# The OptoReg system: A simple and inexpensive solution for regulating water oxygen.

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## ABSTRACT
This paper describes an optocoupler-based regulation apparatus for saturation manipulation of oxygen in water (OptoReg). This system enables control of solenoid valves for oxygen and nitrogen gases using a FireSting-$O_2$ meter, an optocoupler box, and an electronic switch box. The hardware components connect to a computer through USB cables. The control software is free and has a graphical user interface, making it easy to use. With the OptoReg system, any lab with a 4-channel FireSting-$O_2$ meter can easily and cheaply set up four independently controlled systems for regulating water oxygen levels. Here we describe how to assemble and run the OptoReg system and present a data set demonstrating the high precision and stability of the OptoReg system during static acclimation experiments and dynamic warming trials.

## INTRODUCTION
Water oxygen is, like water temperature (Brett, 1971), an “abiotic master factor” because of its effects on aquatic organisms (Vaquer-Sunyer and Duarte, 2008; Chu and Tunnicliffe, 2015; Ern et al., 2016; Ern, 2019; Woods et al., 2022; Ern et al. 2023). Climate change and eutrophication are expanding the oxygen minimum zones (OMZs) in the world's ocean and increasing the number of aquatic oxygen deficient (hypoxic) “dead zones” in costal and estuarine areas (Diaz and Rosenberg, 2008; Rabalais et al., 2010; Breitburg et al., 2018). Additionally, hyperoxic water can occur during aquatic warming and during sunny days in shallow productive waters, but its potential effect on aquatic organisms is understudied (McArley et al., 2020). Investigating the effects of deoxygenation and hyperoxygenation on aquatic organisms is essential for understanding the important role of oxygen, as well as the impacts of climate change, on aquatic ecosystems. Consequently, the impact of water oxygen on aquatic organisms is an increasingly important research topic (IPCC, 2022).

Research on the effects of water oxygen levels on aquatic organisms has been partially hampered by the relative unavailability of systems for controlling water oxygen in laboratory settings. Commercial systems cost tens of thousands of euros, putting them out of reach for many researchers with limited funding. Do It Yourself (DIY) systems based on Arduino and Raspberry Pi platforms have been developed and are cheaper, but require both engineering and programming skills, which can also put them out of reach for many researchers. The ability to regulate water oxygen levels in laboratory settings has therefore required either large funds for available commercial systems or the technical skills to build and program custom systems. To address this issue, we have designed a system for regulating water oxygen levels using the common FireSting-$O_2$ oxygen meter in combination with commercially available optocouplers, electrical switches, solenoid valves, and software with a graphical user interface. Below we describe how to assemble and run this optocoupler-based regulation apparatus for saturation manipulation of oxygen in water (OptoReg), and we present a data set demonstrating precise oxygen regulation during a static acclimation experiment and during a dynamic warming trial.

## MATERIALS AND METHODS
The OptoReg system uses an optocoupler to relay an electrical signal from an oxygen meter to an electrical switch controlling a solenoid valve. When the water oxygen level reaches a predetermined

threshold value, the solenoid valve opens and releases a gas (nitrogen for hypoxia experiments and oxygen for hyperoxia experiments) into the water (Figure 1).

