UNISENSE

O_2 N_2O H_2 NO H_2S Redox Temp Opto pH EP

The Microsensor Company

Field Studies

February 2022
Tage Dalsgaard

UNISENSE

Online Biogeochemistry Workshop

Wednesday 23 February
14:30-15:30 CET - Introduction to Microsensors
15:45-16:45 CET - Lab-based Studies

Thursday 24 February
14:30-15:30 CET - Field Studies
15:45-16:30 CET - Demonstration of Field Microprofiling System
16:45-17:30 CET - Demonstration of Activity Calculation - Software

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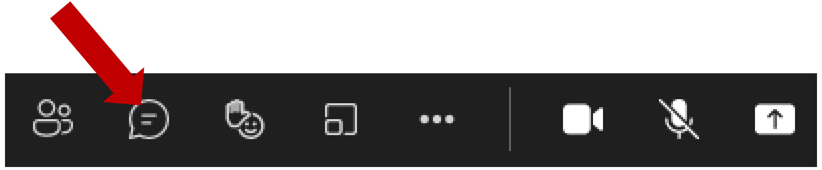
A few rules before we get started

1. Please turn off your microphone

2. Questions: During lecture please use chat.
After lecture you can unmute and ask.



Very application-specific questions may be better answered in a private session afterwards.

You will get access to all the presentations as PDF´s + recordings shortly after the workshop.

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Outline

- In situ UniAmp amplifiers and pressure compensation
- Microprofiles in situ - MiniProfiler
- Flux chamber incubations in situ - MiniChamber Lander
- Non invasive flux measurements - Eddy Covariance
- Deep sea landers
- Measurements in the water column
- Working with very low O_2 concentrations - STOX sensor

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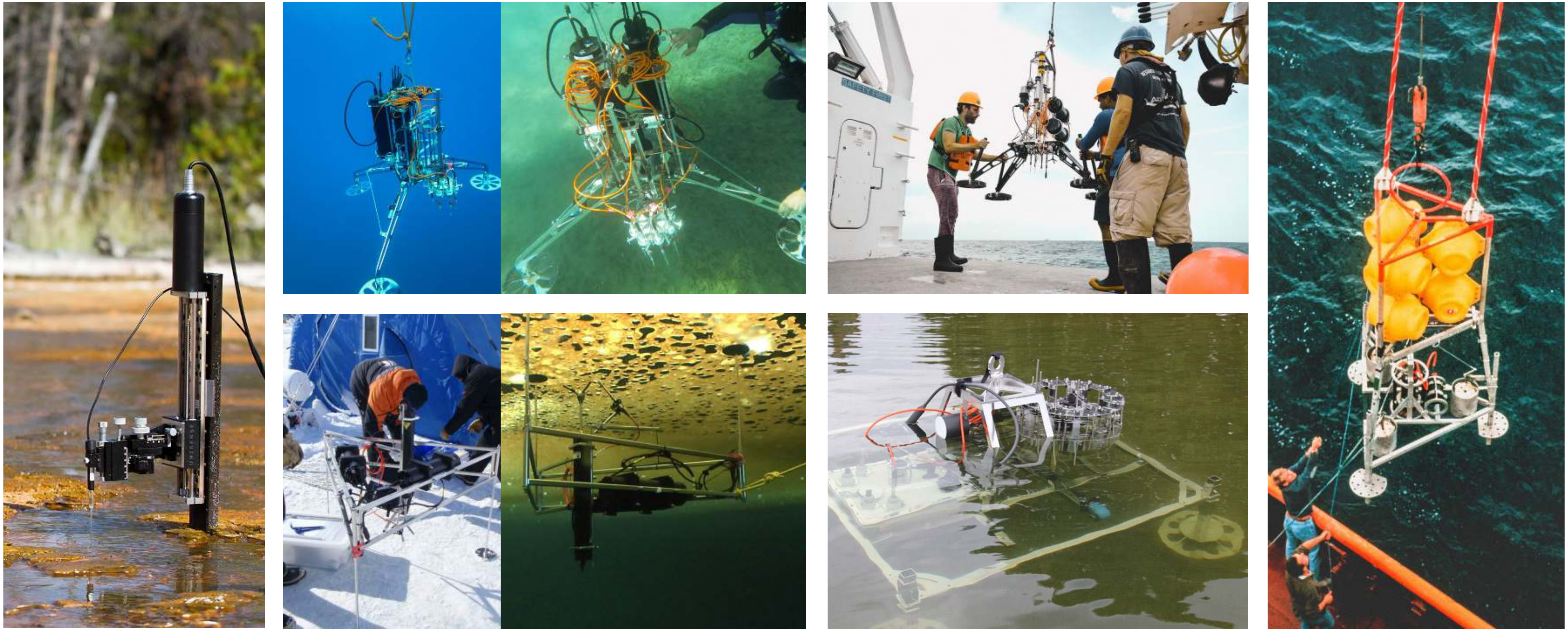
Unisense Field Systems



Microsensors for in situ use

- Any Unisense sensor for in situ use:
  - O_2, H_2S, H_2, N_2O, NO
  - pH, Redox potential, electrical potential
- Tip size: 10 - 500 µm
- Mounted via In Situ Connector
- Pressure compensation (> 50 m)

Deep sea: 6000 m water depth

Lab and Field Multi Meter

Shallow: 300 m water depth

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Application of microsensors

Microprofiles:

- Small tip diameter
- High spatial resolution

Bulk water:

- 500 µm tip diameter
- Protection cap

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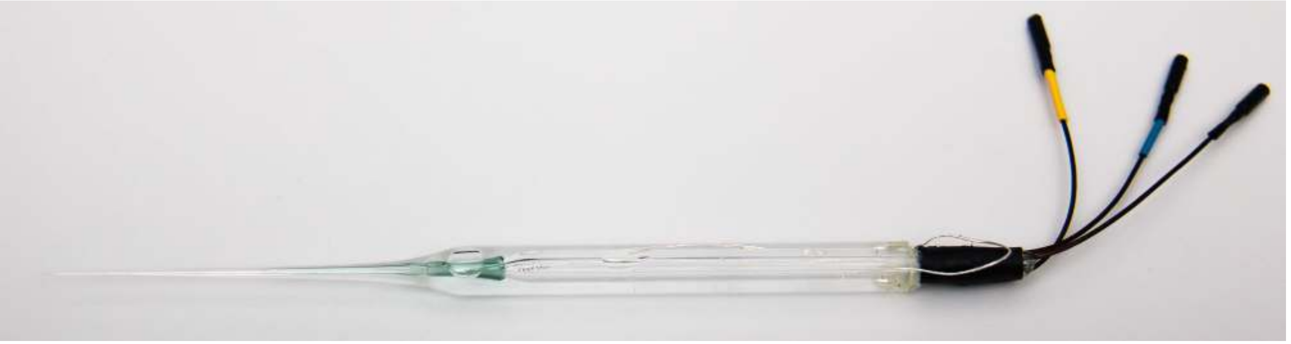
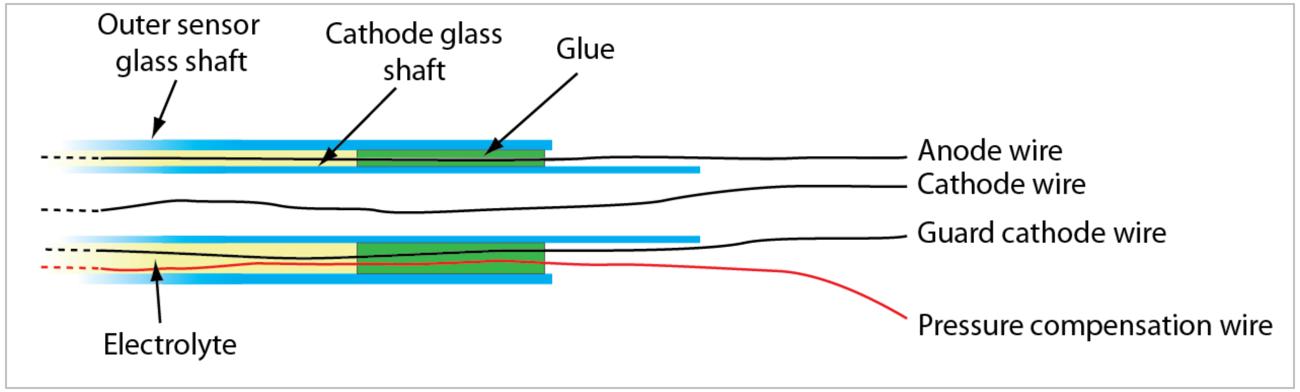
Pressure compensation

Pressure compensation is required for > 50 m water depth





Outer sensor glass shaft

Cathode glass shaft

Glue

Anode wire

Cathode wire

Guard cathode wire

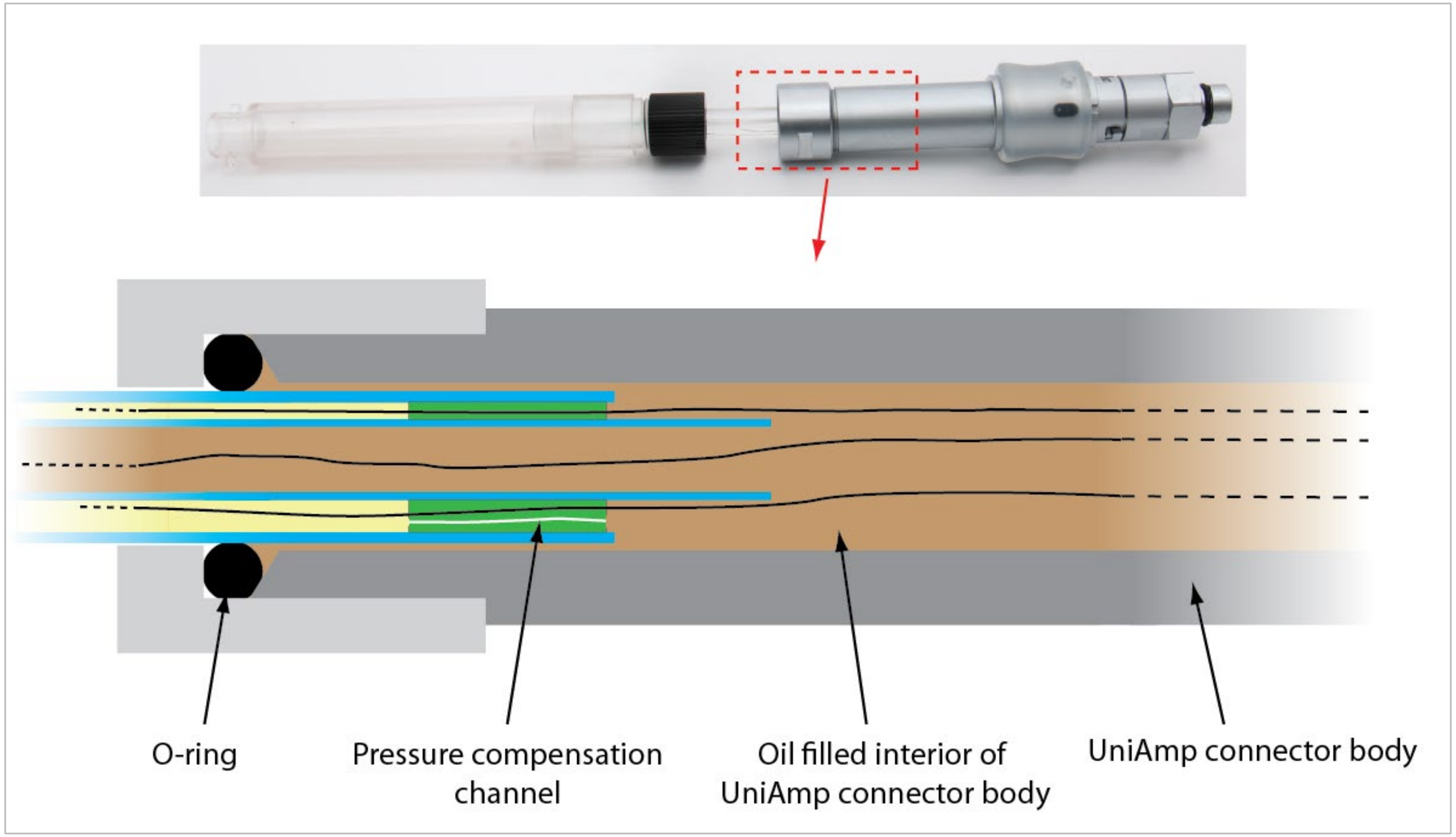
Pressure compensation wire

Electrolyte

In situ connectors

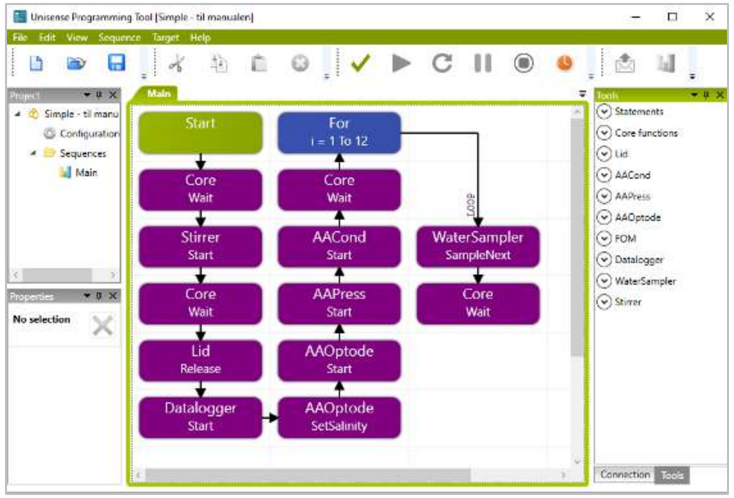
Pressure compensation

Sensor mounted in In Situ Connector



SensorTrace Suite

- Logger (freeware)
- Profile
- Rate
- Photo
- Programming tool



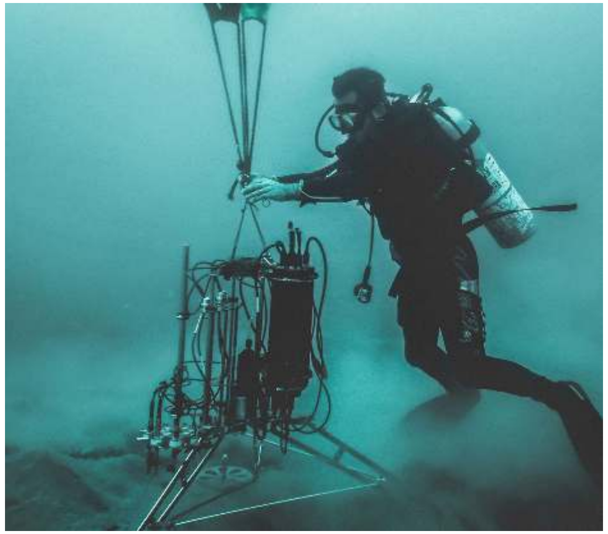
Profile

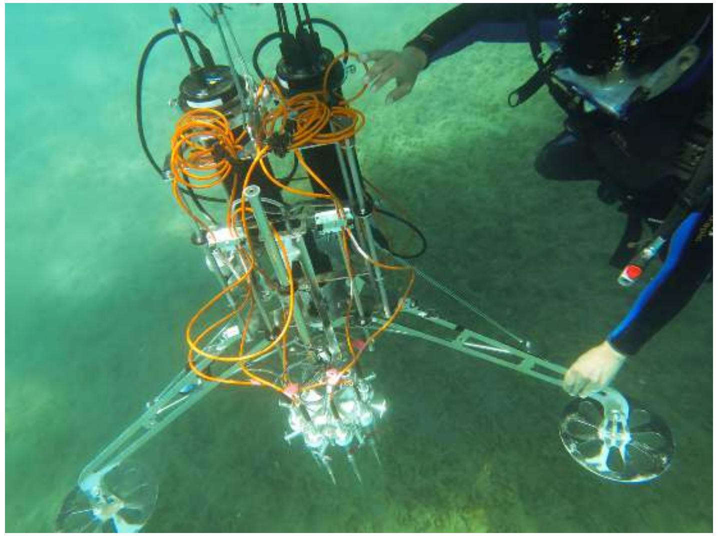
Logger (freeware)

Programming tool

In Situ MiniProfiler

- Depth distribution of analytes, e.g.:
    - O_2 penetration
    - H_2S front
    - pH depth distribution
    - Electrical potential (cable bacteria)
- Fluxes in the analysed spot
- Specific activity per volume of sediment with depth
- Activity of the sediment itself without macro fauna
- Diurnal cycles - non destructive, repeated measurements

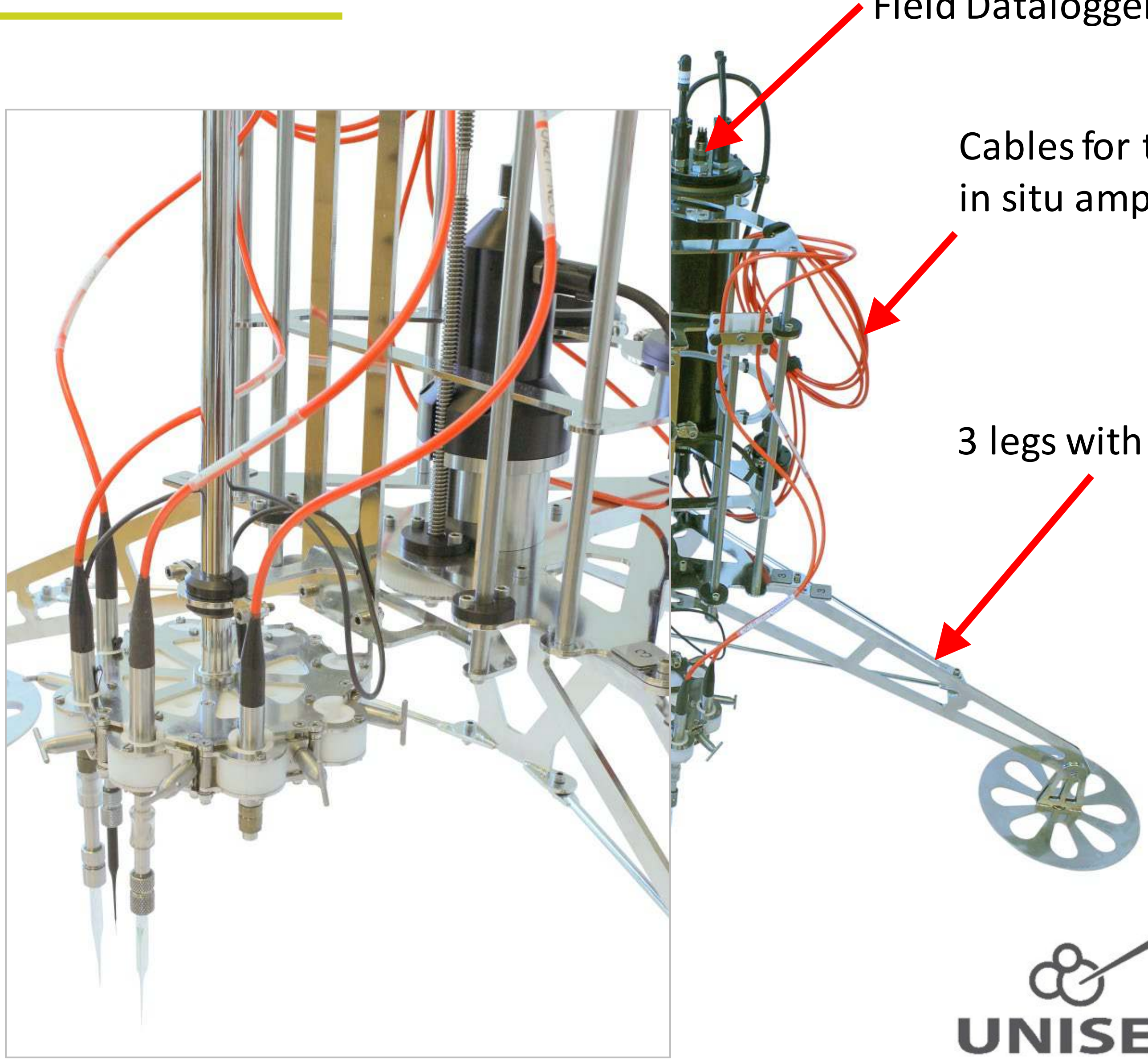




Diver assisted deployment of MiniProfiler

In Situ MiniProfiler – 1D

- Tripod
- 1.5 m between feet
- Controlled by the Field DataLogger
- Room for extra battery pack
- 4 or 8 sensor version
- 300 or 6000 m depth rating
- 50 µm step size



