

USER MANUAL

AutoResp[™]

Version 2.3.0







CONTENTS

С	ОИЛ	ENTS 2
1		GETTING STARTED 3
2		PACKAGES (DAQ-PACs) 4
	2.1	DAQ-PAC-WF1 4
	2.2	DAQ-PAC-WF4 6
	2.3	DAQ-PAC-WF8 8
3		CHAMBERS10
	3.1	Static chambers10
	3.2	Swim tunnels11
4		INSTRUMENTS13
	4.1	Witrox 13
	4.2	DAQ-BT17
	4.3	Bluetooth power strip (NETIO)
	4.5	TEMP-425
5		SENSORS27
	5.1	Flow-through cell mini sensor
	5.2	Dipping probe mini sensor28
	5.3	Sensor spot mini sensor
	5.4	Temperature sensor Pt1000
6		SOFTWARE INSTALLATION31

6.1	AutoResp [™] installation31
6.2	WiBu software protection44
7.	USE AUTORESP [™] 45
8.	SWIM TUNNEL RESPIROMETRY52
9.	AMBIENT WATER QUALITY56
9.1	Oxygen saturation57
9.2	Temperature59
10.	BACKGROUND THEORY62
10.	1 Measuring metabolic rate62
10.	2 What is oxygen used for?62
10.	3 Intermittent respirometry63
10.	4 Dissolved oxygen67
11.	TROUBLESHOOTING68
11.	1 Change Y-scale units68
11.	2 Noise68
11.	3 Previous versions69
11.	4 2-point calibration70
11.	5 Run AutoResp™ always as admin71
12.	INDEX71
13.	APPENDIX72



1. GETTING STARTED

Please follow these few steps below to get started. For more details go to the page numbers listed under each step.

- 1. Uninstall any previous versions of AutoResp[™] software (page 69).
- 2. Install the new AutoResp[™] software on a PC with Windows 10, 8 or 7 (page 31).

Minimum PC requi	rements:
CPU	Duo Core 2,4 GHz or similar
RAM	4 GB
USB ports	2-5 (system dependent)
Monitor	1024 x 768

- 3. Connect the Bluetooth power strip (NETIO) to a grounded wall socket. Connect the USB Bluetooth dongle to the PC (page 22).
- 4. Set up your Witrox instrument(s) (page 13).
- 5. For swim tunnel respirometers set up the DAQ-BT instrument(s) (page 17)
- 6. Mount your chambers with a pump for flushing and a pump for recirculating the chamber using a minimum of tubing (page 10). Remember to thermostate your chamber(s) by placing it submersed in a surrounding (ambient) tank.
- 7. Connect all flush pumps to RE1 on the Bluetooth power strip (NETIO) using a power strip or adapter cable supplied with the system. Connect all recirculating pumps to RE2.
- 8. Insert the green hardkey protection (WiBu) dongle (page 44) containing license information.
- 9. Start the AutoResp[™] software (page 45).

Do not run any other programs at the same time, neither software delivered with $Loligo^{TM}$ instruments, nor software from other vendors. Thus, software delivered with fiber optic instruments and needed for stand alone use, should not be run in parallel with AutoRespTM.

- 10. Set up AutoResp[™] to the instruments you have by using the Auto Configure button and chose one of the system (page 4)
- 11. For reviewing respirometric data or graphs and for post analysis, load raw data files via the File menu in AutoResp[™] (page 50).
- 12. Calculated data is saved in an Excel compatible text file.



2.PACKAGES (DAQ-PACs)

2.1 DAQ-PAC-WF1

This package for measurements of oxygen consumption rates in a single respirometer chamber includes fiber optic oxygen sensing technology suited for any chamber volume, and equipment for monitoring water temperature. With optional equipment water temperature can also be controlled and/or water O_2 saturation can be monitored or controlled.

- Single chamber
- Any chamber volume
- Water temperature measurements (regulation optional)
- Optional measurements and regulation of ambient water oxygen saturation

NB! Please note that no fiber optic oxygen sensors are included in the package since these should match the application. More about fiber optic sensors can be found on page 13.





2.1.1. List of parts

- DAQ-M instrument
 - Power cord
 - \circ Converter piece (230 V/110 V)
 - Device connector (2x)
- AutoResp[™]
 - USB MEMORY STICK
 - $_{\circ}~$ Wibu dongle
- Witrox 1 oxygen instrument for mini sensors
 - AC/DC travel adapter (1.5 m)
 - $_{\odot}\,$ USB power cable for PC (1.5 m)
 - PT1000 sensor (4W,ClassA, 1.9x40mm, 5 m cable)
 - Plastic suitcase (345x285x122 mm)

2.1.2. Optional

For measuring effects of hypoxia/hyperoxia on respiration, we offer an oxygen instrument and an (DO-SET) accessory kit for injection of N_2 or O_2 gas.

For oxygen consumption measurements in two chambers, add a Witrox 1 +sensor, or consider a Witrox 4 +sensors for multiple chambers.

For regulating water temperature, all you need is a TMP-SET accessory kit (#AC10150/#AC10160).



2.2DAQ-PAC-WF4

This package for measurements of oxygen consumption rates in up to four respirometer chambers includes fiber optic oxygen sensing technology suited for any chamber volume, and equipment for monitoring water temperature. With optional equipment water temperature can also be controlled and/or water O_2 saturation can be monitored or controlled.

- 1-4 chambers
- Any chamber volume
- Water temperature measurements (regulation optional)
- Optional measurements and regulation of ambient water oxygen saturation

NB! Please note that no fiber optic oxygen sensors are included in the package since these should match the application. More about fiber optic sensors can be found on page 13.





2.2.1. List of parts

- DAQ-M instrument
 - Power cord
 - $_{\odot}$ Converter piece (230 V/110 V)
 - Device connector (2x)
- AutoResp[™]
 - USB MEMORY STICK
 - \circ Wibu dongle
- Witrox 4 oxygen instrument for mini sensors
 - AC/DC travel adapter (1.5 m)
 - \circ USB power cable for PC (1.5 m)
 - PT1000 sensor (4W,ClassA, 1.9x40mm, 5 m cable)
 - Plastic suitcase (345x285x122 mm)

2.2.2. Optional

For measuring effects of hypoxia/hyperoxia on respiration, we offer an oxygen instrument and an (DO-SET) accessory kit for injection of N_2 or O_2 gas.

For oxygen consumption measurements in 5-8 chambers, add a Witrox 4 oxygen instrument + sensors

For regulating water temperature, all you need is a TMP-SET accessory kit (#AC10150/#AC10160).



2.3DAQ-PAC-WF8

This package for measurements of oxygen consumption rates in up to eight respirometer chambers includes fiber optic oxygen sensing technology suited for any chamber volume, and equipment for monitoring and regulating water temperature. With optional equipment water temperature can also be controlled and/or water O_2 saturation can be monitored or controlled.

- 1-8 chambers
- Any chamber volume
- Water temperature measurements (regulation optional)
- Optional measurements and regulation of ambient water oxygen saturation

NB! Please note that no fiber optic oxygen sensors are included in the package since these should match the application. More about fiber optic sensors can be found on page 13.





2.3.1. List of parts

- DAQ-M instrument
 - Power cord
 - \circ Converter piece (230 V/110 V)
 - Device connector (2x)
- AutoResp[™]
 - USB MEMORY STICK
 - \circ Wibu dongle
- Witrox 4 oxygen instrument for mini sensors (2x)
 - AC/DC travel adapter (1.5 m)
 - USB power cable for PC (1.5 m)
 - PT1000 sensor (4W,ClassA, 1.9x40mm, 5 m cable)
 - Plastic suitcase (345x285x122 mm)

2.3.2. Optional

For measuring effects of hypoxia/hyperoxia on respiration, we offer an oxygen instrument and an (DO-SET) accessory kit for injection of N_2 or O_2 gas.

For regulating water temperature, all you need is a TMP-SET accessory kit (#AC10150/#AC10160).



3.CHAMBERS

3.1 Static chambers

When using AutoResp[™] with horizontal chambers for determining the metabolic rate of resting/inactive animals, the chamber and pumps must be assembled as indicated in the diagram below.



Water tank

Place the outlet hose just above the water surface for visual check of pump flow and to avoid siphon water entering the respirometer when the flush pump is turned off!

Avoid excess tubing to limit volume, gas diffusion and surfaces for bio film.

For more info on intermittent resp. principle see page 63.



3.2Swim tunnels



For SWIM-MINI tunnels assembled as indicated in the diagram below.

When using a DAQ-BT instead of a DAQ-M instrument connect the input and output connectors from the swim tunnel motor controller to the DAQ-BT input and output connectors. For the PULSE output please use the long data cable. For the analog input please use the short data cable.

Connect the flush pump to RE1 on the BlueTooth power strip (NETIO) instead of the DAQ-M.



For large swim tunnels assembled as indicated in the diagram below.



When using a DAQ-BT instead of a DAQ-M instrument connect the input and output connectors from the swim tunnel motor controller to the DAQ-BT input and output connectors.

Connect the flush pump to RE1 on the BlueTooth power strip (NETIO) instead of the DAQ-M.



4. INSTRUMENTS

4.1Witrox



The Witrox is either a 1 channel (Witrox 1) or 4 channel (Witrox 4) oxygen instrument for measuring dissolved oxygen using fiber optic mini sensors (optodes) and monitoring temperature.

NB! Do not use WitroxView software while running AutoResp[™].

SETUP

Connect the Witrox instrument to the power adapter by using the USB cable on the backside of the instrument.

Connect the power adapter to a wall outlet.

Push the Power button, the power LED will turn green. The LINK LED will flash green.

Press the Windows Start button and choose Devices. An overview of the connected devices is listed.

Control Panel + Hardware and Sound + Devices and Printers +	 ✓ ✓ Search Devices and Printers
Add a device Add a printer	E • 0
Devices	
Vertication Vertication Vertication Vertication	



Please click on the "Add a device" button.

Select a c	evice levice to add to this comp ill continue to look for new device	uter 25 and display t	hem here.		
J	CT-CLIENT13 Bluetooth Laptop computer Witrox Bluetooth Other	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Erik Jessen Bluetooth Phone		
What if Win	dows doesn't find my device?			Next	Cancel

Click on the Witrox instrument, then click Next.



Page 14 of 74



Please choose "Enter the device's pairing code", then click Next.



Enter a 0 (zero) in the pairing field, then click Next. The WITROX driver will now be installed.



The instrument is now ready to be used in AutoResp[™].



Right click on the device and choose Properties. Check the assigned COM port number. This COM port number must correspond to the COM port number set in AutoResp[™].

Utrox Properties	
Wtrox Device Functions: Name Type Standard Setial over Bluetooth 1k (COM18) Ports (COM	Oxygen (files) %air saturation
	Instruments Data aquisition instrument Device name DAQ-M ▼ %[dev1] ▼ Fiber optic instrument N° 1 COM port
Device Function Summary Manufacturer: Microsoft	Witrox 1 Image: COM18 -none- Image: Composition of the second sec
Location: on Bluetooth Device (RFCOMM Protocol TDI) #2 Device status: This device is working properly.	TEMP-4 instrument N* 1 Board number Temp-4 instrument N* 2 Board number NO NO Image: State Sta
OK Cancel Apply	

Connect a fiber optic sensor to the front input (marked *CH1-CH4*). Connect the Pt1000 temperature sensor to the front input marked TEMP.

For more information on fiber optic sensors, see page 13.



4.2DAQ-BT



The DAQ-BT instrument is used for wireless data acquisition and automated control of Loligo® swim tunnels in combination with AutoResp^M software. The wireless Bluetooth communication means that the PC can be placed at a distance from the swim tunnel without data cables that can pick up noise in a lab environment.

Apart from the inputs and outputs needed for controlling the swim tunnel motor (RPM) and acquiring data from it, the DAQ-BT instrument has extra channels for analog data acquisition and TLL control of other devices.

SETUP

Connect the DAQ-BT instrument to the power adapter by using the USB cable on the backside of the instrument.

