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O_2 N_2O H_2 NO H_2S Redox Temp Opto pH EP

The Microsensor Company

Lab-based 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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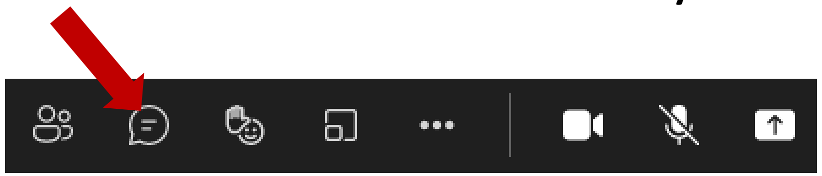
A few rules before we get started

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

- Microprofiles
  - Experimental design
  - H_2 dynamics in microbial mats
  - Electrical potential - Cable bacteria
- Photosynthesis - Light/Dark shift technique
- Microprofile theory - Short
- MicroRespiration System
- Lab vs. Field
  - O_2 variation in seagrass sediment
  - Bioturbation in mangrove sediments

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Microprofiles – Equipment

What you will need

- Microsensor
- Amplifier

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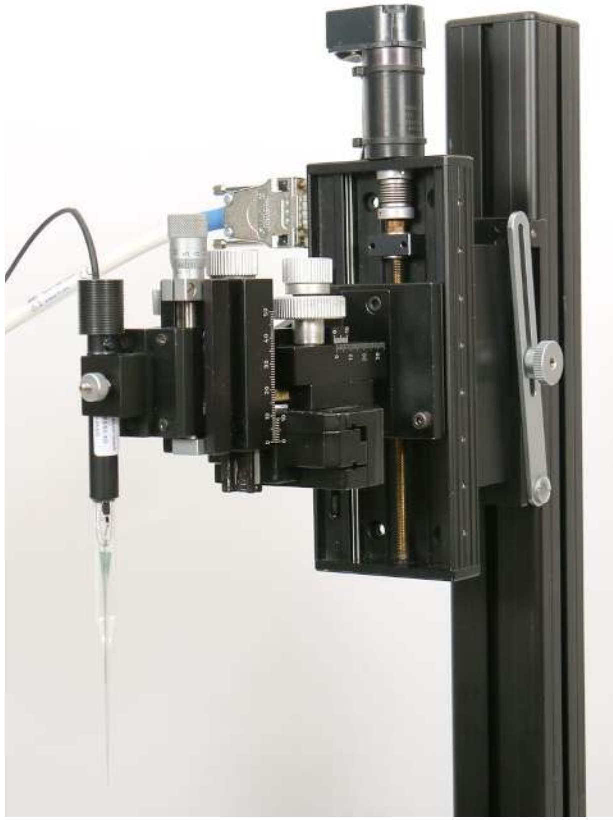
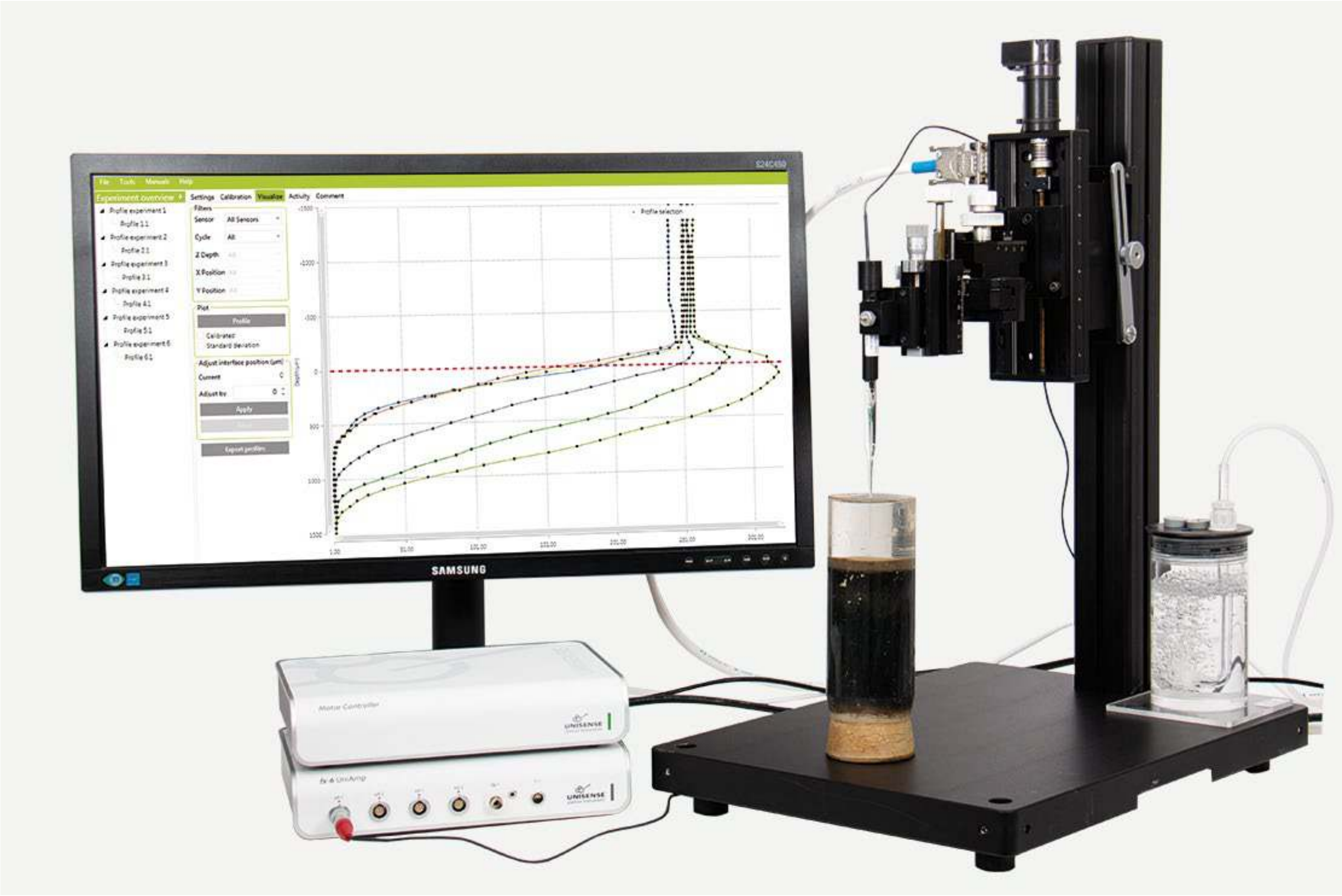
Microprofiles – Equipment



