and convert from mmHg to hPa. Input the new value.
- If you are running a treatment at 4.0 DO, change the "Oxygen (graphs)" setting to mg/L. Set the salinity to 32.0 ppt.
- For intermittent respirometry, set the Flush and Measure periods to 300 seconds and the Wait period to 60 seconds.
- Make sure "recirc pump always ON" box is not checked.

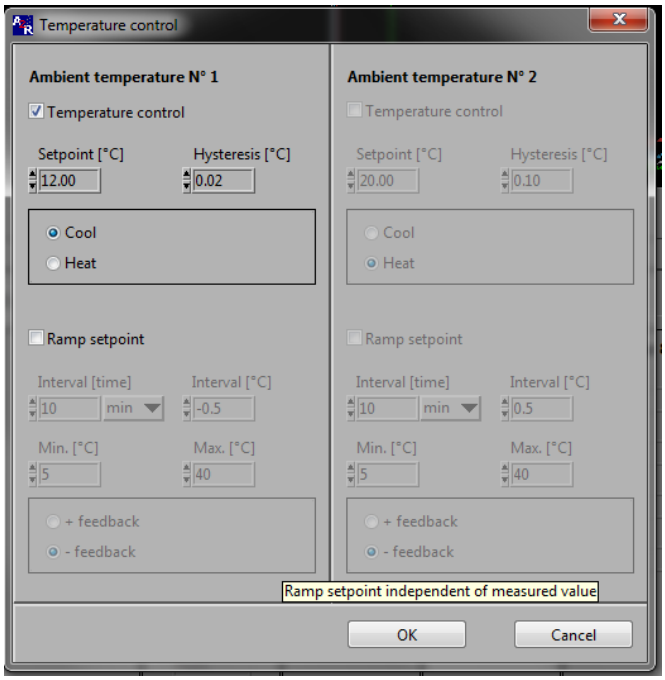
ii. Input the oxygen settings.

- Ensure that the gases are turned off, then click "Ramp setpoint." Set the interval (time) to 3 loops, the interval (O2) to -10% saturation, the O2 minimum to 40%, and the Max to 70% on – feedback. Unclick "Ramp Setpoint."
- If you are running a 4.0 or X treatment, set the O2 setpoint to 5.0 mg/L with a .01 hysteresis. If you are running a control or 7.5 pH treatment, set the setpoint to 70% saturation with a .01 hysteresis, and then unclick oxygen control.




iii. Input the Temperature settings as shown:




3. **Set water to treatment levels**:
   a. To get the dissolved oxygen to treatment levels, turn on the N2 gas. Gently adjust the gas flow rate so that the DO drops slowly to 5.0 without overshooting by more than .2 mg/L. Once the DO level reaches 5.0, lower the setpoint by .3 mg/L at a time to ensure the gas doesn't overshoot until you reach 4.0 mg/L. Confirm the DO levels with the Hach probe and record temperature/pH/DO in the logbook.
   b. To get the pH to treatment levels:
      i. The Apex will be left on at the correct setpoint. However, if you need to change the setpoint:
         - Press the middle button
         - Set Up
         - Outlet Set Up
         - Program Outlet
         - CO2_3_7
         - Change the values if needed

- Save!

ii. Place the gas stone in the cooler. Turn the CO2 gas on and adjust the flow to 2. The gas should be on very low with only a couple small trickles of bubbles emerging. Adjust the flow rate with the fine tuning dial until the levels are stable.

1. **Remember: we are aiming for a pH of 7.45-7.50**

4. **Remove air bubbles from the system:**
   a. Disconnect each pump from its outflow tubing and turn so the outflow port faces up to blow out any air bubbles. Reconnect the pump to the tubing.
   b. Carefully disconnect both end caps from each fish chamber. Hold the chamber vertically and slowly lower it up and down in the cooler water to remove any bubbles. Jiggle the end caps to remove any trapped bubbles.
   c. Turn off the DAQ and reconnect each chamber to its end caps, and check again for bubbles in the system. Repeat any above steps as necessary.

