DIVING-PAM-II/O2PH

Underwater Oxygen and pH Sensor

Manual

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1 Safety Instructions

1.1 General Safety Instructions

- The Underwater Oxygen Sensor DIVING-PAM-II/O2PH (denoted hereinafter as “device”) should be used only by qualified personal.
- Read safety and operating instructions in the manual prior to operation of the device.
- Follow safety and operating instructions of the manual, as well as the appropriate laws and guidelines for safety in the laboratory.
- Keep devices and sensors outside the reach of children.
- Ensure that no liquids, foreign objects, dust, sand, or dirt get inside the unit or into the connector.
- Do not put the devices and sensors near sources of heat.
- Connect the device only to the port indicated in the operating instructions.
- The devices should only be repaired by qualified personnel. There are no serviceable parts inside the device. Opening the housing will invalidate the warranty!
- The devices and sensors are not intended for medical or military purposes or any other safety-critical applications.
- The devices and sensors must not be used for applications in humans; not for in vivo examinations on humans, not for human-diagnostic or any therapeutic purposes.

1.2 Handling hazardous substances

The chemicals used for calibration of the sensor are listed in Table 1. Each chemical is accompanied by its safety data sheet. Read and understand the safety data sheets, before using the chemicals. Contact Walz when safety data sheets are missing.

Order Code	Chemical	Used for calibration of
OXCAL	Sodium sulphite	O_2 Sensor Spot
PHCAL2	Citric acid	pH Sensor Spot
PHCAL11	Sodium carbonate monohydrate	pH Sensor Spot

Table 1: Calibration Chemicals

2 Introduction

2.1 Measuring Principle

This manual introduces the Underwater Oxygen and pH Sensor DIVING-PAM-II/O2PH. The pH sensor was developed for seawater; however, its specifications do not apply for freshwater. The sensor is an accessory for the DIVING-PAM-II but can also be operated by the MINI-PAM-II.

The DIVING-PAM-II/O2PH was designed to study the relationships between the two environmental parameters, O_2 and pH, and marine life. Examples of research are how climate change affects these relationships, or how marine microbes influence bathymetric profiles of oxygen and pH.

The DIVING-PAM-II/O2PH uses optode-type sensor technique. Both the oxygen- and pH responsive element is a sensor spot which carries a luminescent dye. High oxygen concentrations quench the luminescence of the oxygen sensing dye, but by high proton concentrations enhance the luminescence of the pH sensing dye is enhanced (Fig. 1). In practice, not the luminescence intensity is measured but the phase shift of luminescence relative to the sine-modulated excitation light.

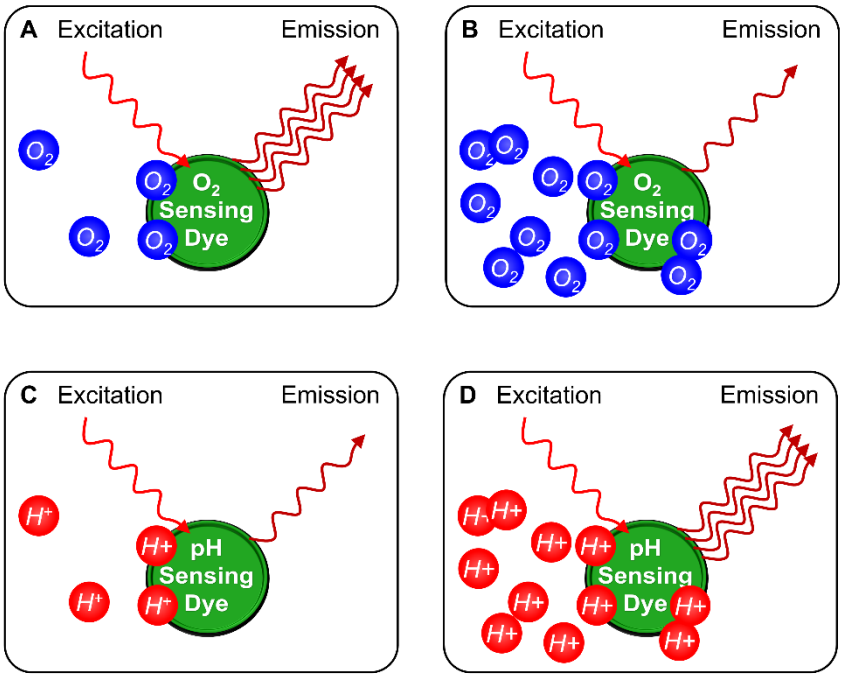

Fig. 1: Principles of O_2 and pH Measurements

The figure illustrates the effect of oxygen and protons on oxygen and pH sensing spots, respectively. A high luminescence intensity at low oxygen concentrations. B, low luminescence intensity at high oxygen concentrations. C, low luminescence intensity at low proton concentrations. D, high luminescence intensity at high proton concentrations. Redrawn after www.pyroscience.com.