What you will need

- Microsensor
- Amplifier
- Micromanipulator
  - Manual
  - Motorized

Microprofiles – Equipment



What you will need

- Microsensor
- Amplifier
- Micromanipulator
  - Manual
  - Motorized
- Software

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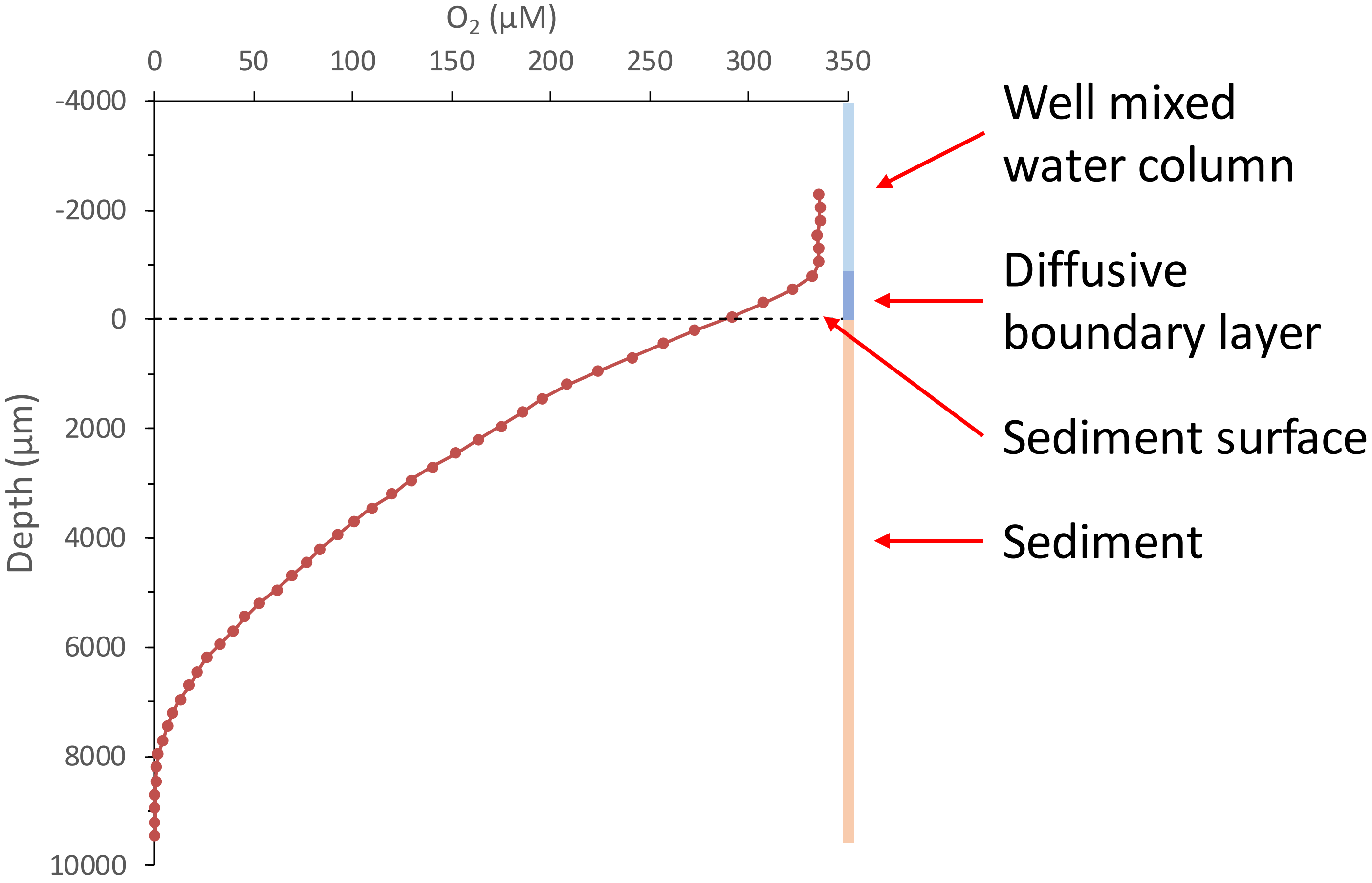
Microprofiles – Experimental design

We would like:

- Nice profiles
- Well defined conditions
- Easy to work with
- Sensor safe setup
- Steady state



Microprofiles – Experimental design

Desirable features of experimental micro-profiling set-ups

- Stirred (well mixed) water phase

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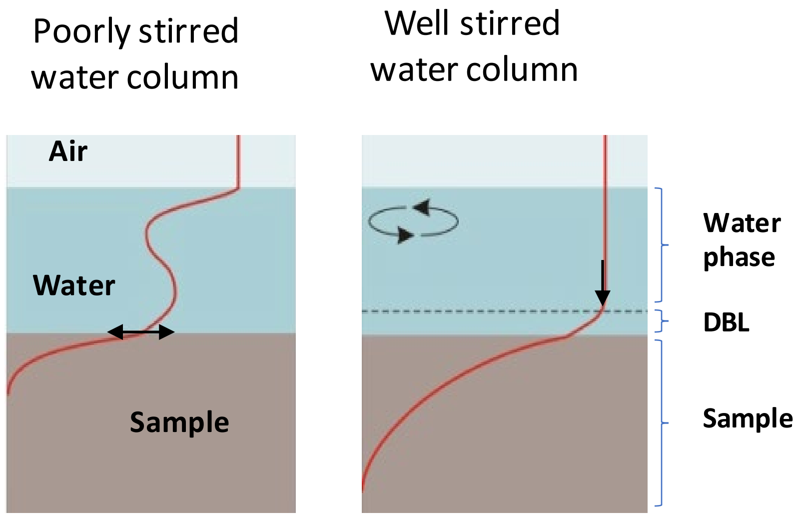
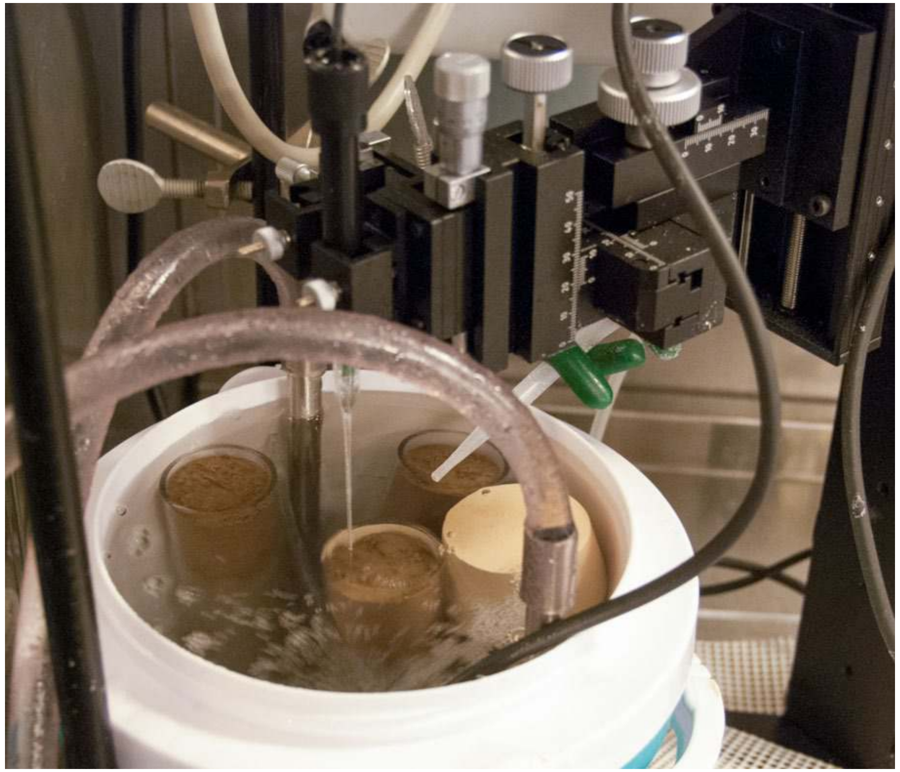
Microprofiles – Experimental design

Stagnant versus stirred conditions



Example: Air blown across the water surface to create stable flow



Characteristics of a stirred set-up:

- Well defined conditions
- Steady-state can be reached
- Data can be interpreted
- Natural conditions (?)