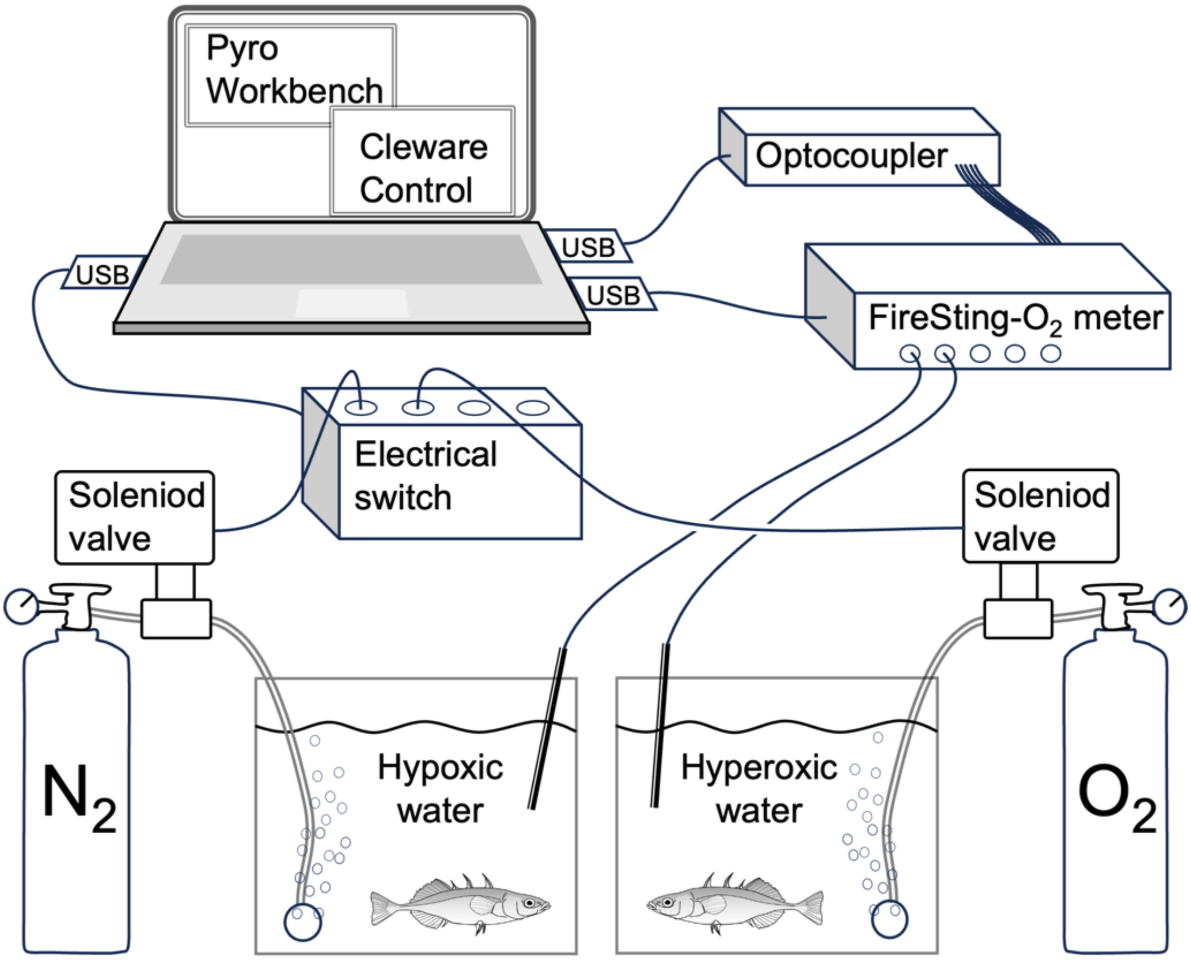


FIGURE 1. A schematic drawing of the OptoReg system when regulating water oxygen levels in two tanks, one with hypoxic water and one with hyperoxic water. The FireSting-$O_2$ oxygen meter measures the water oxygen level using fiber-optic oxygen sensors, and the Pyro Workbench software can send signals depending on the water oxygen levels (Box 1). An optocoupler relays the signal from the oxygen meter to a computer. ClewareControl software (Box 2) on the computer controls an electrical switch with a solenoid valve. The switch opens the solenoid valve if the water oxygen level rises above or decreases below a set threshold value.

A solenoid valve is an electromechanical device that controls the flow of gas (or liquid). In the OptoReg system, solenoid valves control the bubbling of nitrogen or oxygen gas. Nitrogen bubbling reduces the water oxygen level and oxygen bubbling increases the water oxygen level. A solenoid valve has an inlet port marked with a P (Pressure) and an outlet port marked with an A (Air/Atmosphere). In a normally closed valve, the type used in the OptoReg system, the valve is closed when no electric current is applied and opened by applying an electric current. Nitrogen is an inert gas and can, therefore, be controlled using most types of Solenoid valves. By contrast, oxygen gas supports combustion and must only be controlled using Solenoid valves that are suitable for use with oxygen. Controlling the flow of oxygen gas using a solenoid valve that is not recommended for oxygen increases the risk of fires.

An optocoupler is an electronic component that uses light to transmit electrical signals between two electrical circuits – an input circuit and an output circuit – while keeping them electrically isolated from each other. An optocoupler consists of a light-emitting diode (LED) that is connected to the input circuit and a phototransistor that is connected to the output circuit. The LED emits light when a voltage from the input circuit is applied to it. The phototransistor detects the light and generates a current proportional to the light intensity. Keeping the two circuits electrically isolated during signal transmission prevents interference, which would otherwise cause errors in data transmission. An optocoupler is therefore used in the OptoReg system to transmit signals between the low voltage FireSting-O2 system and the high voltage switch.