2.2 Extent of Delivery

- Underwater Oxygen Sensor DIVING-PAM-II/O2PH, with PT100 resistance thermometer.
- 2 m underwater cable 000130204945

- Two spare oxygen sensor spots OXSP5
- Two spare pH sensor spots PHSP5-PK8T
- Holder for DIVING-PAM-II/O2PH consisting of the mounting brackets 000246001714 and 000246003914 and 1 ring holder 000244905514.
- OXCAL O2 Calibration Capsules (10 pieces)
- PHCAL2 pH Calibration Capsules (10 pieces)
- PHCAL11 pH Calibration Capsules (10 pieces)

3 Setup

3.1 Mounting

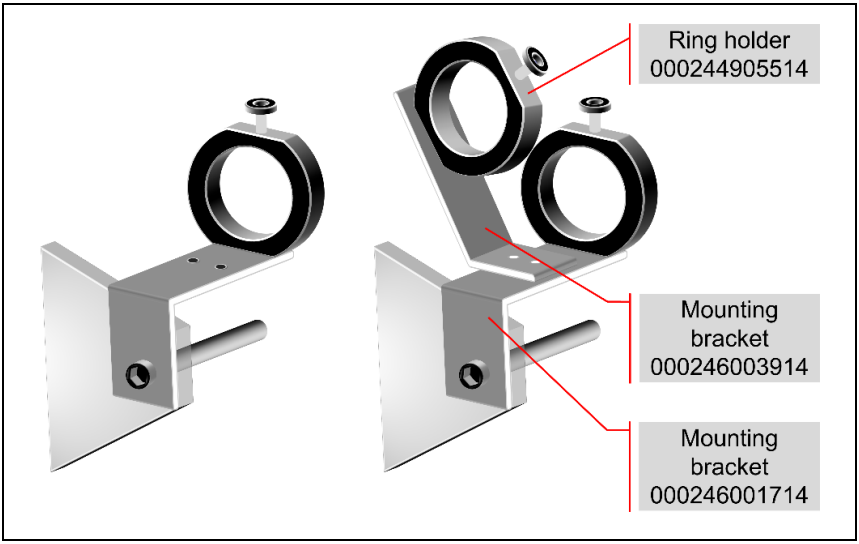

Fig. 2: DIVING-PAM-II/O2PH Holder

Left, standard holder. Right, dual holder for DIVING-PAM-II/O2PH and MINI-SPEC.

The DIVING-PAM-II/O2PH sensor is delivered together with a ring holder (order number 000244905514) and a mounting bracket (order number 000246003914). Both parts form a holder for the DIVING-PAM-II/O2PH. To mount the holder, fasten mounting bracket (#000246003914) to mounting bracket #000246001714 (see Fig. 2). Then fasten second ring holder as shown in Fig. 2.

An additional mounting bracket #000246001714 is part of delivery. This second mounting bracket is provided for owners of the first models in the series. These models have a mounting bracket with only two holes.

The DIVING-PAM-II/O2PH sensor is connected via a 4-pole underwater cable to one of the two AUX ports of the DIVING-PAM-II. For a proper and watertight connection, consider the instructions given in Fig. 3.