Field Datalogger

Cables for the in situ amplifiers

3 legs with feet

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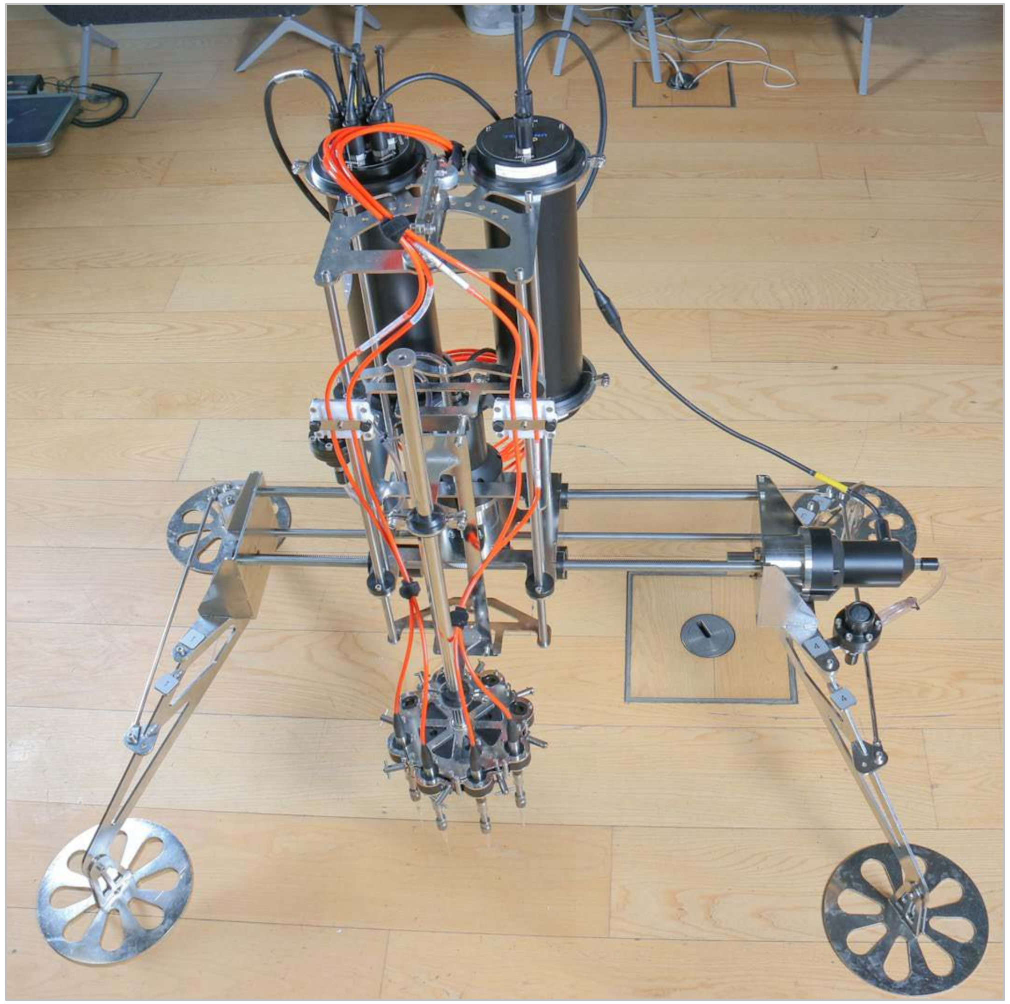
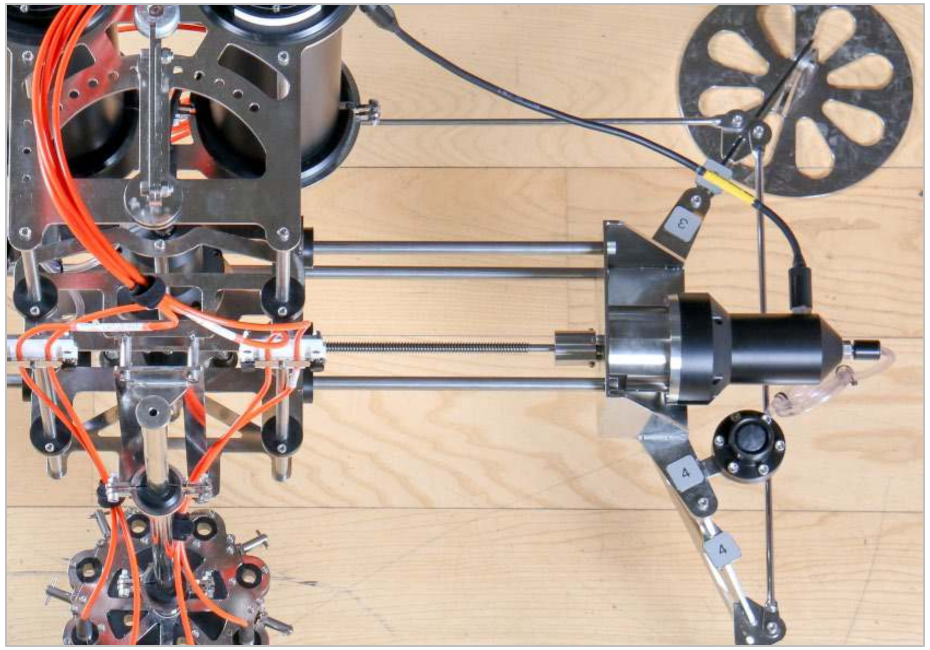
In Situ MiniProfiler – 2D

- Vertical unit same as 1D
- Horizontal movement
- Microprofiles on a line

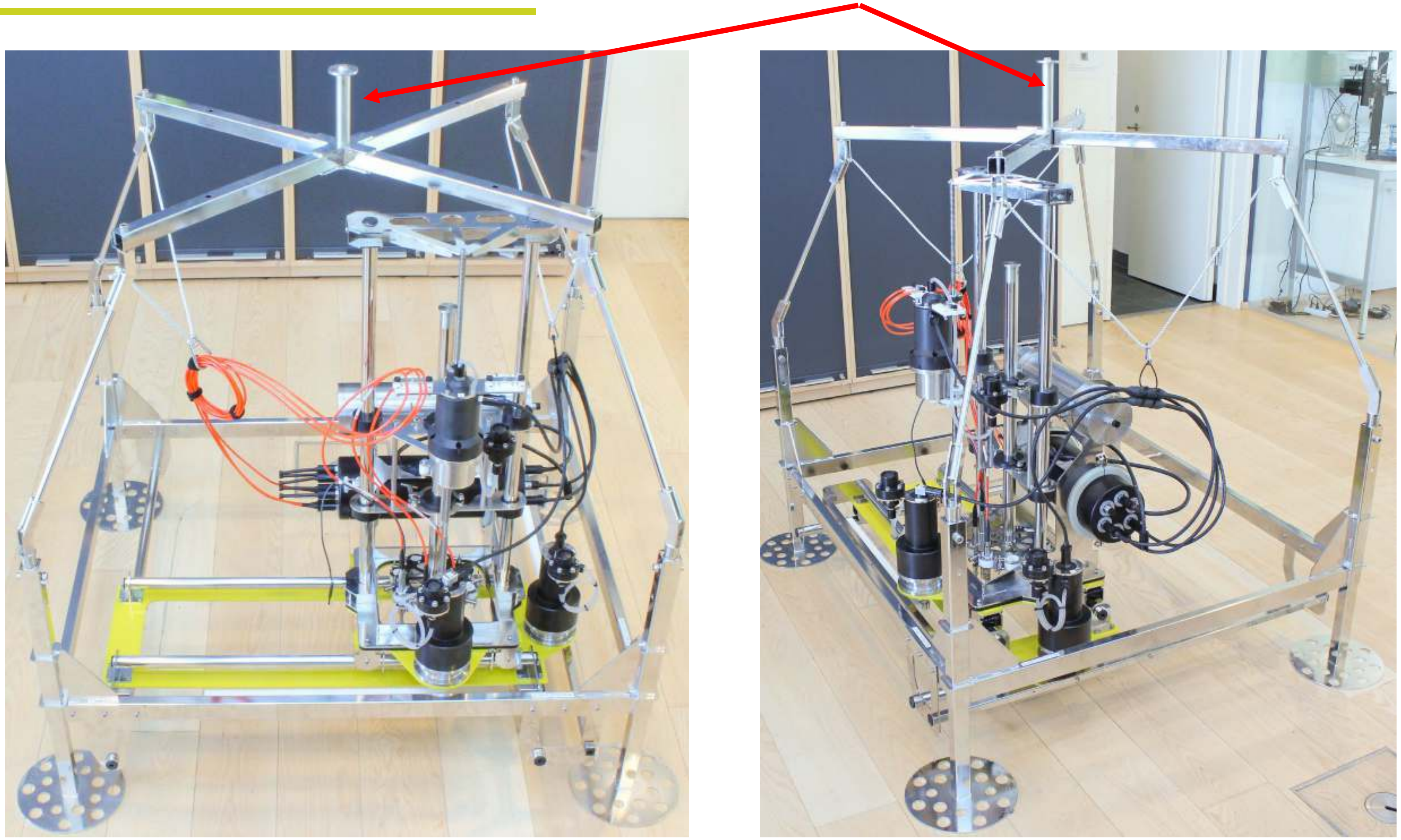




In Situ MiniProfiler – 3D

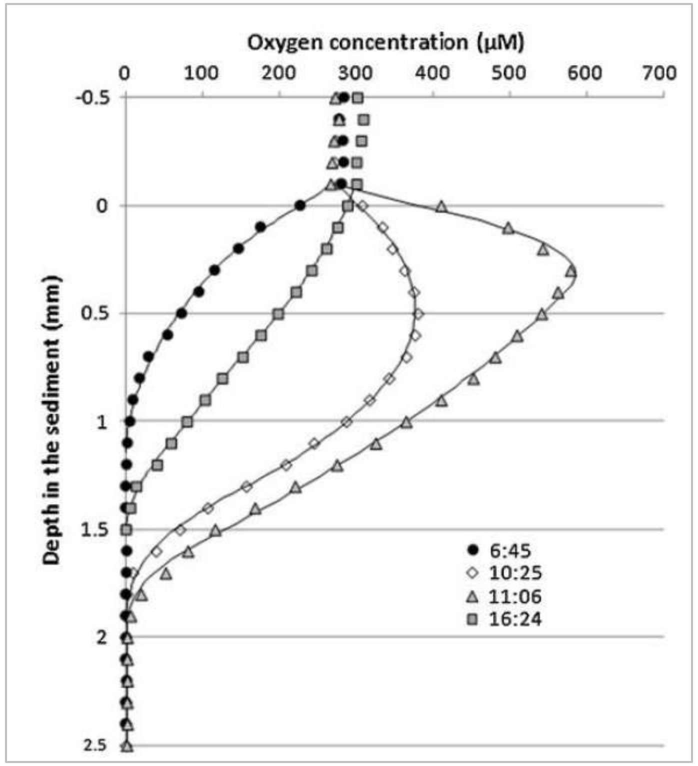
- Vertical and horizontal movement
- Microprofiles in a grid


ROV Switch

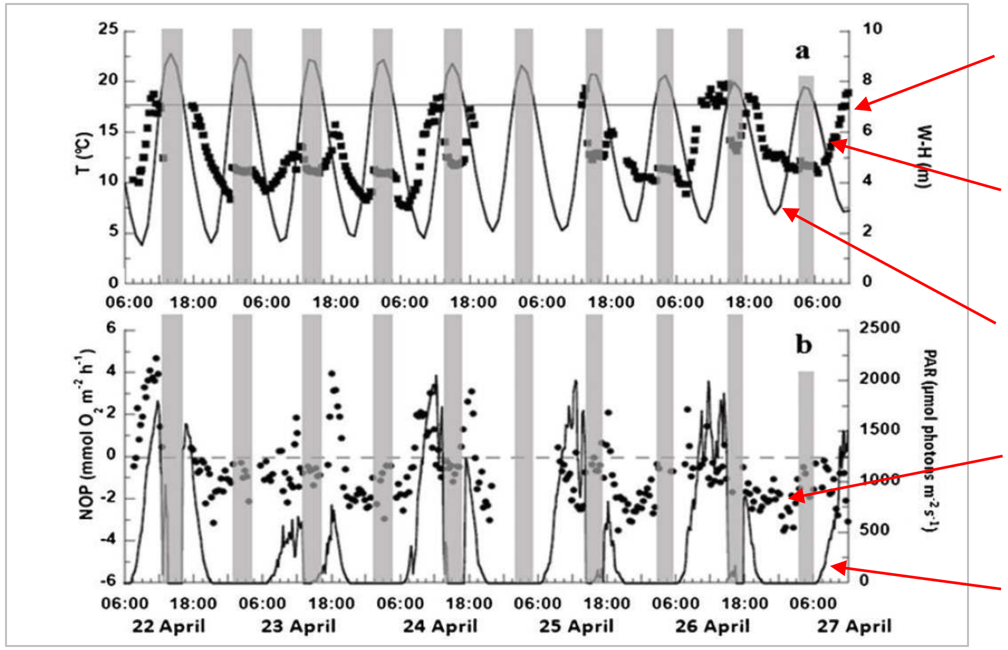

ROV Handle

In Situ MiniProfiler – Examples of data

Oxygen dynamics on intertidal flat in the English Channel, France
6-day experiment, > 900 O_2 microprofiles



Examples of microprofiles



Sediment

Temperature

Water height

Net O_2 Prod.

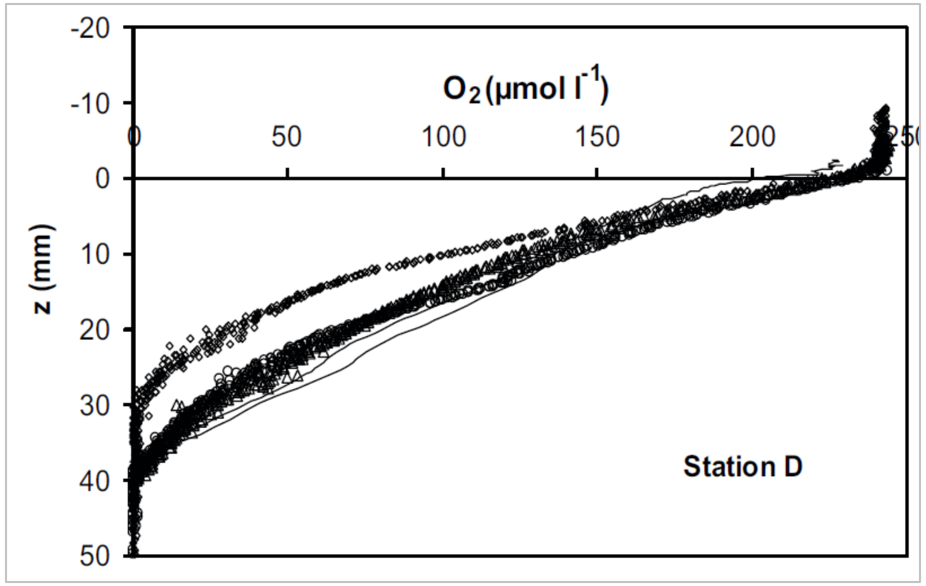
Light

Net Oxygen Production (NOP), light (PAR), temperature and water height

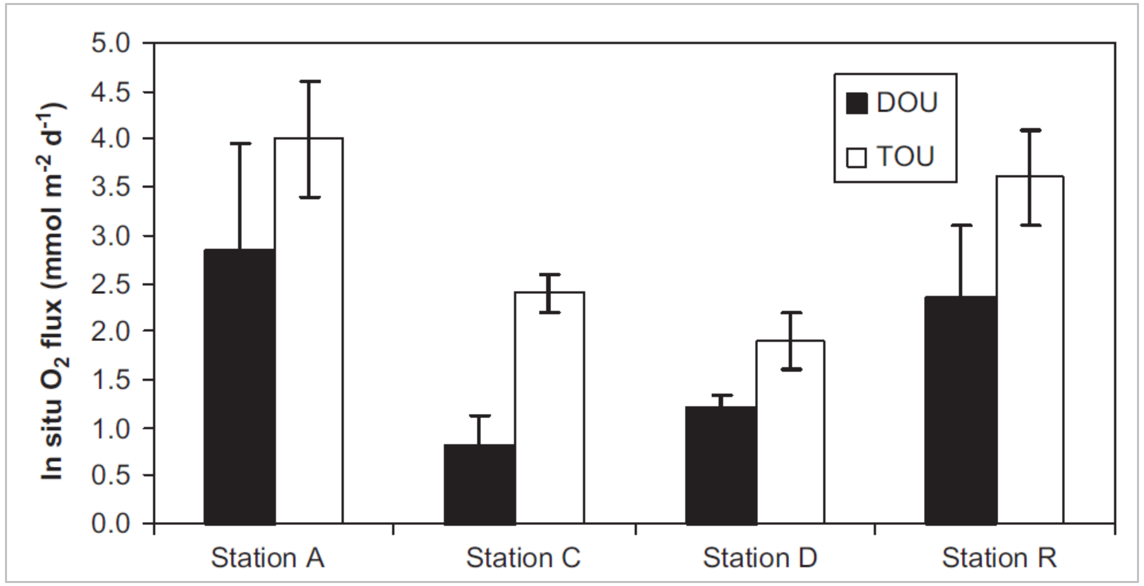
Denis, L., F. Gevaert, and N. Spilmont. 2012. Microphytobenthic production estimated by in situ oxygen microprofiling: Short-term dynamics and carbon budget implications. J. Soils Sediments 12: 1517–1529. doi:10.1007/s11368-012-0588-8

In Situ MiniProfiler – Examples of data

Oxygen penetration and uptake rates in the Southern Atlantic off Congo
Autonomous lander, 1304 – 3994 m water depth



Station D at 3964 m



DOU: Diffusive O_2 uptake from microprofiles
TOU: Total O_2 uptake from flux chamber incubation

Rabouille, C., J. C. Caprais, B. Lansard, P. Crassous, K. Dedieu, J. L. Reyss, and A. Khripounoff. 2009. Organic matter budget in the Southeast Atlantic continental margin close to the Congo Canyon: In situ measurements of sediment oxygen consumption. Deep. Res. Part II Top. Stud. Oceanogr. 56: 2223–2238. doi:10.1016/j.dsr2.2009.04.005

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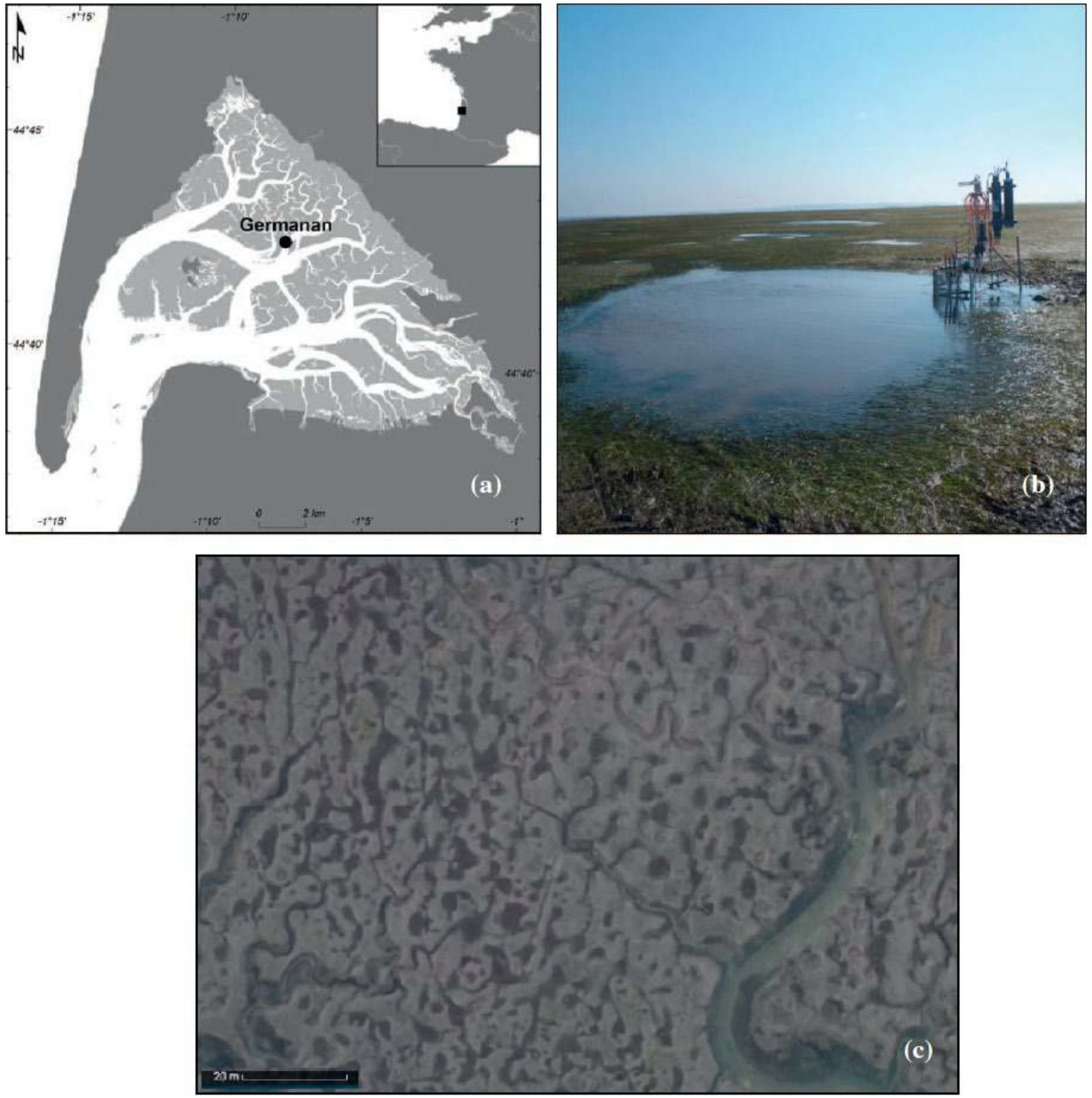
In Situ MiniProfiler – Examples of data

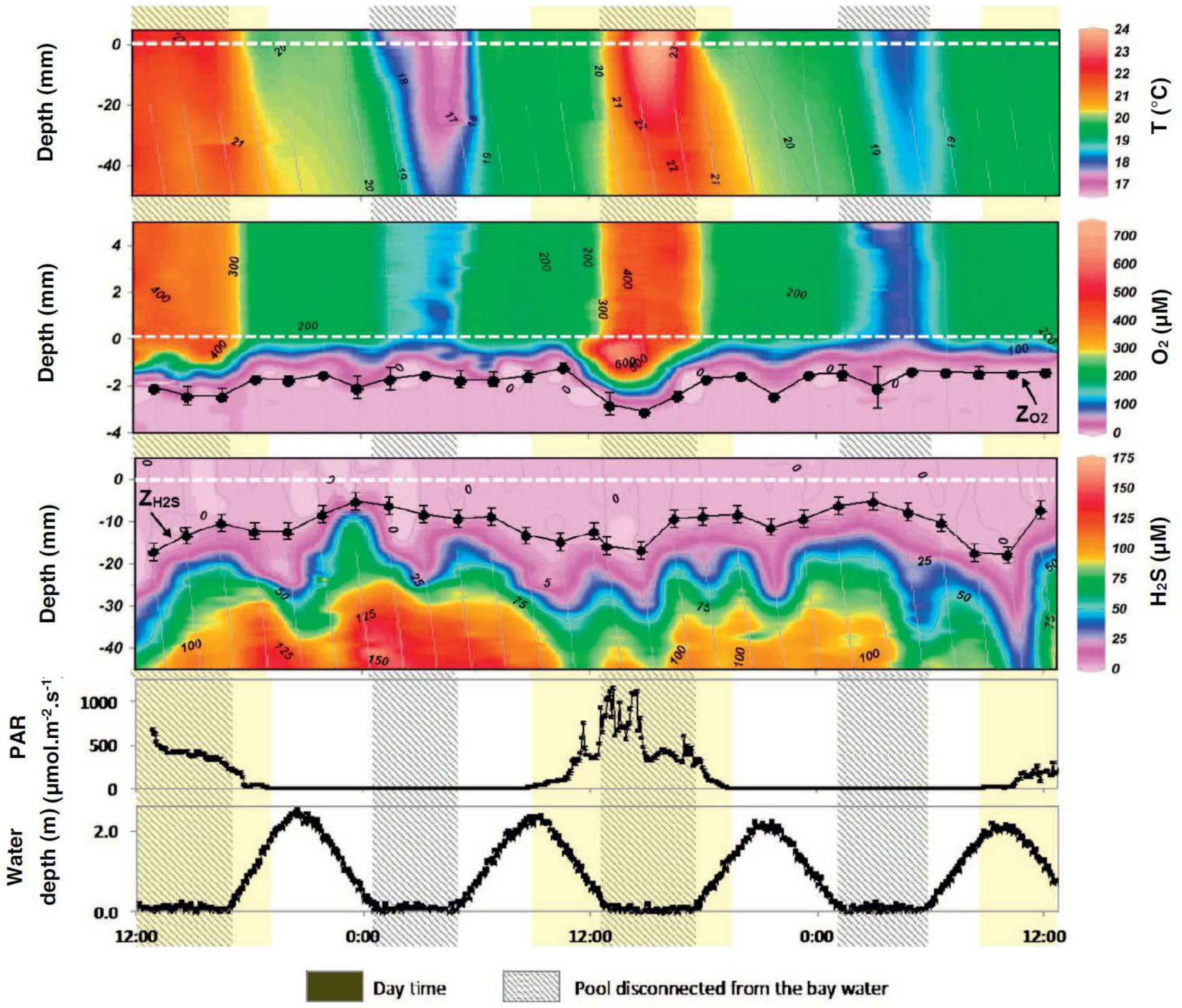


Microprofiles in tidal pools
Arcachon Bay (France)