The OptoReg system described below is built using oxygen meters, sensors, and control software from PyroScience (pyroscience.com); optocouplers, electrical switches, and control software from Cleware (cleware-shop.de); and solenoid valves from Burkert (burkert.com). All components are commercially available and affordable (Table 1).

**Table 1.** Hardware specifications and purchasing details (at the time of publication) on the equipment used to build an OptoReg system and on FireSting-$O_2$ systems.

| Item | Stock No | Price (€) | Quantity |
|---|---|---|---|
| **Cleware-shop.de** | | | |
| USB Optocoupler* (OptoIn) | 54 | 60 | 1 |
| USB Switch 4 | 11-3 | 155 | 1 |
| **RS-online.com** | | | |
| PCB terminal block | 712-4487 | 20 | 5 |
| Wago terminal block | 883-7557 | 10 | 10 |
| Equipment wire | 361-579 | 28 | 100 m |
| Solenoid valve (for Nitrogen) | 307-0248 | 58 | 1 |
| Power cable | 490-245 | 9 | 1 |
| Push-in fitting | 212-9173 | 15 | 10 |
| Tubing | 144-7829 | 123 | 150 m |
| Y tube-to-tube adaptor | 916-0918 | 22 | 10 |
| **Pyroscience.com** | | | |
| FireSting-$O_2$ (1 Channel) | FSO2-C1 | 2.280 | 1 |
| FireSting-$O_2$ (4 Channels) | FSO2-C4 | 4.480 | 1 |

***** *When ordering the USB Optocoupler from Cleware, add a note in the remarks field that you need 2500 mV input. Then they will adapt the resistance for this setting.*

*Hardware*

The FireSting-$O_2$ Oxygen meter from PyroScience comes with up to four channels for fiber-optic oxygen sensors, one temperature sensor and an USB cable for connecting it to a computer (see the official manual for how to operate the FireSting oxygen meter). The back of the FireSting oxygen meter has an integrated Connector X2 extension port with one ground (Pin 1) and four analog outputs (Pin 2-5) (Figure 2). The four analog outputs have a range of 0 mV to 2500 mV. The 8-channel USB Optocoupler from Cleware has eight contacts (1-8) and a USB cable for connecting it to a computer. The contacts go from right to left, with Contact 1 being furthest to the right and Contact 8 being furthest to the left. Each contact has an input (positive, left clamp) and output (negative, right clamp) (Figure 2). A contact is open when no voltage (0 mV) is applied to the input and closed when voltage (2500 mV) is applied to the input. When ordering the USB Optocoupler from Cleware, add a note in the remarks field that you need 2500 mV input. The 4-channel USB-Switch from Cleware has four electrical switches (Socket 1-4), a USB cable for connecting it to a computer, and an electrical cord with a wall plug.

The FireSting-$O_2$ Oxygen meter is connected to the Cleware Optocoupler using a PCB Terminal Block, a Wago Terminal Block, and equipment wires (Figure 2). The signal from the Optocoupler is relayed to the Cleware USB-Switch using the ClewareControl software as detailed below (Box 2).