Connect the power adapter to a wall outlet.

Push and hold the Power button for at least 5 seconds until the Power and Status LED flashes green. The pairing mode for the DAQ-BT is now enabled.

Press the Windows Start button and choose Devices. An overview of the connected devices is listed.



				×
Control Panel + Hardware and Sound + Devices and Printers +	▼ 49	Search Devices and Printer	s	٩
Add a device Add a printer			-	?
Devices				
Vertical point Vertical point Vertical point Vertical point Vertical point Vertical point Vertical				
-				

Please click on the "Add a device" button.

Select a d	evice to add to this com	puter	nem here.	
	BTH-1208LS-5FA4 Bluetooth Other GT-N8000 Bluetooth Phone		Bluetooth LE Device Bluetooth Other	
What if Wind	dows doesn't find my device?			

Click on the BTH-1208LS instrument, then click Next.



M Add a davias	22	
 Select a pairing option Create a pairing code for me The device has a keypad. 	0	
Enter the device's pairing code The device comes with a pairing code. Check for one on the device or in the device manual.	BTH-1208LS-5FA4	
Pair without using a code This type of device, such as a mouse, does not require a secure connection. How can I tell if my device has a pairing code?		
	Next Cancel	

Please choose "Enter the device's pairing code", then click Next.

		23
🕒 📝 Add a device		
Enter the pairing code for	or the device	
This will verify that you are con	necting to the correct device.	
0000		•
The code is either displayed on came with the device.	your device or in the information that	-
		BTH-1208LS-5FA4
What if I can't find the device p	airing code?	
		Next Cancel

Enter 0000 (four zeros) in the pairing field, then click Next. The DAQ-BT driver will now be installed.



			23
\bigcirc	💣 Add a device		
	Add a device Add a device This device has been successfully added to this computer Windows is now checking for drivers and will install them if necessary. You may need to wait for this to finish before your device is ready to use. To verify if this device finished installing properly, look for it in Devices and Printers.	BTH-1208LS-5FA4	
			_
		Clos	;e

Press the Windows Start button and open InstaCal from all programs/Measurement Computing. InstaCal will detect the DAQ-BT and configure it properly.

InstaCal	alibrate Test Help		23
	Plug and Play Board Detection		
PC Board	The following plug and play devices have been detected		
	Check those devices you wish to have added to the configuration file.		
l	OK		
Ready			

The instrument is now ready to be used in AutoRespTM. The assigned board number must correspond to the board number set in AutoRespTM.



InstaCal				
File Install Calibrate Test Help		-1	Instruments	
	📥 🕥 📂		Data aquisition instrument	Device name
PC Board List			DAQ-IM	% devi
Bluetooth Board# 0 - 3TH-1208LS (serial#1B85FA4)			Fiber optic instrument N° 1 Witrox 1	COM port
			Fiber optic instrument N° 3 -none-	COM port
			TEMP-4 instrument N° 1 NO	Board number
Ready			Swim tunnel N° 1 instrumer DAQ-BT	Board number Board 0

To turn the instrument OFF push and hold the power buttons until both LEDs are turned OFF.

To turn the instrument ON without enabling the pairing mode push and hold the power button for approximately 3 seconds until the power LED turns ON.

When the instrument is communicating with the software the status LED will start flashing.

To measure a Loligo swim tunnel connect a data cable from a swim tunnel controllers output labeled OUT to the DAQ-BT instruments input labeled IN.

To control a Loligo swim tunnel connect a data cable from the DAQ-BT instruments output labeled OUT to a swim tunnel controllers input labeled IN.





4.3Bluetooth power strip (NETIO)



The Bluetooth power strip (NETIO) is essentially a 4-fold power strip for wireless software control of equipment connected to one of the four independent electrical sockets, *i.e.*, turning pumps, valves, stirrers etc. on and off from a distance.

SETUP

Connect the Bluetooth power strip to a wall outlet. Turn ON the power strip with the power button. When the power strip is ready for use the Bluetooth LED will light blue.

Connect the USB Bluetooth dongle to the PC. After the dongle has been found a COM port will be assigned. Please open the device manager and check the COM port. This COM port number must correspond to the COM port number set in AutoRespTM.



🚔 Device Manager
File Action View Help
ERIK-PC
⊳ - 🚯 Bluetooth Radios
⊳ -1. Computer
Disk drives
Display adapters
DVD/CD-ROM drives
Floppy drive controllers
👌 🥼 Human Interface Devices
De ATA/ATAPI controllers
> Keyboards
Mice and other pointing devices
Monitors
Network adapters
Ports (COM & LPT)
Bluegiga Bluetooth Low En Irgy (COM2)
Standard Serial over Rhystooth link (COM7)
Standard Serial over Bluetooth link (COMR)
>
Sound, video and game controllers
System devices
🔊 🌐 Universal Serial Bus controllers

ygen (files)			
ir saturation 🛛 🔻			
Data aquisition instrument NETIO	COM port		
Fiber optic instrument N° 1	COM port	Fiber optic instrument N° 2	COM port
Witrox 1 💌	1 ₀	-none-	I/0
Fiber optic instrument N° 3	COM port	Fiber optic instrument N° 4	COM port
-none-	۲ <u>/</u>	-none-	I%
TEMP-4 instrument N° 1	Board number	Temp-4 instrument N° 2	Board number
NO	_	NO	
Swim tunnel A/D N° 1	Board number	Swim tunnel A/D N° 2	Board number
DAO-BT	-	DAO-BT	

The Bluetooth power strip is now ready for use in AutoResp $^{\text{TM}}$.



4.4DAQ-M



The DAQ-M instrument for USB is used with AutoResp[™] software for automated oxygen consumption measurements in up to eight static chambers or two swim tunnels, and for monitoring and regulating ambient water temperature and oxygen saturation.

SETUP

To power up the instrument connect the power cord to a <u>grounded</u> wall outlet and the socket labeled 100-240 VAC 50-60Hz on the back side of the instrument. Remember to switch the instrument ON/OFF using the main power switch. Connect the USB cable to the front input (marked *PC*) and to a free USB port on your PC.

First time the DAQ-M instrument is connected to the PC, the drivers will be installed. This might take some time.

All analog outputs from oxygen and temperature instruments, and swim tunnel motors, must be connected to input channels 1-8 on the DAQ-M instrument using either single or split (double) data cables supplied with the devices.

The digital relays are used to control pumps for flushing/recirculating and pumps or valves for ambient water quality control. See page 56.

SPECIFICATIONS

Supply voltage:	100-240 VAC, 50-60 Hz
PC interface:	USB 2.0 (1.1 compatible)
Input channels:	8
Input voltage:	0-10 V
Output channels:	2
Ouput voltage:	0-10 V
Resolution:	14 bit (single-ended)
Signal noise:	62 dB
Frequency:	1000 Hz
Relays:	4
Relay voltage:	100-240VAC, 50-60 Hz
Relay max. power:	2 A per channel (max 6.3 A for all relays)



4.5 TEMP-4



The TEMP-4 is a four channel instrument for monitoring, recording and controlling water temperature with AutoResp[™] or TempCTRL software.

SETUP

To power up the instrument connect the power cord to a <u>grounded</u> wall outlet and the socket labeled 100-240 VAC 50-60Hz on the back side of the instrument. Connect Pt100 temperature probe(s) to the front inputs (marked $IN \ 1 - IN \ 4$). Then connect the USB cable to the front input (marked PC) and to a free USB port on your PC.

SPECIFICATIONS

Supply voltage:	100-240 VAC, 50-60 Hz
PC interface:	USB 2.0 (1.1 compatible)
Inputs:	4
Resolution:	24 bit
Signal noise:	60 dB
Frequency:	16 Hz
Relays	4
Relay voltage: :	100-240VAC, 50-60 Hz
Max. relay current:	2 A per channel, 6,3 A all channels



First time the TEMP-4 instrument is connected to the PC, the drivers will be installed. This might take some seconds. Then open InstaCal (this software is installed together with AutoResp[™]) and check the assigned board number. This board number must correspond to the board number set in AutoResp[™].

InstaCal	Instruments connected to th	e PC
<u>File</u> Install <u>C</u> alibrate <u>T</u> est <u>H</u> elp		
	Data aquisition instrument	Device name
RC Board List	DAQ-M	O Devi
Universal Serial Bus		
	Fiber optic instrument N° 1	COM port
	-none-	×
	Temperature instrument N°	Board number
	TEMP 4	
		Board I
Ready		



5.SENSORS

5.1Flow-through cell mini sensor



The flow-through oxygen mini sensor is for use with 10 - 13 mm tubing, and requires a fiber cable with bare tip for use with Witrox 1 or Witrox 4 instruments. The re-usable (non-disposable) oxygen sensor has excellent long-term stability. Clean it with ethanol or even ETO, recalibrate, and use it again and again.

Features:

Easy to handle Very robust sensor Excellent long-term stability No self-consumption of oxygen Signal independent on flow velocity Insensible towards electrical interferences Measures oxygen in liquids as well as in gas phase



5.2Dipping probe mini sensor



The dipping probe oxygen mini sensor, consists of a polymer optical fiber (POF) with a polished distal tip which is coated with a planar oxygen-sensitive foil. The end of the polymer optical fiber is covered with a high-grade steel tube, to protect both the sensor material and the POF. The cable has an outer diameter of 2.8 mm. The inner diameter of the POF is 2.0 mm. The steel tube has an outer diameter of 4 mm. Usually, the fiber is coated with an optical isolated sensor material in order to exclude ambient light from the fiber tip and to increase chemical resistance especially against oily samples as well as to reduce bio-fouling on the sensor membrane. This type of oxygen sensor has an excellent long-term stability.

Features:

Easy to handle Usable for process application Limit of detection: 0.15 % air-saturation, 15 ppb dissolved oxygen Measuring range: 0 - 100 % oxygen, 0 - 45 mg/L Very robust sensor with excellent long-term stability Sterilizable by H2O2, ethanol, gamma irradiation, EtO2 No self-consumption of oxygen Signal independent on flow velocity Insensible towards electrical interference and magnetic fields Dry gases applicable Robust sensor body



5.3Sensor spot mini sensor



Sensor spots are thin planar oxygen mini sensors immobilized onto either polyester or glass supports. The latter is autoclavable. The sensor spots are glued inside chambers, flasks or disposables with translucent and non-fluorescent walls (e.g. glass, polyester, acrylic etc.). Then oxygen measurements can be done in a non-invasive and non-destructive way from outside and through the wall of your vessel.

Features:

Excelent long-term stability Easy to handle and robust Non-invasive and non-destructive measurement from outside through the wall of the flask Usable for process application No self-consumption of oxygen Signal independent on flow velocity Insensible towards electrical interferences and magnetic fields Measures oxygen in liquids as well as in gas phase Excellent mechanical stability and long-term stability Online monitoring



5.4Temperature sensor Pt1000



The Pt1000 temperature probe is robustly constructed with a stainless steel protection sheath, moulded mini handle with built in stress relief and complete with 5 metre of cable and connector for our Witrox instruments.

The Pt1000 sensor has a tolerance of 1/3 of Class B sensor giving a very high accuracy of +/- 0.15° C.



6.SOFTWARE INSTALLATION

6.1 AutoResp[™] installation

The following steps will explain how to install AutoResp[™] and instrument drivers on your PC.

Minimum PC requirements:CPUDuo core 2,4 GHz or similarRAM4 GBUSB ports2-5 (system dependent)Monitor1024 x 768

It is important to remove any previous versions of AutoResp[™] before starting to install new AutoResp[™] software:

- 1. Click Start→Control Panel
- 2. Open Programs and Features
- 3. Double click on National Instruments software
- 4. Select all packages, and then click on Remove.



- 5. You will then be notified that AutoResp[™] also will be removed. Click Yes
- 6. Now wait until all packages are uninstalled. This might take some time.
- 7. Windows will now ask for a restart.
- 8. When the computer is restarted, please proceed and install the new AutoResp[™] software.