- Tidal cycles - 2 m
- Temperature
- O_2
- H_2S

Rigaud, S., B. Deflandre, O. Maire, G. Bernard, J. C. Duchêne, D. Poirier, and P. Anschutz. 2018. Transient biogeochemistry in intertidal sediments: New insights from tidal pools in Zostera noltei meadows of Arcachon Bay (France). Mar. Chem. 200: 1–13. doi:10.1016/j.marchem.2018.02.002

In Situ MiniProfiler – Examples of data



Rigaud, S., B. Deflandre, O. Maire, G. Bernard, J. C. Duchêne, D. Poirier, and P. Anschutz. 2018. Transient biogeochemistry in intertidal sediments: New insights from tidal pools in Zostera noltei meadows of Arcachon Bay (France). Mar. Chem. 200: 1–13. doi:10.1016/j.marchem.2018.02.002

MiniChamber Lander

- Sediment-water fluxes
- Use sensors
  - Unisense sensors
  - Aanderaa sensors (O_2, conductivity, depth, turbidity)
- Use water samples
  - 12 samples during incubation
  - 60 ml sample for any analysis
- Flux estimates of larger sediment area
  - Includes activity of flora and fauna
- Ratio of flux from chamber to diffusive flux indicate faunal activity

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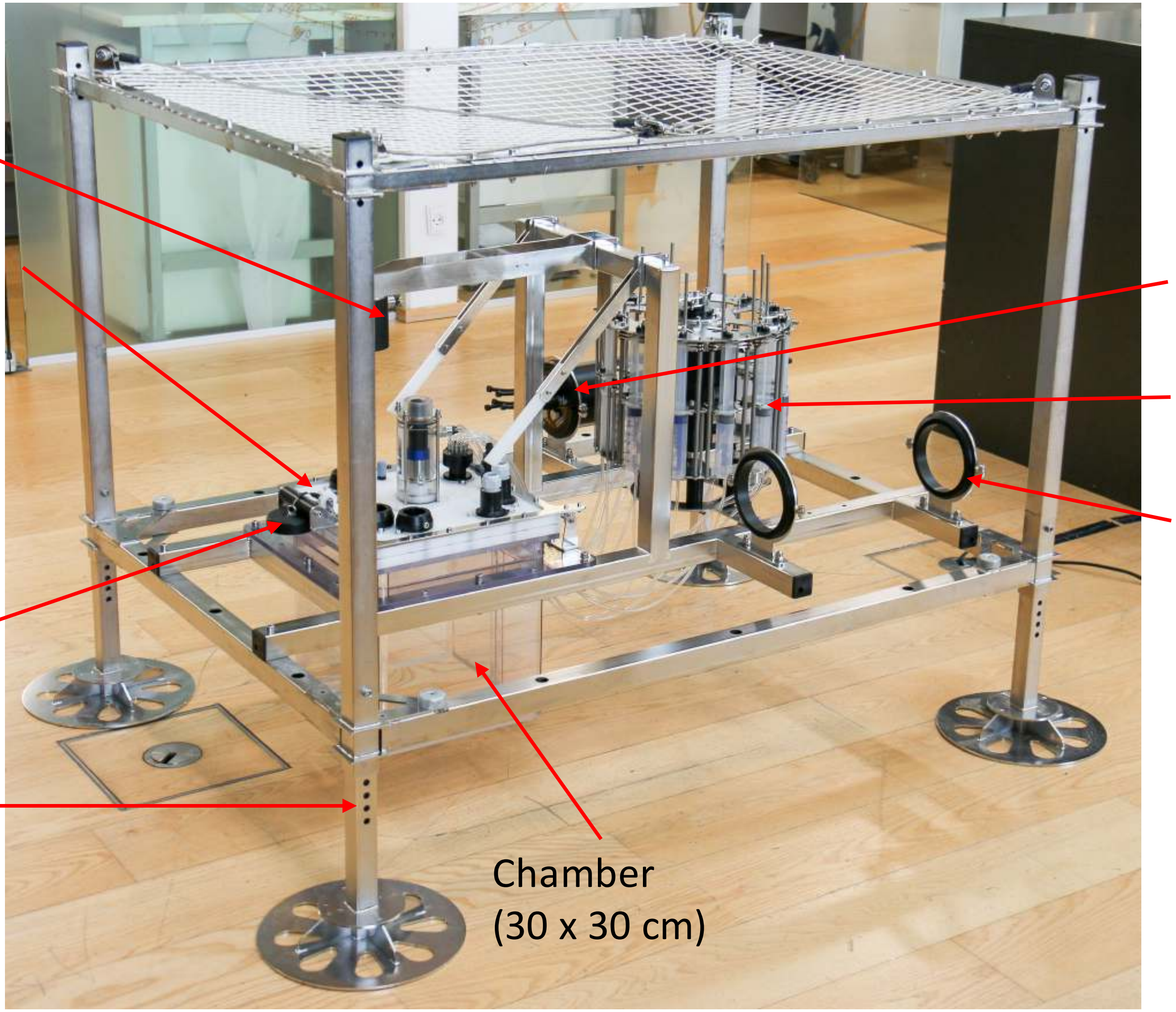
MiniChamber Lander



Magnet

Lid with
sensors

Magnet

Adjustable feet

Chamber
(30 x 30 cm)

Field Datalogger

Water sampler

Holder for extra battery

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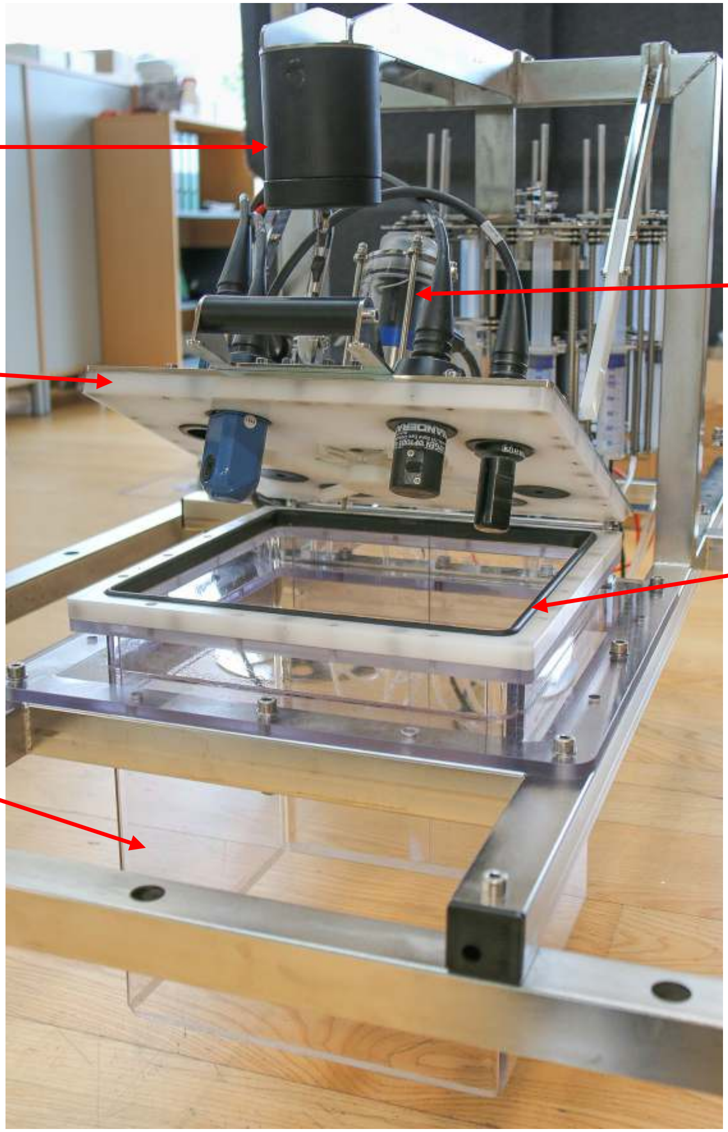
Chamber unit and lid



Permanent magnet &
counter polarized electromagnet

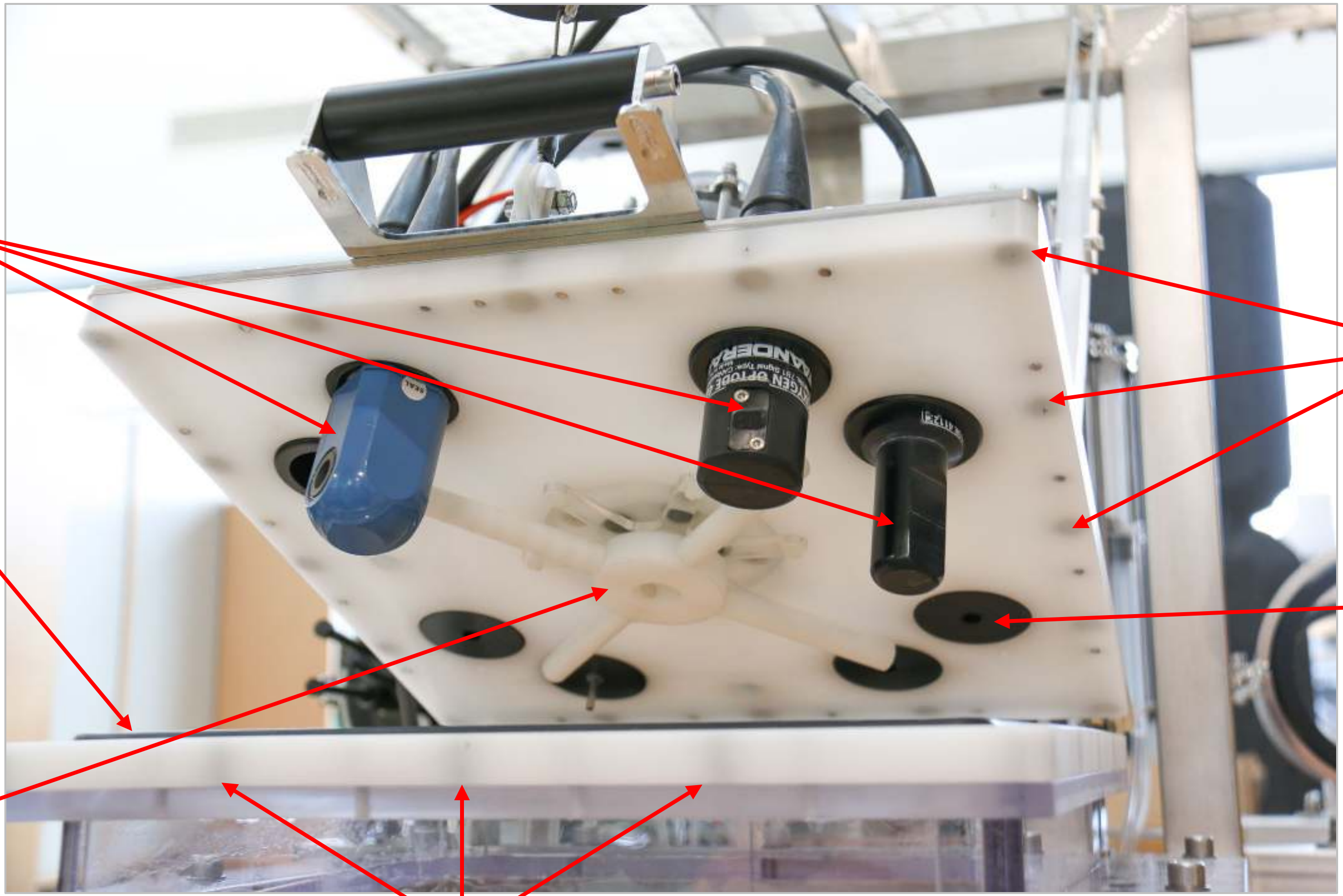
Stirrer motor

Lid (POM, Polyoxymethylene)

Rubber seal

Incubation chamber (Polycarbonate)

Lid - below



Aanderaa sensors

Rubber seal

Stirrer

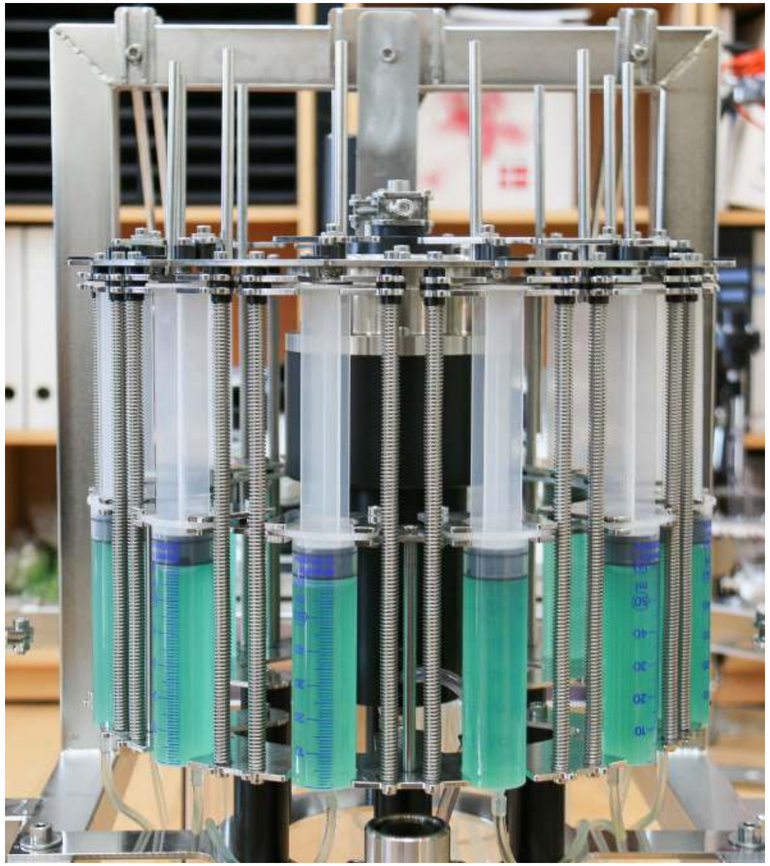
Magnets

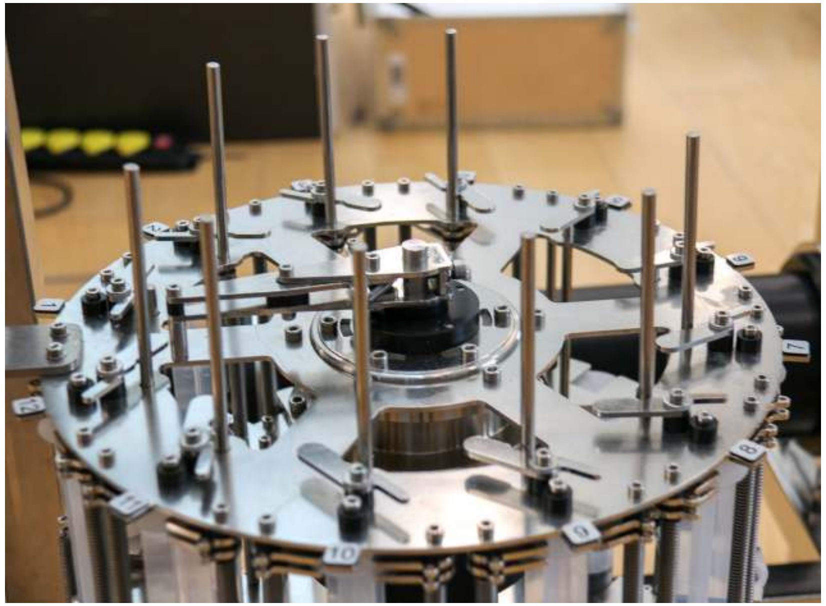
Port for Unisense sensors

Magnets

Water sampler

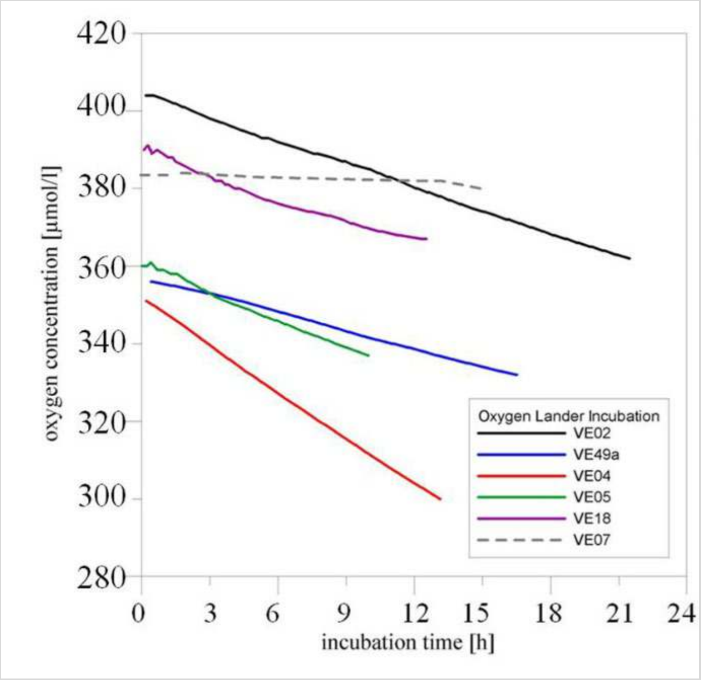
- 12 syringes
- 60 ml
- Sample or inject
- Quantify sediment-water fluxes of e.g. nutrients

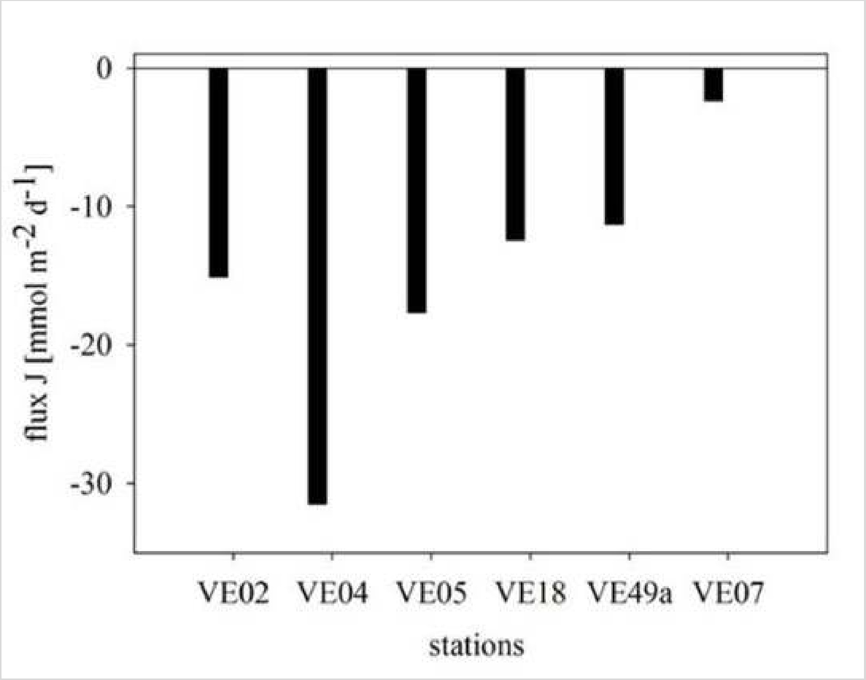




Examples of data

Oxygen uptake rates at different stations in the Baltic Sea





Deployment data

- Chamber penetration into sediment: 5 cm
- Chamber water volume: 27 l
- Incubated sediment area: 900 cm^2
- Stirring: 15 rpm
- Incubation length: 10 - 22 hours

MiniChamber Lander with O_2 optode and water sampler

Thoms, F., C. Burmeister, J. W. Dippner, M. Gogina, U. Janas, H. Kendzierska, I. Liskow, and M. Voss. 2018. Impact of macrofaunal communities on the coastal filter function in the Bay of Gdansk, Baltic Sea. Front. Mar. Sci. **5**: Article no. 205.