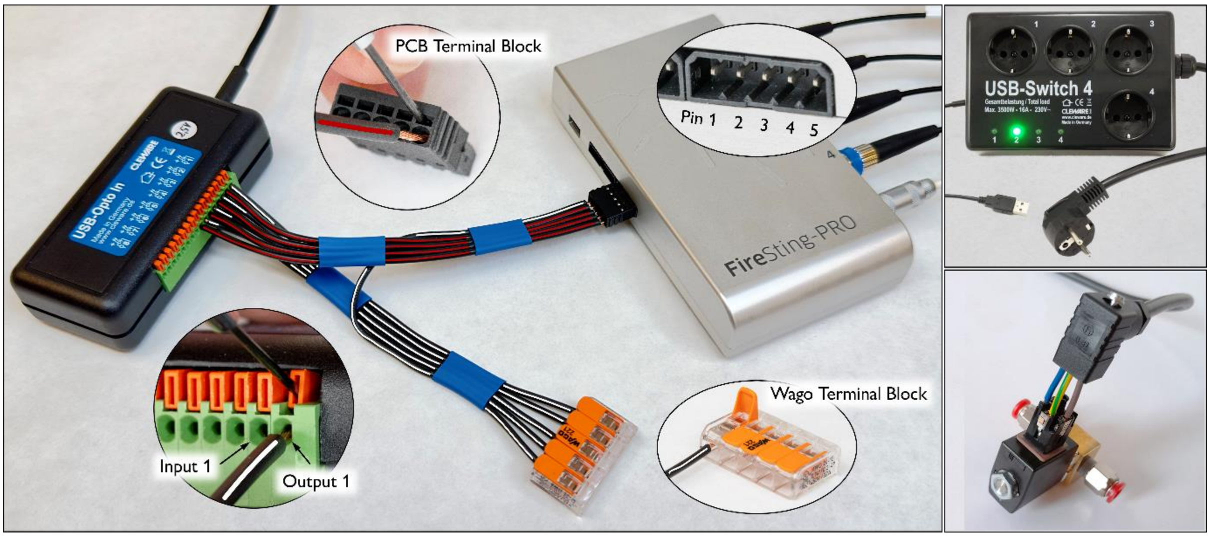


FIGURE 2. How to connect the analog outputs on the FireSting-$O_2$ to the inputs on the Optocoupler, and outputs on the Optocoupler to the ground on the FireSting-$O_2$. The four analog outputs (Pin 2-5) in the Connector X2 extension port on the FireSting are connected to the four inputs (1-4) of the first four contacts on the Optocoupler, using a PCB Terminal Block (red lined wires). The four outputs (1-4) of the first four contacts on the Optocoupler are all connected to the ground (Pin 1) in the Connector X2 extension port on the FireSting, using a Wago Terminal Block (white lined wires). The last four contacts (5-8) on the Optocoupler are not used. Wires are attached to the Optocoupler and the PCB Terminal Block using Spring Clamps, and to the Wago Terminal Block using Spring Cages. The spring clamps are pushed opened using the tip of a 2 mm flathead screwdriver.

*Software*

Pyro Workbench software is used to control the four analog outputs on the FireSting-$O_2$ meter via the 'Alarm if out of range' mode (Box1). In this mode, the outputs can be set to change from 0 mV to 2500 mV if the water oxygen level falls below a Lower alarm limit or rises above an Upper alarm limit (Figure 3). When the voltage of an output changes from 0 mV to 2500 mV, the connected contact on the Optocoupler (c.f. Figure 2) is opened. The ClewareControl software is used to pair the first four contacts on the Optocoupler with the four electrical switches on the USB-Switch, in a manner that turns a switch ON when the paired contact is opened and turns in OFF when the contact is closed (Box 2, Figure 4). Turning on a switch will activate the connected Solenoid valve, which will bubble the water with either nitrogen or oxygen, depending on the desired water oxygen level (as detailed below).

**BOX 1: Settings for the FireSting-$O_2$ meter using Pyro Workbench software.**
The ‘Alarm if out of range’ mode is programmed in the ‘Analog output / broadcast mode’ menu, which is opened via Settings → Firesting Pro (4 Channels) (A) → Analog output / broadcast mode. In the ‘Alarm if out of range’ mode, the four analog outputs in the Connector X2 extension port can output either 0 mV or 2500 mV. The 0 mV is given if the value of the oxygen level measured by the oxygen sensor is within a specific range, which can be adjusted at the Lower alarm limit and the Upper alarm limit. The analog output changes from 0 to 2500 mV if the value of the measured oxygen level falls below the Lower alarm limit or rises above the Upper alarm limit. The ‘Alarm if out of range’ mode is programmed in the ‘Analog output / broadcast mode’ menu, which is opened via Settings → Firesting Pro (4 Channels) (A) → Analog output / broadcast mode. In the window, the four analog outputs (Pin 2-5) in the Connector X2 extension port are designated with A, B, C, and D. Each of the four analog outputs (A-D) can be paired with one of the four optical oxygen sensor channels (Ch. 1-4). The default settings for ‘Channel’ and ‘Value’ should be left unchanged. The default settings for ‘Mode’ should be changed from ‘Standard analog out’ to ‘Alarm if out of range’. The ‘**Lower alarm limit**’ is typed in the box next to the 0 mV and the ‘**Upper alarm limit**’ is typed in the box next to the 2500 mV. The default setting is 0.00 for the ‘Lower alarm limit’ and 5000.00 for the ‘Upper alarm limit’. These settings are changed, depending on the desired water oxygen level (Figure 3).

