

FIRST TIME USE

1

The resting respirometry system comes with either horizontal or vertical acrylic chambers. Each chamber is equipped with two submersible Eheim pumps, tubing, fittings, and a flow-through oxygen cell/sensor (1). One pump is used for flushing (F) the chamber, and the other pump is used for mixing and recirculating (R) water inside the chamber during the closed measurement phase. For vertical chambers, see setup illustrations (SETUP GUIDE 2).

2

All the chambers, pumps, and tubes must be fully submerged during measurements to keep a stable temperature (2). The end of the flush tubing (2) must exit just above the water surface to create a closed chamber system when the flush pump is off.

For the flush pump, use the blue pump filter inside each pump head, and close off the fitting port with the included circular lid (1, red arrow).

Chamber setup

Set up each chamber as illustrated in the setup illustrations at the end of this guide:

3

- Horizontal chambers = **SETUP GUIDE 1**
- Vertical chambers = **SETUP GUIDE 2**

Keep tubing as short as possible to minimize the total water volume.

Remove air inside chamber setup

4

- **Chamber and tubing**
Release any visible air bubbles before closing the chambers and starting measurements.
- **Pumps**
Each pump must be fully purged of air before pump operation. To prime the pump with water, the pump intake must be submerged at all times.
- **Small air bubbles forming?**
An increase in water temperature can force dissolved gasses out of the solution forming numerous small gas bubbles on all solid surfaces. Leave everything at the experiment temperature for a 45-60 min. to reduce the risk of this phenomenon.

Connect flush and recirculation pumps

Power each pump from an available relay on the PowerX4 unit.

5

For independent chamber control, each flush pump and recirculation pump must be connected to their own relay on the PowerX4. The AutoResp™ v3 software will show which relay number each pump should connect to. Alternatively, multiple flush or recirculation pumps can connect to the same relay using a standard power strip (not included) for running chambers in a synchronized way.

Connect oxygen and temperature sensors

6

Insert the fiber optic cable into the flow-through oxygen sensor, so that the cable tip is as close to the sensor spot as possible (6). Fix cable by tightening the blue threaded head (do not overtighten). Connect fiber optic cable and temperature probe to the Witrox. Leave the Witrox temperature probe in the water bath that the chambers are submerged in.