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Microprofiles – Experimental design

Desirable features of experimental micro-profiling set-ups

- Stirred (well mixed) water phase
- Know the position of the sediment surface

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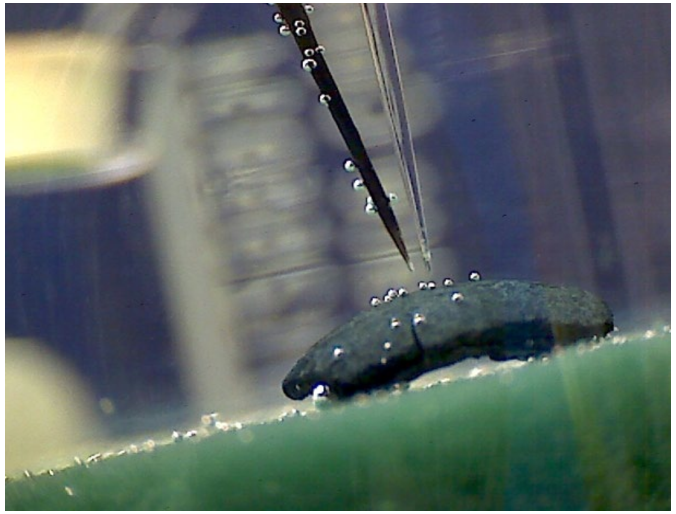
Microprofiles – Experimental design