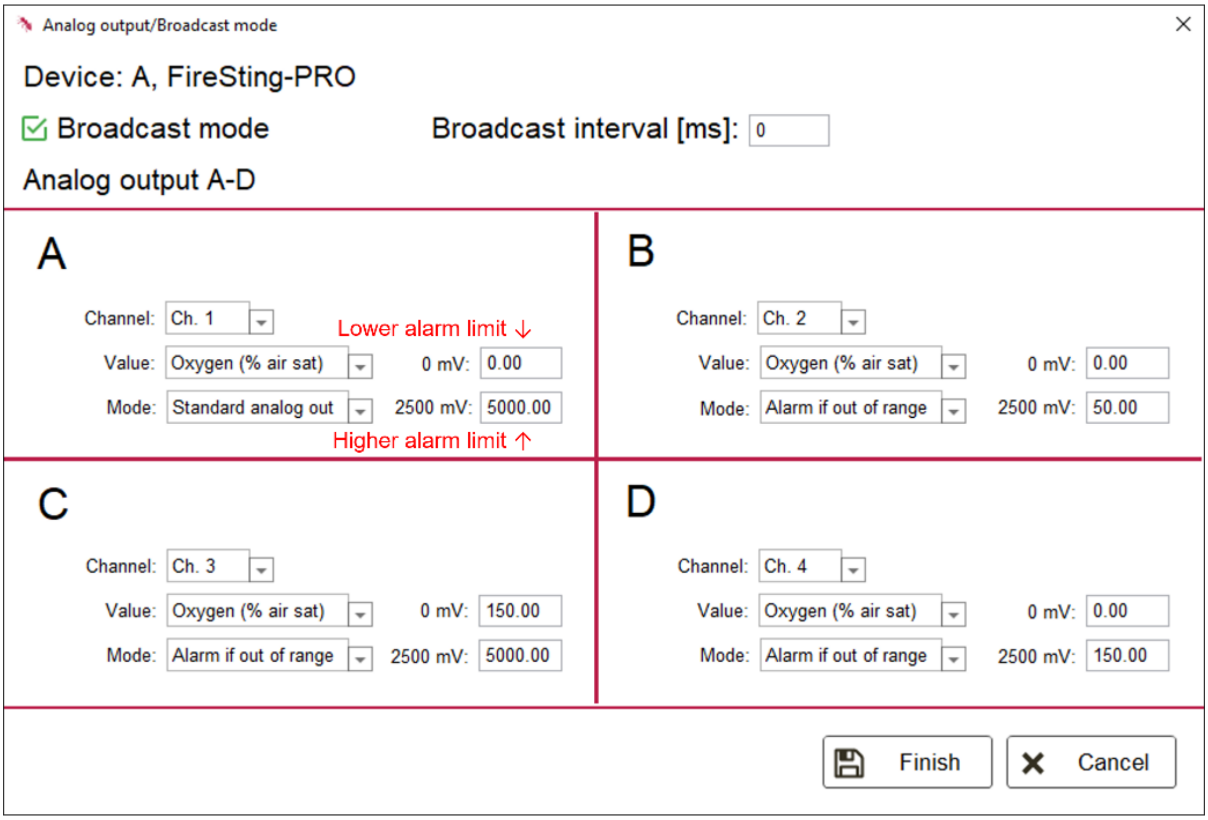


FIGURE 3. Settings for regulating water oxygen using the ‘*Alarm if out of range’* mode in the Pyro Workbench control software for the FireSting-$O_2$ meter. The Lower alarm limit and the Higher alarm limit are indicated for the Analog output A. Output **A** shown default settings; output **B** show settings for maintaining water oxygen level at 50% air saturation using solenoid-controlled nitrogen bubbling; output **C** show settings for maintaining water oxygen level at 150% air saturation using solenoid-controlled oxygen bubbling, and output **D** show settings for maintaining water oxygen level at 150% air saturation using passive oxygen bubbling and solenoid-controlled nitrogen bubbling. See text and Box 1 for details.