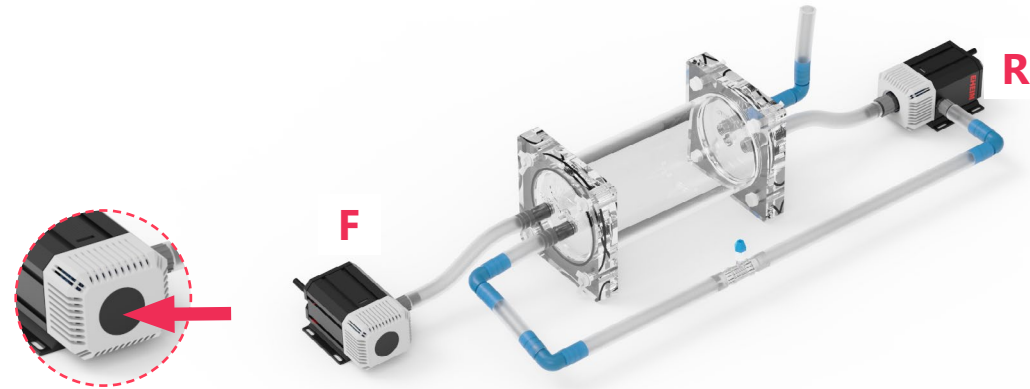
Setup instruments

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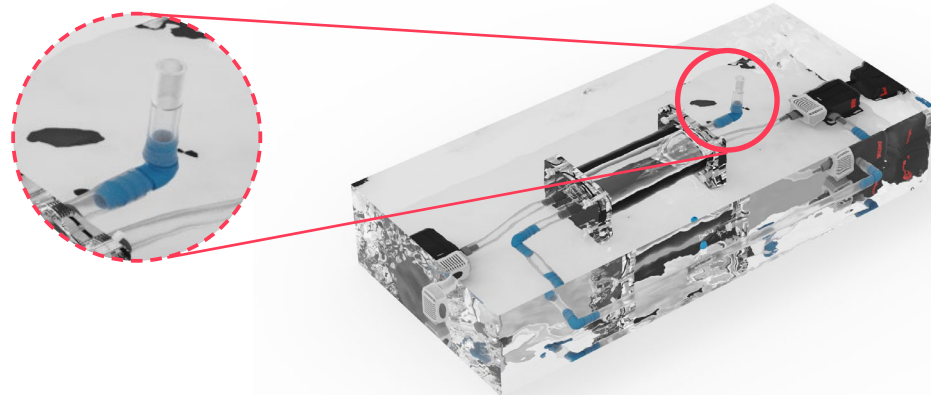
- **PowerX4:** Power each PowerX4 from a grounded wall outlet. Relays may now power on.
- **Witrox oxygen instrument:** Insert the power adapter for the Witrox instrument to a wall outlet and the USB cable to the backside socket (7). Alternatively, power the Witrox directly from a USB port.

Follow the [Quick guide for AutoResp™ v3](#) to set up the instruments.

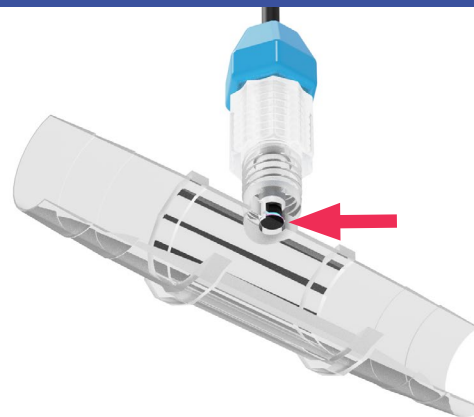
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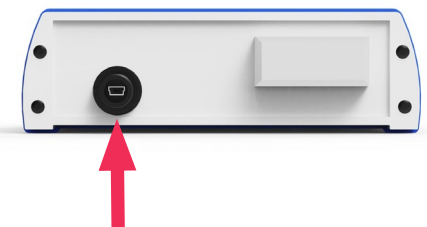
2



6



7



FOR EACH TRIAL

8 Start each trial by following step 2-7.

9 Place the experimental organism in the chamber. Check that fish face flush-end of horizontal chambers, so that their gills are being ventilated (9).
MAKE SURE that the flush pump is on and that water exits the open tube. Otherwise, your organisms may suffocate during setup!