Finding the surface – Solution 1:
Visual guidance

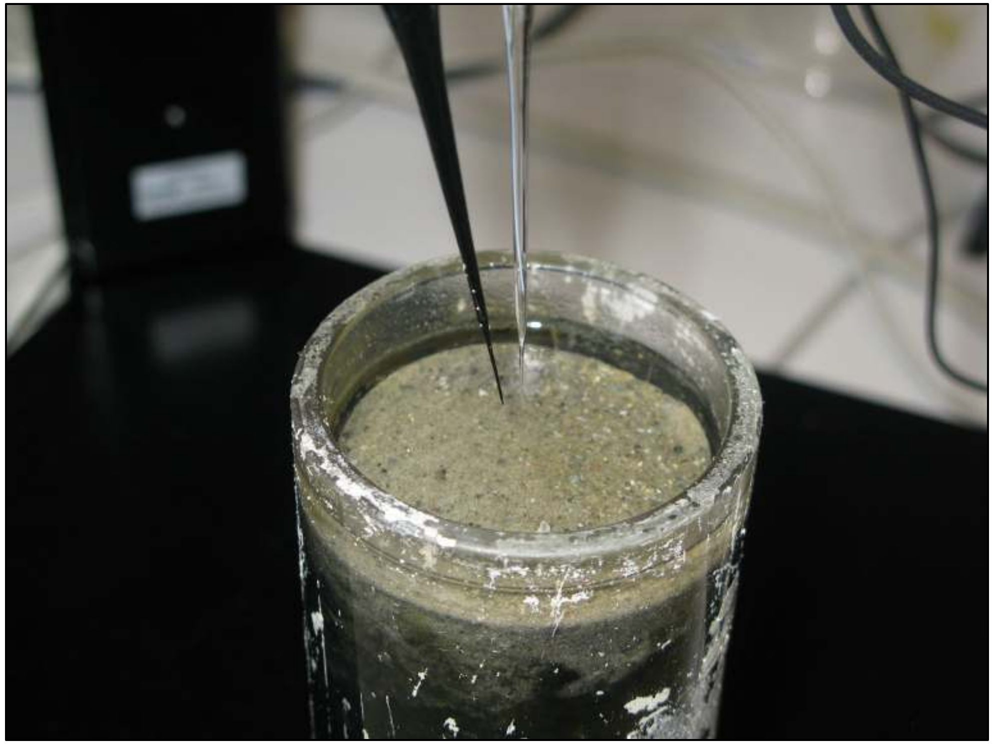
Dissection microscope

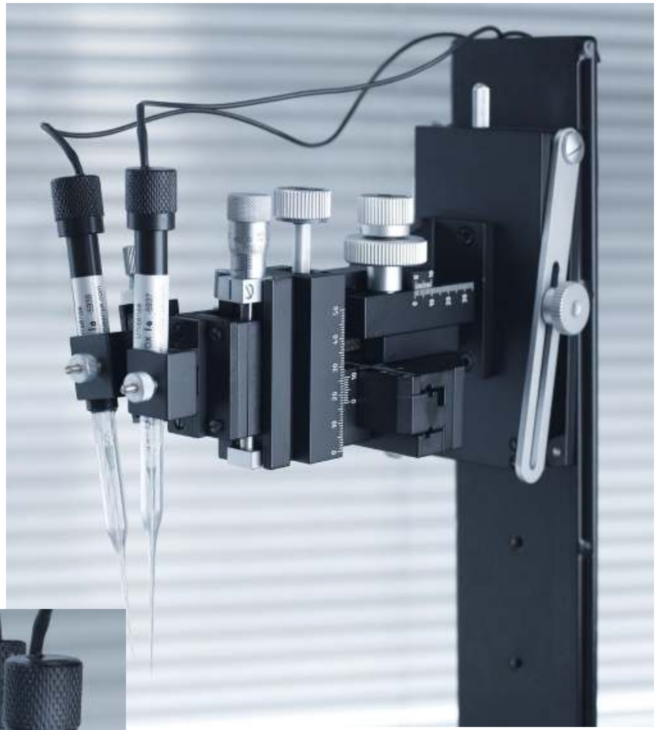


Microprofiles – Experimental design

Finding the surface – Solution 2:
Sensor alignment during profiling

O_2 and H_2S sensors





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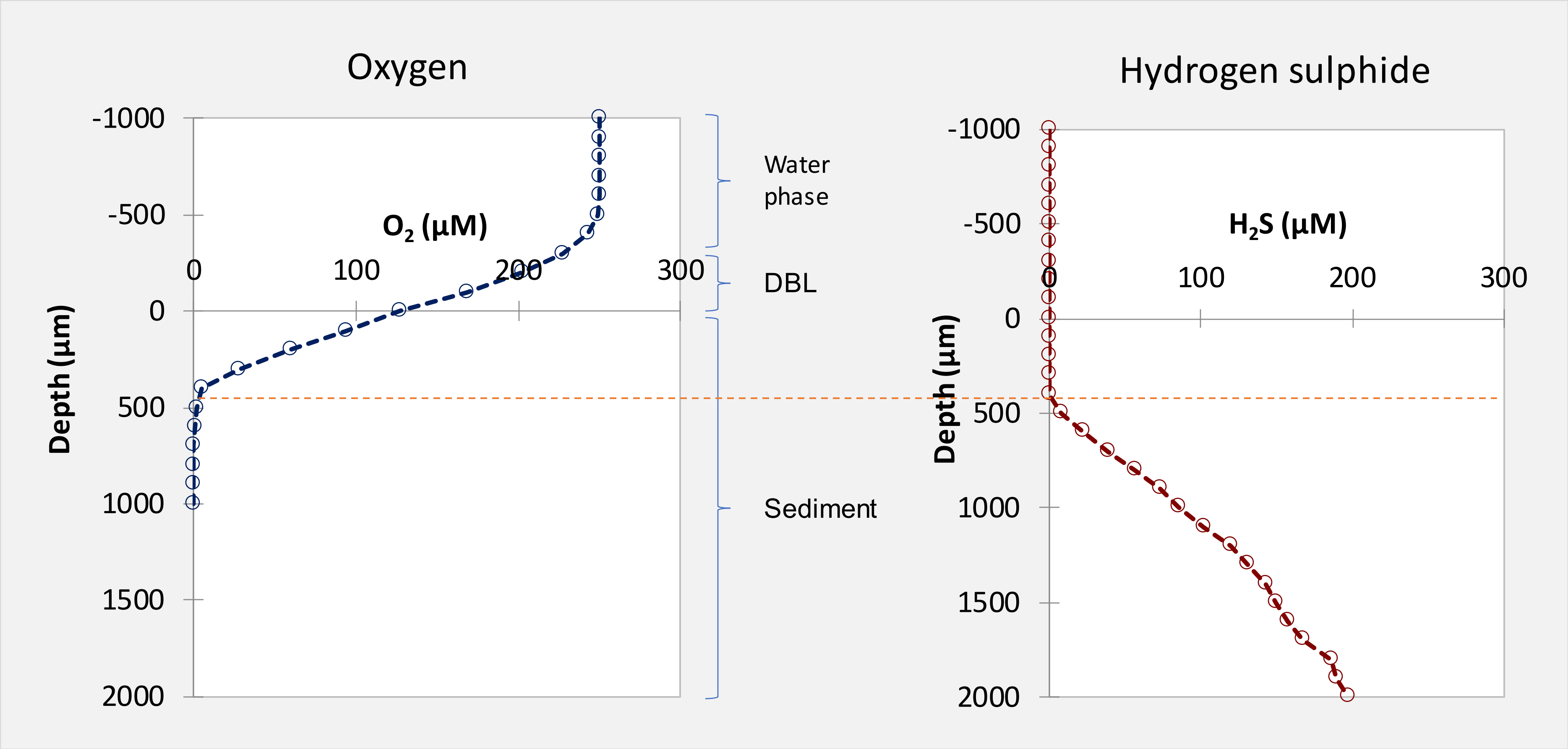
Microprofiles – Experimental design

Surface positioning - the problem of knowing where you are!



Microprofiles – Experimental design

Desirable features of experimental micro-profiling set-ups

- Stirred (well mixed) water phase
- Good visual inspection of the sample
- Proper control of water conditions
  - Temperature
  - Oxygen
  - Nutrients

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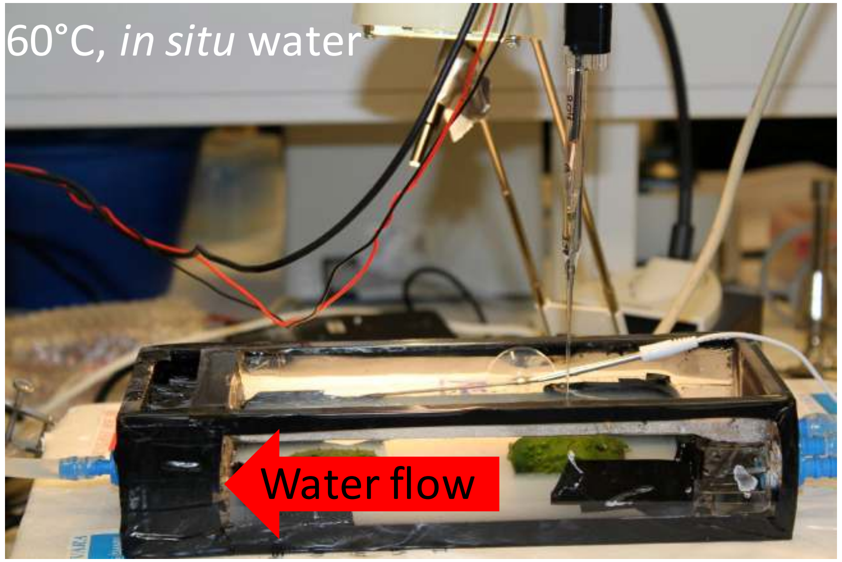
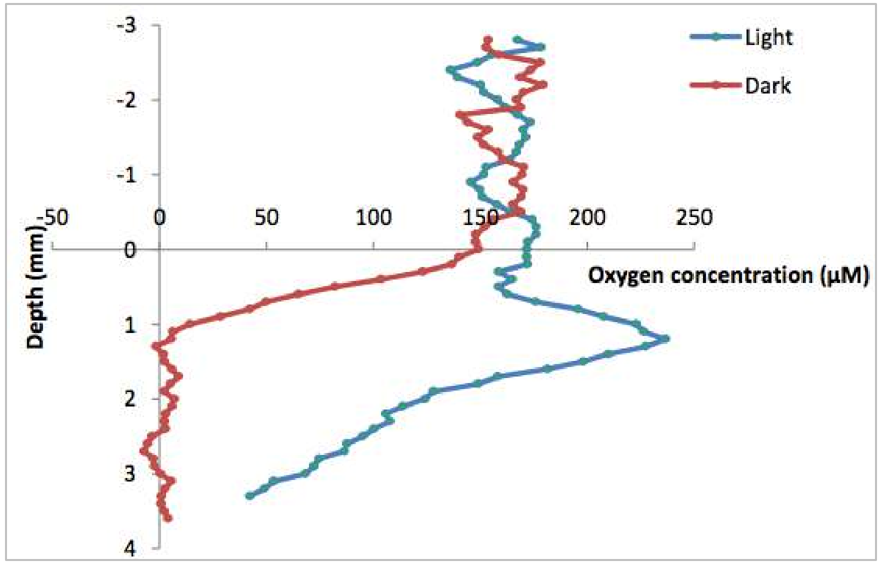
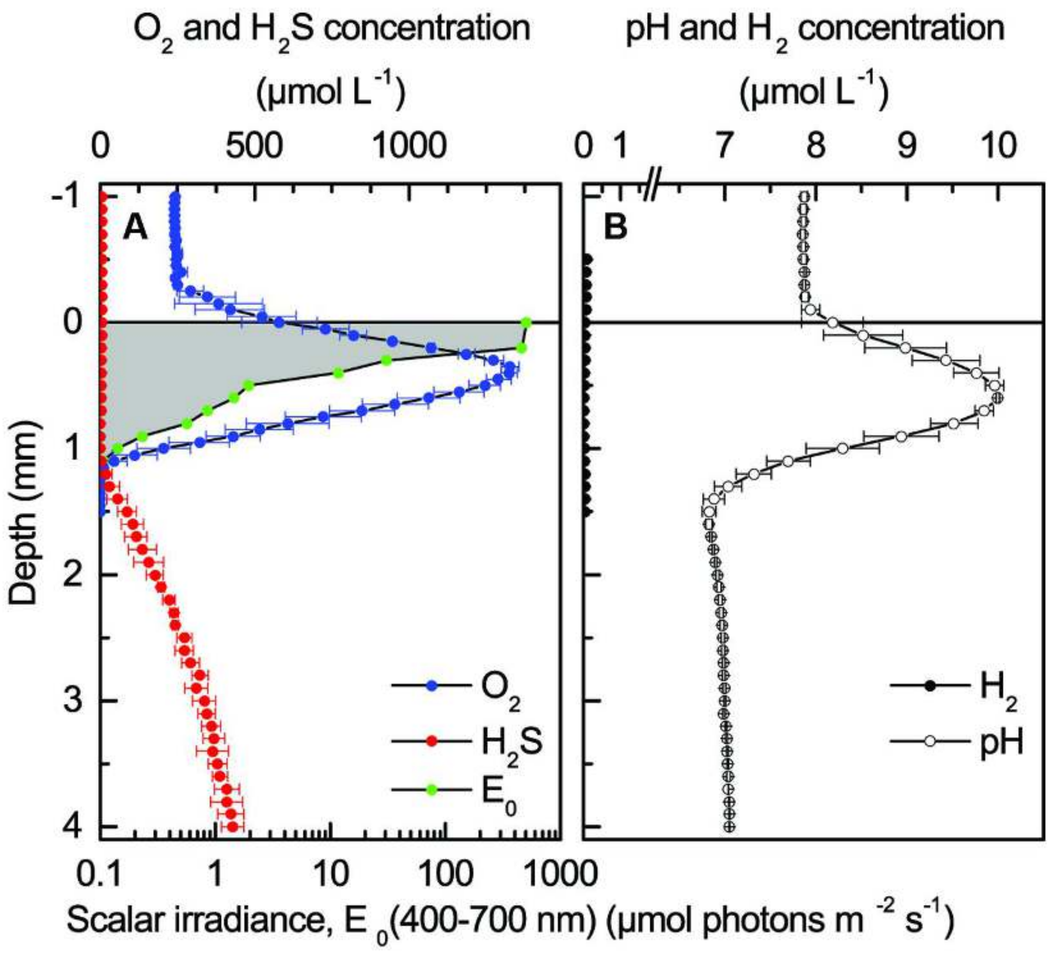
Microprofiles – Experimental design