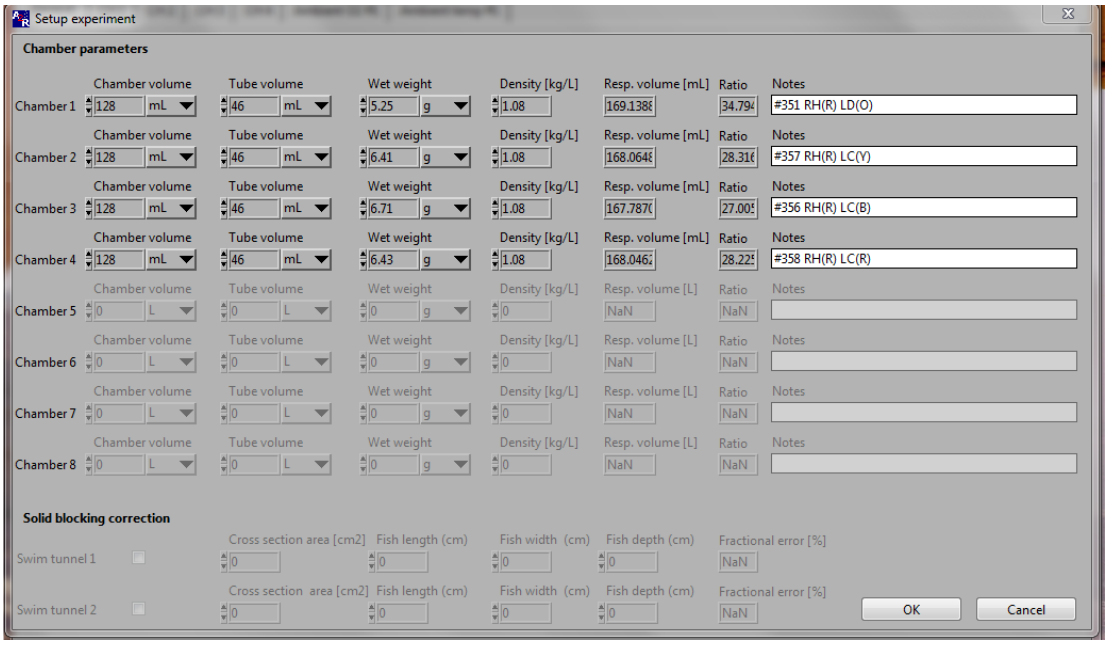
5. **Adjust hose clamps**:
   a. As the system is flushing, water should be flowing from the open ends of the hoses in the PVC pipe. The flow rate from each hose should be the same. If the flow rate of a chamber is too high or low, tighten or loosen the hose clamp on the flushing tubing until the flow reaches the correct rate.
   b. If AutoResp shows that the oxygen levels in the chambers are significantly different from each other after flushing (>.2 off), check the hose clamps on the recirculation hosing and adjust as necessary.

6. **Weigh Fish:**
   a. Ensure that fish in the subset you are running have not yet been fed. "A" fish go in the respirometer on the evening of "A" days and go through pCrit on the morning of "B" days and vise-versa.
   b. Catch all fish in the subset and place in a hang tank filled with treatment water.
   c. Place a small volume of treatment water in a beaker on the hundredths scale. Tare the scale.
      i. Catch a fish in the net and gently shake the net to remove excess water.
      ii. Place the fish in the beaker of water. Record the fish's weight and tag colors in the logbook and assign the fish a chamber #.
      iii. Tare the scale and repeat the process with the next 3 fish.

7. **Loading Fish:**
   a. Once the temperature is down between 12-12.5 degrees and the pH and DO are at the correct levels, begin loading the fish into the chambers.
   b. Remove the end cap closet to you from each chamber. Load each fish into its assigned chamber (using a net or your hand) with its head facing the back of the cooler. Carefully close the end cap without pinching the fish's tail.
   c. Place the cooler lid over the hole.