**BOX 2: Connecting Optocoupler and USB-switch using ClewareControl software.**
The two devices are paired in the 'Device settings' menu, which is opened via View → Device settings. The default name for each contact is the Optocoupler serial number followed by the contact number (e.g., 1401039/1 for contact number one), and the default name for each electrical switch is the USB-Switch serial number followed by the switch number (e.g., 652418/1 for switch number one). For easy overview, it is recommended to replace the Optocoupler serial number of the contacts with 'Contact' (e.g., 1401039/1 is renamed Contact/1) and the USB-Switch serial number of the switches with 'Switch' (e.g., 652418/1 is renamed Switch/1) (c.f. Figure 4). Contact/1 is paired with Switch/1 as follows: Select **Contact/1**. Click **Add** under Switch points. Check **opened** under Contact is. Select **Switch** under Action type. Select **Switch off** under Action. Click **Ok**. Click **Add** under Switch points. Check **closed** under Contact is. Select **Switch** under Action type. Select **Switch** on under Action. Click **Ok**. This path is visualized in the Supplementary materials. With these settings, Switch/1 is turned off when Contact/1 is open (0 mV is applied) and turned on when Contact/1 is closed (2500 mV is applied) (Figure 4). The remaining Contacts (2-4) are paired with the corresponding Switches (2-4) as described for Contact/1 and Switch/1.