Microbial mats - H_2 dynamics

Coastal cyanobacterial mat, Denmark



Sediment cores incubated in light in the lab

Microsensors used:

- O_2
- H_2S
- pH
- H_2
- H_2-X (insensitive to H_2S)
- Light (PAR)
- 10 - 70 µm tip diameter

Nielsen, M., N. P. Revsbech, and M. Kühl. 2015. Microsensor measurements of hydrogen gas dynamics in cyanobacterial microbial mats. Front. Microbiol. **6**: 1–12. doi:10.3389/fmicb.2015.00726

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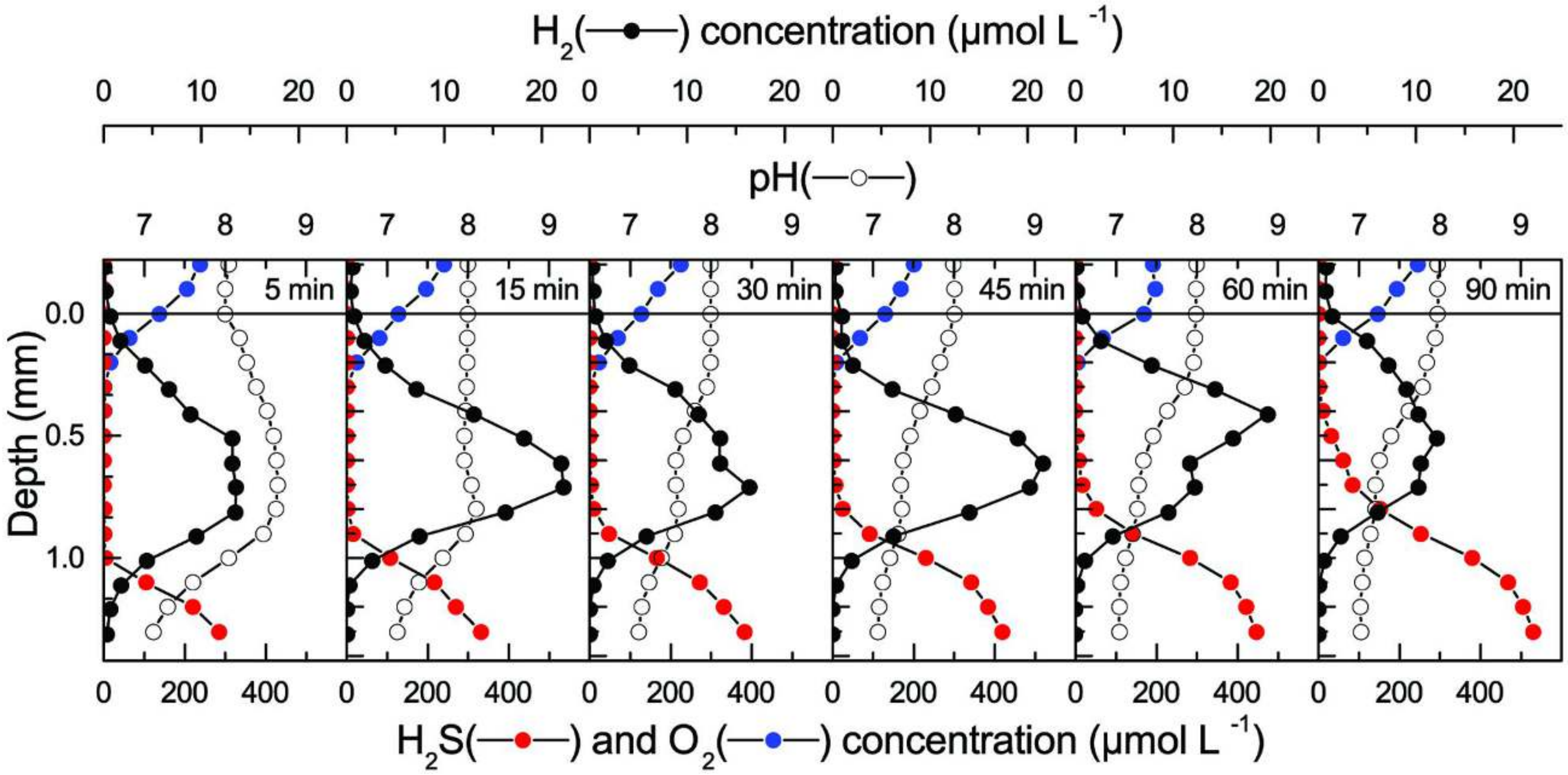
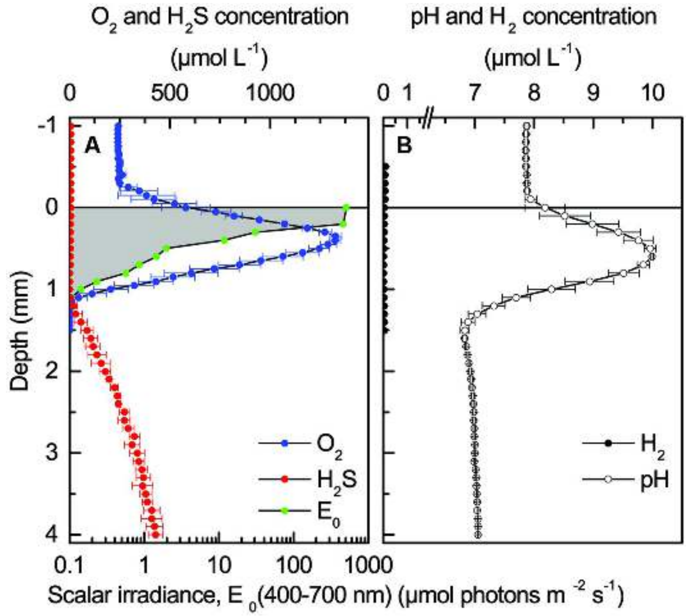
Microbial mats - H_2 dynamics





Same sediment as previous slide.