Insert the USB memory stick labelled Loligo and wait until you see the following screen.

AutoPlay	
Removable Disk (E:)	
General options	
Open folder to view files using Windows Explorer	
View more AutoPlay options in Control Panel	

Click Open folder to view files and double click on the icon labelled AutoResp_Installer.exe.

to Unzipping AutoResp [™] by Loligo Systems	Setup
	Cance
Unzipping VC2005MSMs_x64.msi	About

After the file has been unzipped, the Welcome screen will appear.





oResp					
Desl Se	ination Directory lect the primary installation	directory.			
All so differ	ftware will be installed in th ent location(s), click the Bro	e following location(owse button and sel	s). To install softwa set another directo	re into a ry.	
Dir C:\	ectory for AutoResp Program Files\AutoResp\			Brows	e
Din	ectory for National Instrume	nts products			
					<u></u>

Select destination directory for AutoResp[™] and for the drivers and click Next.

License Agreement You must accept the license(s) displa	yed below to proceed.
NATIONAL INSTRUMEN	TS SOFTWARE LICENSE AGREEMENT
INSTALLATION NOTICE: THIS IS A CONTR AND/OR COMPLETE THE INSTALLATION I DOWNLOADING THE SOFTWARE AND/OF COMPLETE THE INSTALLATION PROCES AGREEMENT AND YOU AGREE TO BE BO BECOME A PARTY TO THIS AGREEMENT. CONDITIONS, CLICK THE APPROPRIATE DO NOT INSTALL OR USE THE SOFTWAR (30) DAYS OF RECEIPT OF THE SOFTWAR ALONG WITH THEIR CONTAINERS) TO TH SHALL BE SUBJECT TO NI'S THEN CURR	ACT. BEFORE YOU DOWNLOAD THE SOFTWARE PROCESS, CAREFULLY READ THIS AGREEMENT. BY R CLICKING THE APPLICABLE BUTTON TO S, YOU CONSENT TO THE TERMS OF THIS UND BY THIS AGREEMENT. IF YOU DO NOT WISH TO AND BE BOUND BY ALL OF ITS TERMS AND BUTTON TO CANCEL THE INSTALLATION PROCESS, RE, AND RETURN THE SOFTWARE WITHIN THIRTY RE (WITH ALL ACCOMPANYING WRITTEN MATERIALS, HE PLACE YOU OBTAINED THEM. ALL RETURNS RENT RETURN POLICY.
	 I accept the License Agreement I do not accept the License Agreement.
	<< Back Next >> Cancel

If you accept the License Agreement, please select "I accept the License Agreement. Then click Next.



AutoResp	
License Agreement You must accept the license(s) dis	splayed below to proceed.
.NET 2.0 IVI	
MICROSOFT SOFTWAR	E SUPPLEMENTAL LICENSE TERMS T .NET FRAMEWORK 2.0
Microsoft Corporation (or based on whe to you. If you are licensed to use Micros you may use this supplement. You ma You may use a copy of this supplement	ere you live, one of its affiliates) licenses this supplement soft Windows operating system software (the "software"), y not use it if you do not have a license for the software. t with each validly licensed copy of the software.
The following license terms describe a and the license terms for the software a these supplemental license terms app	dditional use terms for this supplement. These terms apply to your use of this supplement. If there is a conflict, ly.
By using this supplement, you accept the supplement. If you comply with these li	hese terms. If you do not accept them, do not use this icense terms, you have the rights below.
	 I accept the above 2 License Agreement(s). I do not accept all these License Agreement(s).
	<< Back Next >> Cancel

If you accept the License Agreement, please select "I accept the License Agreement(s). Then click Next.

AutoResp	
Start Installation Review the following summary before continuing.	
Adding or Changing • NI System Configuration 1.1.1 • AutoResp Files • NI-DAQmx 9.2.1 • NI MAX Configuration Support • NI-VISA 5.0 Bun Time Support • LabVIEW C Interface • NI Measurement & Automation Explorer 4.7.1	
Click the Next button to begin installation. Click the Back button to change the installation settings.	
Save File) << <u>B</u> ack Next >>	<u>C</u> ancel

A summary is given of the products to be installed. Click Next.



🐙 AutoResp	
Oursell Dramon 1% Conselate	
Publishing product information	
_	
	<< Back Next >> Cancel

The installation may take a while.

4 AutoResp	
Installation Complete	
The installer has finished updating your system.	
	<< Back Next >> Einish

When installation is complete click Next.



Loligo SP2 Readme Please read the following Inform	ation. Welcome to the Loligo Service Pack 2 installer. The following products will now be installed: • WiBuKey • MCC Dag	
	Print < Back Install	<u>C</u> ancel

Now to the installation of drivers for the WiBu hardkey protection dongle. Click Install.



Click Next.


🛃 WibuKey Setup	
	Please select the languages that WibuKey should support: English Chinese [Simplified] French German Italian Hungarian Japanese Spanish
	< <u>B</u> ack <u>N</u> ext> Cancel

Click Next.



Click Next.





Click Next.

The following components will be installed: WibuKey driver files WibuKey COM control WIBU-SYSTEMS Shell Extension WibuKey network server WibuKey tools Copying files done. Verifying language modules done. Installing files done. Verifying installed language modules done. Storing uninstall information done. Creating shortcuts done. Transfering settings done.	E

Click Next.





Click Finish.



Click OK.

VinZip Self-Extract	or	
Press OK to conti	nue with in	stallation.

Click OK.



202	17	
Installing MCC Daq Software.	<u>S</u> etup Cancel	
	About	

Click Setup to start installing third party (Measurement Computing) device drivers.

MG MCC DAQ	SUREMENT
Select the packages you want to install or modify InstaCal & Universal Library InstaCal & Universal Library IncerDAD ULx for LabVIEW Legacy) Hardware Manuals Required dependencies: IncertX 9.0c	 on your computer. Description Data logger, oscilloscope, and strip chart recorder application. Requirements: Windows 2000(SP4)/XP(SP2) //ista/7, InstaCal for Windows, Microsoft .NET framework version 2.0, DirectX 9.0c
Install	View ReadMe Cancel

Deselect TracerDAQ, then click Install.



B InstaCal and Universal Libra	ary for Windows - InstallShield Wizard
	Welcome to the InstallShield Wizard for InstaCal and Universal Library for Windows
	The InstallShield(R) Wizard will allow you to modify, repair, or remove InstaCal and Universal Library for Windows. To continue, dick Next.
	< Back Next > Cancel

Click Next.

Program Main Modify, repair, o	r remove the program.
<u>Modify</u>	Change which program features are installed. This option displays the Custom Selection dialog in which you can change the way features are installed.
© Repair	Repair installation errors in the program. This option fixes missing or corrupt files, shortcuts, and registry entries.
© <u>R</u> emove	Remove InstaCal and Universal Library for Windows from your computer.

Click Next.



ed.
eature Description Iniversal Library Examples his feature requires 0KB on our hard drive. It has 0 of 5 ubfeatures selected. The ubfeatures require 0KB on your ard drive.

Click Next.

Ready to Modify the Program	
The wizard is ready to begin installation	on.
Click Install to begin the installation	n.
If you want to review or change a exit the wizard.	any of your installation settings, dick Back. Click Cancel to

Click Install.



InstallShield Wizard Completed
The InstallShield Wizard has successfully installed InstaCal and Universal Library for Windows. Click Finish to exit the wizard.

Click Finish.

Loligo SP2	×
Finished Loligo SP2 installation succes	sful.
	To finalize the installation your computer needs to be restarted. Do you want to restart your computer now? • Yes, I want to restart my computer now • No, I will restart it later Click Finish to end the installation.
	K Back Finish Cancel

Choose "Yes, I want to restart my computer now", then click Finish. After the PC has restarted you have installed all AutoResp[™] software and device drivers.



6.2 WiBu software protection

AutoResp[™] is protected with an USB hardkey dongle (WiBu), and will only run if a valid dongle is connected to an USB port on the computer. If not, the error message below will appear.



Plug in the WiBu hardkey dongle and wait to let it be recognized by Windows. Only then can AutoRespTM be used.



7. USE AUTORESP[™]

NB! To enjoy all functions in AutoResp[™] it is necessary that the PC user has administrator status. (page 71)

Start AutoResp[™] from the start menu in Windows. It might take a few seconds to load the program initially. Watch the Windows task bar.

AutoRespTM will start in the screen mode shown below. Here you can connect the different instruments that you have, and configure the input channels on your DAQ instrument. Here you also choose units of measure.

If one of our a DAQ-PACs or systems are used, please use the Auto configure function for a quick setup.

Nuto configure MANUAL SETUP	MO2 mgO2/kg/hr 🗨	Oxygen (files) %air saturation			
Configure		Instruments			
Chamber 1	Chamber 5	Data aquisition instrument	Device name ^I ‰dev1 💌		
Chamber 3 Chamber 4	Chamber 7 Chamber 8	Fiber optic instrument N° 1 -none-	COM port	Fiber optic instrument N° 2 -none-	COM port
Ambient oxygen N° 1	Ambient oxygen N° 2	Fiber optic instrument N° 3 -none-	COM port	Fiber optic instrument N° 4 -none-	COM port
Ambient temperature N° 1	Ambient temperature N° 2 Swim tunnel N° 2	TEMP-4 instrument N° 1 NO	Board number	Temp-4 instrument N° 2 NO	Board number
		,			

NB! In AutoResp[™] users can freely choose between the oxygen units % air saturation, kPa (partial pressure of oxygen) and mgO2/L (oxygen concentration), but this requires a user input for barometric pressure and salinity. Temperature is also required, but can be acquired automatically if connecting a temperature instrument to the system.

- 1. The digital output from the oxygen instrument must be calibrated in the AutoResp[™] software to show correct oxygen values.
- 2. Measure at 0% air saturation, and press LOCK LO. Since fiber optic oxygen sensors are sensitive to temperature, it is also necessary to enter this value.
- 3. Measure at 100% air saturation, and press LOCK HI. Since fiber optic oxygen sensors are sensitive to temperature, it is also necessary to enter this value.
- 4. Choose Online (Ambient) as temperature input channel. It is very important to temperature compensate the fiber optic oxygen values, since the oxygen values are very temperature dependent.
- 5. Repeat this step for every oxygen channel.
- 6. The calibration and configuration is continuously saved to a binary file (AutoResp.conf).
- 7. Now choose Experiment \rightarrow Start to start a respirometry experiment.



Ketup experiment				×
Chamber parameters				
Chamber volume Chamber 1 🕴 1 📃 L 💌	Tube volume Wet weight ∯0 mL ▼ ∯100 g ▼	Density [kg/L]	Resp. volume [L] 0,9	Ratio Notes
Chamber volume	Tube volume Wet weight ↓0 mL ▼ ↓200 g ▼	Density [kg/L]	Resp. volume [L]	Ratio Notes
Chamber volume Chamber 3 🚽 3 🛛 L 🗨	Tube volume Wet weight ↓0 mL ▼ ↓300 g ▼	Density [kg/L]	Resp. volume [L]	Ratio Notes
Chamber volume Chamber 4 📲 4 📃 💌	Tube volume Wet weight ∯0 mL ▼ ∯400 g ▼	Density [kg/L]	Resp. volume [L] 3,6	Ratio Notes
Chamber volume	Tube volume Wet weight ★0 mL ▼ ★100 g ▼	Density [kg/L]	Resp. volume [L] 0,9	Ratio Notes
Chamber volume	Tube volume Wet weight ↓0 mL ▼ ↓100 g ▼	Density [kg/L]	Resp. volume [L]	Ratio Notes
Chamber volume	Tube volume Wet weight	Density [kg/L]	Resp. volume [L] 0,9	Ratio Notes
Chamber volume	Tube volume Wet weight	Density [kg/L]	Resp. volume [L] 0,9	Ratio Notes
Solid blocking correction				
Swim tunnel 1	Cross section area [cm2] Fish length (cm)	Fish width (cm)	Fish depth (cm)	Fractional error [%] NaN
Swim tunnel 2	Cross section area [cm2] Fish length (cm)	Fish width (cm) ≢0	Fish depth (cm)	Fractional error [%] NaN OK Cancel

- 8. Before the experiment starts a number of parameters must be given in order for AutoResp[™] to calculate oxygen consumption rate etc.
- 9. First enter the chamber volume and choose the correct unit.
- 10. Enter the volume of tubes in the recirculating loop only and choose the correct unit of measure. In swim tunnels this tube volume should be set to zero (0).
- 11. Enter the wet weight of the animal(s) and choose the unit of measure.
- 12. Then enter the density of the animal(s) for the software to calculate the respirometric volume.
- 13. The respirometric volume is calculated as:

Resp. voume [*L*] = *chamber volume* + *tube volume* - *volume of organism(s)*

14. The ratio field indicates the ratio between the Resp. volume and the wet weight volume. As rules of thumb, this ratio should be 10-20 for measurements in resting animals, 10-50 for micro respirometry (<<50 mL) and up to 200 for measurements in active animals (swim tunnel respirometry) for reliable MO2 measurements. If experimental temperatures are high the ratio can be higher without compromising the quality of MO" data.