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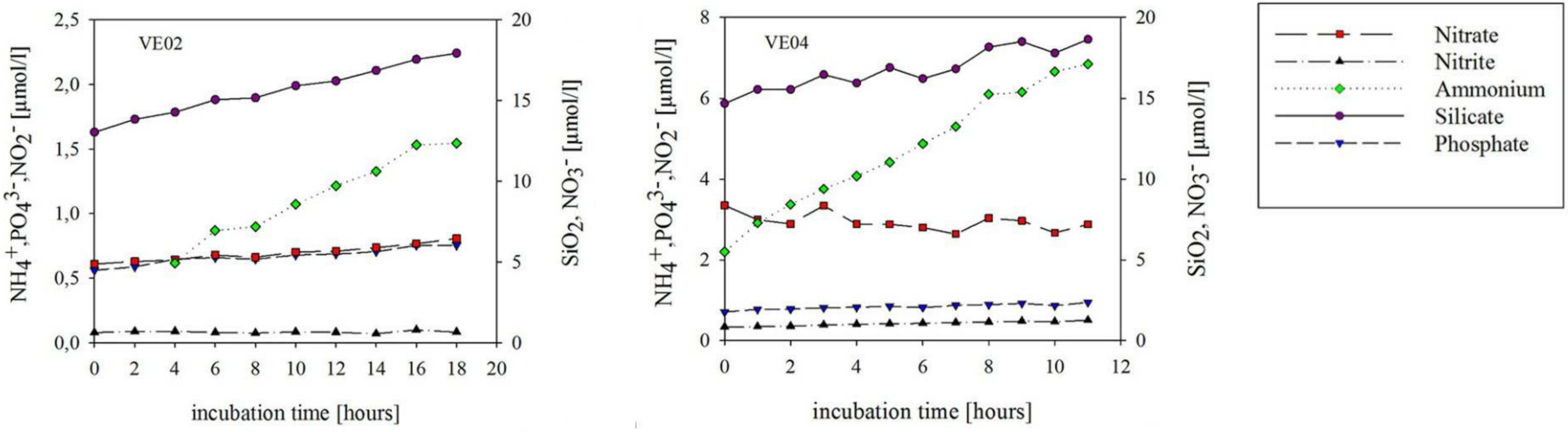
Examples of data

Sediment water fluxes of nutrients at different stations in the Baltic Sea



MiniChamber Lander with O_2 optode and water sampler

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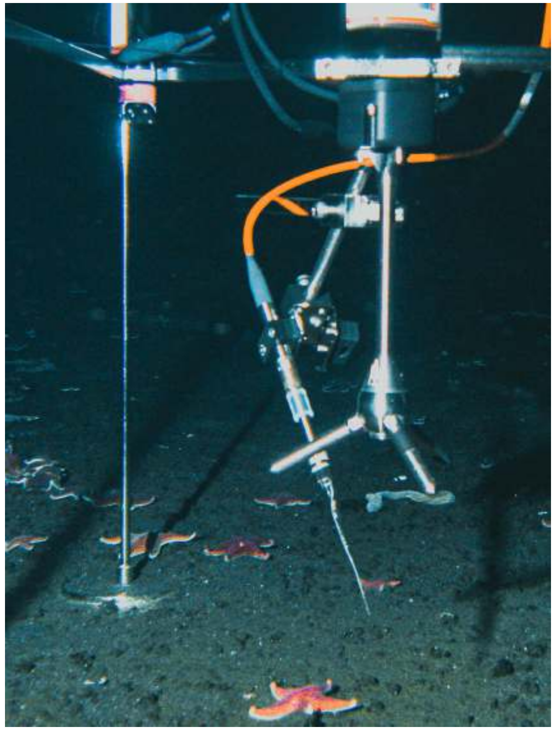
Eddy Covariance Method

- Sediment-water fluxes
- Non-invasive
- Sensors
  - O_2 microsensor (fast)
  - O_2 microoptode (fast)
  - H_2S microsensor (fast)
- Flux estimate from a large sediment area (5 - 100 m^2)
- Includes large flora and fauna e.g. seagrass beds, mussel beds



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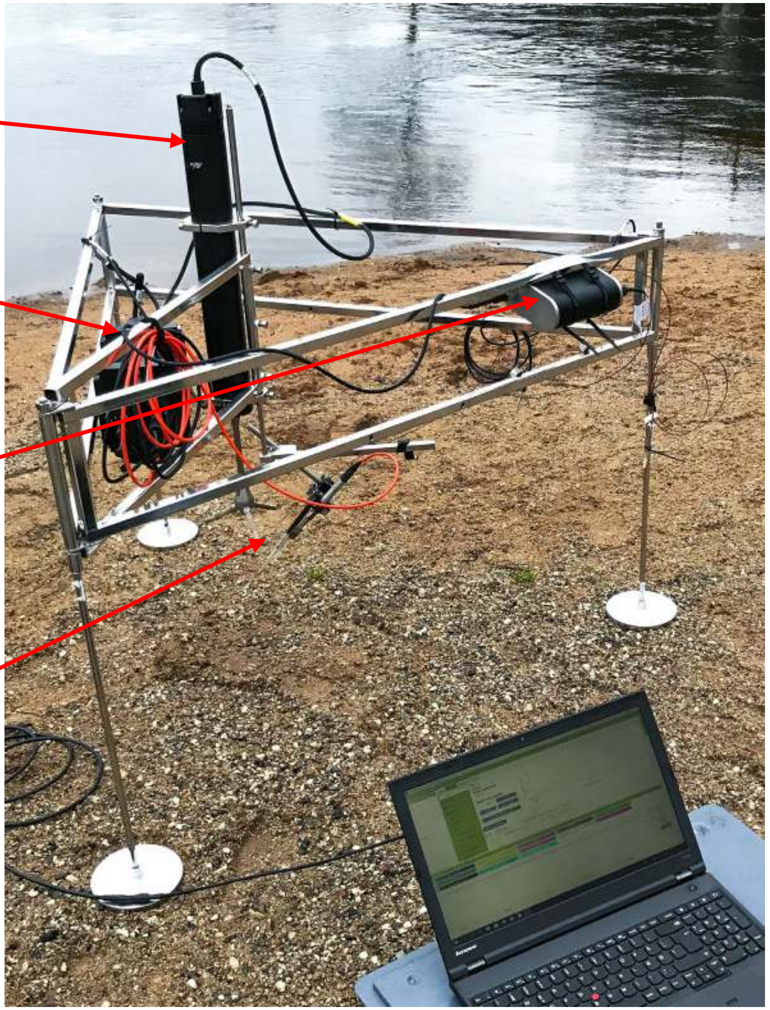
Eddy Covariance Method

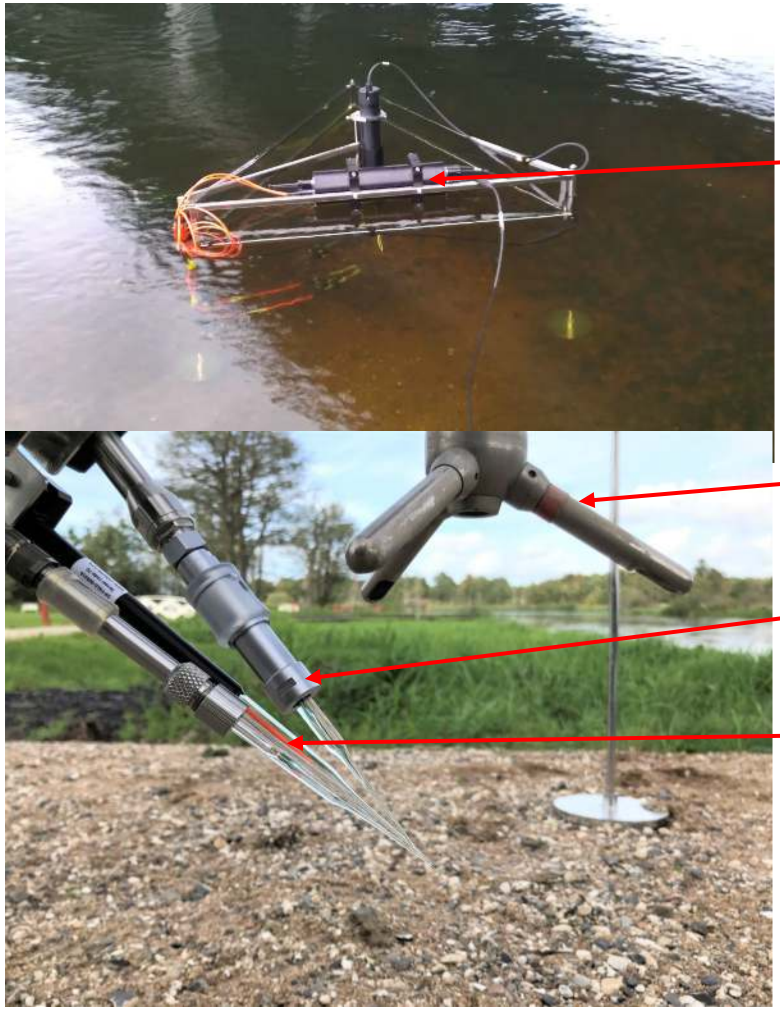


Vector (acoustic 3D current meter)

Field Datalogger

Field Optode Meter

Sensors



Field Datalogger

Vector

Clark type O_2 sensor

O_2 optode

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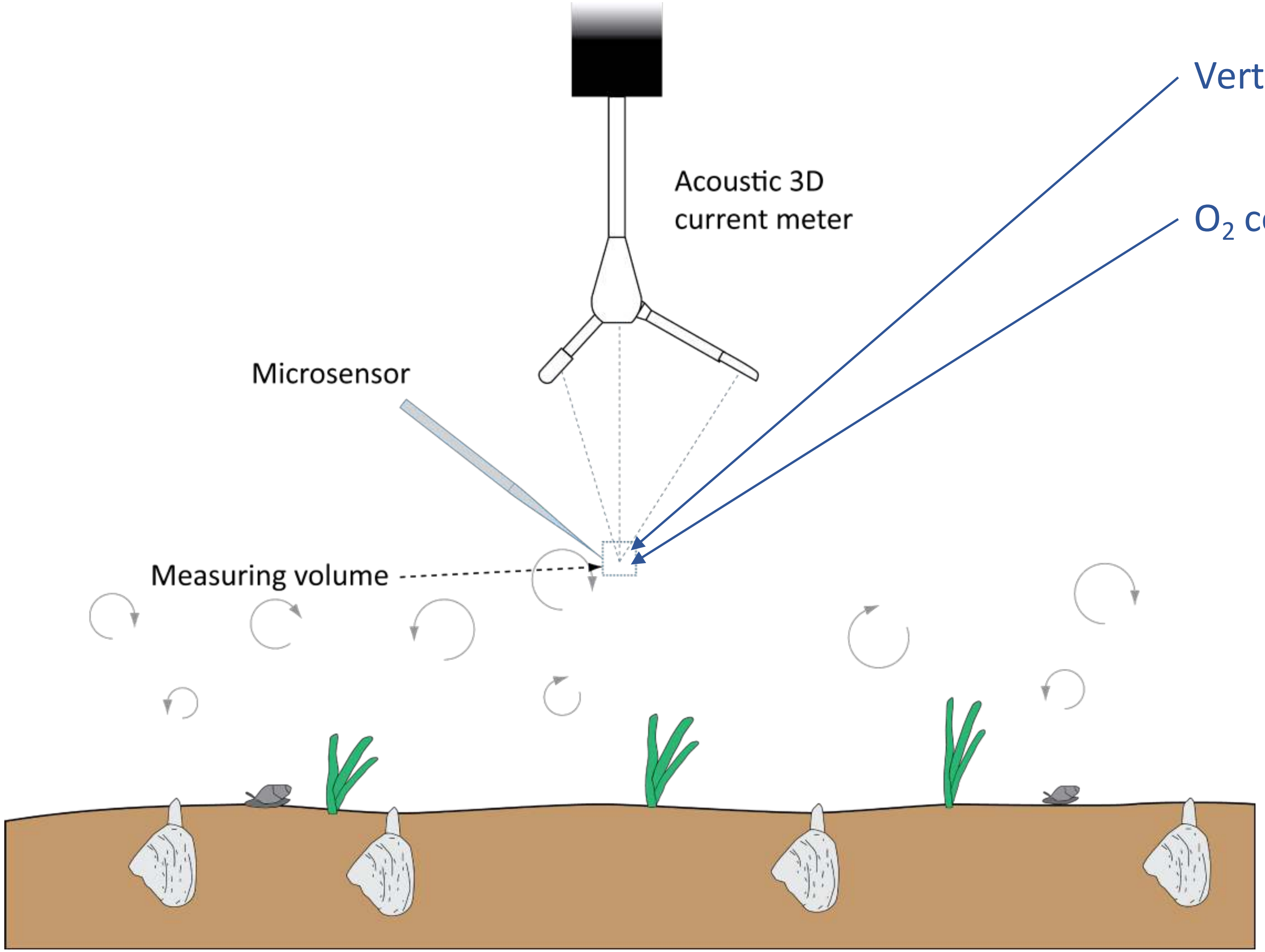
Eddy Covariance Method



Vertical flow rate (z)

O_2 concentration (C)

64 Hz

Principle of flux calculation

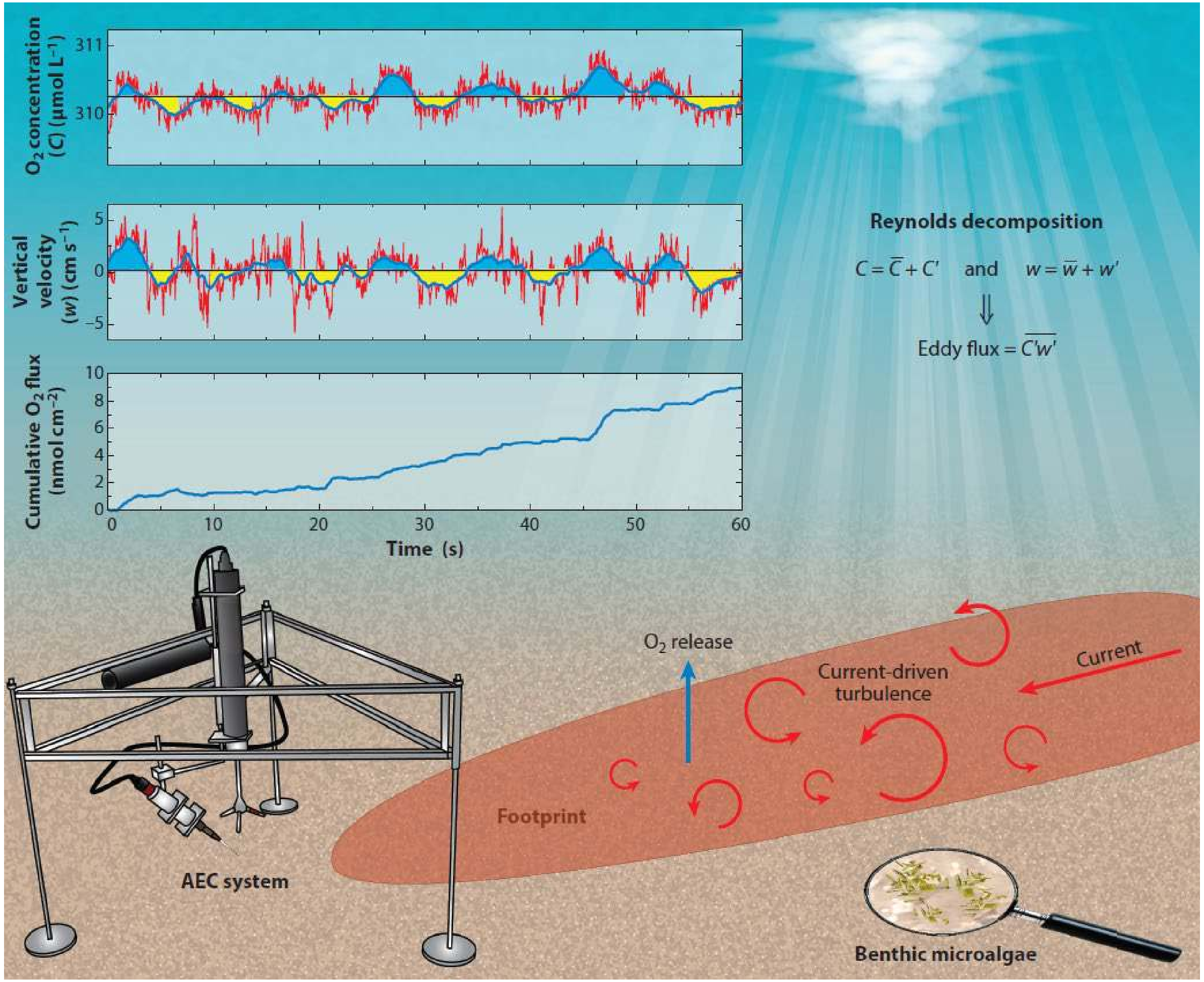
Instantaneous flux each time step (1/64 second)

Flux = z × C

Summarize flux over time to get flux rates

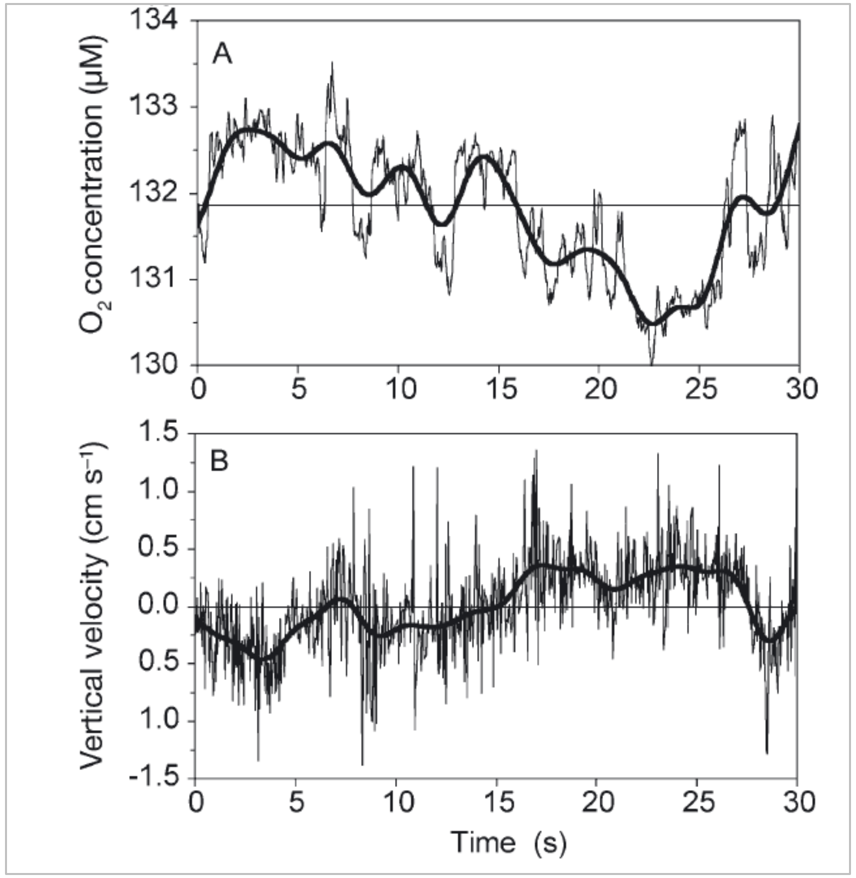
Berg, P., M. L. Delgard, R. N. Glud, M. Huettel, C. E. Reimers, and M. L. Pace. 2017. Non-invasive flux Measurements at the Benthic Interface: The Aquatic Eddy Covariance Technique. Limnol. Oceanogr. e-Lectures **7**: 1–50. doi:10.1002/loe2.10005

Eddy Covariance - Principle

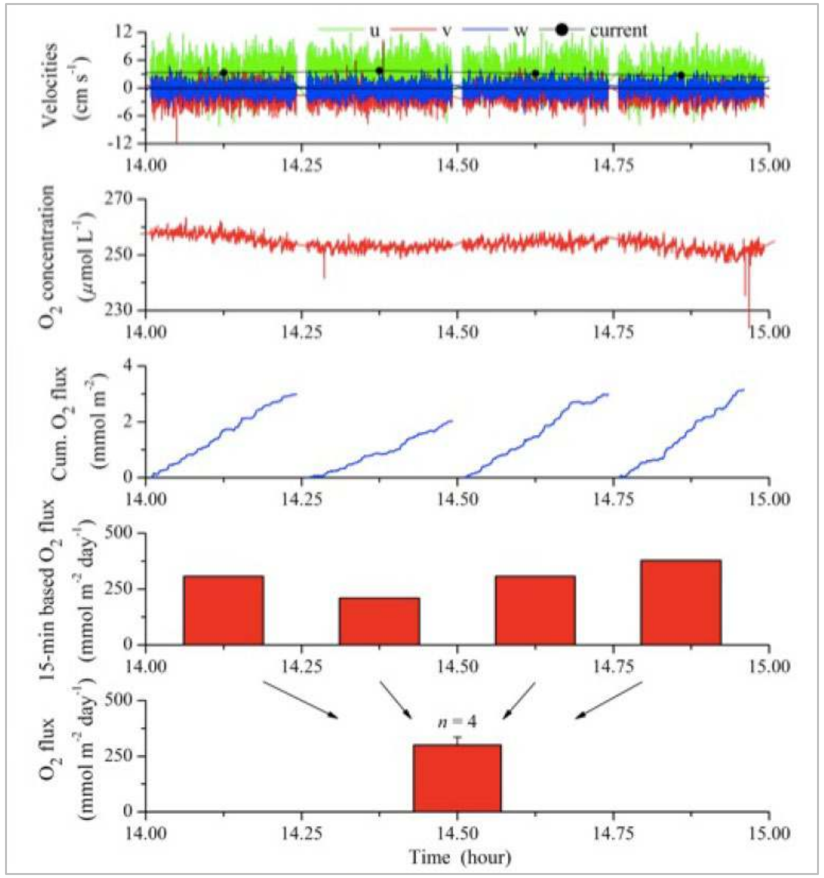


Berg, P., M. Huettel, R. N. Glud, C. E. Reimers, and K. M. Attard. 2022. Aquatic Eddy Covariance: The Method and Its Contributions to Defining Oxygen and Carbon Fluxes in Marine Environments. Ann. Rev. Mar. Sci. 14: 431–455. doi:10.1146/annurev-marine-042121-012329

Eddy Covariance - Principle



Berg, P., H. Roy, F. Janssen, V. Meyer, B. B. Jørgensen, M. Huettel, and D. de Beer. 2003. Oxygen uptake by aquatic sediments measured with a novel non-invasive eddy-correlation technique. Mar. Ecol. Ser. 261: 75–83.