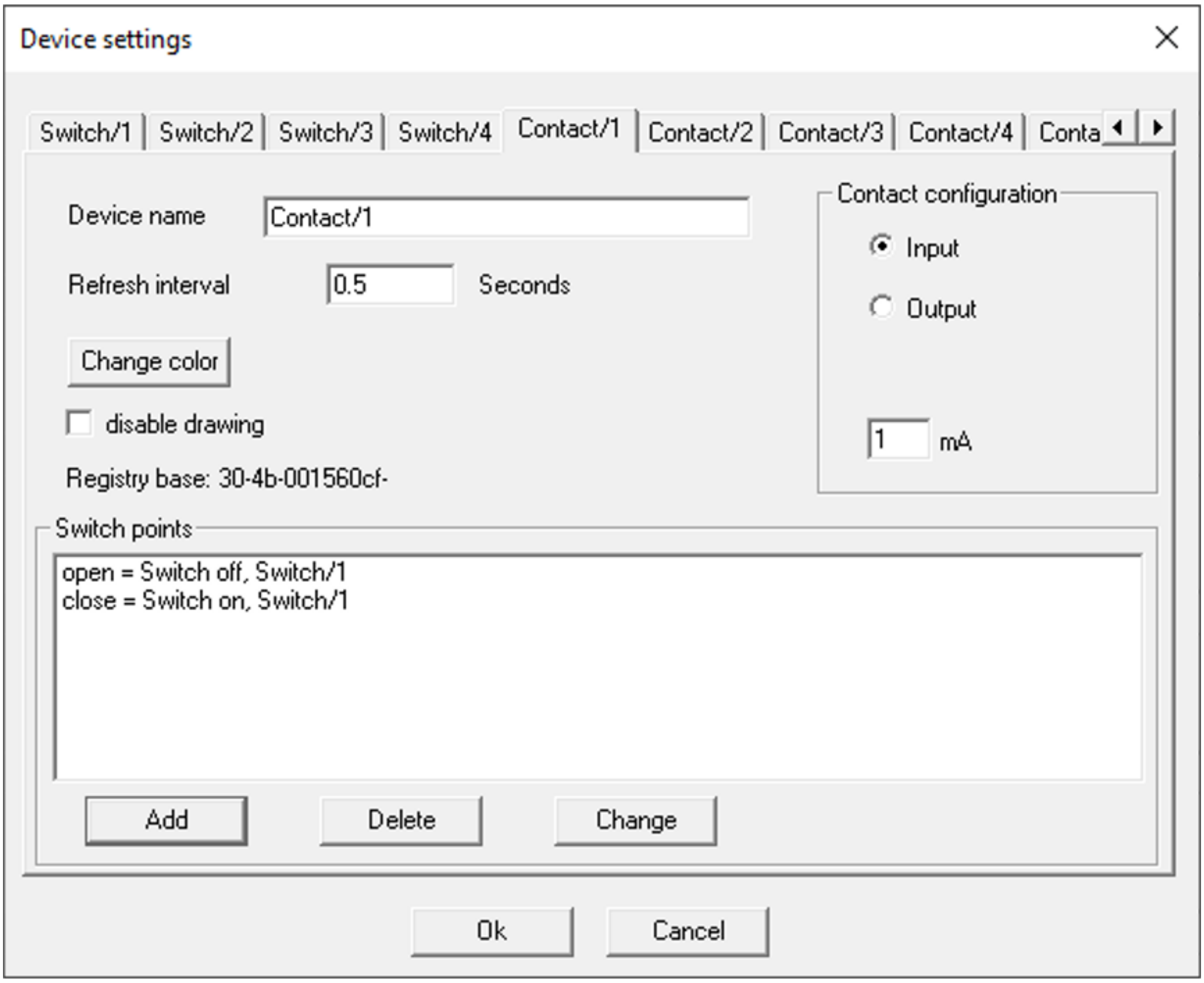


FIGURE 4 Settings for turning Switch/1 off when Contact/1 is open (0 mV is applied) and for turning Switch/1 on when Contact/1 is closed (2500 mV is applied). See test, Box 2, and Supplementary materials for details.

*Regulating water hypoxia using solenoid-controlled nitrogen bubbling*

In the Pyro Workbench software, the Lower alarm level is set to 0% of air saturation and the Upper alarm level is set to the desired water oxygen level (e.g., 50% air saturation) (Figure 3, Output A). With this setting, the voltage from the analog output will be 0 mV when the water oxygen level is between 0% air saturation and 50% air saturation, and it will be 2500 mV when the water oxygen level is above 50% air saturation (or below 0 % air saturation). When activated, and starting with normoxic water, this setting will bubble nitrogen gas into the water until the water oxygen level declines below 50% air saturation, at which point the solenoid valve is closed. Whenever the water oxygen level rises above 50% air saturation, because of the passive diffusion of oxygen into the water, the solenoid valve is opened, and the water is bubbled with nitrogen until water oxygen level again declines below 50% air saturation.

*Regulating water hyperoxia (v1) using solenoid-controlled oxygen bubbling*

When using pure oxygen gas, the solenoid valve must be suitable for use with oxygen. In the Workbench software, the Lower alarm level is set to the desired water oxygen level (e.g., 150% air sat) and the Upper alarm level is set to 5000% air saturation (Figure 3, Output C). With this setting, the voltage from the analog output will be 0 mV when the water oxygen level is between 150% air saturation and 5000% air saturation, and it will be 2500 mV when the water oxygen level is below 150% air saturation. When activated, and starting with normoxic water, this setting will bubble oxygen gas into the water until the water oxygen level rises above 150% air saturation, at which point the solenoid valve is closed. Whenever the water oxygen level decreases below 50% air saturation, for example from diffusion of oxygen out of the water, the solenoid valve is opened, and the water is bubbled with oxygen until water oxygen level again rises above 150% air saturation.

*Regulating water hyperoxia (v2) using passive oxygen bubbling and solenoid-controlled nitrogen bubbling*

Hyperoxia can alternatively be controlled without the use of oxygen solenoid valves using the following setup. In the Workbench software, the Lower alarm level should be set to 0% air saturation and the Upper alarm level should be set to the desired water oxygen level (e.g., 150% air sat) (Figure 3, Output D). With this setting, the voltage from the analog output will be 0 mV when the water oxygen level is between 0% air saturation and 150 % air saturation, and it will be 2500 mV when the water oxygen level is above 150% air saturation. When activated, and starting with normoxic water, the passive oxygen bubbling will raise the water's oxygen level until the oxygen level rises above 150% air saturation. When this happen, the solenoid valve controlling the flow of nitrogen gas is opened, and the water is bubbled with nitrogen until oxygen level decreases below 150% air saturation, at which point the valve is closed and the water oxygen begins to increase because of the passive oxygen bubbling. The rate of nitrogen bubbling should be large enough, that the water oxygen level decreases when the solenoid valve controlling the flow of nitrogen gas is opened, despite the passive oxygen bubbling.

*Validation experiments*

The accuracy and stability of the OptoReg system were tested during a static temperature acclimation experiment (10 days) and two dynamic warming trials (0.3°C per min). The acclimation experiment was conducted using a 50L tank and the warming trials were performed using 12L tanks. Water mixing during trials was achieved using Eheim universal 300 water pumps (eheim.com). Water temperature was regulated using a SmartPID CUBE - Smart Thermostat application (smartpid.com) and Aqua Medic 500 W titanium heating elements (aqua-medic.de).