CALIBRATION, SERVICE & MAINTENANCE

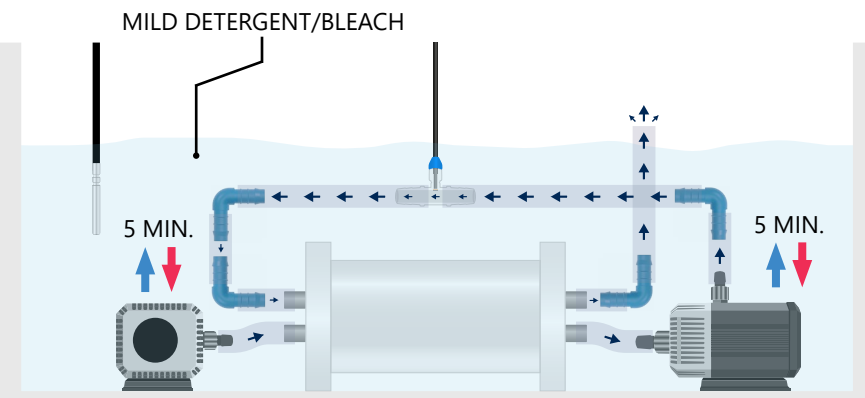
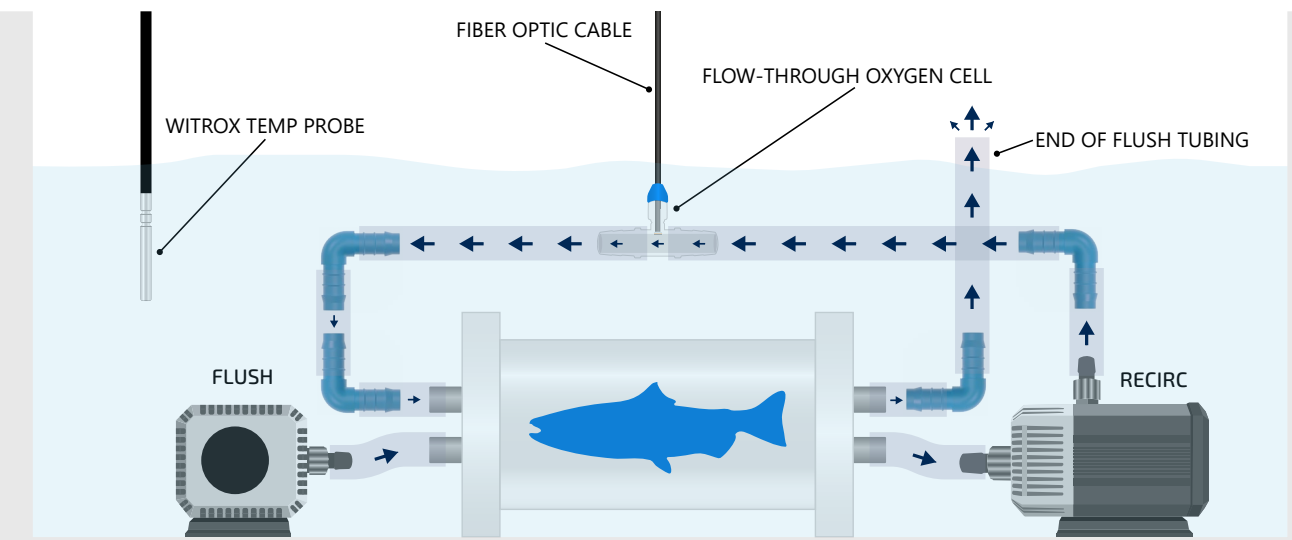
10 **Calibrating oxygen sensors**
 Follow instructions according to:
[Quick guide for AutoResp™ v3](#)
 Alternatively, watch the YouTube tutorial:
[AutoResp™ v3 - How to calibrate your oxygen sensor](#)

11 **Cleaning and storing between trials**
 Pumps, tubes, and chambers should be stored clean, empty, and dry between use. Empty the bath/vessels/chambers and refill with tap water adding mild detergent or bleach, if needed. Let pumps run with solution for a few minutes (11). Repeat this step with clean tap water. Empty pumps and bath/vessels/chamber of the tap water.

12 **Oxygen sensor performance**
 Oxygen sensors should be stored in a dark place between trials to avoid exposing the fluorescent sensor dye to UV light. UV light will bleach the sensor and cause signal drift.
 Sensor spot performance check (amplitude value):