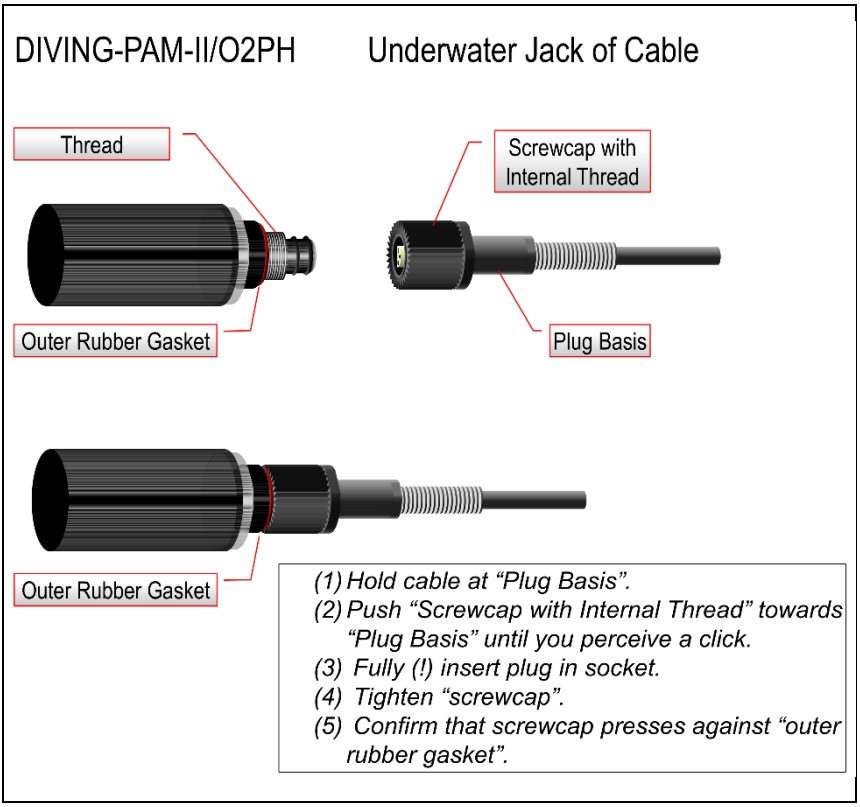

Fig. 3: Connection of DIVING-PAM-II/O2PH

3.2 Sensor Spot Properties

The DIVING-PAM-II/O2PH sensor measures pH and oxygen optically. Sensor spots carrying a luminescent dye, change their yield for luminescence dependent on oxygen or proton concentration (Fig. 1). Excitation light reaches the sensor spot via fiberoptics.

Dye luminescence is guided by the same fiberoptics to the high-precision oxygen or pH meter.

Each sensor spot is provided with a code that contains information for oxygen or pH meter (Table 2). At Walz, the sensor codes are written to the respective meter inside the DIVING-PAM-II/O2PH sensor. The influence of temperature on dye luminescence is automatically compensated using the temperature measured by a PT 100 resistance thermometer (Fig. 4, page 10).

CODE	**SA**	**E**	**6**	**-**	**540 - 216**
MEANING	Sensor Type	LED Intensity	Amplification Factor		Pre-Calibration Values

Table 2: Sensor Code (Example)

3.3 Sensor Spot Replacement

Long-term use decreases signal strength of the sensor spots. At a certain level of degradation, measurements fail and the corresponding data fields display a dash and an error message pops up (compare Table 3, page 18). In this case, the sensor spot must be renewed.

The minimum number of measurements by a single oxygen sensor is estimated to be 1,000,000 corresponding to almost 300 hours of measuring time for 1 Hz measuring frequency. Switching off the oxygen sensor (by turning off the DIVING-PAM-II) between experiments extents the sensor lifetime. Different from the O_2 sensor spot, the manufacturer did not specify the minimum number of measurements for the pH sensor spot.

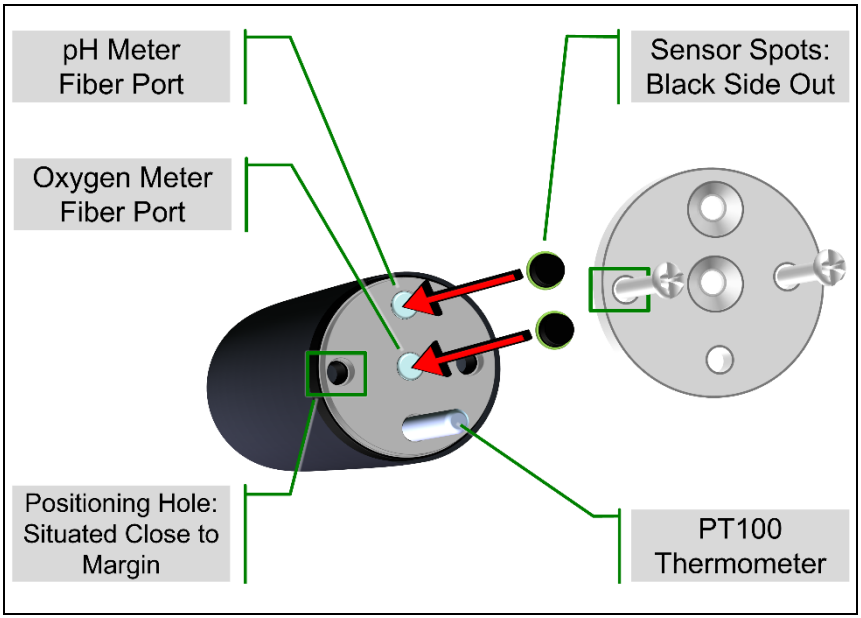

Fig. 4: Underwater Oxygen Sensor DIVING-PAM-II/O2PH

To change a sensor spot, remove the two plastic screws at the end of the DIVING-PAM-II/O2PH sensor and lift off the acrylic glass disk (see top right side of Fig. 4, page 10). Remove the degraded sensor spot. Place the new sensor spot exactly on the corresponding fiber port. Make sure that the black (optically isolating) side is outward directed. Incorrect orientation will render the sensor spot inoperable.

Note that one hole in the acrylic glass disk is moved more to the edge than the other hole (Denoted “Positioning Hole in Fig. 4, page 10, marked by green rectangle). When replacing the acrylic disc, ensure that this hole is positioned over the corresponding hole in the oxygen meter.

4 Preparing for Calibration

4.1 General Advice

Visually control that the sensor pads are free of bubbles.

Stir efficiently but not vigorously.

Calibrate at constant temperature. Keep sensor and calibration liquids in constant environment for at least 30 minutes.

Flush sensor with distilled or deionized water when changing calibration liquids and after calibration.