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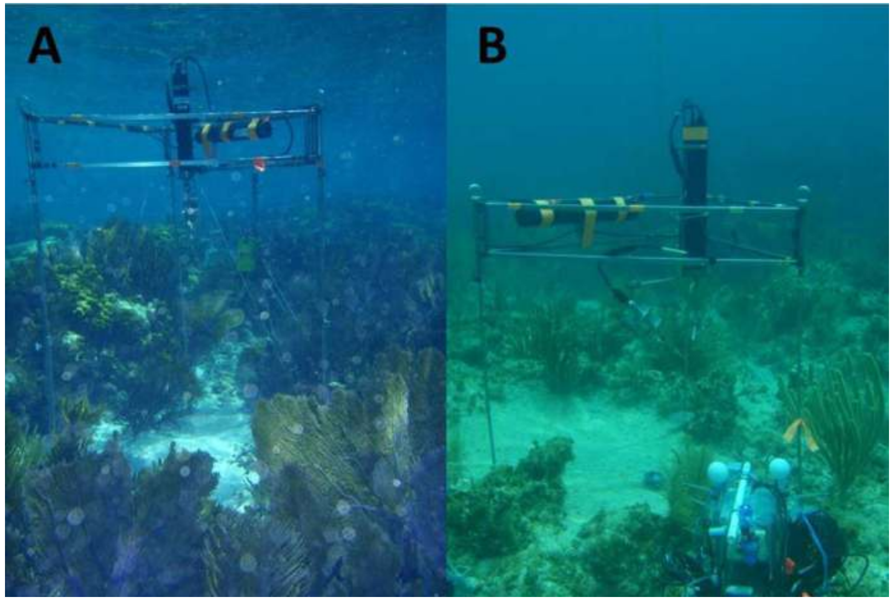
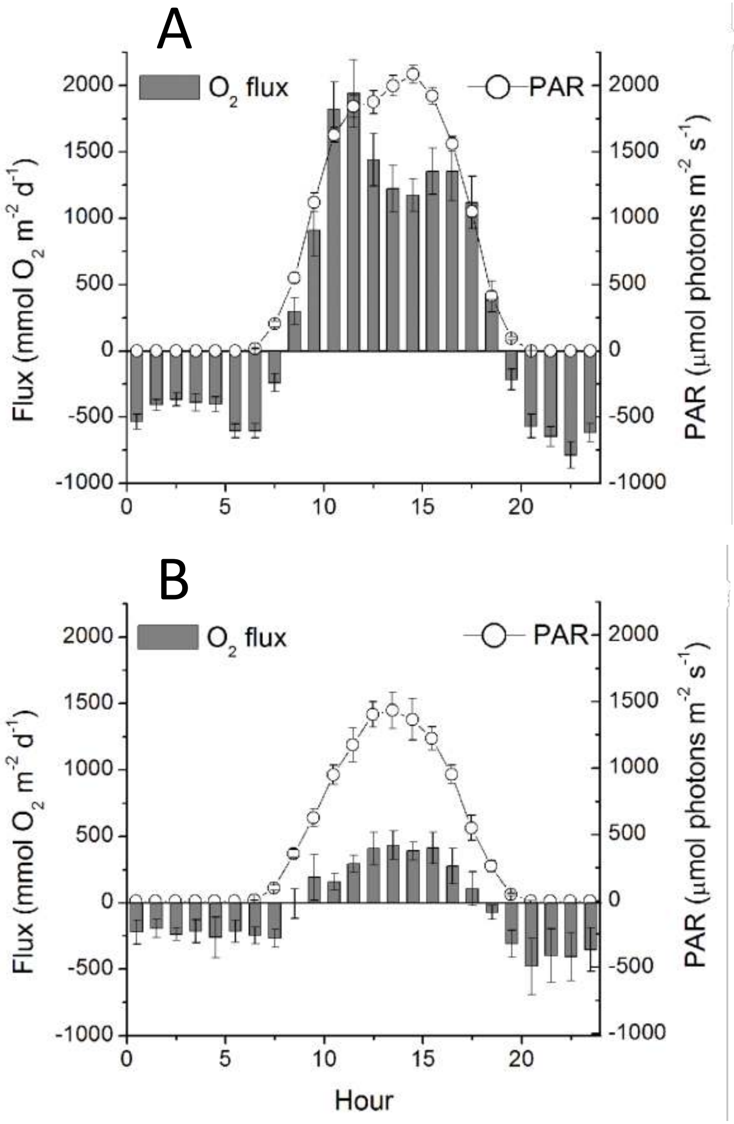
Eddy Covariance - Example of data

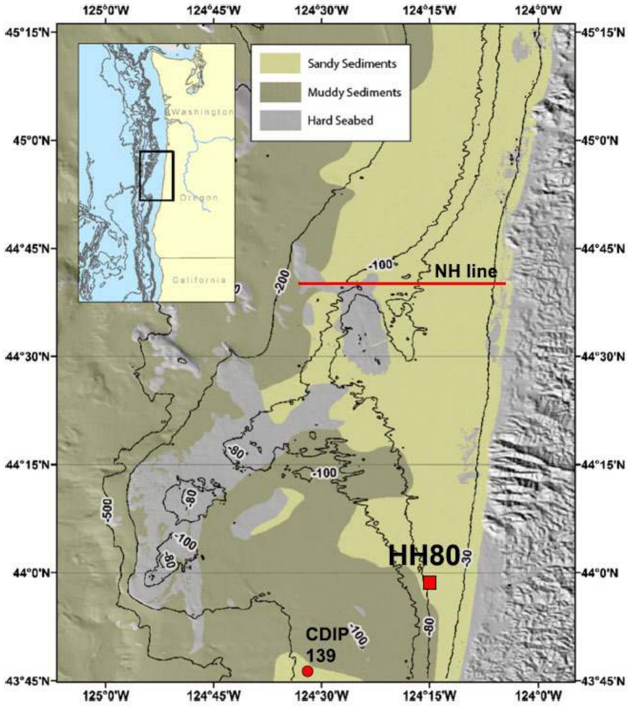
Coral reef – Oxygen fluxes

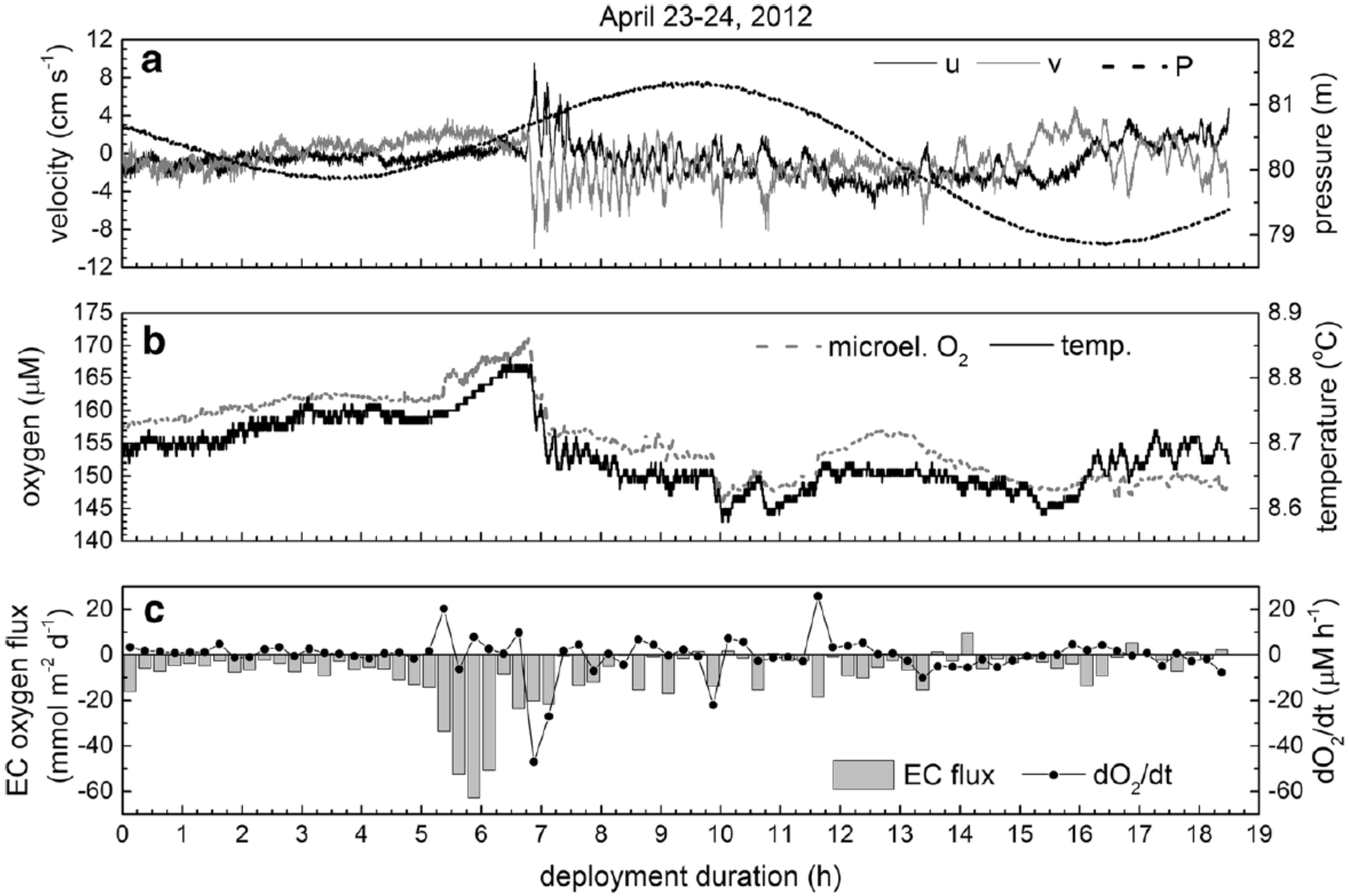


Long, M. H., P. Berg, D. de Beer, and J. C. Zieman. 2013. In Situ Coral Reef Oxygen Metabolism: An Eddy Correlation Study. PLoS One 8. doi:10.1371/journal.pone.0058581



Eddy Covariance - Example of data

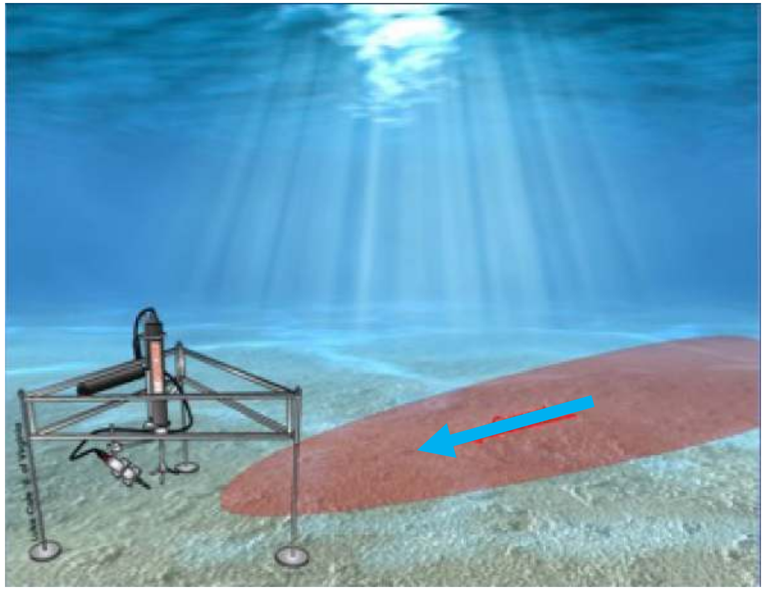


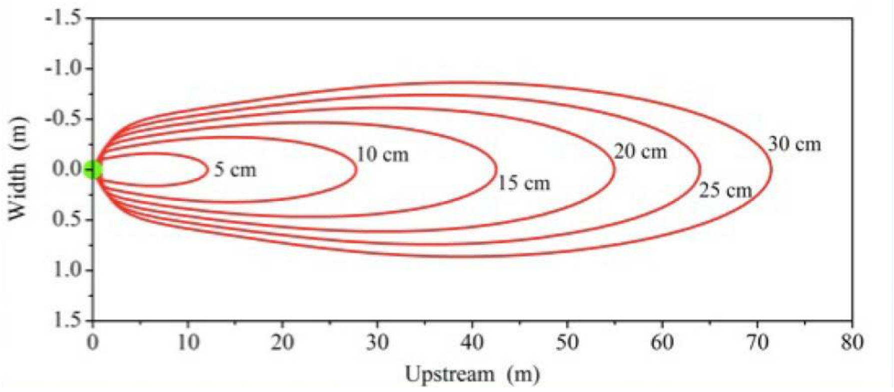


Reimers, C. E., H. T. Ozkan-Haller, R. D. Sanders, K. McCann-Grosvenor, P. J. Chace, and S. A. Crowe. 2016. The Dynamics of Benthic Respiration at a Mid-Shelf Station Off Oregon. Aquat. Geochemistry **22**: 505–527. doi:10.1007/s10498-016-9303-5

Eddy Covariance - Footprint

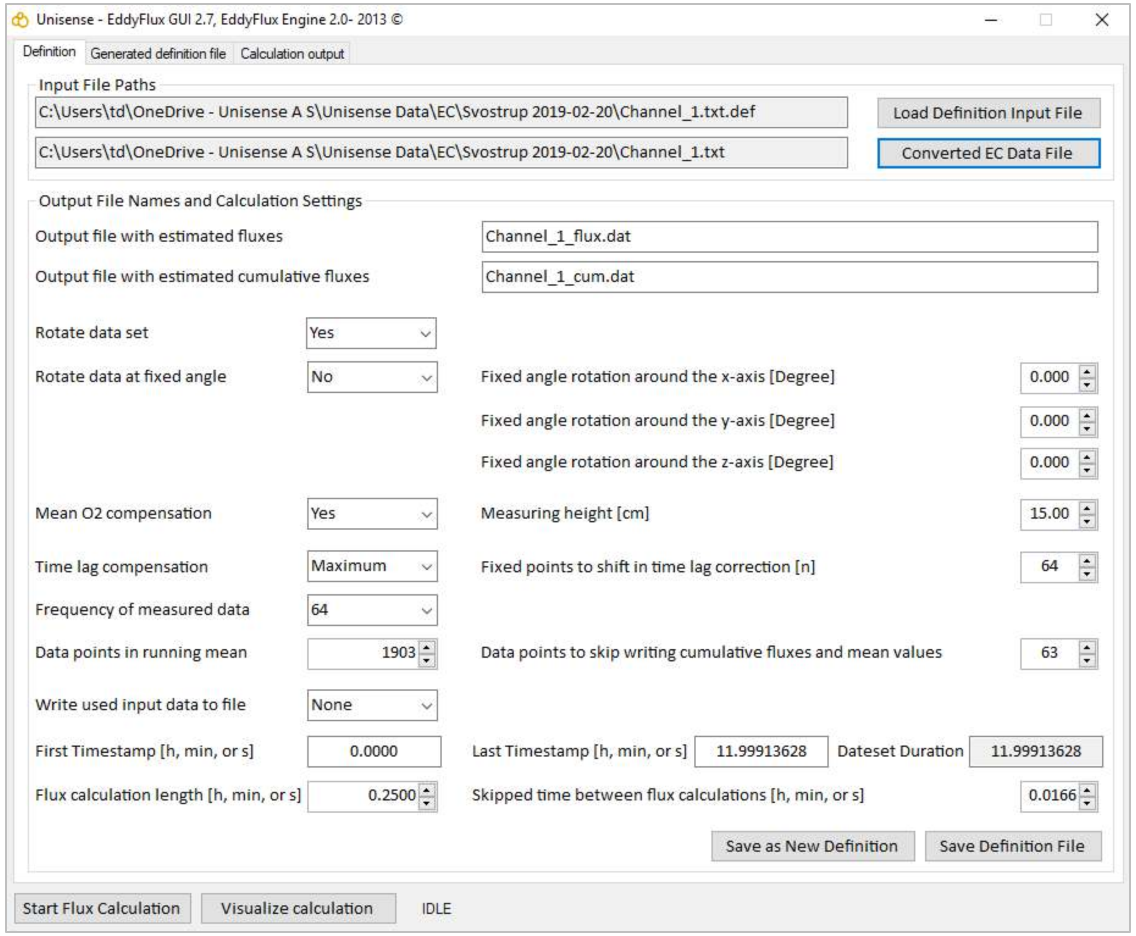
- Measured flux originates from downstream – The *footprint*
- Typical size 5 to 100 m^2
- Calculated according to Berg et al., 2007
- Affected by
  - Sensor height over surface
  - Flow conditions
  - Sediment roughness





Berg, P., H. Roy, and P. L. Wiberg. 2007. Eddy correlation flux measurements: The sediment surface area that contributes to the flux. Limnol. Oceanogr. 52: 1672–1684.

Unisense EddyFlux Graphical User Interface



Input File Paths

C:\Users\td\OneDrive - Unisense A S\Unisense Data\EC\Svostrup 2019-02-20\Channel_1.txt.def

C:\Users\td\OneDrive - Unisense A S\Unisense Data\EC\Svostrup 2019-02-20\Channel_1.txt

Output File Names and Calculation Settings

Output file with estimated fluxes	Channel_1_flux.dat		
Output file with estimated cumulative fluxes	Channel_1_cum.dat		
Rotate data set	Yes		
Rotate data at fixed angle	No	Fixed angle rotation around the x-axis [Degree]	0.000
		Fixed angle rotation around the y-axis [Degree]	0.000
		Fixed angle rotation around the z-axis [Degree]	0.000
Mean O2 compensation	Yes	Measuring height [cm]	15.00
Time lag compensation	Maximum	Fixed points to shift in time lag correction [n]	64
Frequency of measured data	64		
Data points in running mean	1903	Data points to skip writing cumulative fluxes and mean values	63
Write used input data to file	None		
First Timestamp [h, min, or s]	0.0000	Last Timestamp [h, min, or s]	11.99913628
		Dateset Duration	11.99913628
Flux calculation length [h, min, or s]	0.2500	Skipped time between flux calculations [h, min, or s]	0.0166

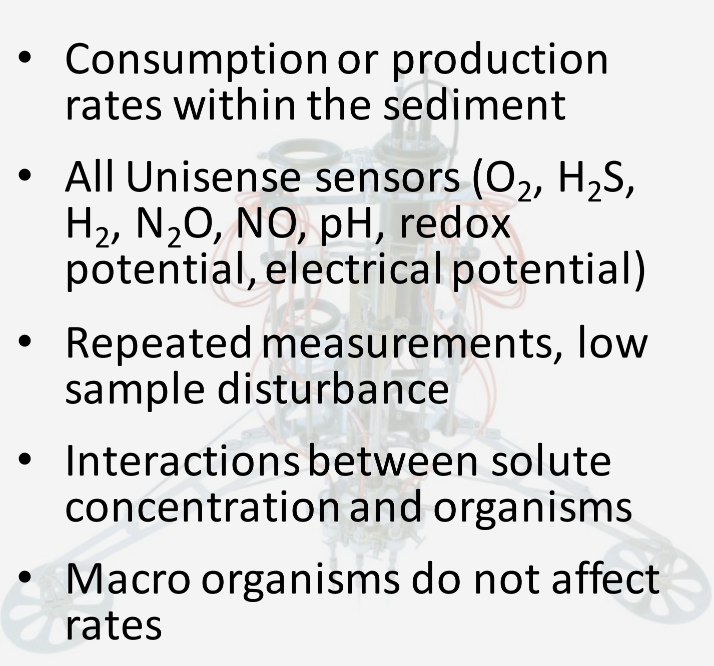
IDLE

Data sets: Data set 7

Flux 1 -2.539 mmol/m²/day **Flux 2** -16.746 mmol/m²/day **Flux 3** -20.821 mmol/m²/day

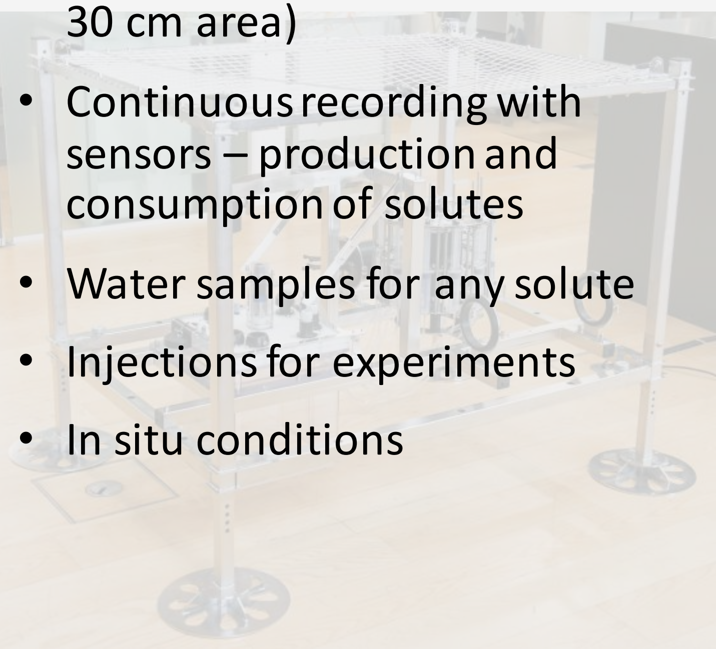
Three complementary methods

Microprofiles



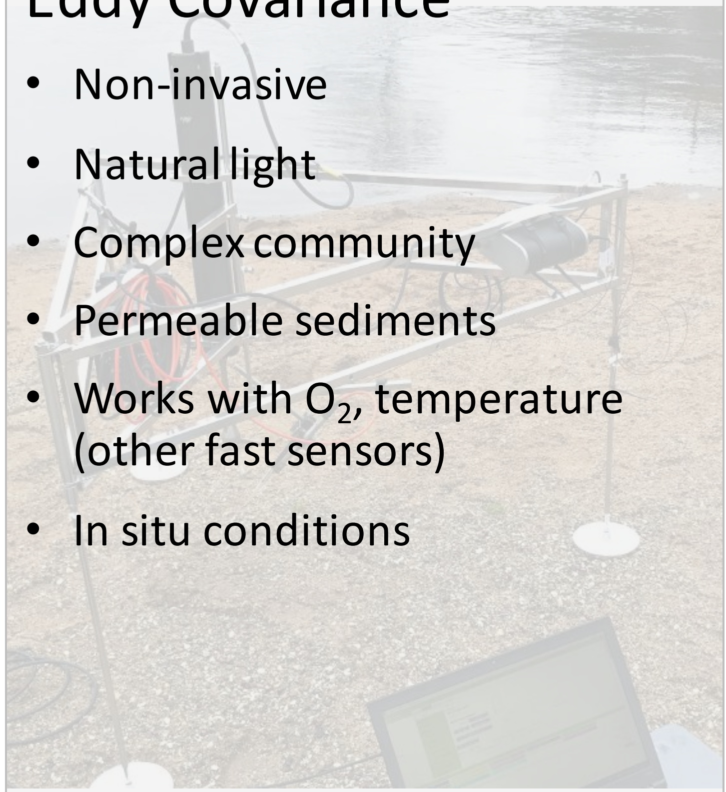
- Vertical distribution of solutes
- Consumption or production rates within the sediment
- All Unisense sensors (O_2, H_2S, H_2, N_2O, NO, pH, redox potential, electrical potential)
- Repeated measurements, low sample disturbance
- Interactions between solute concentration and organisms
- Macro organisms do not affect rates
- In situ conditions

MiniChamber



- Community metabolism (30 x 30 cm area)
- Continuous recording with sensors – production and consumption of solutes
- Water samples for any solute
- Injections for experiments
- In situ conditions