NB! The solid blocking correction fields are only available when running with a swim tunnel, see SWIM TUNNEL RESPIROMETRY, page 52.



- 15. Now enter correct values for chamber 2.
- 16. When done, press OK.
- 17. Now a file dialog pops up, where the user is asked a file path for the (calculated) data file and raw data file. The raw data file will get the same name as the data file but with "_raw" as an extension to the file name.

AutoResp™ Flu	shed for 13	8 seconds							
File Experiment	Settings	Help							
Flush 😐	Close 🔿	Intermitten	nt O Skip pha	Add fi	le note			🔾 RE 1 🔘	RE 2 🔘 RE 3 🔘 RE 4
110-									
100 -									
90 -									
등 80-									
1 Te 70-									
- 00 -									
<u>%</u> 50 -									
40 -									
õ 30-									
20 -									
10-									
0-									
14100114									14:07:14
14:00:14					Time				14:07:14
					Time				14:07:14
Data MO2 R	t^2 MO2	2 histogram MO	12 vs. O2 MO2 vs. tem	np Ambient oxygen	Time				14:07:14
Data MO2 R Chamber 1	t^2 MO2	2 histogram MO Chamber 2	02 vs. 02 MO2 vs. tem	p Ambient oxygen	Time Ambient temp Chamber 5	Chamber 6	Chamber 7	Chamber 8	14:07:14
Data MO2 R Chamber 1 Oxygen	t^2 MO2	2 histogram MO Chamber 2 Oxygen	2 vs. 02 MO2 vs. tem Chamber 3 Oxygen	p Ambient oxygen Chamber 4 Oxygen	Time Ambient temp Chamber 5 Oxygen	Chamber 6 Oxygen	Chamber 7 Oxygen	Chamber 8 Oxygen	14:07:14
Data MO2 R Chamber 1 Oxygen 98,45	1^2 MO2	2 histogram MO Chamber 2 Oxygen 95,14	02 vs. O2 MO2 vs. tem Chamber 3 Oxygen 89,14	p Ambient oxygen Chamber 4 Oxygen 85,76	Time Ambient temp Chamber 5 Oxygen NaN	Chamber 6 Oxygen NaN	Chamber 7 Oxygen NaN	Chamber 8 Oxygen NaN	14:07:14 Ambient #1 Oxygen [99,92]
Data MO2 R Chamber 1 Oxygen 98,45 MO2	R^2 MO2	2 histogram MO Chamber 2 Oxygen 95,14 MO2	02 vs. O2 MO2 vs. tem Chamber 3 Oxygen 89,14 MO2	p Ambient oxygen Chamber 4 Oxygen 85,76 MO2	Time Ambient temp Chamber 5 Oxygen NaN MO2	Chamber 6 Oxygen NaN MO2	Chamber 7 Oxygen NaN MO2	Chamber 8 Oxygen NaN MO2	Ambient #1 Oxygen 99.92 Temperature
Data MO2 R Chamber 1 Oxygen 98,45 MO2 0	1^2 MO2	2 histogram MO Chamber 2 Oxygen 195,14 MO2 0	02 vs. 02 MO2 vs. tem Chamber 3 Oxygen 189,14 MO2 0	p Ambient oxygen Chamber 4 Oxygen 85,76 MO2 0	Time Ambient temp Oxygen NaN MO2 0	Chamber 6 Oxygen NaN MO2 0	Chamber 7 Oxygen NaN MO2 0	Chamber 8 Oxygen NaN MO2 0	Ambient #1 Oxygen 99.92 Temperature 20.05
Data MO2 R Chamber 1 Oxygen 98,45 MO2 0 Slope	1^2 MO2	2 histogram MO Chamber 2 Oxygen 95,14 MO2 0 Slope	2 vs. O2 MO2 vs. tem Chamber 3 Oxygen 89,14 MO2 0 Slope	p Ambient oxygen Chamber 4 Oxygen 85,76 MO2 0 Slope	Time Ambient temp Chamber 5 Oxygen NaN MO2 D Slope	Chamber 6 Oxygen NaN MO2 0 Slope	Chamber 7 Oxygen NaN MO2 0 Slope	Chamber 8 Oxygen NaN MO2 0 Slope	Ambient #1 Oxygen 99.92 Temperature 20.05
Data MO2 R Chamber 1 Oxygen 98,45 MO2 0 Slope 0	K^2 MO2	2 histogram MO Chamber 2 Oxygen 95,14 MO2 0 Slope 0	02 vs. 02 MO2 vs. tem Chamber 3 Oxygen 189,14 MO2 0 Slope 0 0	p Ambient oxygen Chamber 4 Oxygen 85,76 MO2 0 Slope 0	Time Ambient temp Chamber 5 Oxygen NaN MO2 0 Slope 0	Chamber 6 Oxygen NaN MO2 0 Slope 0	Chamber 7 Oxygen NaN MO2 0 Slope 0	Chamber 8 Oxygen NaN MO2 0 Stope 0	14:07:14 Ambient #1 Oxygen 199.92 Temperature 20.05
Data MO2 R Chamber 1 Oxygen 98,45 MO2 0 Slope 0 R^2	K^2 MO2	2 histogram MO Chamber 2 Oxygen 95,14 MO2 0 Slope 0 R^2	2 vs. 02 MO2 vs. tem Chamber 3 Oxygen [89,14 MO2 [0 Slope [0 R^22	p Ambient oxygen Chamber 4 Oxygen 85,76 MO2 0 Slope 0 R^2	Time Ambient temp Chamber 5 Oxygen NaN MO2 0 Slope 0 R^2	Chamber 6 Oxygen NaN MO2 0 Slope 0 R^2	Chamber 7 Oxygen NaN MO2 0 Slope 0 R^2	Chamber 8 Oxygen NaN MO2 0 Slope 0 R^2	14:07:14 Ambient #1 Oxygen [99,92 Temperature [20,05
Data MO2 R Chamber 1 Oxygen 98,45 MO2 0 Slope 0 R^2 0 0	(^2 MO2	2 histogram MO Chamber 2 Oxygen 95,14 MO2 0 Slope 0 R^2 0	2 vs. 02 MO2 vs. tem Chamber 3 Oxygen [89,14 MO2 [0 Slope [0 R^2 [0]	p Ambient oxygen Chamber 4 Oxygen 85,76 MO2 0 Slope 0 R^2 0	Time Ambient temp Chamber 5 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 6 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 7 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 8 Oxygen NaN MO2 0 Slope 0 R^2 0	14:07:14 Ambient #1 Oxygen 199,92 Temperature 20,05 Ambient #2
Data MO2 R Chamber 1 Oxygen 98,45 MO2 0 0 Slope 0 0 R^2 0 0 Uspeed 0 0	(^2 MO2	2 histogram MO Chamber 2 Oxygen 95,14 MO2 0 Slope 0 R^2 0	22 vs. O2 MO2 vs. tem Chamber 3 Oxygen 189,14 MO2 0 Slope 0 R^2 0 0 0	p Ambient oxygen Chamber 4 Oxygen 85,76 MO2 0 Slope 0 R^2 0	Time Ambient temp Chamber 5 Oxygen NaN MO2 0 Slope 0 R^2 0 Uspeed Uspeed	Chamber 6 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 7 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 8 Oxygen NaN MO2 0 Slope 0 R^2 0	14:07:14 Ambient #1 Oxygen 199,92 Temperature 20,05
Data MO2 R Chamber 1 Oxygen 98,45 MO2 0 Slope 0 Slope 0 R^2 0 Uspeed NaN	1/2 MO2	2 histogram MO Chamber 2 Oxygen 95,14 MO2 0 Slope 0 R^2 0	2 vs. 02 MO2 vs. tem Chamber 3 Oxygen 89,14 MO2 0 Slope 0 R^2 0 0	p Ambient oxygen Chamber 4 Oxygen B5,76 MO2 0 Slope 0 R^2 0	Time Ambient temp Chamber 5 Oxygen NaN MO2 0 Slope 0 R^2 0 Uspeed NaN Uspeed NaN	Chamber 6 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 7 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 8 Oxygen NaN MO2 0 Slope 0 R^2 0	14:07:14 Ambient #1 Oxygen 199,92 Temperature 20,05 Ambient #2 Oxygen NaN
Data MO2 R Chamber 1 Oxygen 98,45 MO2 0 Slope 0 R^2 0 Uspeed NaN Usvim	1/2 MO2	2 histogram MO Chamber 2 Oxygen 95,14 MO2 0 Slope 0 R^2 0	2 vs. 02 MO2 vs. tem Chamber 3 Oxygen 189,14 MO2 0 Slope 0 R^2 0 0	p Ambient oxygen Chamber 4 Oxygen [85,76] MO2 0 Slope 0 R^2 0	Time Ambient temp Chamber 5 Oxygen NaN MO2 0 Slope 0 R^2 0 Uspeed NaN Uswim	Chamber 6 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 7 Oxygen NaN MO2 0 Slope 0 R^2 0	Chamber 8 Oxygen NaN MO2 0 Slope 0 R^2 0	Ambient #1 Oxygen 199,92 Temperature 20,05 Ambient #2 Oxygen NaN Temperature

- 18. Now an experiment is started, but in a continous flush mode so that no oxygen consumption measurements will take place until the user sets the operation to either "Close" (closed respirometry) or Intermittent (intermittent respirometry).
- 19. Move the mouse over the relay indicator diodes in the upper right corner. A Help text will pop up and help the user which relay is used as recirc, flush, ambient control etc.
- 20. The upper screen shows the oxygen values for the chambers. Right clicking on the graph will show a submenu. It is here possible to hide/show chamber graphs, pause graphs, scale axes etc.

NB! Please note, that AutoResp[™] will continue collecting data even though graphs are set on pause (Show cursor). While set on pause the x-scrollbar appears and it is possible to go back to the start time of the experiment using the scrollbar.



NB! If using the cursor and having troubles that others graphs values are shown, use the sticky function. The sticky function will attach a cursor to the curve from one chamber specified by the user.

- 21. The lower graph panel shows the data and graphs for calculated values.
- 22. The Data tab will show all current values. Some fields are gray, depending on the configuration mode. So in the current example case, chamber 1 and 2 are shown and the ambient temperature only.
- 23. Because the ambient temperature was activated, an ambient Temp tab is also accessable, showing ambient water temperature in real time or average values over time (right click). Depending on the configuration, different tabs may appear, such as ambient oxygen, Uspeed (swimming speed) etc.
- 24. In the upper right corner, the kind of experiment currently running can be chosen.

Flush – This means that the flush is always on. The object will get fresh water constantly, but this also means that no MO2 values are calculated.

Close – This means the chamber is closed, and every time a measure period is finished a MO2 is calculated. After the measure period is finished a new measure period will begin immediately. If choosing this mode of operation, take care not to leave the set up to avoid severe oxygen depletion inside the chamber due to animal respiration.