4.2 O_2

4.2.1 Zero % Calibration Liquids

OXCAL

Part of delivery are OXCAL capsules containing sodium sulphite, Na_2SO_3 (PyroScience, Aachen, Germany). One capsule gives 50 mL 0% calibration standard.

Use a 50 mL sealable wide-neck container. Add magnetic stir bar and capsule. Fill up to the rim with demineralized water. DO NOT use saline water (e.g., seawater). Close container. Avoid headspace and air bubbles in container.

Place on a magnetic stirrer and stir until the salt is completely dissolved. Stop stirring. Leave to stand for about 15 minutes. Immerse sensor into 0% calibration solution. Let equilibrate and perform calibration.

Do not store the sensors in this solution and rinse carefully after the calibration with demineralized water.

OTHERS

<> Water thoroughly bubbled with nitrogen gas (pass gas through an air stone).

<> Procedure according to: Delieu T, Walker DA (1972) An improved cathode for the measurement of photosynthetic oxygen evolution by isolated chloroplasts. New Phytologist 71: 201-225

"A few small crystals of sodium dithionite are added to stirred water in the cells which are then closed with the plungers. This reacts with dissolved oxygen according to the equation

$Na_2S_2O_4 + O_2 + H_20 \rightarrow NaHSO_4 + NaHSO_3$

and since the reaction goes rapidly to virtual completion this procedure is equivalent, but more convenient, than prolonged flushing with nitrogen."

<> Carbonated water can be used for a quick zero-point check.

4.2.2 Hundred % Calibration Liquids

<> Water bubbled with air (pass air through an air stone connected to an air pump, e.g., an aquarium pump).

<> If an air pump is not available, fill water into a flask leaving about 50% air in the head space and shake it strongly for about 3 minutes.

4.3 pH

4.3.1 Principle

For two-point calibration, the optical pH sensor is brought into the fully protonated and fully deprotonated state (Fig. 5). This procedure differs from other sensor calibrations in which calibration occurs in the dynamic range of the sensor.

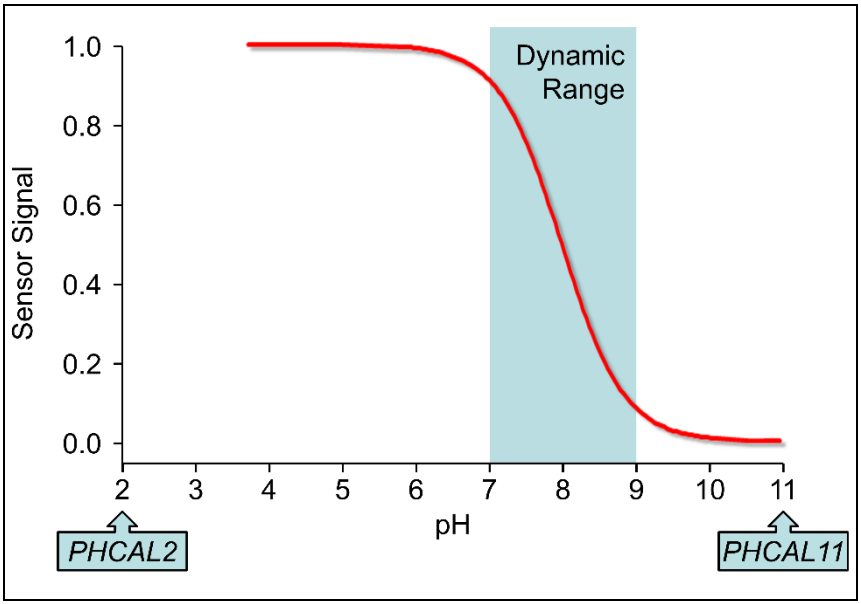

Fig. 5: Signal Versus pH Curve of the pH Sensor

4.3.2 Specific Instructions

Calibrate the pH sensor before each measurement campaign.

Do NOT use commercially available buffer solutions as most contain anti-microbial agents that irreversibly affect sensor performance.

The sensor must be wet. If stored in dry conditions, place the sensor spot in distilled or deionized water overnight or for at least 60 minutes. Do not use tap water for soaking.

If possible, calibrate at the temperature of the actual pH measurements.

4.3.3 pH Calibration Liquids

First Calibration Point (acidic pH)

pH 2 buffer solution prepared with PyroScience buffer capsules (PHCAL2) or a custom calibration buffer.

Second Calibration Point (alkaline pH)

pH 11 buffer solution prepared with PyroScience buffer capsule (PHCAL11) or a custom calibration buffer.

Open the PHCAL2 or PHCAL11 capsule by holding both ends and pulling them apart. Add 100 mL deionized water to the contents. Mix well until the chemical is completely dissolved.

Completely immerse the sensor end of the DIVING-PAM-II/O2PH into the stirred buffer. The sensor end must be free of air bubbles. Gently shake the sensor to remove any air bubbles. Let the sensor equilibrate and perform the calibration. Wait for a stable sensor signal (this can take several minutes depending on the temperature and stirring rate). For best results, wait at least 15 minutes.

