PM Resting Respirometry Checklist

☐ Gas tank volumes checked (should be >500 psi)
☐ System rinsed with DI water
☐ Chambers rinsed with hot water
☐ Chiller filled with packed ice/water and hoses not kinked
☐ Confirm MO2s don't decrease during measurement period with no fish (microbial respiration)
☐ Probes in correct lines

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

☐ Water bath recirculation pump plugged in
☐ N2 and CO2 gas flow adjusted and monitored
☐ N2 gas tank turned off for night if running Control or 7.5 pH fish
☐ pH/O2 treatment levels confirmed with Hach probe
☐ Water temperature at 12° C
☐ Barometric pressure updated
☐ Flush/Wait/Measure periods set to 300/60/300
☐ "Recirc pump always on" box **UNCHECKED**
☐ Ramp setpoint inputs ready for Pcrit and unchecked
☐ Air bubbles removed
☐ Flushing outflow rate visually checked and equal between chambers
☐ Hose clamps checked (don't adjust clamps unless something looks off)
☐ Double check fish IDs and weight inputs in AutoResp
☐ Chamber and tube volumes correct (128/46) and in mL
☐ Double check for air bubbles after loading fish
☐ Chambers closed tightly
☐ MO2 levels checked (should be 80-200 mgO2/kg/hr)
☐ Chambers reaching correct and similar DO levels after flushing (within 1% saturation)
☐ AutoResp set to Intermittent Flow
☐ Gas tank psi re-checked
☐ Lights off in respirometry area
computer monitor off

AM Pcrit Respirometry Checklist

<mark>** 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.</mark>

☐ Check MO2, DO, pH, and temp levels in AutoResp
☐ Chiller refilled with ice
☐ Barometric pressure updated
☐ Water bath recirculation pump plugged in
☐ Do not quit AutoResp program without first choosing "End Experiment"

Control or 7.5 pH treatment:
☐ New experiment started and run for 3 loops at ambient
☐ Carefully lower DO to 70% while adjusting N2 flow rate during 4th flush period

4.0 DO or X treatment:
☐ New experiment started and DO raised to 70% with air stone
☐ Acclimate 1 hour on intermittent flow
☐ Start new experiment

Ramping Setpoints:
☐ Chamber DO's at 70- if lower, raise water bath setpoint to ~72
☐ DO levels not overshooting by more than 2%
☐ Ramp setpoint to 40 by -10% every 3 loops (takes about 1 hour 10 mins)
☐ Chambers rising to correct and similar DO during wait periods (within 1% saturation)
☐ O2 interval changed to -5 and minimum to 15% during one of the three 40% loops (takes ~2 hr 45 mins to reach 15% loops)
☐ Chiller ice refreshed
☐ System monitored during 15% loops – make sure chamber DOs reading above 8% during wait and measurement phase

Clean Up
☐ After last loop at 15% take screenshot of MO2 vs time and MO2 vs DO graphs and save to Pictures -> OA/DO Respirometry folder
☐ Click "End Experiment"
☐ Remove fish as soon as possible, checking each tag to confirm chamber locations
☐ Trial data backed up to external hard drive
☐ Respirometry cooler drained, rinsed with DI
☐ Cooler, tubing and chambers dried thoroughly if not reloading
☐ Chiller ice drained