NB! The object will remove all oxygen from the chamber after some time, and then the object will **DIE**! Use with care.

Intermittent – This means the experiment runs in a loop (flush, wait and measure) as explained in the back ground theory chapter (page 62). During each flush period, the flush pump is turned on to flush chambers with ambient water. The wait periods allow time for steady state before measurements start during the measure period.

In Intermittent mode, the skip phase button allow users to jump between these three modes (flush, wait measure) immediately and on-the-fly for special applications or situations, e.g. to start flushing the chamber immediately after reaching Ucrit during a swim trial.

LOOP

 $F1 \rightarrow W1 \rightarrow M1 \rightarrow F2 \rightarrow W2 \rightarrow M2$ etc.

For more information about the Intermittent principle, see page 63.

25. Now choose Settings \rightarrow General to set the settings for the experiment.



General settings		×
Oxygen		
Unit (graphs) <u>%air saturation</u>	Salinity [‰]	BP [hPa] ♥ 1013,0
Intermittent respirometry		
Flush [s]		
Wait [s]		
♥ 30		
Measure [s]		
Recirc pump always ON		
MO2 analysis		
SMR estimation	Min O2 [%a.s.]	pCrit estimation [N]
		A TO
	ОК	Cancel

26. In the General settings it is possible to change oxygen units, water salinity or barometric pressure during experiments. It is also possible to change flush, wait and measure times.

Flush times should be set to allow complete renewal of water inside each chamber. This will restore oxygen values to ambient between each measurement. As a rule of thumb, cylindrical chambers should be flushed with a volume five times the chamber volume for 99% wash-out, but this depends on chamber dimensions.

The measurement times should allow enough data for a reliable determination of the slope of the oxygen curve to be estimated. The slope is determined from a linear regression, and the regression coefficient r^2 express the statistical validity of the calculated slope, e.g. r^2 >0.95 indicates a sound linear relationship (oxygen vs. time), and thus a reliable MO2 value.

Notice that the recirculating pump can be set to constant activity. This will double the flow during flush periods potentially affecting animal behavior/locomotion, but will provide better oxygen readings if the oxygen probe is placed outside the chamber in a recirculating loop.

- 27. The MO2 analysis buttons are gray during measurements, but can be used during post analysis when loading saved raw data files form past experiments.
- 28. Press OK to close the general settings.
- 29. Now run an experiment for min/hours/days depending on application. Adjust your settings during measurements if needed. Any changes will be written in the raw and data file.



AutoResp [™]	
File Experiment Settings Help	
Flush O Close O Intermittent O Skip phase Add file note	🔵 RE 1 🜑 RE 2 🜑 RE 3 🔘 RE 4
95-	09:11:03 85,41
92,5-	
⁸ 90- 5 × × × × × × × × × × × × × × × × × × ×	
	A A A À À A À A À
5- 6	
82,5-	
80-1 04:37:42	08:37:42
Time	
MO2 R^2 MO2 histogram MO2 vs. 02 MO2 vs. temp Stats	
180-	
160-	05:16:10 36,01
140-	
9-120- 2 100-	
2 80-	
40- -	······································
12.05.44 13:00:00 14:00:00 15:00:00 16:00:00 17:00:00 18:00:00 19:00:00 20:00:00 21:00:00 22:00:00 00:00:00 01:00:00 02:00:00 03:00:00 04:00:00 05:00:00 Time	0 06:00:00 07:00:00 08:00:00 09:11:09

Your graph should look like this. This is a graph for one chamber only.

- 30. To end the experiment, click Experiment \rightarrow Stop.
- 31. Now press File \rightarrow Load to analyze saved data. The file loading might take a while.
- 32. When the file is loaded, a new tab labeled Stats are now available for the user. Use this tab for calculations and statistics.





33. The analysis settings are available in Settings→General. Choose SMR estimation method and choose a minimum average oxygen level for estimating standard metabolic rate (SMR) to exclude any hypoxic values. For hypoxia experiments, the critical point for metabolic homeostasis (P_{crit}) can be calculated automatically. Click the button to set the number of low oxygen values that should be part of the oxy-conforme curve when the animal is no longer able to regulate respiration rate independently of ambient water oxygen.





8. SWIM TUNNEL RESPIROMETRY

If you purchased a Loligo[™] swim tunnel respirometer to measure oxygen consumption in swimming animals the AutoResp[™] software has several features for real-time data acquisition, analysis and control. The AutoResp[™] is able to operate one or two swim tunnels at a time.

8.1 Motor output

In AutoResp[™] it is possible to acquire an analog DC voltage signal or a tacho signal from a Loligo[™] swim tunnel motor controller. The tacho signal will give the more precise measurement of water speed (RPM), so we recommend collecting this type of signal if possible.

8.2Motor input

In AutoResp[™] it is possible to generate an analog DC voltage signal to control a Loligo[™] swim tunnel motor controller.

8.3 AutoResp[™] settings

To set up AutoResp[™] to collect a swim tunnel motor output signal, the motor must be running. It is possible to measure oxygen consumption rates and swimming speed from up to two swim tunnels simultaneously. Data from swim tunnel N°1 will appear as chamber 1 and data from any second swim tunnel will be saved under chamber 5 in files, graphs etc.

Configure	
Chamber 1	Chamber 5
Chamber 2 🗌	Chamber 6
Chamber 3 🗌	Chamber 7
Chamber 4	Chamber 8 📃
Ambient oxygen N° 1	Ambient oxygen N° 2
Ambient temperature N° 1	Ambient temperature N° 2
Swim tunnel N 1 🔽	Swim tunnel N° 2 📃

If Swim tunnel N°1 is activated a tab labeled Velocity (CH 1) will appear. If Swim tunnel N°2 is activated another tab labeled Velocity (CH 5) will be active.

AutoResp[™] will also be asking if a DAQ-BT is used. If a DAQ-BT is used please enter the board number. No further in or output settings are necessary then.

Click the tab to calibrate the swim tunnel motor output to show correct water and swim speeds in the software.



elocity input char AQ-M, channel 1	nel	Input type Voltage 🗸	Velocity control Disabled 🛛 👻	10- 9-					
Moving average No 💌	Input [V] 4,88	Velocity [cm/s] 9,76	Output [V]	8- 7- 6-		_	_	-	
WO-POINT CALIE	RATION			4-			~~~~~~		
Lock LO	Input [V] 0,00	Velocity [cm/s]	Output [V]	2-					
Lock HI	Input [V]	Velocity [cm/s]	Output [V]	1-	I	I	1	1	1
				13:44:15	13:44:25	13:44:35	13:44:45 Time	13:44:55	13:45:05

First click on the input channel and choose the input channel. Any of the eight input channels on your DAQ-M instrument can be used. Then choose between a voltage type or tacho type of signal from the motor. If choosing the tacho type of signal, it will take about 10 seconds before the tacho signal starts in the graph.

If AutoRespTM has to control the motor, enable Velocity control. For Velocity (CH 1) the OUT 1 output is used, for Velocity (CH 5) OUT 2 is used. The output [V] can manually be set by entering a value.

Now perform a 2-point calibration at two known velocities and Output [V] values for the software to show correct velocity units (e.g. cm/sec).

E.g. set the LO value to 0,75 V, wait until the Input [V] value gets stable, then press Lock LO, and enter the velocity in the Velocity [cm/s] field.

After the flow calibration, click start experiment. In the setup experiment screen it is now possible to enter the fish length and to choose if solid blocking correction should be used.

Setup er	xperiment						
Chamber	parameters						
Chamber 1	Chamber volume	Tube volume	Wet weight	Density [kg/L]	Resp. volume [L]	Ratio Notes	
Chamber I	Chamber volume	Tube volume	Wet weight	Density [kg/L]	Resp. volume [L]	Ratio Notes	
Chamber 2	▲2 L ▼	📲 0 mL 💌	‡200 g ▼	1	1,8	9	
Chamber 3	Chamber volume	Tube volume	Wet weight	Density [kg/L]	Resp. volume [L]	Ratio Notes	
Chamber 4	Chamber volume	Tube volume	Wet weight	Density [kg/L]	Resp. volume [L]	Ratio Notes	
Chamber 5	Chamber volume	Tube volume	Wet weight	Density [kg/L]	Resp. volume [L]	Ratio Notes	
Chamber 6	Chamber volume	Tube volume	Wet weight	Density [kg/L]	Resp. volume [L]	Ratio Notes	
Chamber 7	Chamber volume	Tube volume	Wet weight	Density [kg/L]	Resp. volume [L]	Ratio Notes	
Chamber 7	Chamber volume	Tube volume	Wet weight	Density [kg/L]	Resp. volume [L]	Ratio Notes	
Chamber 6			Altoo G A	A L	0,9	9	
Solid bloc	king correction	Cross section area [cr	n21 Fish length (cm)	Fish width (cm)	Fish depth (cm)	Fractional error [%]	
Swim tunne	el 1	0	10	0		NaN	
Swim tunne	el 2	Cross section area [cr	m2] Fish length (cm)	Fish width (cm)	Fish depth (cm)	Fractional error [%]	OK Cancel

Page 53 of 74



Solid blocking correction:

An animal swimming in a (closed) channel obstructs the flow of water, causing the water to run faster past the swimming animal. This results in a fractional error, i.e., a difference in water velocity depending on the size of the animals and the dimensions of the channel.

Formula (Bell & Terhune, 1970):

Fractional error = $0.8 \cdot 0.5$ (Fish length/fish radius) \cdot (fish square area/cross area)^{3/2}

Fish length Fish radius ("Thickness")	: Body length of fish : (fish width + fish depth)/4
Fish square area	: $\pi \bullet (fish radius)^2$
Cross sectional area	: cross area of swim tunnel working section

The fractional error is used to convert water velocity in cm/s (as measured during flow calibration with no fish in the working section) into corrected relative swimming speed in BL/s during experiments with swimming fish.

Reference

Bell, W.H. & Terhune, L.D.B. (1970). Water tunnel design for fisheries research. Fish.Res.Bd.Can.Tech.Rep. 195, 1-69.

itoResp™ Flushed f	or 85 seconds							
Experiment Setti	ings Help							
h o Close	ntermitte	ent O Skip pha	se Add fil	le note			🔾 RE 1 🔘	RE 2 🔘 RE 3 🔘 RE
110-								
100								
90 -								
80 -								
70 -								
60 -								
50 -								
40-								
30-								
10-								
10								
0-								
0- 14:15:09				-				14:16:0
0- 14:15:09				Time				14:16:09
0-14:15:09				Time				14:16:09
0 - 14:15:09	MO2 histogram M	02 vs. 02 MO2 vs. tem	p MO2 vs. Uwater	Time MO2 vs. Uswim Uv	vater Uswim			14:16:09
0- 14:15:09 MO2 R^2 Chamber 1	MO2 histogram M	02 vs. 02 M02 vs. tem	p MO2 vs. Uwater Chamber 4	Time MO2 vs. Uswim Uv Chamber 5	vater Uswim	Chamber 7	Chamber 8	14:16:05
0	M02 histogram M Chamber 2 Oxygen	02 vs. 02 M02 vs. tem Chamber 3 Oxygen	p MO2 vs. Uwater Chamber 4 Oxygen	Time MO2 vs. Uswim Uv Chamber 5 Oxygen	vater Uswim Chamber 6	Chamber 7 Oxygen	Chamber 8 Oxygen	14:16:09 Ambient #1 Oxygen
0_ 14:15:09 MO2 R^2 Chamber 1 Oxygen 99,27	M02 histogram M Chamber 2 Oxygen NaN	02 vs. 02 M02 vs. tem Chamber 3 Oxygen NaN	p MO2 vs. Uwater Chamber 4 Oxygen NaN	Time MO2 vs. Uswim Uv Chamber 5 Oxygen NaN	vater Uswim Chamber 6 Oxygen NaN	Chamber 7 Oxygen NaN	Chamber 8 Oxygen NaN	14:16:09 Ambient #1 Oxygen NaN
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Uwater:	This graph plots uncorrected water velocity versus time. By right clicking the graph it is possible to show average values for each measuring period instead of real-time values.
Uswim:	This graph plots swimming speed versus time. The unit of measure for Uswim is body lengths per second (BL/s). When solid blocking correction is activated, corrected swim speed in BL/s will be shown. By right clicking the graph it is possible to show average values for each measuring period instead of real-time values
MO2 vs. Uwater:	This graph plots the MO2 value for each measuring period against average Uwater.
MO2 vs. Uswim:	This graph plots the MO2 value for each measuring period against average Uswim.