5 Calibration

Perform the calibration process either using the user interface of the fluorometer, or by WinControl-3. Using the fluorometer, sequentially select Main Menu → Sensors → O_2/pH Sensor. The menu of the now visible window "O_2/pH Sensor Settings" concern settings and calibration of the DIVING-PAM-II/O2PH sensor (Fig. 6).

The menu items are:

Meas. Interval (s)

Sets the time between measurements. Values from 1 to 60 seconds are allowed.

Blank Out LED

When active, the fluorometer's light source (measuring and actinic light) is switch off when O2/pH is measured. This feature is only needed if the internal light source of the fluorometer distorts O2/pH measurements.

Settings and Output Format

See 5.1, page 21.

Calibrate O_2 and Calibrate pH

These items open calibration dialogs for oxygen and pH calibration, respectively.

The current pH and oxygen concentration are displayed on window "Primary Data" (Fig. 7).

When using the software WinControl-3, go to window "Sensors", to get access to settings and calibration of the O_2 and pH sensors (Fig. 8). All messages and settings related to the oxygen sensor

are explained in Table 3. The button Start Calibration opens the calibration window (Fig. 9).

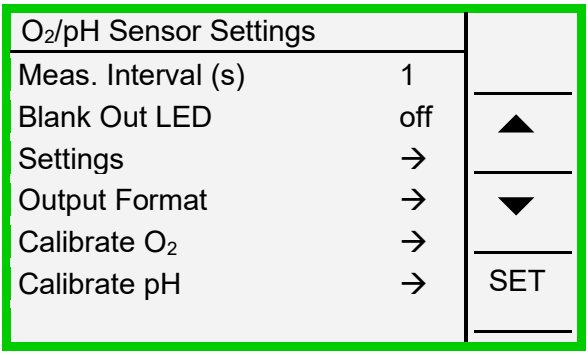

Fig. 6: Oxygen Sensor Settings

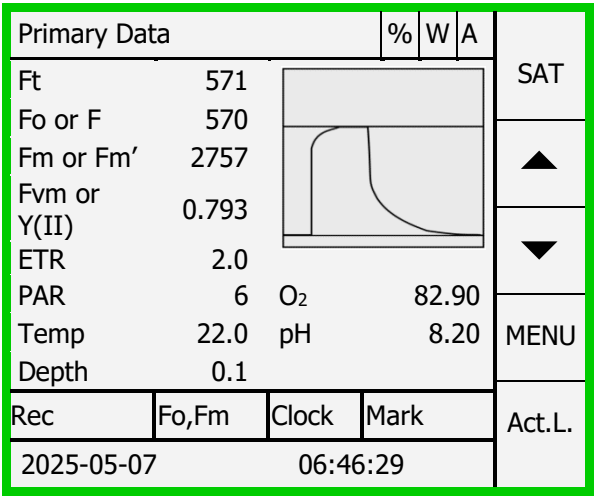

Fig. 7: Primary Data

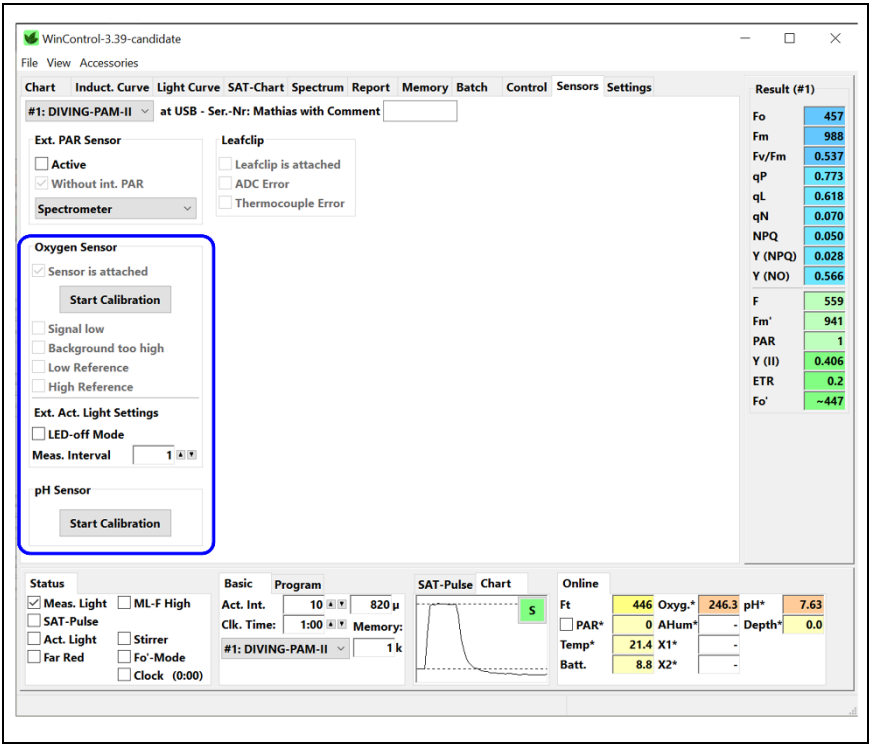

Fig. 8: Sensor Window of WinControl-3

The blue box highlights the area concerning the pH and oxygen sensors.