8. **Run Autoresp:**
   a. Start a new experiment with the name "Trial #_Species_Treatment_Rest" in the 2016 OADO Respirometry Folder
   b. Input the fishes' weights in the appropriate chambers when prompted and record the fish numbers and tag colors.

i. Double check the tag numbers and weight information! Ensure chamber and tube volumes are in mL and not L.




c. Set the experiment to "Intermittent Flow."

9. **Checks:**
   a. Ensure that chambers are closed tightly and all hose connections are tight and secure.
   b. Monitor the first couple loops. Ensure that MO2 levels fall within the 80-200 mgO2/kg/hr range. Agitated fish may rise above this rate and then drop back down.
   c. Watch the chamber readings for the first loop to ensure that all chambers rise to the same DO levels after the flush period. If they do not, try loosening the hose clamps.
   d. Check for any bubbles in the chambers and remove them if found.
   e. Turn off the light in the respirometry area and leave the fishes overnight. Refill the chiller's ice if necessary before you leave.

**AutoResp General Tips:**

- When set on intermittent flow, the top of the AutoResp screen will read F for flush, M for Measure and W for wait.
- The MO2 vs. O2 graph provides the clearest picture of which loop you are on in each cycle.
- The O2 probes in the chambers do not read accurately during the flush period and often show a large drop in DO. The probes will read correctly once the wait period begins.
- Relay assignments are as follows:
  - RE1 = flushing pumps
  - RE2 = recirculation pumps
  - RE3 = N2 Gas
  - RE4 = chiller pump

## PCrit Procedures: (~5 hours of periodic monitoring)

Begin around 8am, or after the fish have acclimated for at least 12 hours.

1. Check MO2 levels in AutoResp on the Data or MO2 tab.
   a. Normal resting MO2s range from 80-200 mgO2/kg/hr.
2. Refill the chiller with packed ice and water.
3. Input the day's barometric pressure in hPa.

4. **Make sure the water bath recirculation pump is plugged in.**
5. **If you are running a Control or 7.5 treatment:**
    a. End the experiment and start a new experiment entitled "Trial #_Species_Treatment_pCrit" in the 2016 OADO Respirometry Folder.
    b. Set the system to Intermittent Flow.
    c. Let the new experiment run for 3 loops (11 minutes each, 33 mins total) at resting levels.
    d. Slowly lower the DO to 70% saturation, gently adjusting the N2 flow rate if necessary. The DO should not overshoot by more than 2%.
6. **If you are running a 4.0 mg/L or X treatment:**
    a. Check the N2 gas flow rate to make sure it is adequate
    b. End the experiment and start a new experiment entitled "Trial #_Species_Treatment_Acc" in the 2016 OADO Respirometry Folder.
    c. Set the system to Intermittent Flow.
    d. Change the DO units back to % saturation in the General Settings tab.
    e. Set the DO to 70% saturation
    f. Insert the air stone into the cooler until DO levels rise to 70%
    g. Set the system to Intermittent Flow.
    h. Acclimate 1 hr at 70% saturation
    i. End the experiment and start a new experiment entitled "Trial #_Species_Treatment_pCrit" in the 2016 OADO Respirometry Folder.
    j. Set the system to Intermittent Flow.
7. **10% Intervals:**
    a. Go to Oxygen Control and click "Ramp Setpoint." Ensure that settings are as follows:
        i. Interval (time): 3 loops
        ii. Interval (O2): -10% saturation
        iii. O2 Min: 15%,
        iv. O2 Max: 70%
        v. – feedback.
    b. The DO should drop by 10% every three loops until it reaches 40% over the course of about 1 hour and 40 minutes.
8. Set a timer for 1 hr 10 mins, at which time the experiment will begin 40% DO loops
9. Watch the chamber readings for the first loop to ensure that all chambers rise to the same DO levels after the flush period. Continue to check the graphs periodically.
10. **5% Intervals:**
    a. During one of the 3 loops at 40% DO (after about 1 hour and 10 mins), adjust the Oxygen settings as follows:
        i. Change the O2 interval to -5%. The DO will now drop by 5% every three loops in order to capture pCrit.
    b. Check the chiller and add more ice if necessary
11. **In case of an emergency (if the DO levels drop too far or overshoot), press "skip phase" to skip a measurement phase and return to flushing without stopping the experiment.**
12. Monitor the system during the 15% DO loops, which should take place 2 hours and 45 minutes after setting the interval to 5%.
    a. Let the system run for 3 loops at 15% DO. You should see a clear drop-off in MO2 where pCrit occurs on the AutoResp graphs.
    b. If the DO levels in a chamber drop below ~8% during a measurement period, skip the measurement phase, flush the chambers, and raise the setpoint and check on the fish.
13. Take a screenshot of AutoResp graphs on the MO2 vs. time graph and save in the OADO Respirometry folder.
14. **End Experiment** and get fish out as soon as possible