The acclimation experiment was conducted to assess the OptoReg system's ability to maintain stable oxygen levels over prolonged periods. At the start of the experiment, the system was set to maintain the water oxygen level in the acclimation tank at 50% air saturation using solenoid-controlled nitrogen bubbling (c.f., Figure 3B). A second, separate, Firesting system was used to independently record oxygen levels. The results showed that the water oxygen level (mean ± 1 SD) during the 10-day acclimation period was maintained at 50.0 ± 0.4% air saturation (Figure 5A).

The warming trials were conducted to assess the OptoReg system's ability to simultaneously maintain distinct oxygen levels in different tanks (Figure 1) and to sustain oxygen levels during fluctuations in water temperature and oxygen solubility. The ramping rate was 0.3°C per minute, which is commonly used to assess the acute upper thermal tolerance of aquatic organisms (Becker and Genoway, 1979; Morgan et al. 2018)

At the start of the trials, the OptoReg system was set to maintain one trial tank at 30% air saturation using solenoid-controlled nitrogen bubbling (see Figure 3B) and to maintain another trial tank at 200% air saturation using solenoid-controlled oxygen bubbling (see Figure 3C). The results showed that the water oxygen level (mean ± 1 SD) during the warming trials was 198.9 ± 2.4% air saturation in the hyperoxia tank and 29.8 ± 0.6% air saturation in the hypoxia tank (Figure 5B).

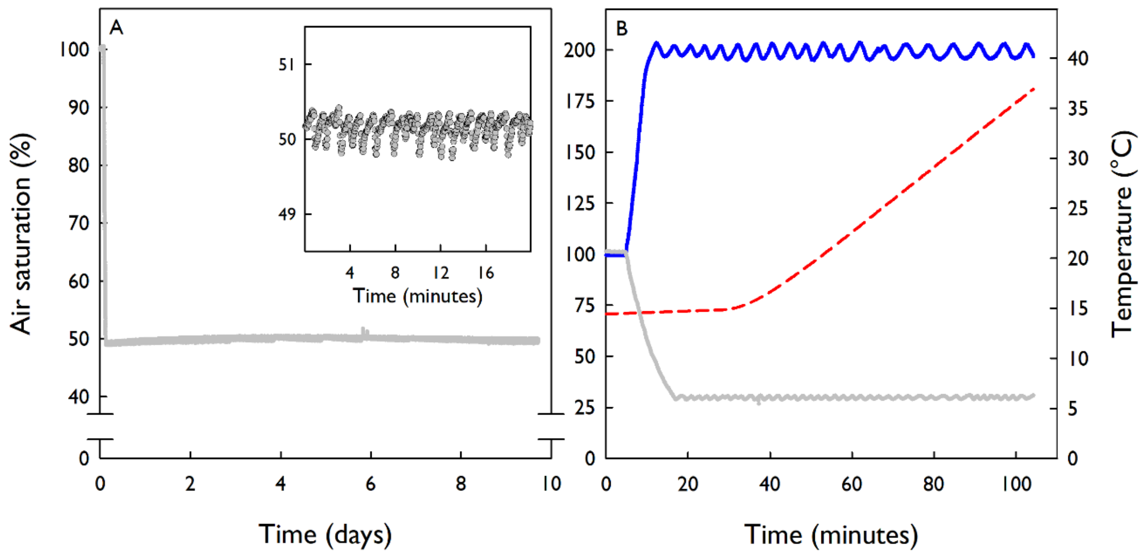


FIGURE 5 Recorded water oxygen levels during (A) a static acclimation experiment (10 days at 20°C) and (B) two dynamic warming trials (0.3°C per min). The embedded graph (A) shows individual $O_2$ recordings over a typical 20-min period during the acclimation experiment.

## CONCLUSION

The OptoReg system offers a simple and inexpensive solution for regulating water oxygen using the FireSting-O2 meter and sensors. Assembling and operating the system requires neither engineering nor programming skills. The system effectively maintains stable water oxygen levels over extended periods and during acute changes in water temperature. By enhancing the capabilities of laboratories to manipulate water oxygen levels, the OptoReg system is poised to facilitate an increase in studies on the responses of aquatic organisms to changes in water oxygen levels. Consequently, this will contribute valuable data to the field of conservation physiology.

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