☑ **Sensor is attached**	The marked checkbox indicates detection of the pH/oxygen sensor by the fluorometer.
☐ **Signal low**	The marked checkbox indicates that the signal intensity is below the threshold for required for reliable pH or oxygen determination. Check the correct position of sensor spots (see Fig. 4, page 10). If no errors are found, one of the sensor spots may be exhausted and needs to be replaced.
☐ **Background too high**	The marked checkbox indicates that external light interferes with pH or oxygen measurements. Possible reasons are light-reflecting air bubbles reflecting external light into the sensor, or light sources shining sideward on the oxygen sensor.
☐ **Low reference**	Indicates severe error when checked. Send to manufacturer.
☐ **High reference**	Indicates severe error when checked. Send to manufacturer.
Ext. Act. Light Settings	
☐ **LED-off mode**	Activate checkbox when an external light source (e.g., External LED Light Source 2054-L) causes high signal noise. Thereafter, the external light source will be switched off when oxygen is measured.
Meas. Interval (s) 1 ▲▼	The number specifies the interval between oxygen measurements. Default value is 1 second. Adjustable values range from 1 – 60 seconds.

Table 3: Sensor Messages and Settings

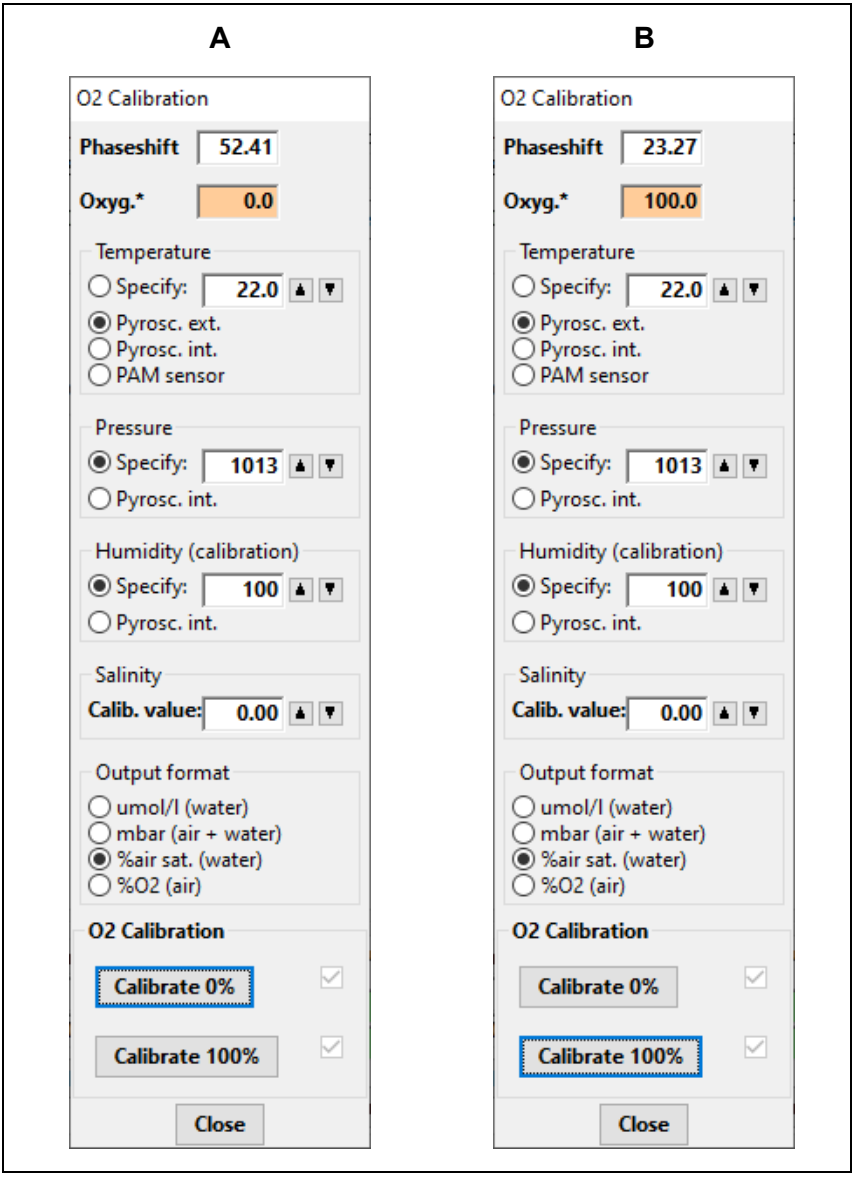

Fig. 9: O2 Calibration Window.