To control the velocity enter the menu Settings \rightarrow Velocity control.

Velocity control	X
Swim tunnel N° 1	Swim tunnel N° 2
Velocity control	Velocity control
Setpoint [cm/s] Velocity unit	Setpoint [cm/s] Velocity unit
Ramp setpoint	Ramp setpoint
Interval [time] Interval [cm/s]	Interval [time] Interval [cm/s]
Min. [cm/s] Max. [cm/s]	Min. [cm/s] Max. [cm/s]
	OK Cancel

Enable the Velocity control, and enter the setpoint value. A ramp function allow users to let the software change set points up or down (use negative step value). When activating the ramp function AutoResp[™] will automatically increase/decrease the setpoint by a given interval and after a given time interval. The duration of the time interval can be set in minutes or in number of loops, During a loop, set points are ramped (changed) at the onset of a flush period.



9. AMBIENT WATER QUALITY

Two relays are available for ambient water quality control (e.g. temperature or oxygen), but if using a TEMP-4 instrument also two extra relays will be available for temperature control only (see table below).

Ambient/Instrument	DAQ-M/NETIO	TEMP-4
Oxygen #1	RE 3	
Oxygen #2	RE 4	
Temperature #1	RE 4	RE 1
Temperature #2	RE 3	RE 2

For further help on what instrument relays to use for the different devices, try to place the mouse over any of the four LEDs in the upper right corner in AutoResp®. This will give you a help text depending on the system configuration (see below).

e Experiment	Settings Help									
ush 🔍	Close O Inter	mittent 🔿	Skip phase	Add file note			🔾 RE 1 🔘	RE 🔘 E 3 🕥 RE 4		AQ-I
100,1 -							A	mbient O2 N° 1		
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69,4308-7 12:19:41	MO2 histogram Chamber 2 Oxygen NaN MO2 0	MO2 vs. O2 M Chamber 3 Oxygen NaN MO2 0	O2 vs. temp Ambie Chamber 4 Oxygen NaN MO2 0	Time ant oxygen Ambien Chamber 5 Oxygen NaN MO2 0	t temp Chamber 6 Oxygen NaN MO2 0	Chamber 7 Oxygen NaN MO2 0	Chamber 8 Oxygen NaN MO2 0	12:24:41	← 1	ЕМР
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9.10xygen saturation

To control the oxygen saturation in the ambient water used for flushing respirometer chambers, a DO-SET is needed. This DO-SET can either be connected to the DAQ-M instrument for software control and monitoring of ambient oxygen levels or an OXY-REG instrument for standalone operation. The DO-SET includes:

- Solenoid valve
- Air stone
- Tubing



To use the DO-SET assemble the system shown in the figure below. The power cord is connected to the DAQ-M or OXY-REG.



For using a DO-SET with AutoResp^M, connect the solenoid directly to one of the DAQ-M/NETIO relays. Now activate ambient oxygen N°1 in the software (see below). After the calibration and experiment settings is finished, the ambient oxygen control settings can be found under Settings \rightarrow Oxygen control.



Ambient oxygen N° 1	Ambient oxygen N° 2
Oxygen control	Oxygen control
Setpoint [%a.s.] Hysteresis [%a.s.] 50,00 1,00	Setpoint [%a.s.] Hysteresis [%a.s.]
● Hypoxic ○ Hyperoxic	HypoxicHyperoxic
Ramp setpoint	Ramp setpoint
Interval [time] Interval [%a.s.]	Interval [time] Interval [%a.s.]
Min. [%a.s.] Max. [%a.s.]	Min. [%a.s.] Max. [%a.s.]
+ feedback• feedback	+ feedback• feedback

Activate oxygen control to start controlling the ambient oxygen saturation. Now set the setpoint and hysteresis and if the you want hypoxic (inject nitrogen gas) or hyperoxic (inject clean oxygen gas) control. Hypoxic control will keep ambient oxygen saturation below normoxic levels to a set point value choosen by the user and visa versa. This set point value can be altered by the user at any point in time.



Graphic depiction of the relay function setpoint:

A ramp function allow users to let the software change set points up or down (use negative step value) in a step-wise way for both temperature or oxygen control. When activating the ramp function AutoResp[™] will automatically increase/decrease the setpoint by a given interval



and after a given time interval. The duration of the time interval can be set in minutes or in number of loops, e.g. change the set point for ambient oxygen by 10% air sat. after each third measuring loop. During a loop, set points are ramped (changed) at the onset of a flush period.

The user can choose between ramping setpoints with or without feedback. Feedback means that setpoints are not ramped (changed) until the measured value has reached the setpoint value. This to avoid problems with large volumes of water and/or large steps of increment/decrement in values, in which case it can take considerable time to change water temperature or oxygen saturation.

Finally, the user can set minimum and maximum setpoint values to avoid severe changes or harmfull conditions when using the automated ramp functions.

NB! Please note, if changing oxygen units, the ramp function settings should be adjusted accordingly by the user.

9.2 Temperature

To control the temperature of the ambient water used for flushing and thermostating respirometer chambers, a TMP-SET is needed. This TMP-SET can either be connected to the DAQ-M instrument for software control and monitoring of ambient oxygen levels or an TMP-REG instrument for stand alone operation. The TMP-SET includes:

- Stainless coil
- Pump
- Tubing



To use the TMP-SET, assemble the system as shown in the figure below. The power cord for the pump is connected to a DAQ-M or TMP-REG relay using a power strip or adapter cable supplied with the system.



Water tank

For using a TMP-SET with AutoResp^M, connect the pump to one of the DAQ-M/NETIO relays as explained above. Now activate ambient temperature N°1 in the software (see below). After the calibration and experiment settings is finished, the ambient temperature control settings can be found under Settings \rightarrow Temperature control.



Temperature control	×
Ambient temperature N° 1	Ambient temperature N° 2
Temperature control	Temperature control
Setpoint [°C] Hysteresis [°C]	Setpoint [°C] Hysteresis [°C]
Cool Heat	○ Cool ● Heat
Ramp setpoint	Ramp setpoint
Interval [time] Interval [°C] ▲ 10 min ▲ Min. [°C] Max. [°C] ▲ 40	Interval [time] Interval [°C] ★10 min ★0,5 Min. [°C] Max. [°C] ★5 ★40
+ feedback• feedback	 + feedback - feedback
	OK Cancel

Activate temperature control to start controlling the ambient temperature. Now set the setpoint and hysteresis and how the relay has to work. The control can either keep the ambient temperature above the setpoint (Heat) or below the setpoint (Cool). If heating the bath in which the stainless coil is submerged must be filled with heated water. If cooling the bath must be filled with chilled water.

For help using the ramp function, see explanation under ambient oxygen control above.



10. BACKGROUND THEORY

10.1 Measuring metabolic rate

Metabolic rate is one of the most important physiological variables for comparing performance and adaptations of different organisms, and it is a measure of the total energy consumption over time. However, it is only possible to measure the amount of energy consumed for metabolism by one of four indirect methods:

- 1. Energy contents of food minus energy content of waste products
- 2. Oxygen consumed or CO₂ excreted (respirometry)
- 3. Heat production (calorimetry)
- 4. Metabolic water produced (isotopic techniques)

Of these oxygen consumption (MO_2) is the easiest to measure in water, and MO_2 is closely linked to the heat production no matter what organic substrates are burned (in average 20 kJ per litre O_2).

Anaerobic metabolism cannot be determined through MO₂ measurements, causing misleading results in some organisms.

10.2 What is oxygen used for?

All organisms use energy at a rate that can be determined and expressed in different ways. The energy is used for maintaining homeostasis, and for vital and energy consuming processes like osmo regulation, acid-base balance, protein synthesis etc.

The metabolism of higher animal cells (eukaryotes) is primarily based on aerobic respiration requiring oxygen in order to generate energy (ATP).

Simplified reaction:

 $C_{6}H_{12}O_{6}\;(aq)\,+\,6O_{2}\;(g)\rightarrow\,6CO_{2}\;(g)\,+\,6H_{2}O\;(I)$

 $\Delta G = -2880 \text{ kJ per mole of } C_6 H_{12} O_6$

Oxygen is used in the reaction, thus measured oxygen consumption rate of an organism mirrors the aerobic metabolism.

Oxygen molecules are used as the "terminal electron acceptor" in the respiratory chain during oxidation of organic molecules. The product of this process is energy in the form of ATP (Adenosine Triphosphate), by substrate-level phosphorylation, NADH and FADH2.



10.3 Intermittent respirometry

The measuring principle behind **AutoResp™** is so-called intermittent respirometry, one of three widespread methods for oxygen consumption measurements (respirometry) in aquatic breathers:

1. Closed respirometry (or constant volume respirometry)

2. Flow-through respirometry (or open respirometry)

3. Intermittent respirometry (or stop-flow respirometry)



10.3.1. Closed respirometry

Measurements in a sealed chamber of known volume, ie. a closed respirometer. The oxygen content of the water is measured initially (t0), then the respirometer is closed and at the end of the experiment (t1) the oxygen content is measured again.

Knowing the body weight of the animal, the respirometer volume and the oxygen content of the water at time t0 and t1 the mass specific oxygen consumption rate can be calculated as follows:

$$VO_2 = \left(\left[O_2 \right]_{t_0} - \left[O_2 \right]_{t_1} \right) \cdot \frac{V}{t} \cdot \frac{1}{BW}$$

VO₂ = oxygen consumption rate (mg O₂/kg/hour)

 $[O_2]t_0 = oxygen concentration at time t_0 (mg O_2/liter)$

 $[O_2]t_1 = oxygen concentration at time t_1 (mg O_2/liter)$

V = respirometer volume minus volume of experimental animal (liter)

 $t = t_1 - t_0$ (hour)



BW = body weight of experimental animal (kg)

An advantage of this method is its simplicity. A disadvantage is that the measurements are never made at a constant oxygen level, due to the continuous use of oxygen by the animal inside the closed chamber. This might cause problems when interpreting data, since animal respiration often changes with ambient oxygen partial pressure.

Furthermore, metabolites from the experimental animal (e.g. CO₂) accumulate in the water, thus limiting the duration of measurements. The limited time for measurements prevents the experimental animal to recover from initial handling stress that often increase animal respiration significantly and for several hours, thus overestimating oxygen consumption rates.

10.3.2. Flow-through respirometry

This is a more sophisticated method for oxygen consumption measurements. The experimental animal is placed in a flow-through chamber, with a known flow rate. Oxygen content is measured in both the inflowing and outflowing water, and oxygen consumption rate can then be calculated as:

 $VO_2 = \left(\left[O_2 \right]_{in} - \left[O_2 \right]_{out} \right) \cdot \frac{F}{BW}$

 $VO_2 = oxygen consumption rate (mg O_2/kg/hour)$

F = water flow rate (l/hour)

 $[O_2]$ in = oxygen content in water inflow (mg O₂/liter)

[O₂]out = oxygen content in water outflow (mg O₂/liter)

BW = body weight of experimental animal (kg)

The advantages of this method are several:

1) the duration of the experiment is in principle unlimited

2) no accumulation of CO2 and other metabolites

3) its possible to measure at a constant oxygen level

4) by controlling the quality of the inflowing water its possible to measure metabolism at different desired levels of oxygen, salinity etc.