- Good performance = 10000 ≤ 50000
- Bad performance = << 10000

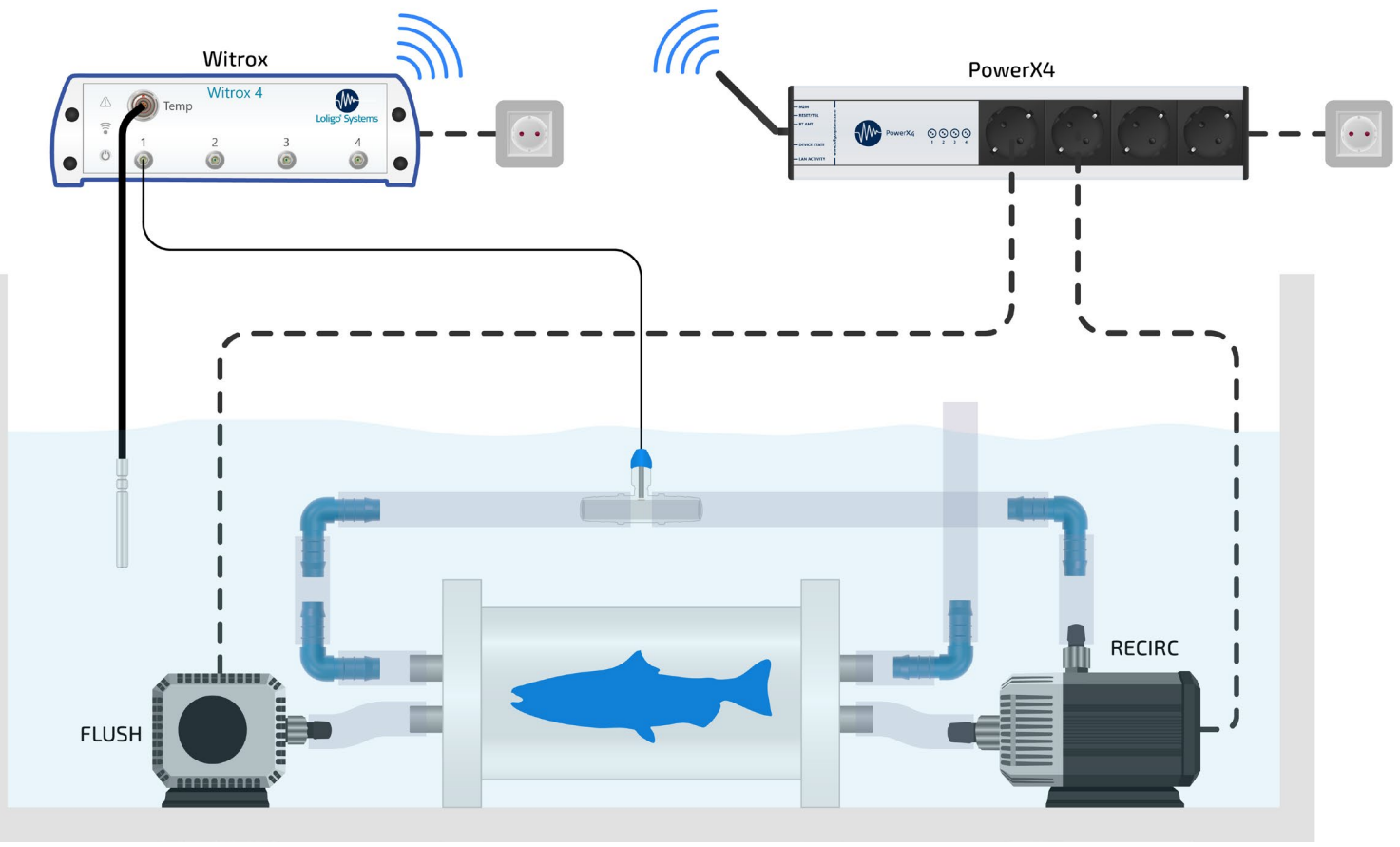
13 **WARNING**
DO NOT use alcohol on acrylic parts (chamber) as it can cause cracks (13). Use instead bleach or detergents for cleaning.



DO NOT USE ALCOHOL FOR CLEANING

Acrylic chamber horizontal setup







-  = Power cable
-  = Temperature probe
-  = Fiber optic cable
-  = Bluetooth connection
-  = 110V/60Hz or 230V/50Hz (wall outlet)
-  = Flow-through oxygen cell