A, 0 % calibration. **B**, 100 % calibration

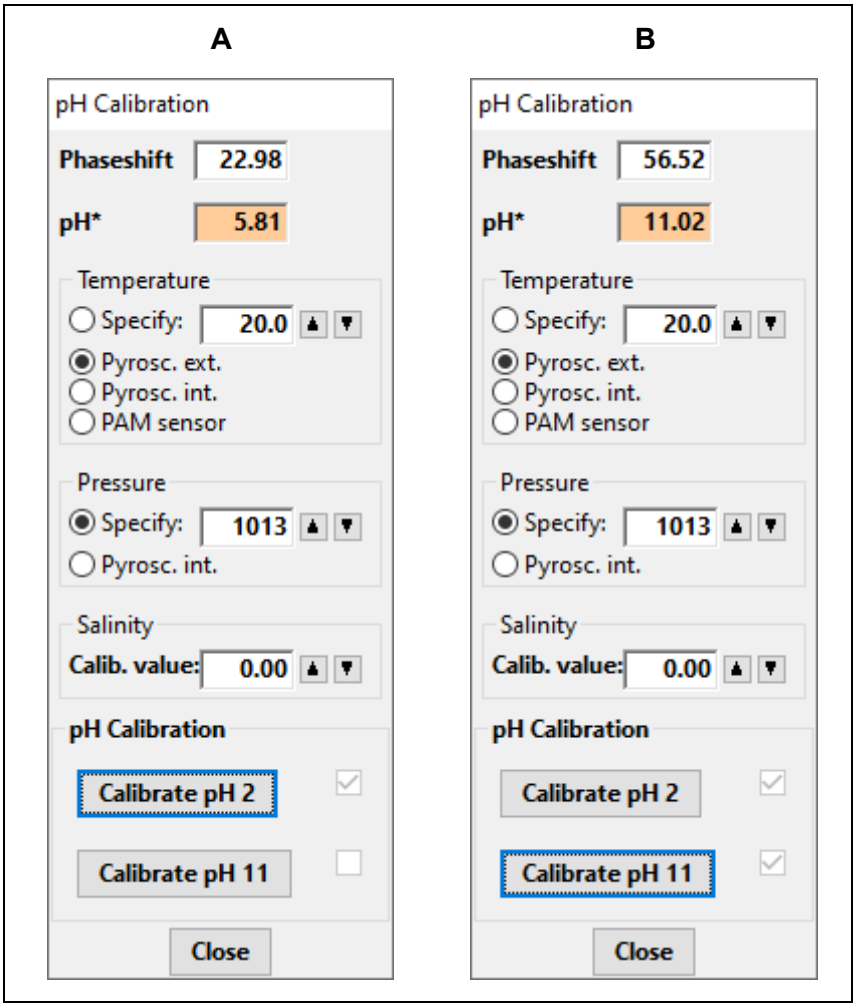

Fig. 10: pH Calibration Window.

A, pH 2 calibration (perform first). **B**, pH 11 calibration

5.1 Settings

The following settings are recommended for measurements with suspensions.

Temperature: Select “Pyrosc. ext”.

Pressure: Select “Specify:” and enter current atmospheric pressure. When a barometer is not available, take the information from the internet (e.g., https://barometricpressure.app/).

Humidity (calibration): For calibration in aqueous solutions, choose “Specify” and enter 100 (%).

Salinity:

For pH measurements, the salinity [g/L] must be entered. The ionic strength can differ between the calibration buffer and the medium of the measurements. For O_2 measurements, salinity plays a role only when the output format is µmol/L (see Oxygen Sensors, Fiberoptic and Contactless, USER MANUAL, OPERATING INSTRUCTIONS, https://www.pyroscience.com/en/).

To measure oxygen in media containing high sugar contents, do not choose µmol/L as format as the Software does not deal with the “salting-out effect” of sugars. A paper dealing with sugar-dependent solubility of O_2 is:

Rischbieter E, Schumpe A, Wunder V (1996) Gas solubilities in aqueous solutions of organic substances: Journal of Chemical & Engineering Data 41: 809-812.

In the Pyroscience manual for optical pH sensors, four general categories of salinity ranges are considered as listed in Table 4.

Category	Salinity [g/l]	Conductivity [mS/cm]	Ionic Strength [mM]
Wastewater	0.5 - 1.5	1 - 3	10 - 30
Intermediate	1.5 - 5	3 - 10	30 - 100
Physiological	5 - 15	10 - 30	100 - 300
Seawater	15 - 40	30 - 80	300 - 800

Table 4: Salinity Ranges

For most accurate pH measurements, enter the salinity [g/l] of the sample. If the salinity of the sample is unknown, estimate salinity using Table 4.

Units of calibration: This item applies to O_2 calibration. Choose one of the three options available for the water phase:

- Dissolved O_2 concentration, µmol/L (applicable for water phase).
- Partial pressure pO_2, mbar = hPa (applicable for gas and water phase).
- Percent air saturation, % (applicable for water phase).
- The option “Volume percent O2, %” is solely applicable for the air phase.