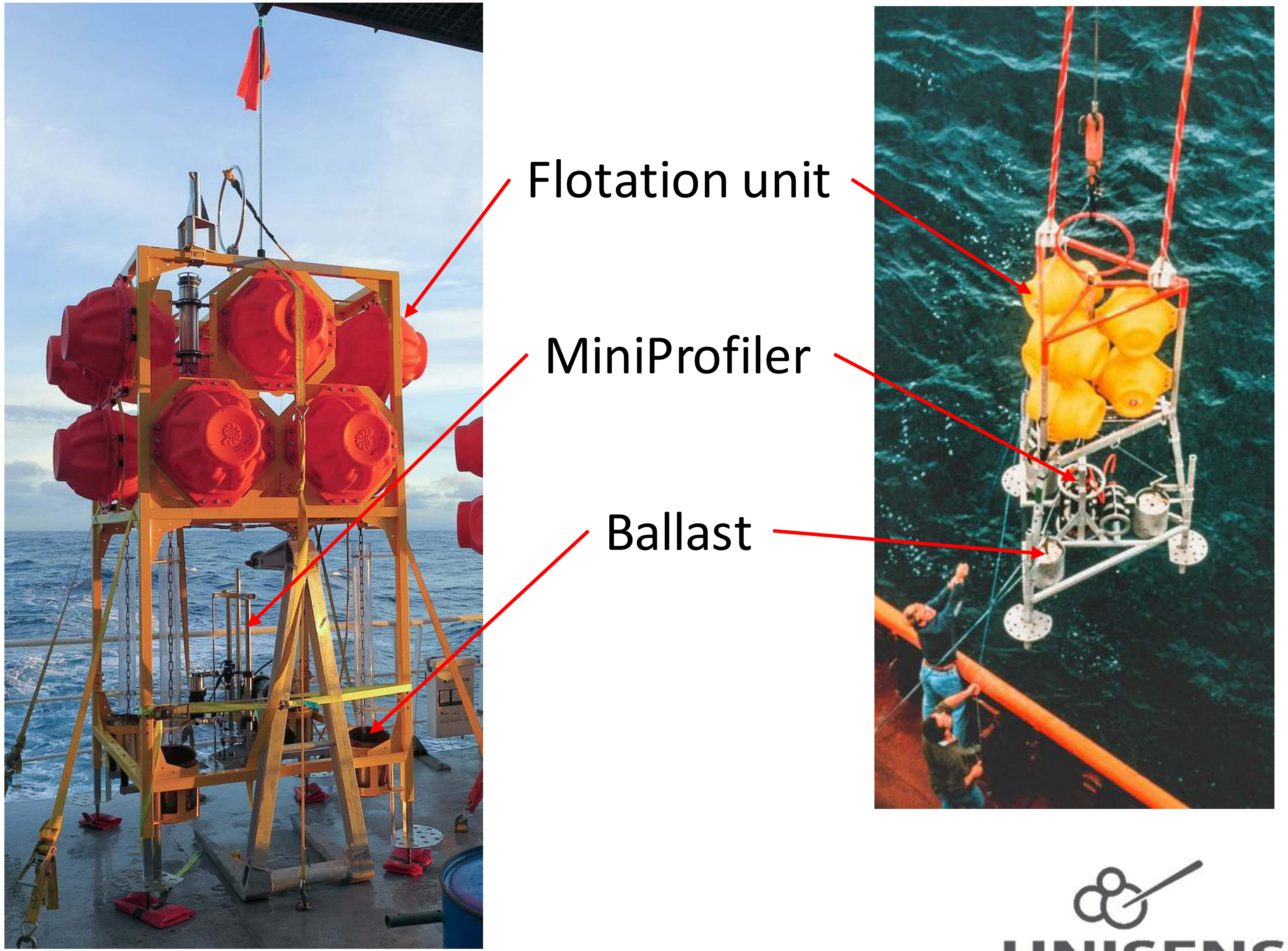
Eddy Covariance



- Non-invasive
- Natural light
- Complex community
- Permeable sediments
- Works with O_2, temperature (other fast sensors)
- In situ conditions

Autonomous free falling deep sea landers

- Flotation unit
- Releasable ballast
- Chamber, Profiler or Eddy Covariation system
- Release ballast for positive buoyancy
  - Burn wire for programmed ascent
  - Acoustic release, redundant
  - Magnesium bolt for safety
- GPS transmitter
- Radio beacon
- Radar reflector



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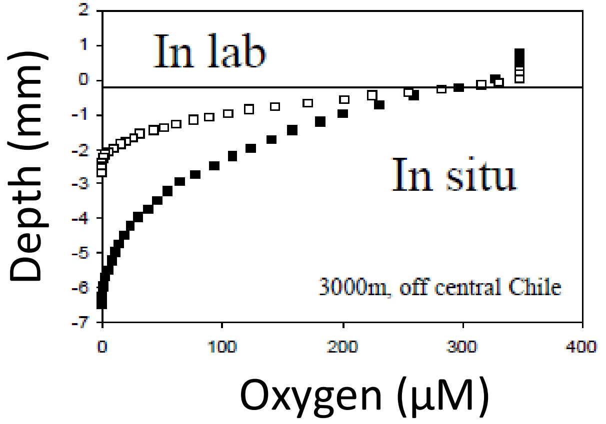
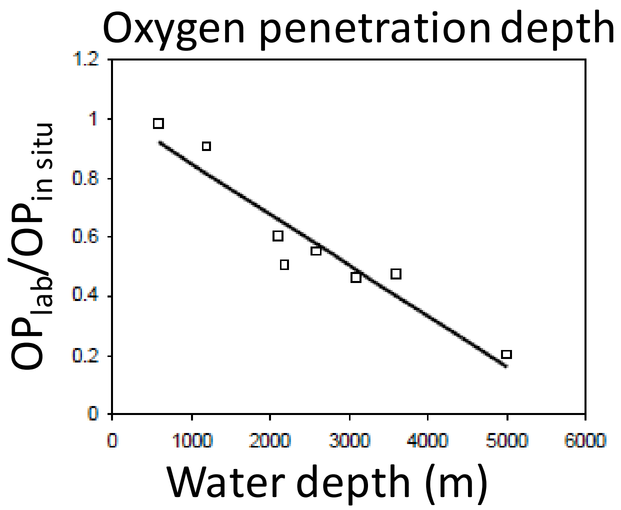
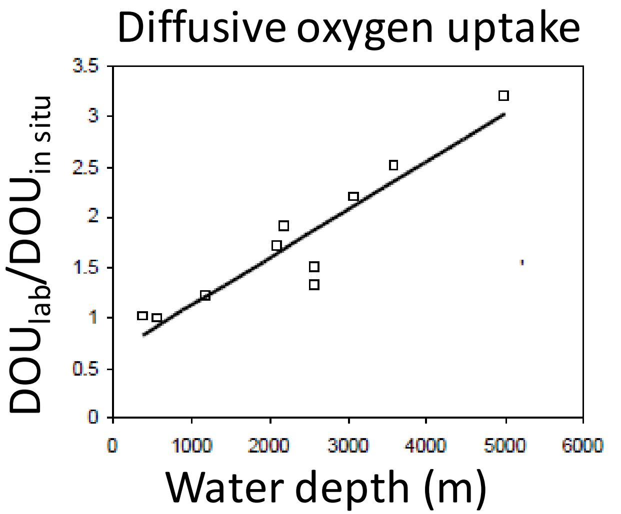
In situ lander and lab







- O_2 penetration underestimated in lab
- O_2 diffusive uptake overestimated in the lab

Glud et al., 1994, Glud et al. 1999

In situ data logging

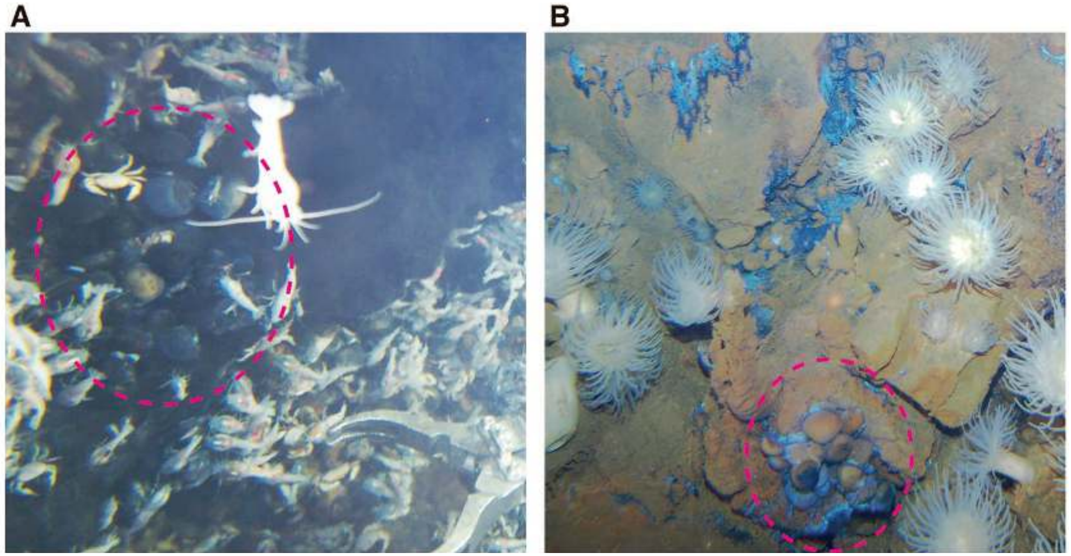
Field Datalogger Mini

- 2 or 4 channels
- All Unisense sensors
- STOX support
- 300 or 6000 m depth rating
- Up to 20 days battery life
- Interface with CTD or other equipment
- 9.5 cm OD x 28 cm, 4.4 kg

UNISENSE

Field Datalogger Mini – Data example

- H_2S and H_2 around Hydrothermal vents
- 2.4 to 3.3 km water depth
- Operated with ROV



Miyazaki, J., T. Ikuta, T. o. Watsuji, and others. 2020. Dual energy metabolism of the Campylobacterota endosymbiont in the chemosynthetic snail Alviniconcha marisindica. ISME J. 14: 1273–1289. doi:10.1038/s41396-020-0605-7

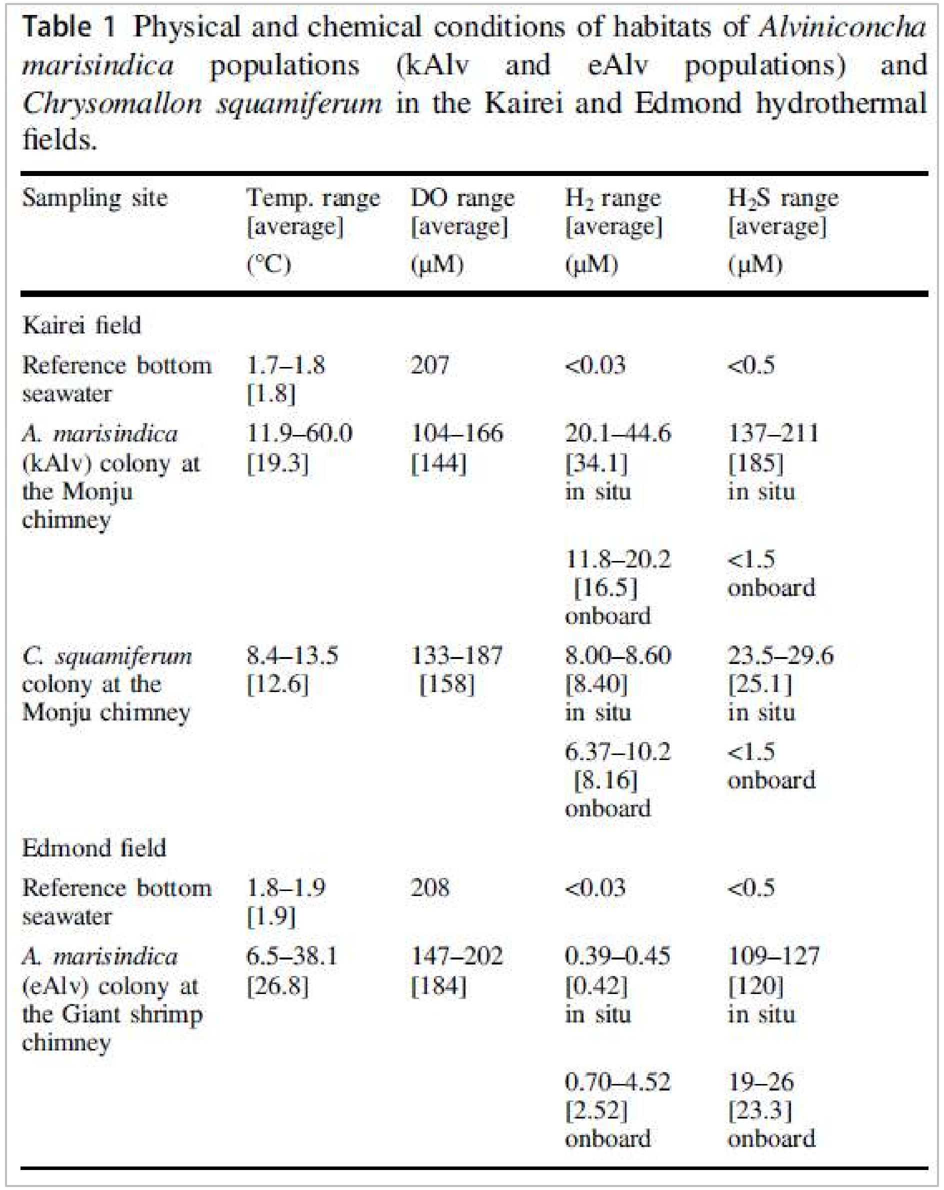


Table 1 Physical and chemical conditions of habitats of *Alviniconcha marisindica* populations (kAlv and eAlv populations) and *Chrysomallon squamiferum* in the Kairei and Edmond hydrothermal fields.

Sampling site	Temp. range [average] (°C)	DO range [average] (μM)	H_2 range [average] (μM)	H_2S range [average] (μM)
Kairei field				
Reference bottom seawater	1.7–1.8 [1.8]	207	<0.03	<0.5
A. marisindica (kAlv) colony at the Monju chimney	11.9–60.0 [19.3]	104–166 [144]	20.1–44.6 [34.1] in situ	137–211 [185] in situ
			11.8–20.2 [16.5] onboard	<1.5 onboard
C. squamiferum colony at the Monju chimney	8.4–13.5 [12.6]	133–187 [158]	8.00–8.60 [8.40] in situ	23.5–29.6 [25.1] in situ
			6.37–10.2 [8.16] onboard	<1.5 onboard
Edmond field				
Reference bottom seawater	1.8–1.9 [1.9]	208	<0.03	<0.5
A. marisindica (eAlv) colony at the Giant shrimp chimney	6.5–38.1 [26.8]	147–202 [184]	0.39–0.45 [0.42] in situ	109–127 [120] in situ
			0.70–4.52 [2.52] onboard	19–26 [23.3] onboard

STOX sensor

- Switchable Trace Oxygen sensor
- Trace amounts of O_2 can be measured reliably with a STOX sensor
- Detection limit down to 5-10 nM O_2

STOX sensor

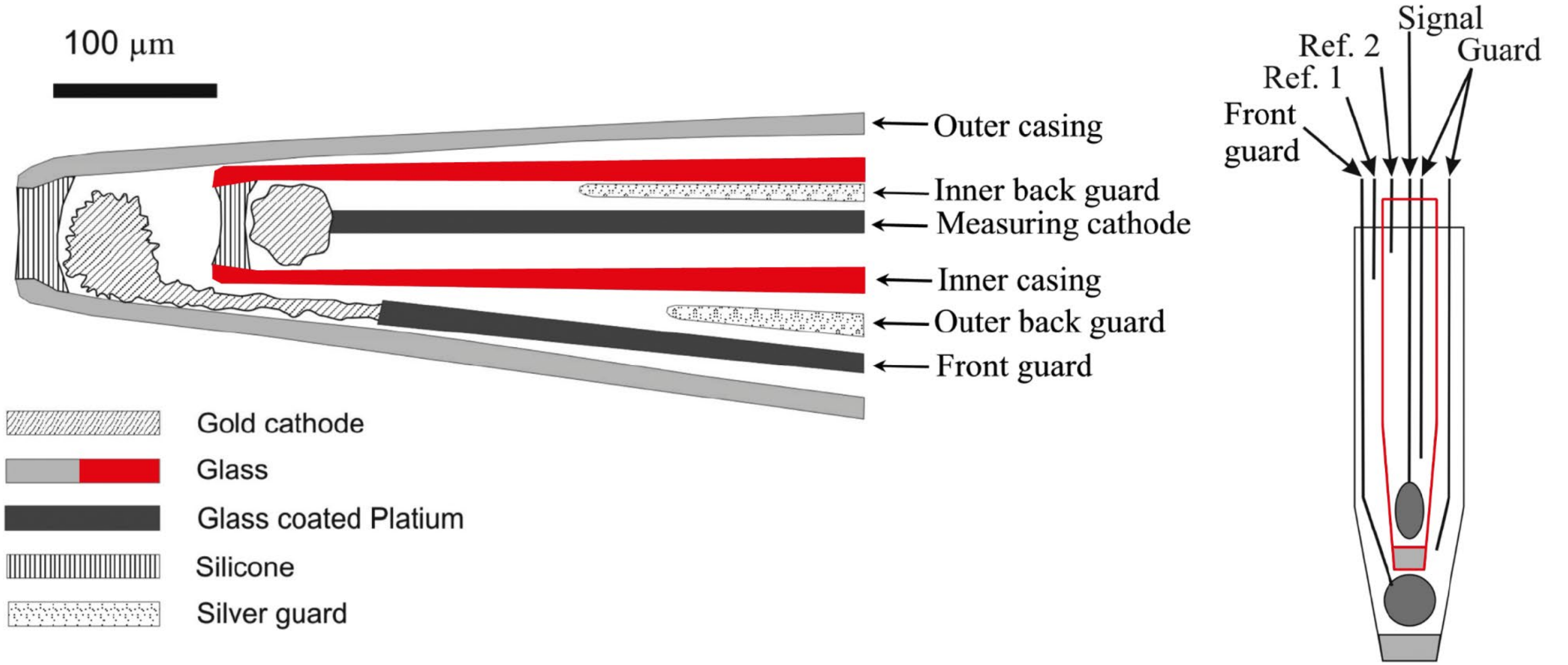
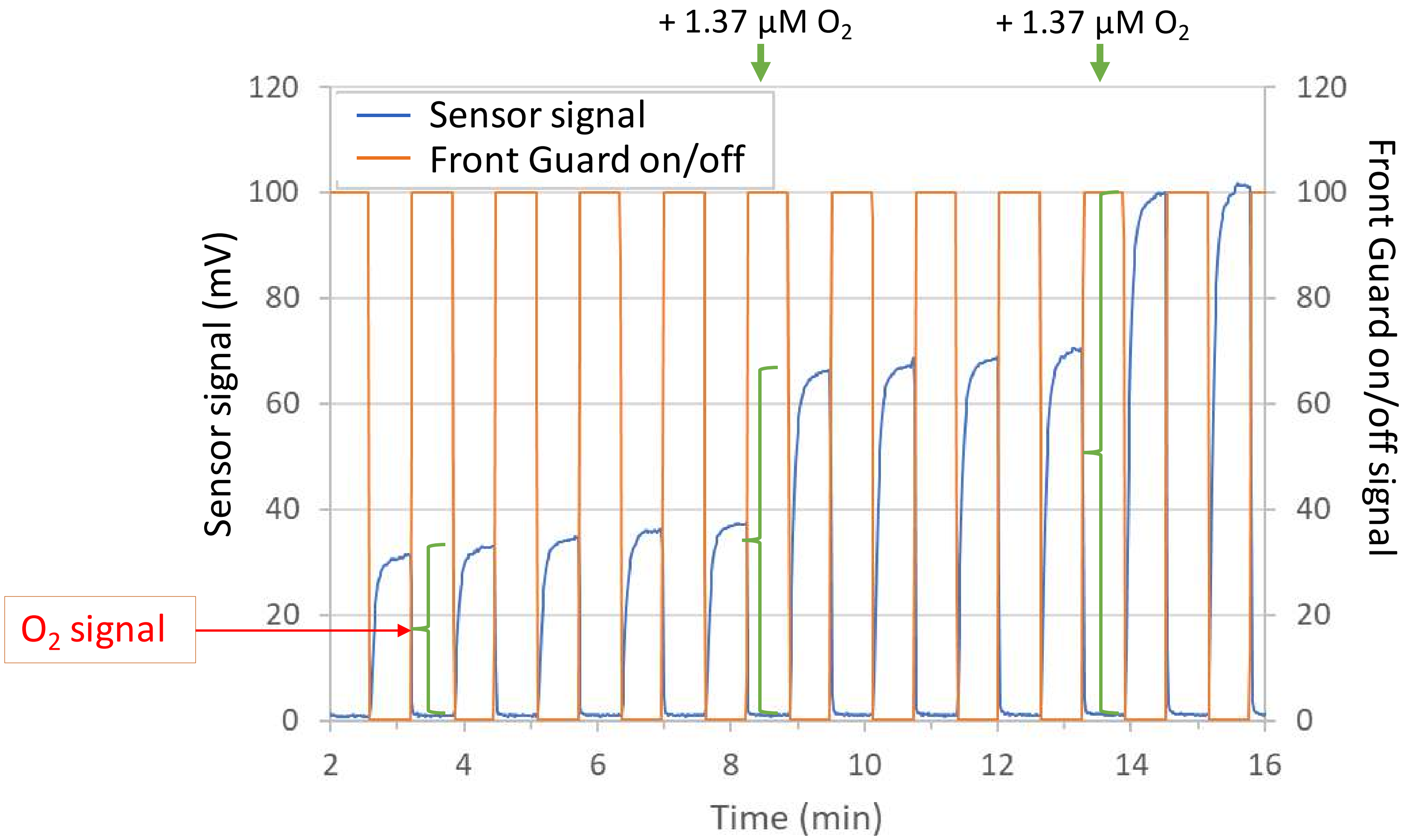


Fig. 1. Left: Schematic drawing of STOX microsensor tip. Not visible are the Ag/AgCl internal references in both inner and outer electrolyte reservoirs. Right: Diagram showing all anodes and cathodes in a STOX sensor.