Light turned off at T = 0

Fast measurements needed to resolve the dynamics

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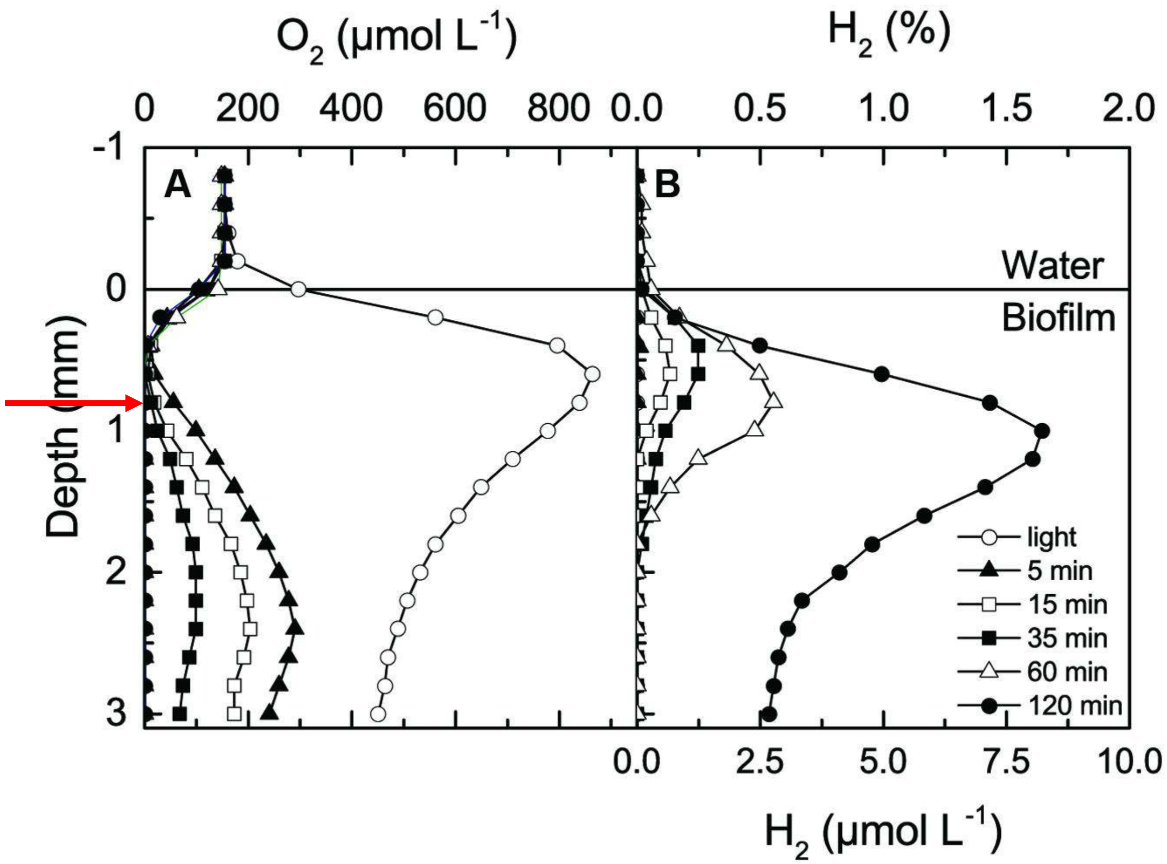
Microbial mats - H_2 dynamics

Hypersaline microbial mat, France



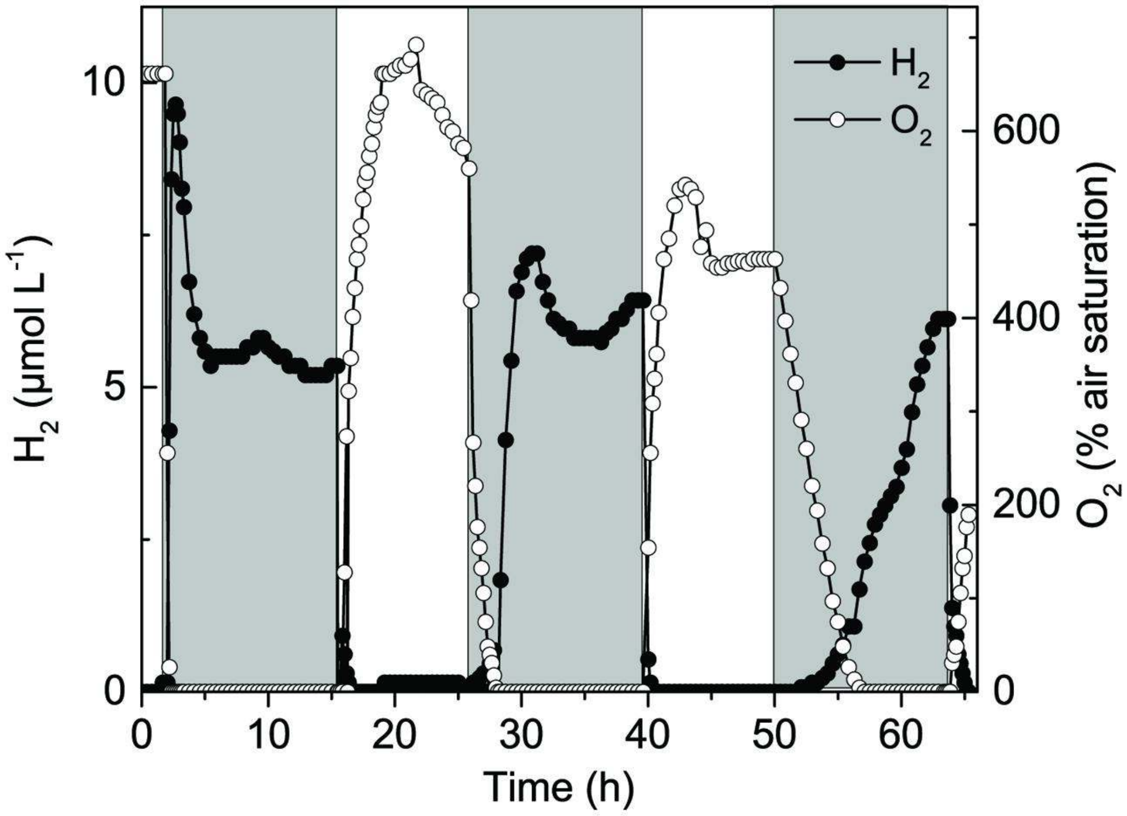
Sediment cores incubated in light in the lab. Light then turned off.

Study O_2 and H_2 dynamics over time. Placed O_2 and H_2 sensor at 0.8 mm depth.

Nielsen, M., N. P. Revsbech, and M. Kühl. 2015. Microsensor measurements of hydrogen gas dynamics in cyanobacterial microbial mats. Front. Microbiol. **6**: 1–12. doi:10.3389/fmicb.2015.00726

Microbial mats - H_2 dynamics

Hypersaline microbial mat, France



Sediment cores incubated in the lab in light/dark cycles. Measured at 0.8 mm depth.