5.2 O_2

Zero calibration: Immerse sensor in Zero % calibration liquid, wait for constant oxygen signal and press Calibrate 0%.

100 % calibration: After careful rinsing, immerse in 100 % calibration liquid, wait for constant signal, press Calibrate 100 %.

After calibration, rinse thoroughly with distilled water

Stability: The calibration of the sensor is rather stable. Still, it is good practice to check the calibration at regular intervals.

Expected phase shift: Dphi is the phase shift of light emission by the sensor spot relative to excitation light. dphi is not linearly related to oxygen abundance. Increasing oxygen levels correspond to decreasing dphi values. Anoxic conditions will give about dphi=53, ambient air will give about dphi=20. (Fig. 9, page 19).

5.3 pH

pH 2 calibration (first): Immerse sensor in pH 2 calibration liquid, wait for constant signal and press Calibrate pH 2.

pH 11 calibration (second): After careful rinsing with distilled water, immerse in pH 11 calibration liquid, wait for constant signal, press Calibrate pH 11.

After calibration, rinse thoroughly with distilled water

Stability: Calibrate sensor prior to each measuring campaign.

Expected phase shift: Dphi is the phase shift of light emission by the sensor spot relative to excitation light. pH 2 will give about dphi=22, pH 11 will give about dphi=56 (Fig. 10, page 20).

6 Specifications

DIVING-PAM-II/O2PH Underwater Oxygen and pH Sensor

Design:

POM plastic tube of 3.25 cm diameter and 17.5 cm length, with optical an Oxygen and a pH sensor spot, both fixed by a Perspex disc, a PT100 resistance thermometer, and a 4-pole underwater socket. Temperature-compensated oxygen and pH measurements by high precision optical meters, connected by fiberoptics to the sensor spot. Including a 2 m underwater cable (000130204945), two oxygen and two pH spare sensor spots,and a holder (weight 75 g, maximum dimensions 6.5 cm x 6 cm x 12 cm, L x W x H) to attach both the DIVING-PAM-II/O2PH and the spectrometer MINI-SPEC to the DIVING-PAM-II optoelectronic unit, consisting of the mounting brackets 000246001714 and 000246003914 and 1 ring holder 000244905514.

Maximum diving depth: 50 m

Dimensions: 3.25 cm diameter, 17.5 cm length

Weight: 135 g

7 Guarantee

7.1 Manufacturer's Guarantee

Under this Manufacturer's Guarantee ("Guarantee"), subject to the Conditions and Instructions below, Heinz Walz GmbH, Germany ("Manufacturer"), guarantees (§443 BGB) to the end customer and user ("Customer") that all products supplied by it shall substantially conform in material respects to the Specifications for 24 months from the delivery date (date on invoice). In this Guarantee, "Specifications" means the product's features (as may be amended by Manufacturer from time to time), which are set out under the headings "specifications" and/or "technical specifications" within the product's respective brochure, data sheet, or respective tab on the Manufacturer's website for such product, and which may be included with the documents for the product when delivered. In case of an eligible guarantee claim, this Guarantee entitles the Customer to repair or replacement, at the Manufacturer's option, and this Guarantee does not include any other rights or remedies.

7.2 Conditions

This Guarantee shall not apply to:

- Any defects or damage directly or indirectly caused by or resulting from the use of unauthorized replacement parts and/or service performed by unauthorized personnel.
- Any product supplied by the Heinz Walz GmbH, Germany which has been subjected to misuse, abuse, abnormal use, negligence, alteration or accident.

- Damage caused from improper packaging during shipment or any acts of God.
- Batteries, cables, calibrations, fiberoptics, fuses, gas filters, lamps, thermocouples, and underwater cables.
- Defects that could reasonably have been detected upon inspection of the product when received by the Customer and not promptly noticed within ten (10) days to Heinz Walz GmbH.
- Submersible parts of the DIVING-PAM or the underwater version of the MONITORING-PAM have been tested to be watertight down to the maximum operating depth indicated in the respective manual. Guarantee shall not apply for diving depths exceeding the maximum operating depth. Further, guarantee shall not apply for damage resulting from improper operation of devices, in particular, the failure to properly seal ports or sockets.

7.3 Instructions

- To obtain guarantee service, please follow the instructions below:
- The Walz Service Information Form available at https://www.walz.com/support/repair_service.html must be completed and returned to Heinz Walz GmbH, Germany.
- The product must be returned to Heinz Walz GmbH, Germany, within 30 days after Heinz Walz GmbH, Germany has received written notice of the defect. Postage, insurance, and/or shipping costs incurred in returning equipment for guarantee service are at customer expense. Duty and taxes are covered by Walz.

- All products being returned for guarantee service must be carefully packed and sent freight prepaid.
- Heinz Walz GmbH, Germany is not responsible or liable for missing components or damage to the unit caused by handling during shipping. All claims or damage should be directed to the shipping carrier.

7.4 Applicable law

- This Guarantee is governed by German law. Place of jurisdiction is Bamberg, Germany.

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