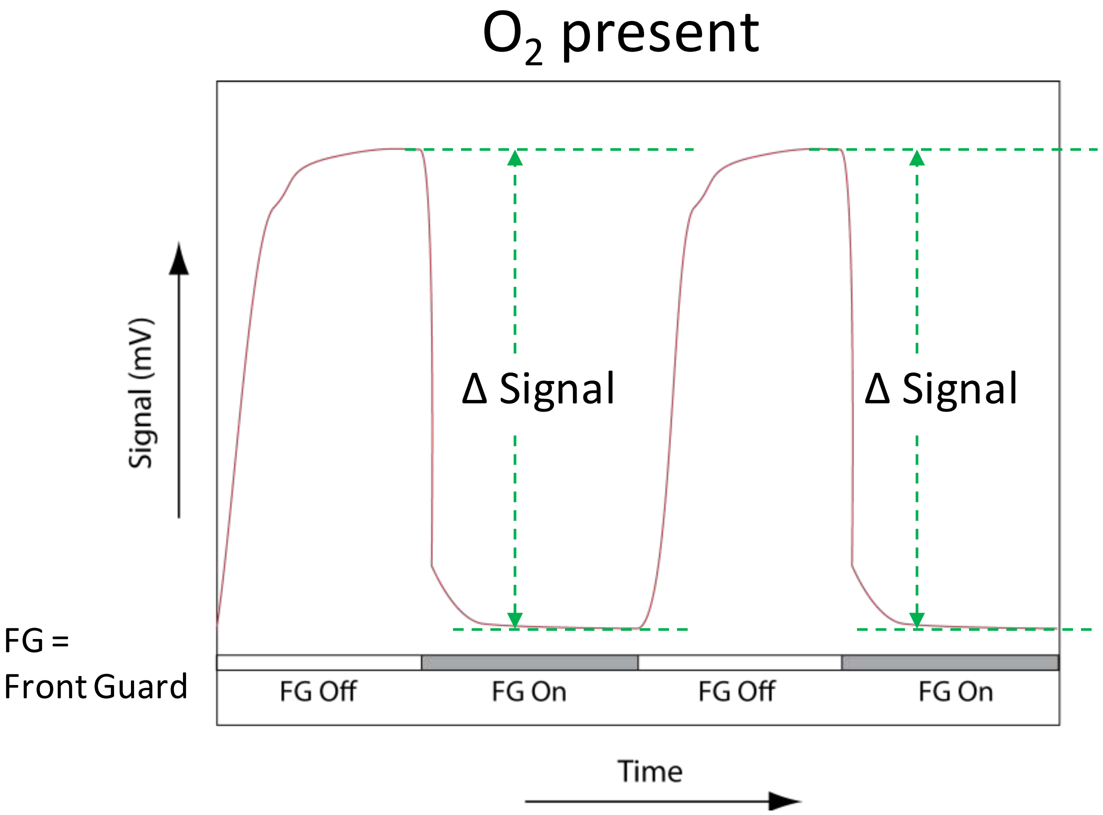
Revsbech, N. P., L. H. Larsen, J. Gundersen, T. Dalsgaard, O. Ulloa, and B. Thamdrup. 2009. Determination of ultra-low oxygen concentrations in oxygen minimum zones by the STOX sensor. Limnol. Oceanogr. 7: 371–381

STOX sensor - signal



O_2 signal = Sensor signal$_{\mathrm{FG\ off}}$ - Sensor signal$_{\mathrm{FG\ on}}$ = Δ O_2 signal

STOX sensor



Δ Signal at zero O_2:

- Signal FG On = Signal FG Off
- Δ Signal = 0 mV

STOX sensor - calibration

At zero O_2 :

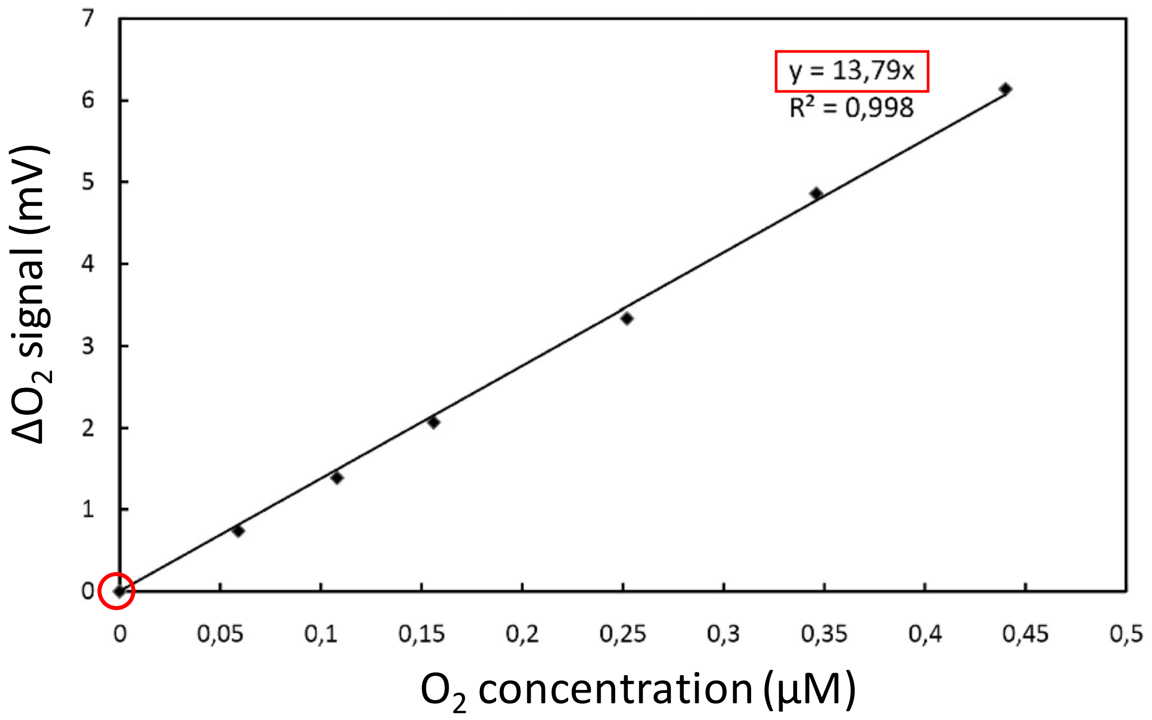
Sensor signal$_{\text{FG off}}$ - Sensor signal$_{\text{FG on}}$ = 0

Sensor response is linear

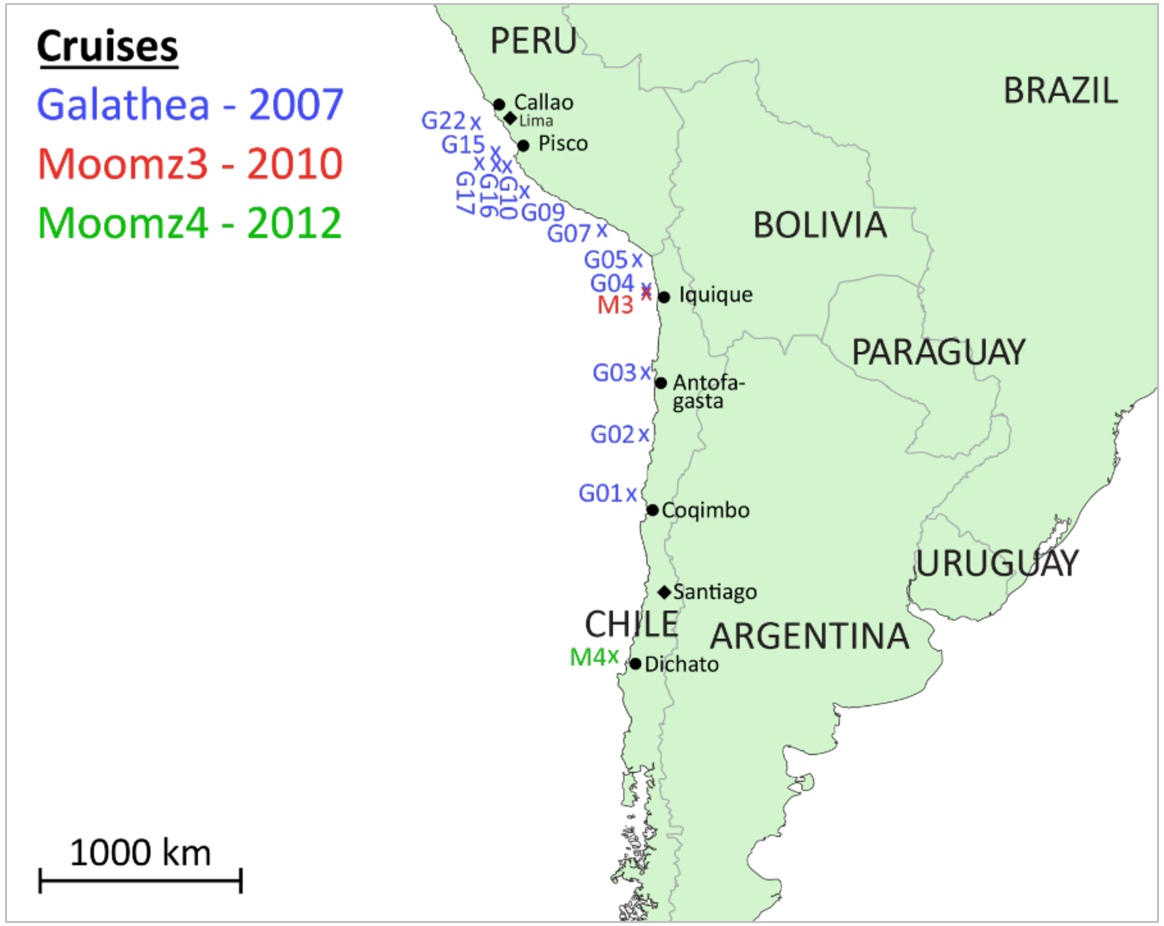
O_2 conc. is proportional to Δ O_2 signal

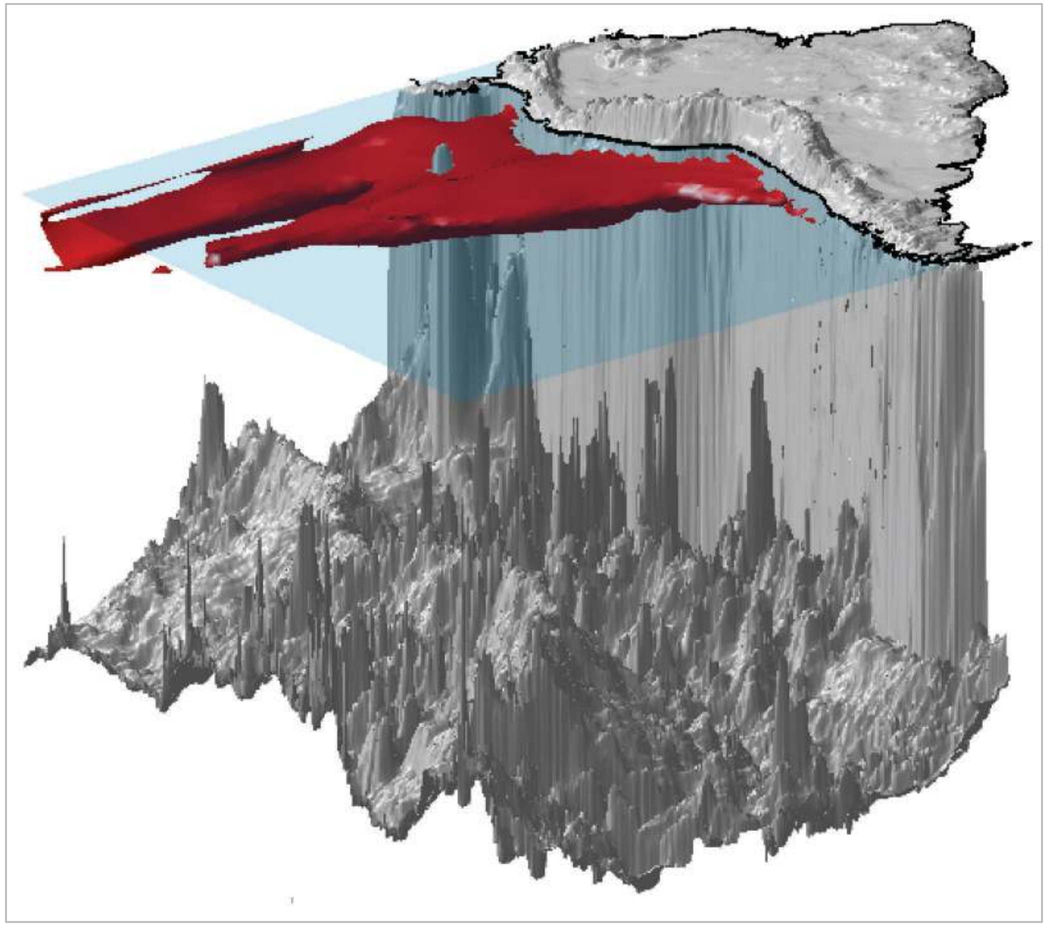
1-point calibration

O_2 conc. = Δ O_2 signal/y

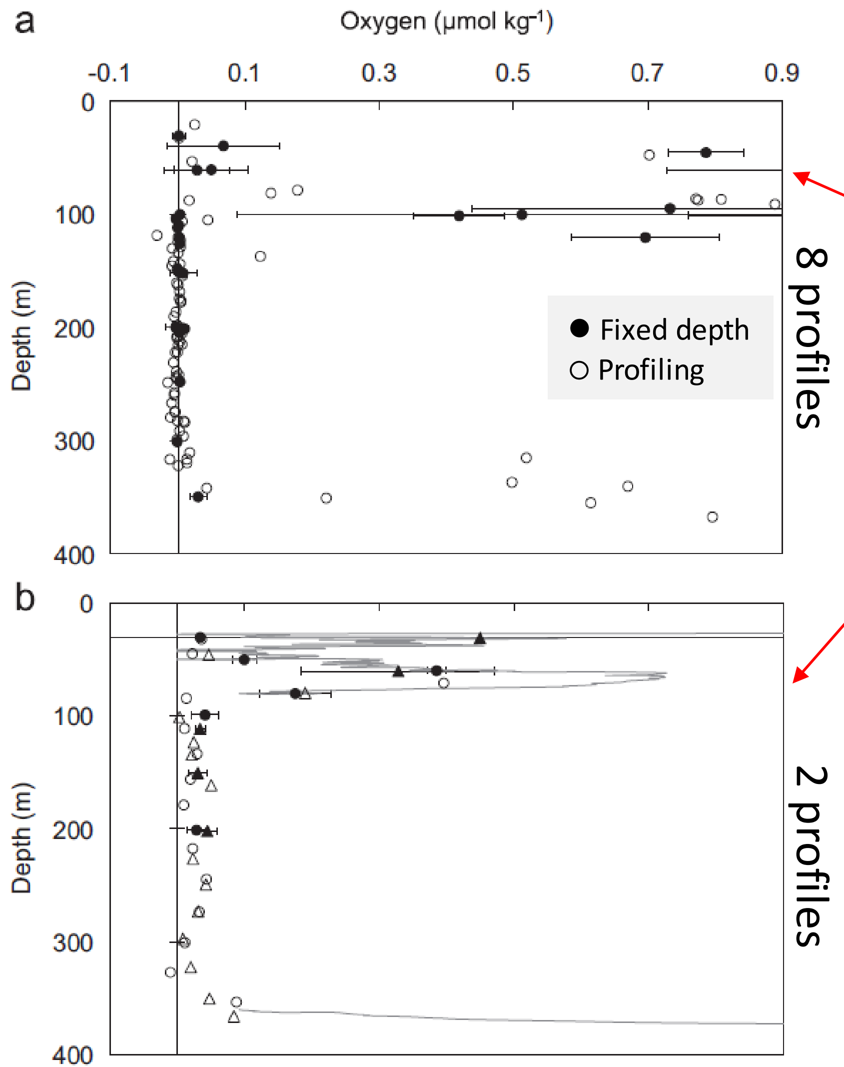


STOX sensor – Eastern South Pacific



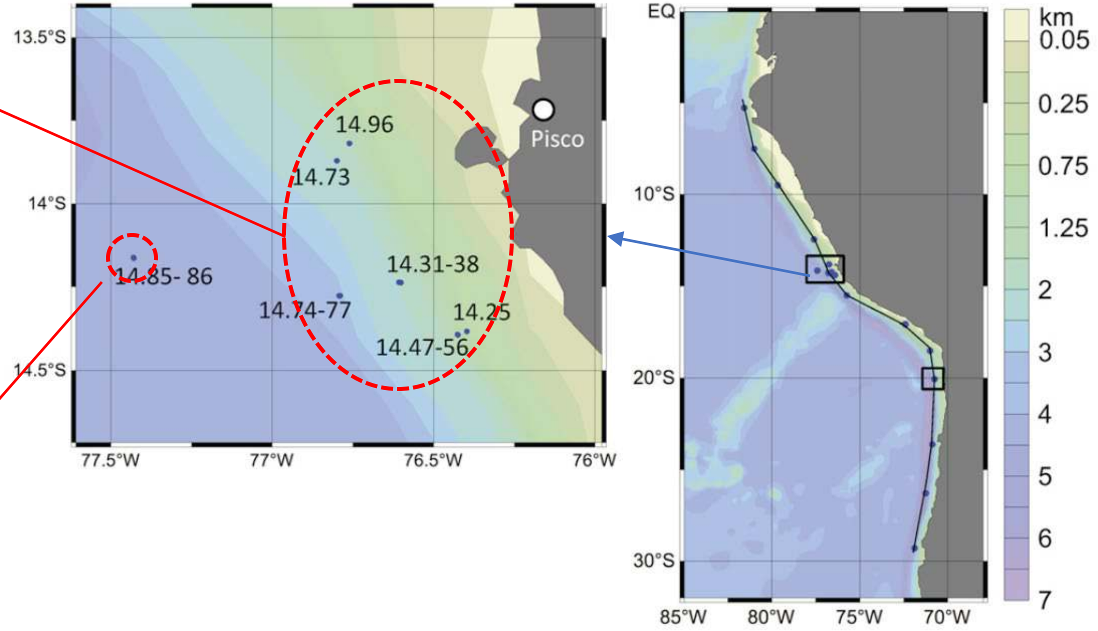


STOX [O_2] – Along Chile and Peru



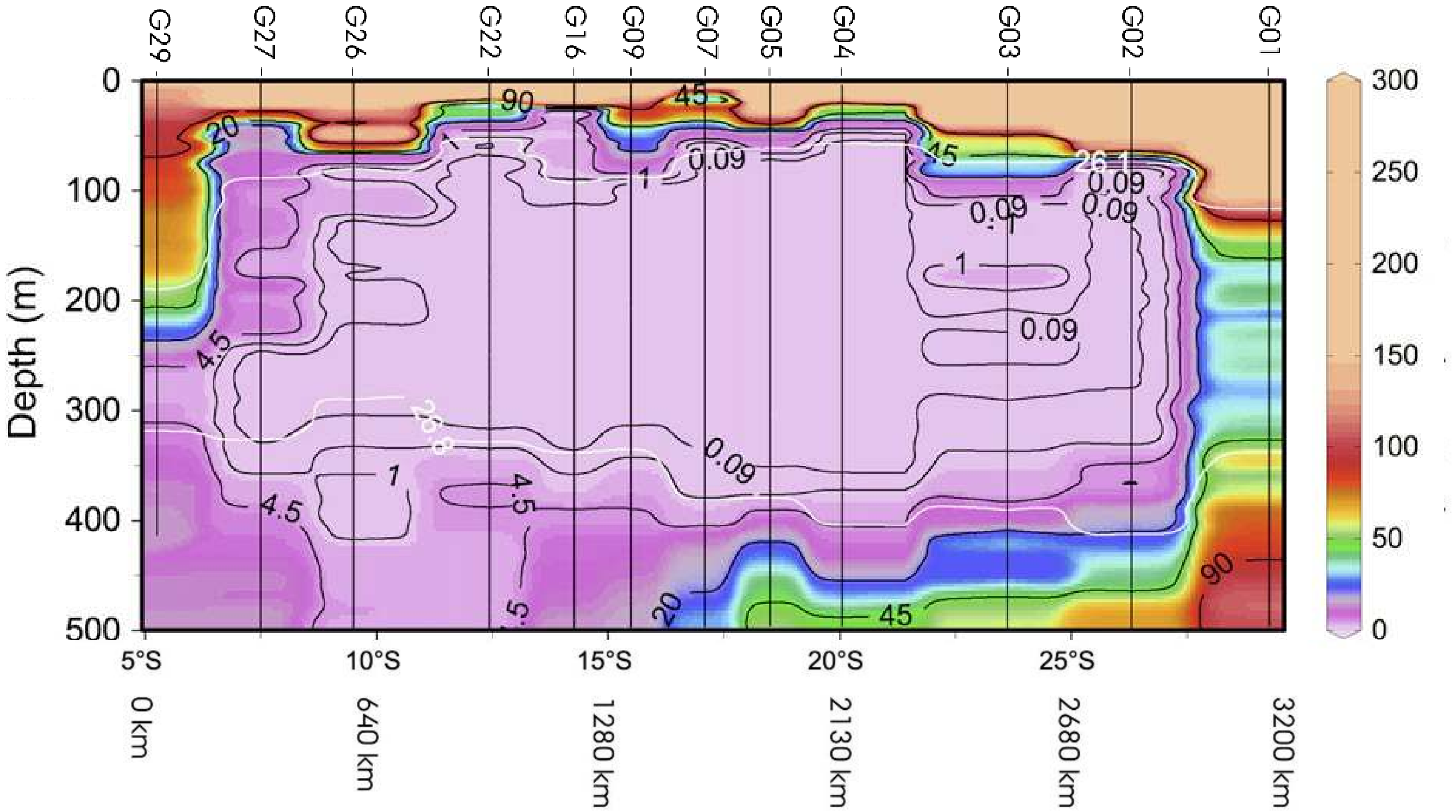
8 profiles

2 profiles

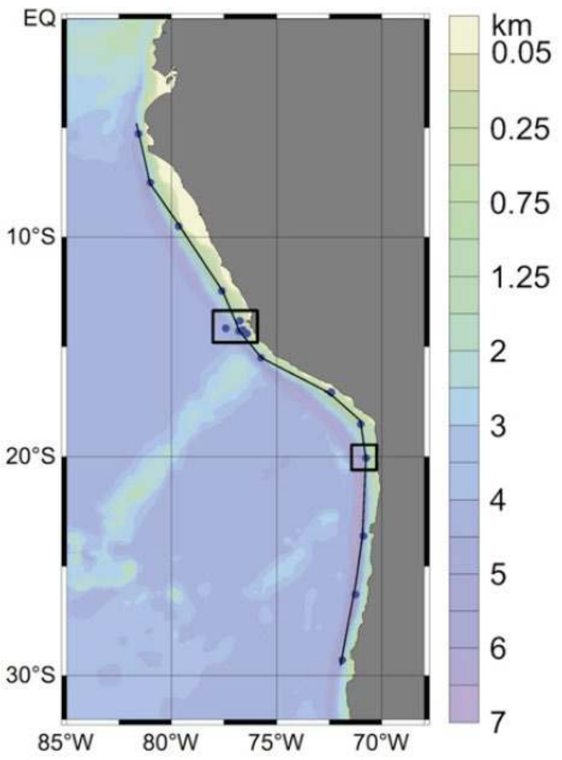


Thamdrup, B., T. Dalsgaard, and N. P. Revsbech. 2012. Widespread functional anoxia in the oxygen minimum zone of the eastern South Pacific. Deep Sea Res. Part I **65**: 36–45.