However, this method bring along one significant disadvantage: in order to determine oxygen consumption by open respirometry it is crucial that the system is in steady state. This means that the oxygen content of the in flowing and out flowing water, AND the oxygen consumption of the animal have to be constant.

If the oxygen consumption of the animal for some reason changes during the experiment, steady state will not exist for a while. Not until the system is in steady state again will the above formula give the correct oxygen consumption rate. The duration of the time lag depends



on the relationship between chamber volume, wash-out and flow rate. Thus, open respirometry measurements have poor time resolution and are not suitable for determination of oxygen consumption on organisms with a highly variable respiration, e.g. like fish that can triple their respiration rate or more within a few minutes.

10.3.3. Intermittent respirometry

AutoResp^m is based on the principle of intermittent respirometry aiming at combining the best of both of the above methods 1) closed and 2) flow-through respirometry.

The experimental animal is placed in a sealed chamber with ports for recirculating the (closed) volume of water inside the chamber during measurements to avoid gradients (mixing) and to maintain adequate flow past oxygen probes with self-consumption of oxygen.

A second set of ports are used to flush the water inside the respirometer chamber intermittently with water from the ambient tank (temperature bath) in which the chamber is submerged to avoid severe oxygen depletion inside the chamber. Hence the name *intermittent respirometry*.

A computer actuated (flush) pump connected to the latter ports is turned on and off intermittently to flush the chamber, When the flush pump is turned off the systems operates like closed respirometry.

When the pc controlled flush pump turns on, it pumps ambient water into the respirometer chamber thus bringing the oxygen content to the level of the surrounding ambient water. In this way, problems with accumulating metabolites and severe changes in oxygen level due to animal respiration are avoided.

As with open (or flow-through) respirometry, the duration of the experiment is in principle unlimited.

However, the most important advantage is the great time resolution of this method. Oxygen consumption rates of animals can be determined for every 5-10 minutes over periods of hours or days, making automated systems extremely well suited for uncovering short and long term variations in respiration.

In summary, AutoResp[™] systems for automated oxygen consumption measurements have been developed for prolonged respirometry experiments in a controlled laboratory environment.

To summarize, the automated measuring procedure runs in three phases:

- 1. Measuring period (M)
- 2. Flush period (F)
- 3. Wait period (W)

In the Measuring period (M) the flush pump is off, and the chamber is closed. Fish respiration rate is calculated from the decline in oxygen. During this time the recirculation pump is active to mix the water inside the respirometer and to ensure proper flow past the oxygen sensor.

The measuring period is followed by a Flush period (F) where the flush pump is active pumping water from the ambient temperature bath and into the respirometer. During this period the



recirculation pump is inactive and the oxygen curve will raise to approach the level of the ambient water.



Finally, the flush pump stops and the loop ends with a short Wait period (W) before starting a new measuring period. This waiting period is necessary to account for a lag in the system response resulting in a non-linear oxygen curve. During the Wait period the recirculation pump is active.



Examples of raw MO₂ data can be seen from the above diagram. Standard metabolic rate of juvenile Rainbow Trout was determined in a static respirometer chamber and with an automated respirometry system from Loligo[™] Systems during approximately 24 hours.

Initial high oxygen consumption rates due to handling stress, were followed by a gradual decline to lower and more stable values indicating standard metabolic rate for the specimen. Notice the high temporal resolution (10 min) of the system revealing sudden changes in MO₂.



10.4 Dissolved oxygen

The oxygen capacitance coefficient β in water is only 1/30 of that in atmospheric air, depending on barometric pressure. The concentration of oxygen in air-equilibrated water depends on water temperature and salinity. Even more importantly, the diffusion velocities of oxygen molecules are 10.000 times lower than in air. Thus oxygen is scarce and fluctuates due to environmental changes in pressure, temperature and salinity.

 $\left[O_{2}\right] = pO_{2} \cdot \beta$

 $[O_2]$ = concentration of oxygen in water (mgO₂/l)

pO₂ = partial pressure of oxygen in water (kPa)

 β = capacitance coefficient of oxygen in water (mg O₂/l/kPa)

Atmospheric air contains c.21% oxygen, e.g. 210 mlO₂/l and in general terrestrial animals is rarely challenged by any significant changes in oxygen availability.

In comparison one litre of "saturated" water in equilibrium with atmospheric air, contains only c.10 mlO₂/l, and oxygen solubility will decrease with increasing water temperature and salinity and *visa versa*.

Thus, many aquatic organisms experiences significant pertubations in oxygen levels in their natural environment, and not only due to anthropogenic effects (eutrofication). In environments with high biological activity, algae and plants can add high amounts of oxygen to the water during daytime photosynthesis, whereas oxygen in the water is consumed during night by the respiration of the same organisms and bacteria in some case causing severe hypoxia.

<u>Formulas</u>

$$[O_2] = PwO_2 \cdot \beta$$

Where

PwO2 = oxygen partial pressure in water (kPa)

 β = oxygen solubility in water (mgO₂/l/kPa)

See appendix for $\ensuremath{\ensuremath{\mathsf{B}}}$ -values in relation to temperature and salinity.

From barometric pressure (BP) and vapor pressure (pH_2O) the partial pressure of oxygen in fully saturated water can be calculated as:

$$pO_2 = (BP - pH_2O) \cdot 0,2094$$

where 0,2094 is the fraction of oxygen in the atmosphere at sea level.

See appendix for pO₂- and pH₂O-values in relation to barometric pressure and temperature



11. TROUBLESHOOTING

11.1 Change Y-scale units

To change the scaling of graph y-axes, double click the upper or lower value of the Y-scale and type a new value using the keyboard numerics.





11.2 Noise

If a noise problem on input signals exist, users can choose a moving average function to smoothen signals.



Example. Graph showing raw sensor signal (red curve) and the same data with a moving average of 10 points (white curve).



11.3 Previous versions

It is important to remove any previous versions before installing the new AutoResp[™] software:

- 9. Click Start→Control Panel
- 10. Open Programs and Features
- 11. Double click on National Instruments software
- 12. Select all packages, and then click on Remove.

Instruments Software		
Products Patches NI 985x Software 1.3.5 NI CompactRIO 3.5.0 NI CompactRIO Module Support 3.5.0 NI FlexRIO 1.5.0		Modify Change which application features are installed. Displays the Select Features dialog, which lets you configure individual features.
NI FlexRIO Adapter Module Support 1.8.0 NI Hierarchical Waveform Storage 1.4.8 NI IVI Compliance Package 4.2 NI Instrument I/O Assistant		Reinstall missing or corrupt files, registry keys, and shortcuts. Preferences stored in the registry may be reset to default values.
NI LabVIEW 2009 NI LabVIEW 2010	X	Remove Remove product from this computer.
		Close

- 13. You will then be notified that AutoResp[™] also will be removed. Click Yes
- 14. Now wait until all packages are uninstalled. This might take some time.
- 15. Windows will now ask for a restart.
- 16. When the computer is restarted, proceed to install the new AutoResp[™] software.



11.4 2-point calibration

If using an oxygen meter with an analog output connected to a data acquisition instrument (e.g. DAQ-M/-S/-1/-4), start by calibrating the sensor against two known standard solutions. This is often done by equilibrating a stirred water sample with atmospheric air to reach 100% air saturation. Use an air pump to bubble the water sample and allow enough time for full equilibration. Then place your oxygen sensor in the sample and wait for the sensor signal to stabilize. Then follow the operating instructions for the meter to set the measured (HI) value to 100%. Repeat this procedure for a zero oxygen solution, setting the measured (LO) value to 0%. Bubble with nitrogen gas or use a sodium sulphite solution to remove all oxygen from the sample.

Now the oxygen meter will convert the oxygen sensor signal into % air saturation and the correct values is displayed on the screen. Alternatively the engineering unit could be changed to mmHg or kPa.

If the oxygen meter has an analog output, this can be connected to an A/D (analog-to-digital) instrument, e.g. DAQ-M/-S/-1/-4 instrument, for PC data acquisition. A typical analog output signal is a 0-5V DC voltage over the entire measuring range, e.g. when the oxygen meter screen reads 0% oxygen saturation the analog output is 0 VDC, and when the meter reads 100% the analog output is approximately 2.5 VDC if the measuring range is 0-200% air saturation.

To convert the 0-5VDC signal into engineering units (e.g. % air sat.) in AutoResp[™], a second two-point calibration is necessary, e.g. to convert the output signal from the instrument into % air saturation or another unit of measure.

Place your sensor into a low solution, wait until the voltage stabilizes, then click LOCK LO and read the value on the oxygen meter and write the value in the corresponding LOCK LO oxygen field. Now the voltage is calibrated against an oxygen saturation.

Do the same for a high solution, but press LOCK HI.

When done, the output from the instrument is calibrated in AutoRespTM.



11.5 Run AutoResp™ always as admin

- 1. Right click on the AutoResp[™] icon.
- 2. Choose properties.
- 3. Go to compatibility.
- 4. Enable "Run this program as an administrator"
- 5. Click OK.
- 6. Now open AutoResp^m by double clicking the icon.
- 7. Choose Yes, to confirm that you want to start the software as admin.

Next time you want to open AutoResp^m as admin only do step 6 and 7.

12. INDEX

AMBIENT WATER QUALITY	56
AutoResp [™]	45
AutoResp [™] installation	31
Change Y-scale units	68
Closed	63
DAQ-M	24
DAQ-PAC-F1	4
DAQ-PAC-F4	6
DAQ-PAC-F8	8
Dipping probe	28
Flow-through	64

Intermittent	65
Loligo [™] Service Pack	36
Noise problems 68; 69; 70;	71
OXY-4 mini13;	17
Sensor spots	29
Solid blocking correction	54
Static chambers	10
Swim tunnels	11
TEMP-4	25
WiBu	44



13. APPENDIX

Table 1. Table of oxygen saturation in water

Temperature	Air saturated water	Atmospheric pressure
(deg C)	(ppm or mgO₂ I⁻¹)	(mmHg)
10	11.3	157.3
11	11.1	157.1
12	10.8	156.9
13	10.6	156.7
14	10.4	156.5
15	10.2	156.3
16	10.0	156.0
17	9.7	155.8
18	9.5	155.6
19	9.4	155.4
20	9.2	155.2
21	9.0	154.9
22	8.8	154.7
23	8.7	154.4
24	8.5	154.1
25	8.4	153.8
26	8.2	153.5
27	8.1	153.2
28	7.9	152.8
29	7.8	152.5
30	7.6	152.2
35	7.1	150.0
40	6.6	148.0
45	6.1	145.5


Table 2. Partial pressure of oxygen (pO2) at different barometric pressures and temperatures