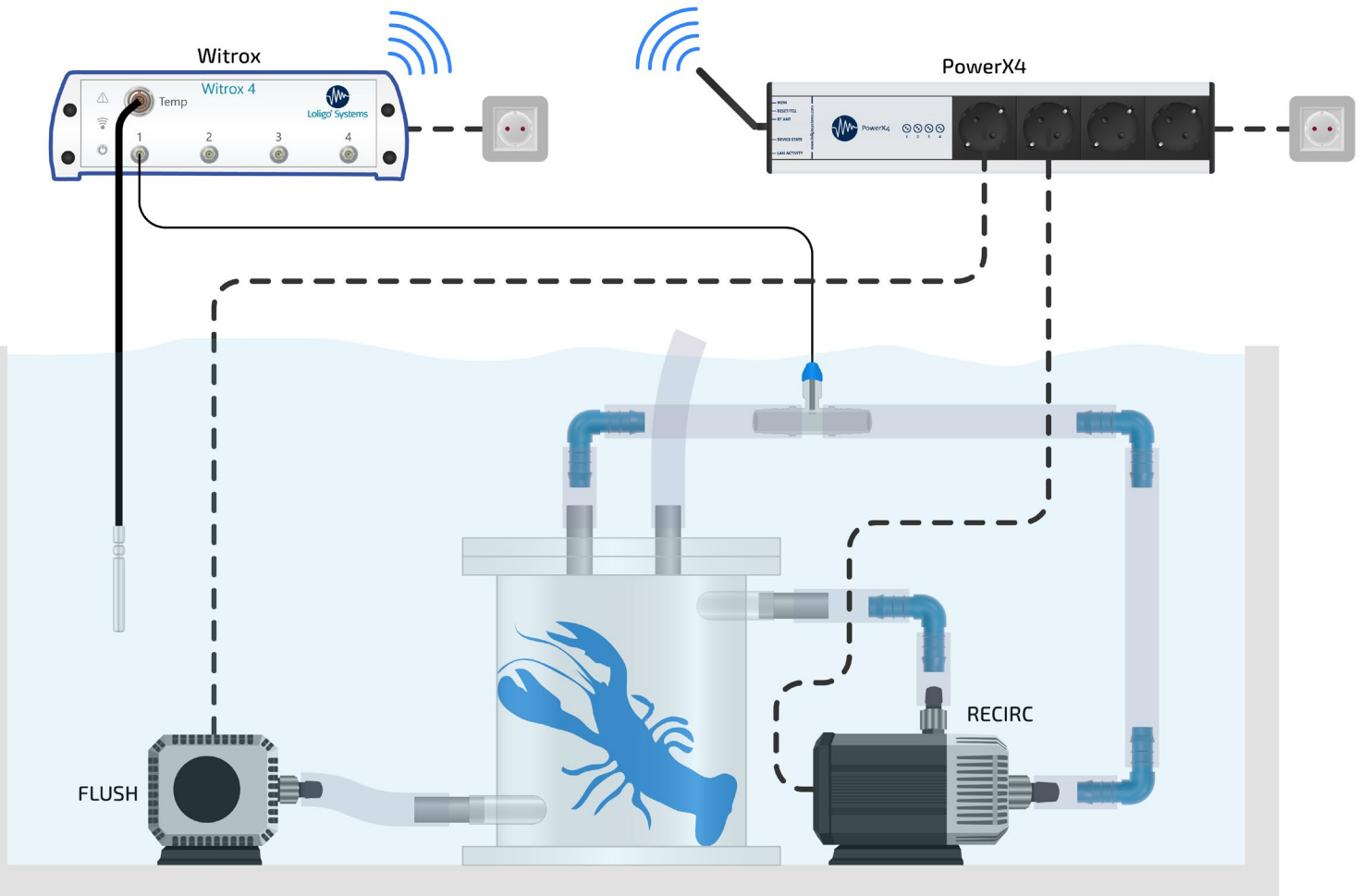


NOTES

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Acrylic chamber vertical setup

-  = Power cable
-  = Temperature probe
-  = Fiber optic cable
-  = Bluetooth connection
-  = 110V/60Hz or 230V/50Hz (wall outlet)
-  = Flow-through oxygen cell



NOTES

Blank area for notes.