

Protocol for Ventilation Experiment

Materials needed:

- Ventilation chambers (included)
- GoPro's (2)
- GoPro stand (we used PVC to make a stand and a cage to hold the blinds up.)
- Waterproof paper blind (included)
- Waterproof paper "fish hammocks" (included)
- Water reservoirs (our design allows for 2 treatments to be run at a time. One treatment in chambers 1-5 and a second in chambers 6-10)
- Water pump with airline tubing to bring water to chambers
- Chiller apparatus with temperature control

Setup:

1. Bring water in tank reservoirs to treatment levels and correct temperature (it can take a long time to get the temperatures to the correct level so it is important to start with that). The temperature in the chambers should be at 12°C ($\pm 2^\circ\text{C}$). We had to have our reservoirs between 10.5-11°C due to the warming of the water through the pump and airline tubing.
2. Once the reservoirs are at treatment levels turn on pumps and make sure no air or debris is entering the airline tubing.
3. Adjust flows to the chambers so that they are flowing at a rate of 5ml/s.
4. Check temperature, DO, and pH levels in the chambers once the flows are set correctly and make any adjustments needed to the reservoirs.
5. Insert "fish hammocks" into the chambers. (The hammocks are used to keep the fish from resting in the corners, which can be out of the GoPros sight.)
6. Identify the fish, assign them to a chamber, and place them in the chamber.
7. On the data sheet enter the information for the temperature, DO, pH levels, along with the fish ID and tag in the corresponding places (data sheet example attached)
8. Place the GoPro (with the wifi on so that they can be controlled remotely) stand above the chambers and insert GoPros into the holders and adjust them so that the chambers are clearly visible. (We had each go pro be able to see 6 of the chambers clearly with the middle two chambers overlapping on each GoPro. The GoPros were set at a medium recording angle, video shot at 60 fps, 1080i resolution.)
9. Once the GoPros are set turn them off and place the blinds over the stand. (The three paper section goes over the GoPros and chambers, parallel to the PVC supports. The two individual papers go on the sides. If they are wet they will stick to the side of the chambers.)
10. Place a headlamp or other light source on top of the waterproof paper and GoPros so that it can illuminate the chambers (it is important that the light is not too bright that it disturbs the fish, but also not too dim so that the video turns out well. Additional light sources may be needed in the setup area to insure full illumination of the chambers.) Turn the go pros on and adjust

headlamp position accordingly to illuminate chambers as completely as possible.

Running the experiment:

1. Once the fish are in the chambers, GoPros aligned, and blind set up with illumination let the fish acclimate for 2 hours.
2. When the 2 hour acclimation is finished turn on the GoPros and record the fish for 30 minutes. (Should be able to turn on GoPros through app on phone)
3. When the trial is over take a picture of the data sheet with the GoPros so that the data is logged in with the videos.
4. Return the fish to their tanks (be careful, they like to jump out of the chambers and try to escape!), turn off the pumps and chillers, and empty the chambers of water.

Video Analysis:

(We noticed that the GoPro videos segmented themselves into multiple files. Be sure to note which files belong to which GoPro and the chronological order of the files that respond to the video. We renamed the files to make it easier to go through and take data later on. Eg. We renamed the files Left Video 1, Left Video 2, Left Video 3, Right Video 1, etc.)

1. The 3 time points to be counted should last for 1 minute and start around 5 minutes, 15 minutes, and 25 minutes.
2. Pull up the video of the trial and chose a chamber to follow.
3. Take the video to the 5 minute marker
4. Follow the video for the next 60 seconds and make sure that the operculum movements are visible, if so record the start time (ST) and end time (ET) of the clip and count each beat for that 60 second period for the first measurement (BPM M1). If the 5-6 minute time point for that fish doesn't work find a 60 sec clip around that time point to make the counts.
5. Repeat step 4 at 15 minutes and 25 minutes and record the ST, ET, and PBM on the data sheet.

Ventilation Experiments 2016

Trial Info	Fish ID #	Tag	Position	Notes	ST/ET M1	BPM M1	ST/ET M2	BPM M2	ST/ET M3	BPM M3
Date:				1						
Species:				2						
Tank:				3						
pH/DO/Temp:				4						
File Name:				5						
Date:				6						
Species:				7						
Tank:				8						
pH/DO/Temp:				9						
File Name:				10						
Date:				1						
Species:				2						
Tank:				3						
pH/DO/Temp:				4						
File Name:				5						
Date:				6						
Species:				7						
Tank:				8						
pH/DO/Temp:				9						
File Name:				10						
Date:				1						
Species:				2						
Tank:				3						
pH/DO/Temp:				4						
File Name:				5						
Date:				6						
Species:				7						
Tank:				8						
pH/DO/Temp:				9						
File Name:				10						