	40	.38		.895	.947	.999	,050	.102	.154	.206	.257	.309	.361	.413	.464	.516	.568	.620	.672	.723	.775	.827	.879	.930	.982	.034	.086
		- ⁻		7 18,	9 18,	1 18,	4 19,	5 19,	3 19,	1 19,	3 19,	5 19,	3 19,	0 19,	3 19,	5 19,	7 19,	0 19,	2 19,	5 19,	7 19,	9 19,	2 19,	4 19,	5 19,	9 20,	1 20,
	37	6,28		19,113	19,16	19,22:	19,27	19,326	19,378	19,43	19,480	19,536	19,588	19,64(19,69	19,749	19,793	19,850	19,903	19,959	20,003	20,059	20,113	20,16	20,216	20,269	20,32:
	35	5,62		19,248	19,301	19,353	19,406	19,459	19,512	19,564	19,617	19,670	19,722	19,775	19,828	19,881	19,933	19,986	20,039	20,092	20,144	20,197	20,250	20,303	20,355	20,408	20,461
	30	4,24		19,526	19,579	19,633	19,686	19,739	19,793	19,846	19,900	19,953	20,007	20,060	20,114	20,167	20,221	20,274	20,328	20,381	20,435	20,488	20,542	20,595	20,649	20,702	20,756
	25	3,17		19,742	19,796	19,850	19,904	19,958	20,012	20,066	20,121	20,175	20,229	20,283	20,337	20,391	20,445	20,499	20,553	20,607	20,661	20,715	20,770	20,824	20,878	20,932	20,986
	20	2,34		19,909	19,963	20,018	20,072	20,127	20,182	20,236	20,291	20,345	20,400	20,454	20,509	20,563	20,618	20,672	20,727	20,782	20,836	20,891	20,945	21,000	21,054	21,109	21,163
	0	2,06		19,964	20,019	20,073	20,128	20,183	20,237	20,292	20,347	20,402	20,456	20,511	20,566	20,620	20,675 :	20,730	20,784	20,839	20,894	20,948	21,003	21,058	21,113	21,167	21,222
	16	1,82	_	20,013	20,068	20,123	20,178	20,233	20,288	20,342	20,397	20,452	20,507	20,562	20,617	20,671	20,726	20,781	20,836	20,891	20,946	21,000	21,055	21,110	21,165	21,220	21,275
	4	1,60	-	20,058	20,113	20,167	20,222	20,277	20,332	20,387	20,442	20,497	20,552	20,607	20,662	20,717	20,772	20,827	20,882	20,937	20,992	21,047	21,102	21,157	21,212	21,267	21,321
	12	1,40		20,097	20,152	20,207	20,262	20,317	20,372	20,427	20,482	20,537	20,593	20,648	20,703	20,758	20,813	20,868	20,923	20,978	21,033	21,088	21,143	21,198	21,253	21,308	21,363
	U F	1,23		20,132	20,187	20,242	20,298	20,353	20,408	20,463	20,518	20,573	20,628	20,684	20,739	20,794	20,849	20,904	20,959	21,015	21,070	21,125	21,180	21,235	21,290	21,346	21,401
	0	1,07		20,163	20,218	20,274	20,329	20,384	20,439	20,495	20,550	20,605	20,660	20,716	20,771	20,826	20,881	20,937	20,992	21,047	21,102	21,158	21,213	21,268	21,323	21,379	21,434
)*.2U94) 6	0,93		20,191	20,246	20,302	20,357	20,412	20,467	20,523	20,578	20,633	20,689	20,744	20,799	20,855	20,910	20,965	21,021	21,076	21,131	21,187	21,242	21,297	21,353	21,408	21,463
ent -	Pbp-Pvap	0,81		20,215	20,271	20,326	20,382	20,437	20,492	20,548	20,603	20,658	20,714	20,769	20,825	20,880	20,935	20,991	21,046	21,102	21,157	21,212	21,268	21,323	21,378	21,434	21,489
t differ	res = ((0,71	-	20,237	20,292	20,348	20,403	20,459	20,514	20,570	20,625	20,681	20,736	20,791	20,847	20,902	20,958	21,013	21,069	21,124	21,180	21,235	21,290	21,346	21,401	21,457	21,512
gen (p02) a	d temperatu	0,61		20,256	20,312	20,367	20,423	20,478	20,534	20,589	20,645	20,700	20,756	20,811	20,866	20,922	20,977	21,033	21,088	21,144	21,199	21,255	21,310	21,366	21,421	21,477	21,532
re of oxy	ssures an			7,32	7,59	7,85	18,12	8,39	8,65	8,92	9,19	9,45	9,72	9,99	0,25	0,52	0,79	1,05	1,32	1,59	1,85	2,12	2,39	2,65	2,92	3,19	3,45
Partial pressur	barometric pres Temperature (de	Pvap (kPa)	Pbp (kPa)	6	6	6	6	6	6	6	6	6	6	6	10	10	10	10	10	10	10	10,	10	10	10	10	10



Table 3. Oxygen solubility in mg O2/liter/kPa at different temperatures and salinities

Oxygen solubility i different temperatu	.n mg 02/ .res and	/liter/kP(salinitie	a at es				ы П	rom Gree	en & Car	crit (19	67). J.	. Mar.]	Biol. 29	; 140-1	147.	1 kPa =	7,501	gHmm	
Salinity (o/oo)		2	4	9	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
Temperature (deg C)																			
0	0,6976	0,6878	0,6781	0,6685	0,6591	0,6498	0,6406	0,6316	0,6227 C	0,61390	,6053 (J,5967 I	,5883 (,5800 0	,5719 (0,5638 (,5559 0	,5480 0	,5403
1	0,6788	0,6694	0,6600	0,6509	0,6418	0,6329	0,6241	0,6154	0,6068 C	,5984 0	,5900 (0,5818	0,5737 0	,5657 0	,5579 (0,5501 (,5424 0	,5349 0	,5274
2	0,6608	0,6517	0,6428	0,6339	0,6252	0,6166	0,6082	0,5998	0,5916 C	0,58340	,5754 (0,5675	,5597 (,5520 0	,5444 (0,5369 (0,5295 0	,5223 0	,5151
ŝ	0,6436	0,6349	0,6263	0,6178	0,6094	0,6011	0,5929	0,5849	0,5769 C	,5691 0	,5614 (0,5537	,5462 0	,5388 0	,5315 (0,5243 (0,5171 0	,5101 0	,5032
4	0,6272	0,6188	0,6104	0,6022	0,5942	0,5862	0,5783	0,5706	0,5629 C	,5553 0	,5479 (0,5405	,5333 (,5261 0	,5190 (0,5121 (,5052 0	,4984 0	,4917
	0,6114	0,6033	0,5953	0,5874	0,5796	0,5719	0,5643	0,5568	0,5494 C	,5421 0	,5349 (0,5278	,5208 0	,5139 0	,5071 0	0,5004 (0,4937 0	,4872 0	,4807
9	0,5963	0,5885	0,5808	0,5731	0,5656	0,5582	0,5508	0,5436	0,5365 C	0,5294 0	,5225 (J,5156 I	,5089 (,5022 0	,4956 (0,4891 (0,4827 0	,4763 0	,4701
7	0,5818	0,5743	0,5668	0,5595	0,5522	0,5450	0,5379	0,5310	0,5241 C	,5172 0	,5105 (0,5039 1	,4974 0	,4909 0	,4845 (0,4782 (,4720 0	,4659 0	1,4598
80	0,5680	0,5607	0,5535	0,5463	0,5393	0,5324	0,5255	0,5188	0,5121 C	,5055 0	,4990 (1,4926	,4863 (,4800 0	,4739 (0,4678 (0,4618 0	,4558 0	,4500
6	0,5547	0,5476	0,5406	0,5338	0,5270	0,5203	0,5136	0,5071	0,5006 C	,4943 0	,4880 (0,4818	,4756 (,4696 0	,4636 (0,4577 (,4519 0	,4461 0	,4405
10	0,5419	0,5351	0,5283	0,5217	0,5151	0,5086	0,5022	0,4959	0,4896 C	0,4834 0	,4774 (0,4713	,4654 (,4595 0	,4537 (0,4480 (,4424 0	,4368 0	,4313
11	0,5297	0,5231	0,5165	0,5101	0,5037	0,4974	0,4912	0,4851 (0,4790 C	0,4730 0	,4671 (0,4613 1	,4555 (,4498 0	,4442 (0,4387 0	0,4332 0	,4278 0	,4224
12	0,5179	0,5115	0,5052	0,4989	0,4928	0,4867	0,4806	0,4747 (0,4688 C	0,4630 0	,4573 (0,4516	,4460 (,4405 0	0,4351 (0,4297 (0,4244 0	,4191 0	,4139
13	0,5067	0,5005	0,4943	0,4882	0,4823	0,4763	0,4705	0,4647	0,4590 C	0,4534 0	,4478 0	1,4423	,4369 (,4315 0	,4262 (0,4210 0	0,4158 0	,4107 0	,4057
14	0,4959	0,4898	0,4839	0,4780	0,4721	0,4664	0,4607	0,4551	0,4496 C	0,44410	,4387 0	0,4333 1	0,4281 0	,4229 0	,4177 (0,41260	0,4076 0	,4026 0	,3977
15	0,4855	0,4796	0,4738	0,4681	0,4624	0,4568	0,4513	0,4459	0,4405 C	0,43520	,4299 (0,4247	,4196 0	,4145 0	,4095 (0,4046	3997 0	,3948 0	,3901
16	0,4755	0,4698	0,4641	0,4586	0,4531	0,4476	0,4423	0,4370	0,4317 C	0,4266 0	,4214 (0,4164 1	,4114 0	,4065 0	,4016 (0,3968	,3920 0	,3873 0	,3827
17	0,4659	0,4603	0,4549	0,4494	0,4441	0,4388	0,4336	0,4284 1	0,4233 C	0,41830	,4133 (0,4084 1	,4035 0	3987 0	,3940 (0,3893 (3846 0	,3800 0	,3755
18	0,4567	0,4513	0,4459	0,4407	0,4354	0,4303	0,4252	0,4202	0,4152 C	0,4103 0	,4054 (0,4006	,3959 (,3912 0	,3866 (0,3820 0	0,3775 0	,3730 0	,3686
19	0,4478	0,4426	0,4374	0,4322	0,4271	0,4221	0,4171	0,4122	0,4074 C	0,40260	, 3979 (J, 3932 I	,3886 (,3840 0	,3795 (0,3750 0	3706 0	,3662 0	,3619
20	0,4393	0,4342	0,4291	0,4241	0,4191	0,4142	0,4094	0,4046	0,3999 C	,3952 0	,3906 (0,3860	,3815 0	,3770 0	,3726 (0,3683 (,3640 0	,3597 0	,3555
21	0,4311	0,4261	0,4212	0,4163	0,4114	0,4066	0,4019	0,3972 1	0,3926 C	,3880 0	,3835 (0,3791	3747 0	,3703 0	,3660 (0,3617 (3575 0	,3534 0	,3493
22	0,4233	0,4184	0,4135	0,4087	0,4040	0,3993	0,3947	0,3901	0,3856 C	,3812 0	,3767 (0,3724 1	,3681 (,3638 0	,3596 (0,3554 (,3513 0	,3473 0	,3432
23	0,4157	0,4109	0,4062	0,4015	0,3969	0,3923	0,3878	0,3833 1	0,3789 C	37450	,3702 (0,3659 1	3617 0	3575 0	,3534 (0,3494 (3453 0	,3413 0	,3374
24	0,4084	0,4037	0,3991	0,3945	0,3900	0,3855	0,3811	0,3767	0,3724 C	0,3681 0	,3639 (0,3597	3556 (3515 0	3475 (0,3435 (0,3395 0	,3356 0	,3318
25	0,4014	0,3968	0,3923	0,3878	0,3833	0,3790	0,3746	0,3703 1	0,3661 C	,36190	,3578 (0,3537 1	3497 0	3457 0	3417 (0,3378 0	0,3339 0	,3301 0	,3263
26	0,3947	0,3902	0,3857	0,3813	0,3770	0,3727	0,3684	0,3642	0,3601 C	0,3560 0	,3519 (3479 1	3439 (,3400 0	,3361 (0,3323 (0,3285 0	,3248 0	,3211
27	0,3882	0,3838	0,3794	0,3751	0,3708	0,3666	0,3624	0,3583 1	0,3542 C	0,3502 0	,3462 (0,3423 1	,3384 (,3346 0	,3308 (0,3270 (0,3233 0	,3196 0	,3160
28	0,3819	0,3776	0,3733	0,3691	0,3649	0,3608	0,3567	0,3526	0,3486 C	34470	,3408 0	J, 3369 I	,3331 0	3293 0	3256 (0,3219 (0,3182 0	,3146 0	,3110
29	0,3759	0,3717	0,3674	0,3633	0,3592	0,3551	0,3511	0,3471	0,3432 C	0,3393 0	, 3355 (0,3317	,3279 (,3242 0	,3205 (0,3169 (0,3133 0	,3098 0	,3063
30	0,3701	0,3659	0,3618	0,3577	0,3537	0,3497	0,3457	0,3418 (0,3380 C	0,3341 0	, 3304 (0,3266	,3229 0	,3193 0	3157 (0,3121	0,3086 0	,3051 0	,3017