Nielsen, M., N. P. Revsbech, and M. Kühl. 2015. Microsensor measurements of hydrogen gas dynamics in cyanobacterial microbial mats. Front. Microbiol. **6**: 1–12. doi:10.3389/fmicb.2015.00726

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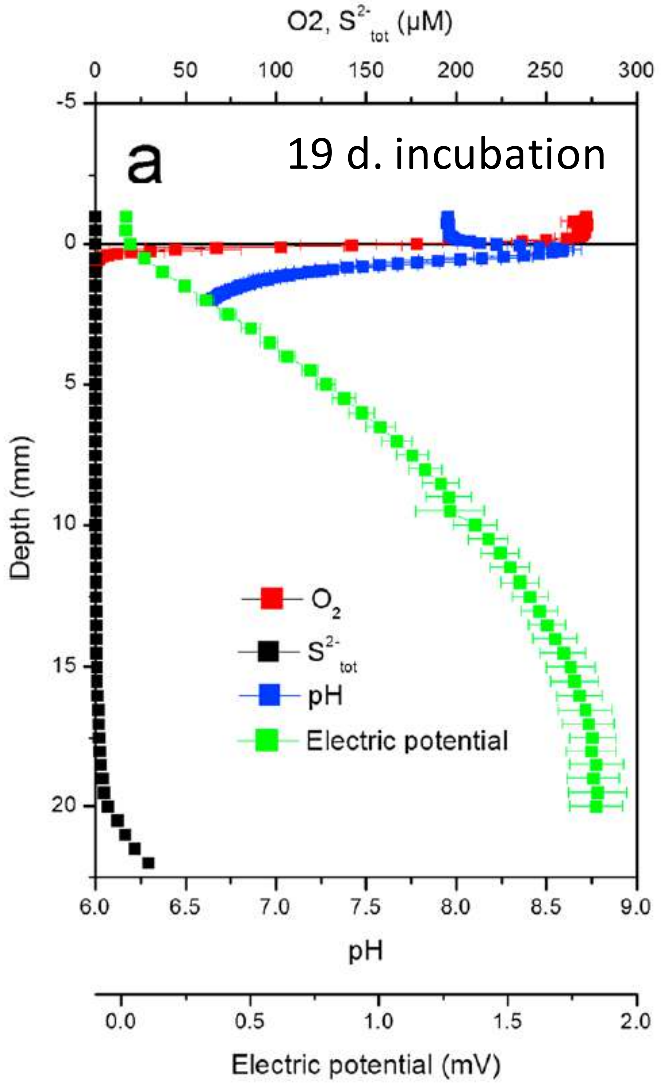
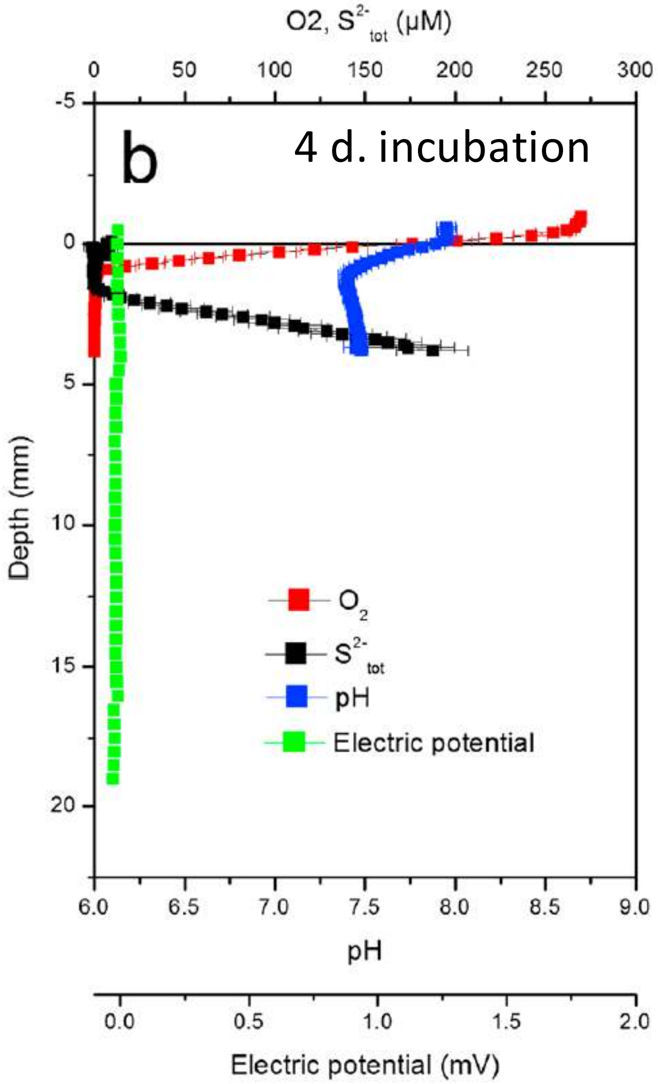
Cable bacteria - Electric potential microsensor





Damgaard, L. R., N. Risgaard-Petersen, and L. P. Nielsen. 2014. Electric potential microelectrode for studies of electrobiogeophysics. J. Geophys. Res. Biogeosciences 119: 1906–1917. doi:10.1002/2014JG002665

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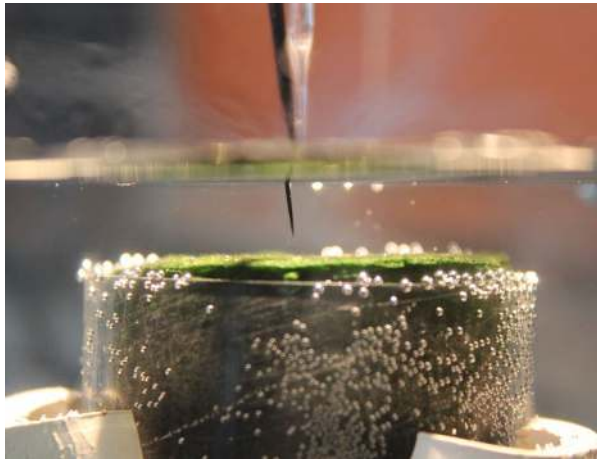
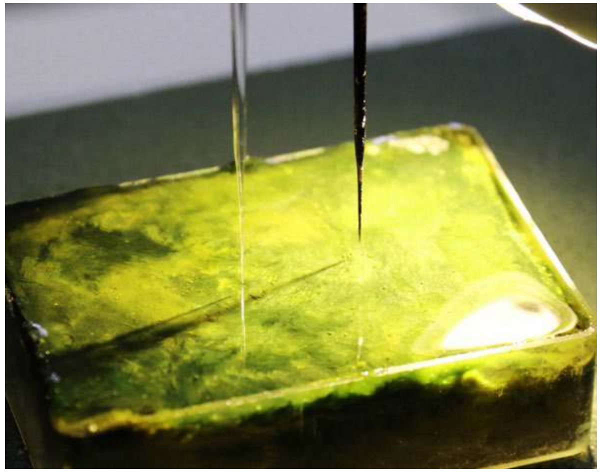
Photosynthesis - Light/dark shift technique