15. Turn off the N2 Gas. Then turn off the DAQ.
16. Carefully remove the fish from their chambers and place into a hang tank of treatment water, checking each fish's tag to confirm. Return the fish to their treatment tank without feeding.
17. Drain all water from the cooler, tubing and chambers. Rinse everything with DI water and dry the cooler with a sponge to prevent microbial growth.
18. Drain leftover ice/water from chiller
19. Back up the trial data on the external hard drive

## **Bleaching Procedures:** (~ 3 Hours)

Bleach the system after every 3rd respirometry trial to prevent the growth of microbes, which can consume oxygen and impact the experiment.

1. Bleaching:
    a. Unscrew the cooler cap and prop up the back of the cooler on the wooden block to empty the cooler of salt water. Briefly rinse all components with the fresh water hose to remove any debris.
    b. ***REMOVE ALL PROBES FROM THE COOLER*** including 4 chamber DO probes with their plastic casings, ambient DO probe, and temperature probe.
    c. Connect all hoses and chambers to pumps and close chambers to prepare for flushing the system.
    d. Fill cooler with fresh water up to just below the level of the PVC pipe across the center using the fresh water hose (be sure not to fill it high enough so that water can enter the holes in the cooler and get inside the insulation)
    e. Measure 1 L bleach (NOT the splash-proof type!) in the beaker labeled bleach (or use a whole 1L Clorox bottle) and pour into the cooler.
    f. Fill the chiller with packed ice and open the AutoResp program. Go to Settings->General->recirc pump always on to keep the chiller pump running continuously during bleaching.
    g. Turn on the DAQ, plug the recirculation pump into the surge protector, and set the system to "Intermittent" in Autoresp.
    h. Leave the system to bleach for 1 hour.
2. Fresh water rinse:
    a. Turn off the DAQ power and unplug the recirculation pump.
    b. Find the two large blue buckets labeled "Bleach." Place one below the cooler drain hole. Prop up the back of the cooler with the wooden block and drain the bleach water into the bucket. When the bucket is ¾ full, replace it with the second bucket and empty the bleach water into the sink. Repeat until the cooler is empty.
    c. Close the cooler cap and refill the cooler with fresh water. Turn the DAQ back on and plug in the recirculation pump. Unscrew the cooler cap and turn the freshwater hose on to a medium flow rate so that the water level in the cooler remains approximately constant. Secure the hose in the cooler by closing the cooler lid and continue to check on the water level for one hour. Don't let the water level fall below the level of the pumps to prevent them from burning out, and don't let the water level rise to the holes in the cooler. 1hr
3. Amquel:

    a. Close the cooler outflow cap and pour 20 mL Amquel into the cooler. This will neutralize any leftover chlorine from the bleaching process. Let the system run on intermittent flow for 30 mins
4. Final Rinse
    a. Drain the Amquel water from the cooler using the bleach buckets. Run the system on Intermittent flow with fresh water for another 30 minutes. Turn off the system and drain the cooler of fresh water once complete.
5. Dry
    a. If you are not using the respirometry system after bleaching it, tip the cooler to fully drain it of fresh water. Open all hose connections and drain the hoses of water as well. Use the large sponges to soak up any remaining water.