STOX [O_2] – Along Chile and Peru



Detection limits:
STOX O_2 = 5 - 9 nM
SBE corrected = 90 nM

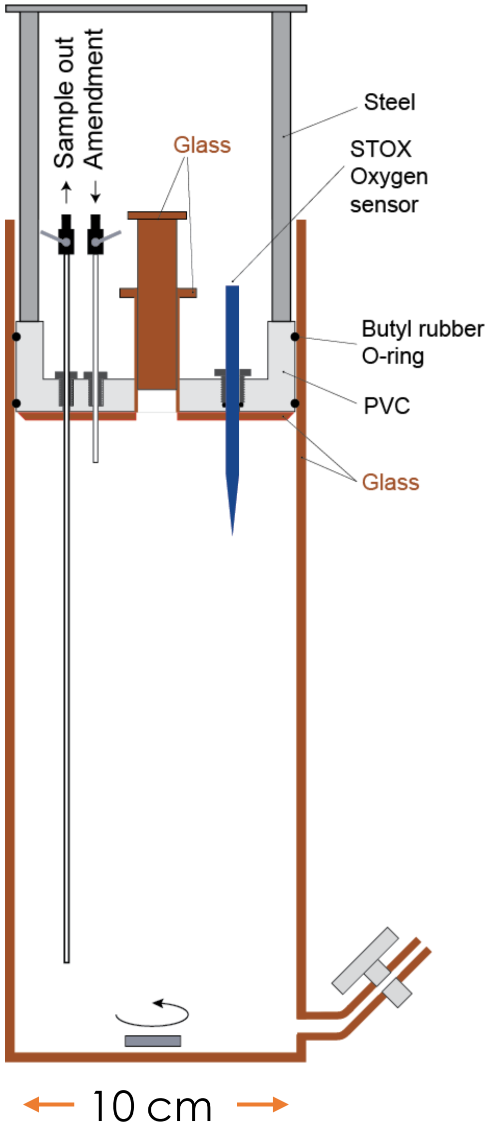


Thamdrup, B., T. Dalsgaard, and N. P. Revsbech. 2012. Widespread functional anoxia in the oxygen minimum zone of the eastern South Pacific. Deep Sea Res. Part I **65**: 36–45.

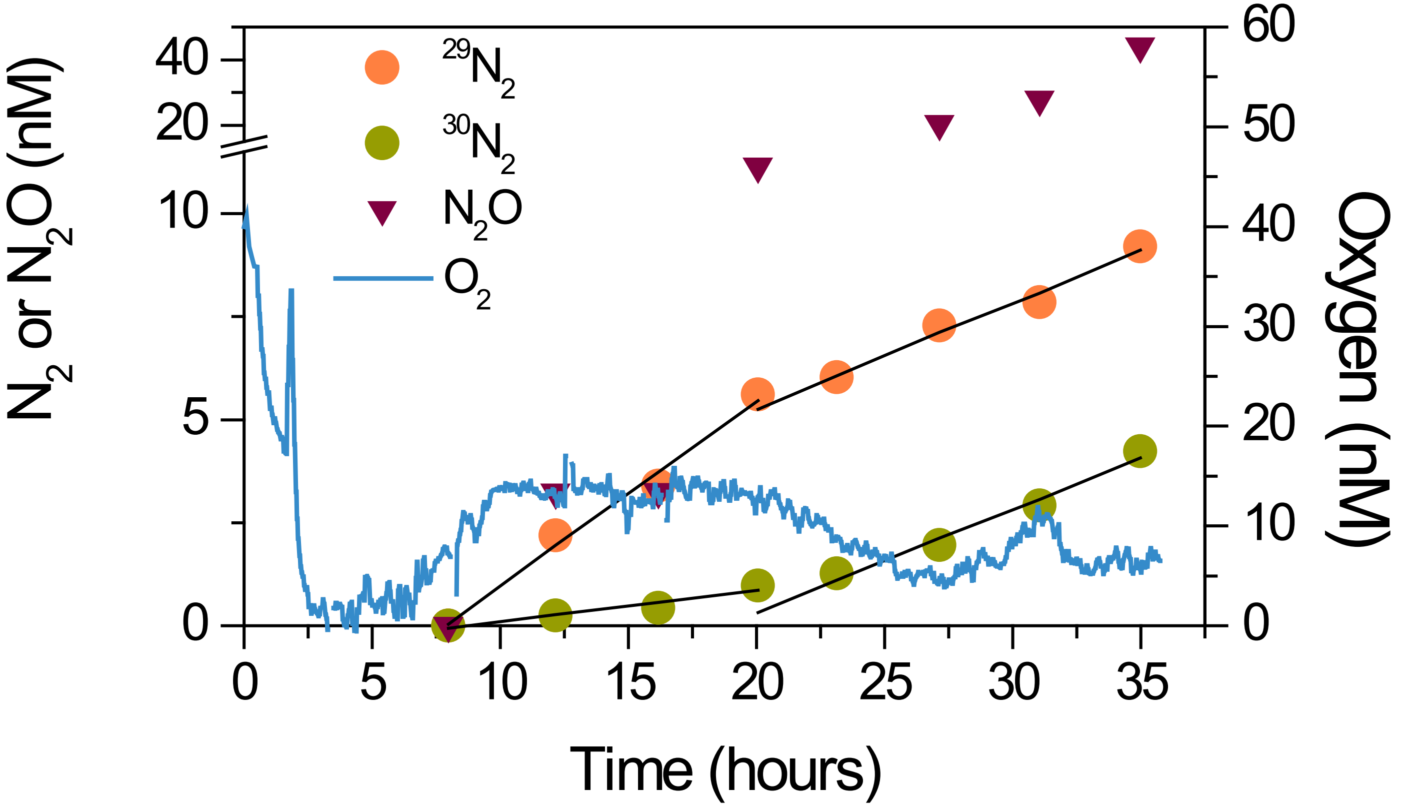
STOX sensor – Controlled reactor experiments

Reactor experiments

- Volume = 2 l
- ^{15}N tracer addition
  - Denitrification
  - Anammox
- Manipulate and monitor O_2



STOX sensor – Monitoring in reactor

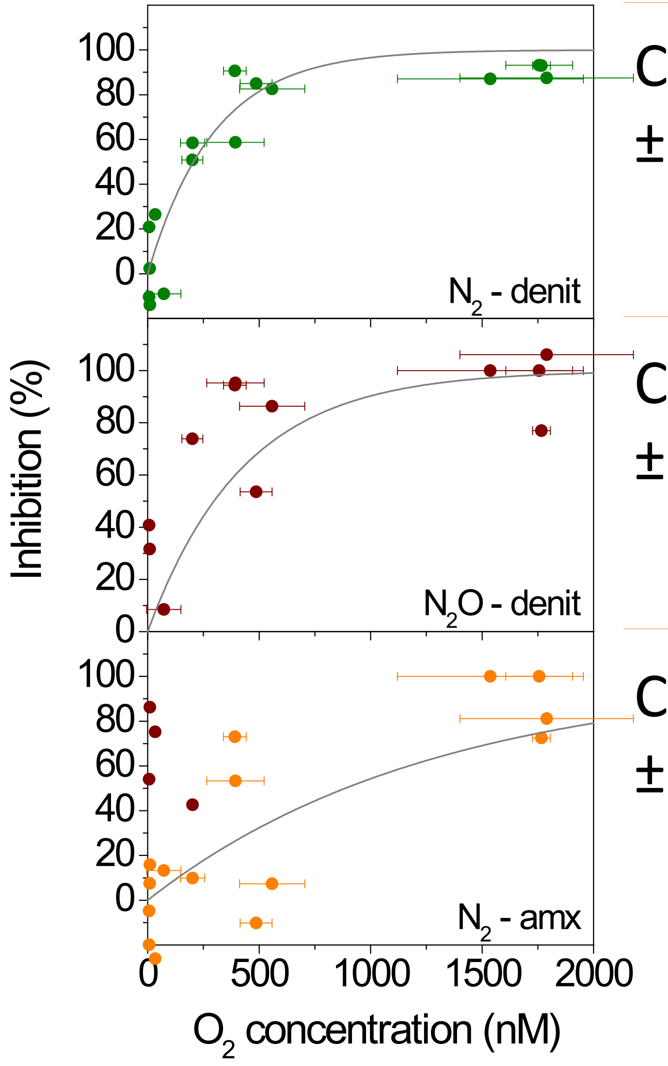


<u>$^{15}NO_2^-$ added:</u>

Anammox:	$^{15}NO_2^- + {}^{14}NH_4^+ \rightarrow {}^{29}N_2$
Denitrification:	$^{15}NO_2^- + {}^{15}NO_2^- \rightarrow {}^{30}N_2$
	$^{15}NO_2^- + {}^{14}NO_2^- \rightarrow {}^{29}N_2$

Dalsgaard, T., F. J. Stewart, B. Thamdrup, L. De Brabandere, N. P. Revsbech, O. Ulloa, D. E. Canfield, and E. F. DeLong. 2014. MBio.

STOX - Inhibition by O_2



C_{50} = 205 nM O_2
±SE = 37

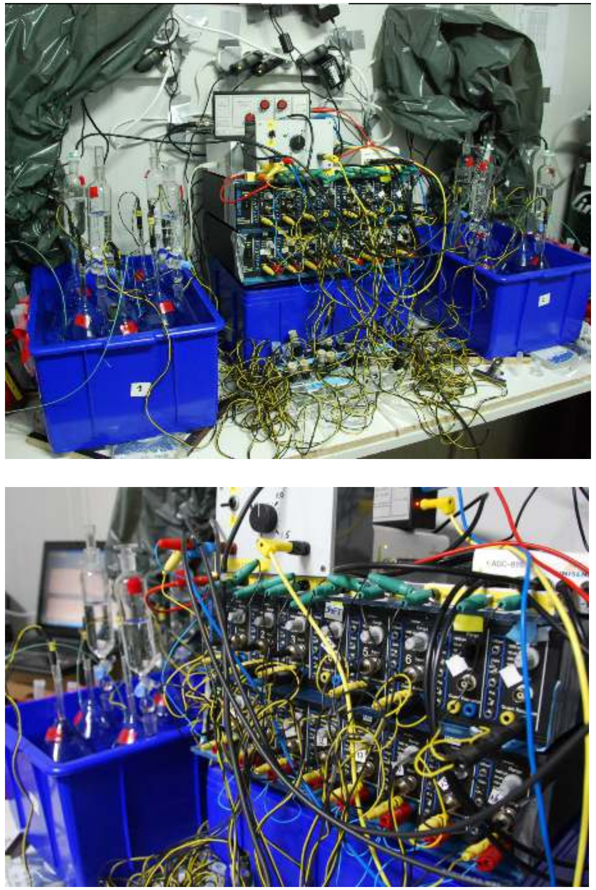
C_{50} = 297 nM O_2
±SE = 139

C_{50} = 886 nM O_2
± = 418

Dalsgaard, T., F. J. Stewart, B. Thamdrup, L. De Brabandere, N. P. Revsbech, O. Ulloa, D. E. Canfield, and E. F. DeLong. 2014. MBio 5.

O_2 is important in the nM range

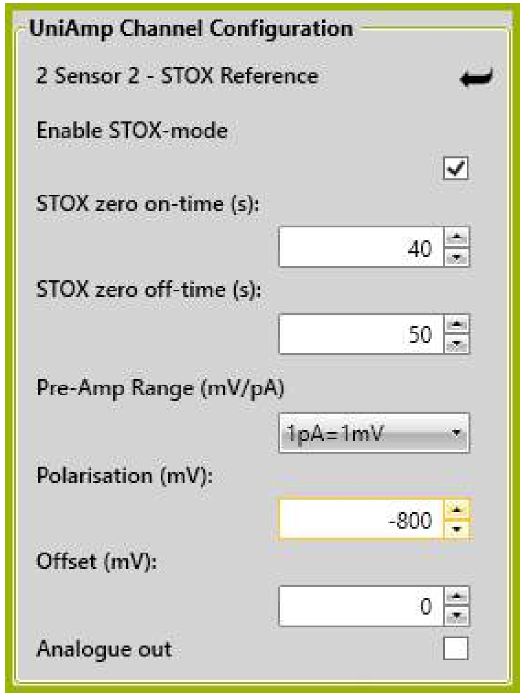
	Process	Product	C_{50} nM O_2	K_m nM O_2
Anaerobic	Denitrification	$\rightarrow N_2$	205	
	Denitrification	$\rightarrow N_2O$	297	
	Anammox	$\rightarrow N_2$	886	
	NO_3^- reduction	$\rightarrow NO_2^-$	740	
Aerobic	NH_4^+ oxidation	$\rightarrow NO_2^-$		330
	NO_2^- oxidation	$\rightarrow NO_3^-$		780



UNISENSE

Unisense STOX in the lab

- UniAmp series of amplifiers has built in STOX support
- Plug and play
- STOX on/off timing adjustable in software



UniAmp Channel Configuration

2 Sensor 2 - STOX Reference

Enable STOX-mode ☑

STOX zero on-time (s): 40

STOX zero off-time (s): 50

Pre-Amp Range (mV/pA): 1pA=1mV

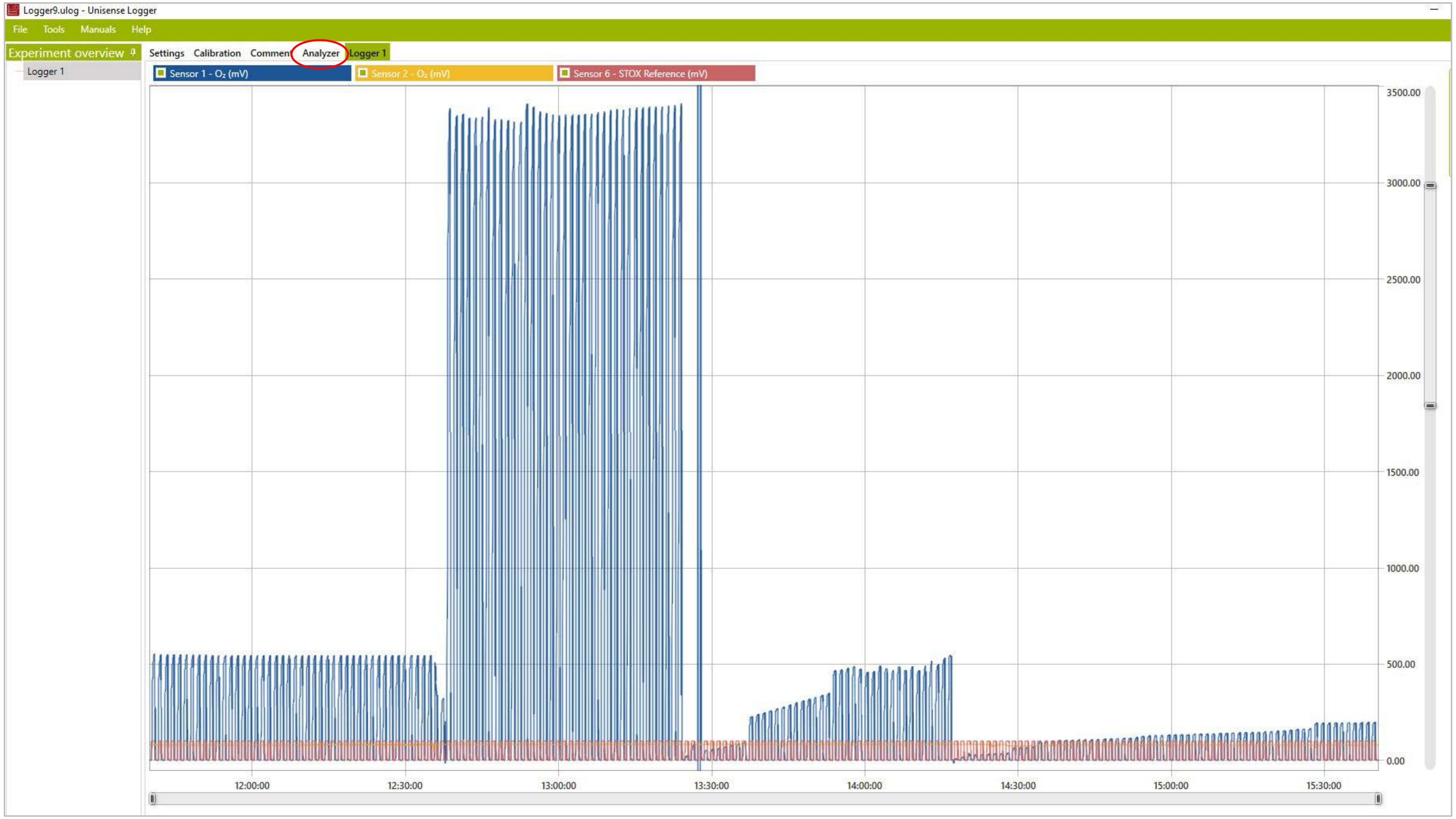
Polarisation (mV): -800

Offset (mV): 0

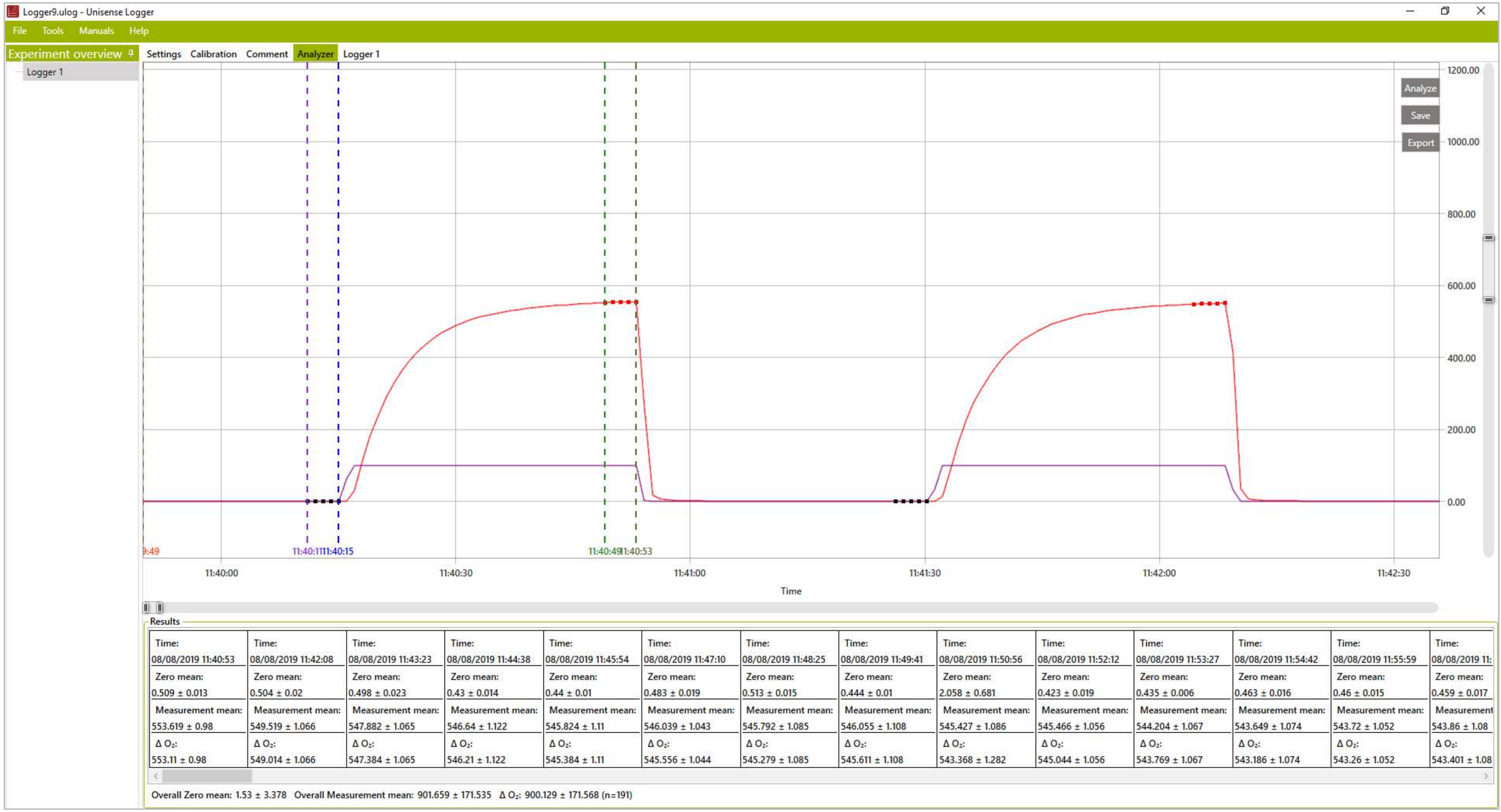
Analogue out ☐

UNISENSE

Unisense STOX data analysis



Unisense STOX data analysis



Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:	Time:
08/08/2019 11:40:53	08/08/2019 11:42:08	08/08/2019 11:43:23	08/08/2019 11:44:38	08/08/2019 11:45:54	08/08/2019 11:47:10	08/08/2019 11:48:25	08/08/2019 11:49:41	08/08/2019 11:50:56	08/08/2019 11:52:12	08/08/2019 11:53:27	08/08/2019 11:54:42	08/08/2019 11:55:59	08/08/2019 11:
Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:	Zero mean:
0.509 ± 0.013	0.504 ± 0.02	0.498 ± 0.023	0.43 ± 0.014	0.44 ± 0.01	0.483 ± 0.019	0.513 ± 0.015	0.444 ± 0.01	2.058 ± 0.681	0.423 ± 0.019	0.435 ± 0.006	0.463 ± 0.016	0.46 ± 0.015	0.459 ± 0.017
Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement mean:	Measurement
553.619 ± 0.98	549.519 ± 1.066	547.882 ± 1.065	546.64 ± 1.122	545.824 ± 1.11	546.039 ± 1.043	545.792 ± 1.085	546.055 ± 1.108	545.427 ± 1.086	545.466 ± 1.056	544.204 ± 1.067	543.649 ± 1.074	543.72 ± 1.052	543.86 ± 1.08
Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:	Δ O_2:
553.11 ± 0.98	549.014 ± 1.066	547.384 ± 1.065	546.21 ± 1.122	545.384 ± 1.11	545.556 ± 1.044	545.279 ± 1.085	545.611 ± 1.108	543.368 ± 1.282	545.044 ± 1.056	543.769 ± 1.067	543.186 ± 1.074	543.26 ± 1.052	543.401 ± 1.08

Overall Zero mean: 1.53 ± 3.378 Overall Measurement mean: 901.659 ± 171.535 Δ O_2: 900.129 ± 171.568 (n=191)

Time for questions !

Unisense Microsensor Academy:
https://www.unisense.com/support/knowledge

Contact us: sales@unisense.com