In light

At any point in the sediment, O_2 concentration is constant over time (steady state) =>

Production by photosynthesis equals removal by respiration and diffusion





Initially in darkness:

- O_2 removing processes are unchanged
  - Respiration
  - Diffusion away
- ΔO_2 concentration = O_2 production in light

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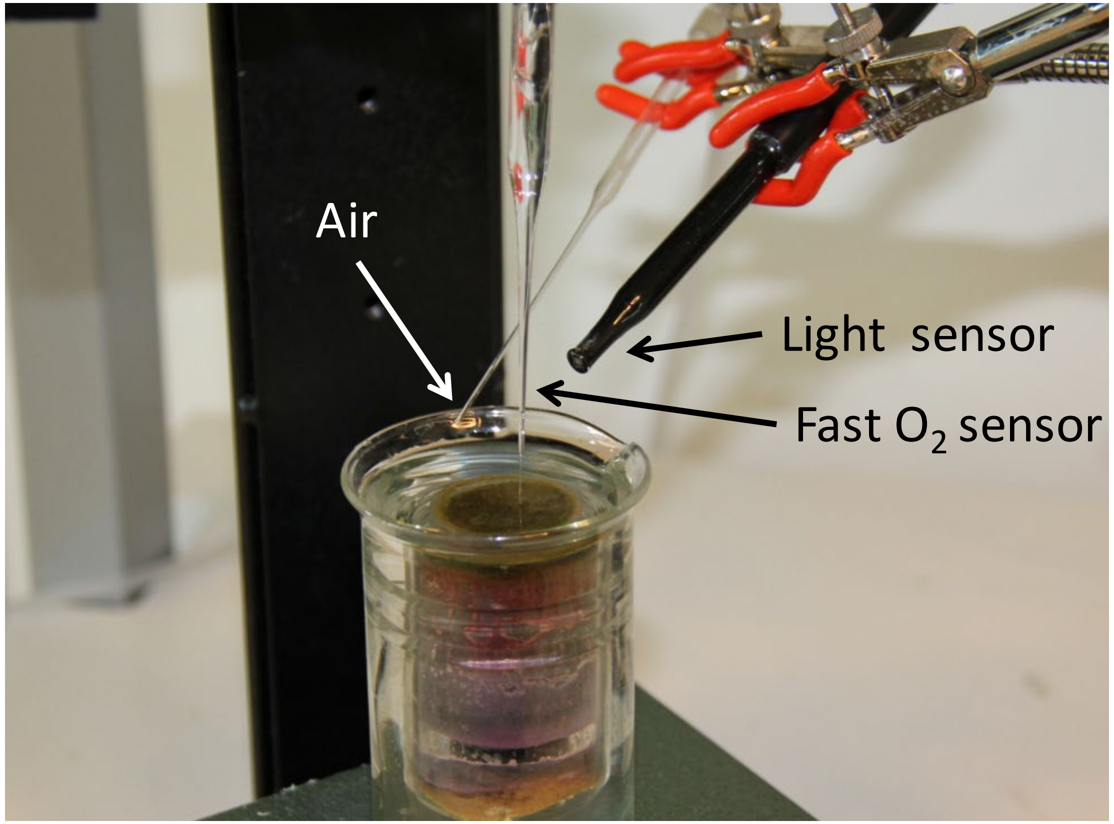
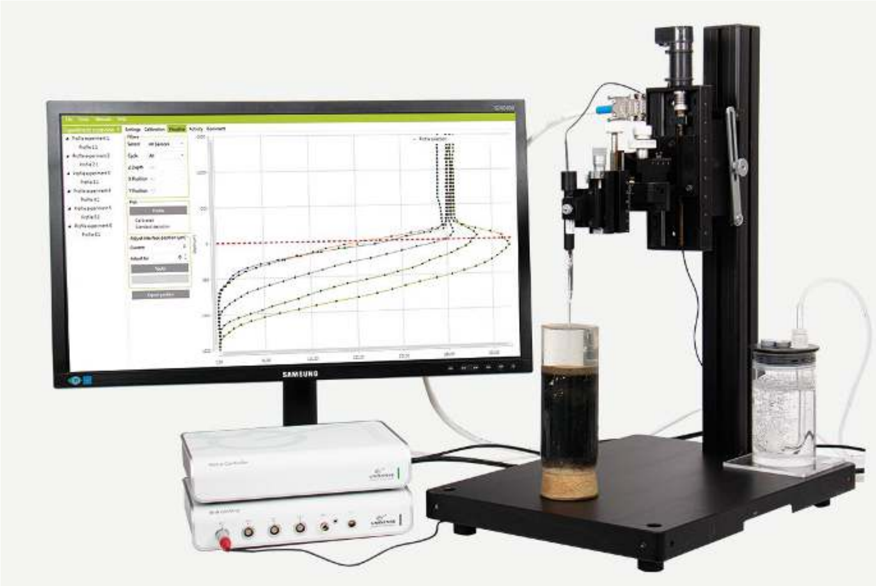
Photosynthesis - Light/dark shift technique

The setup





Photo

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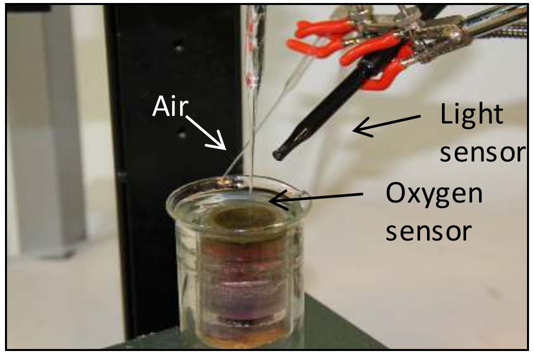
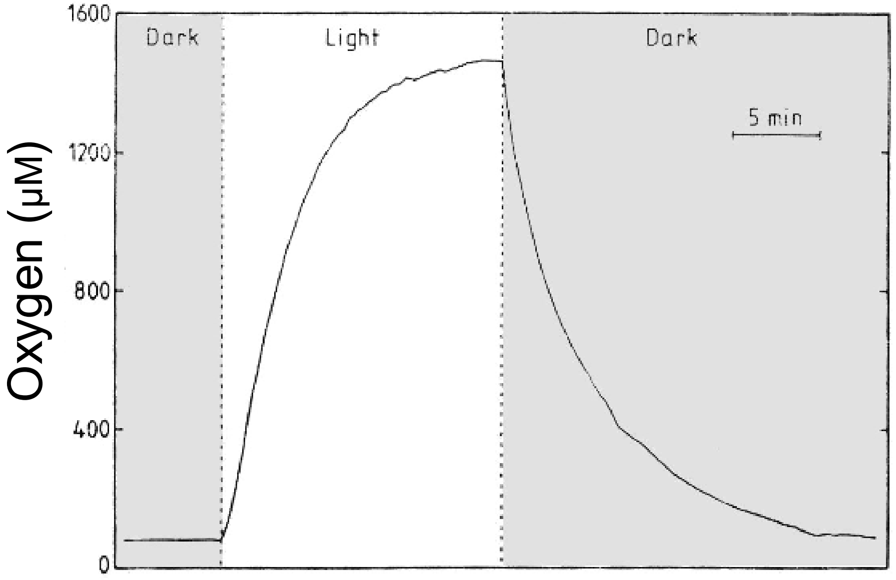
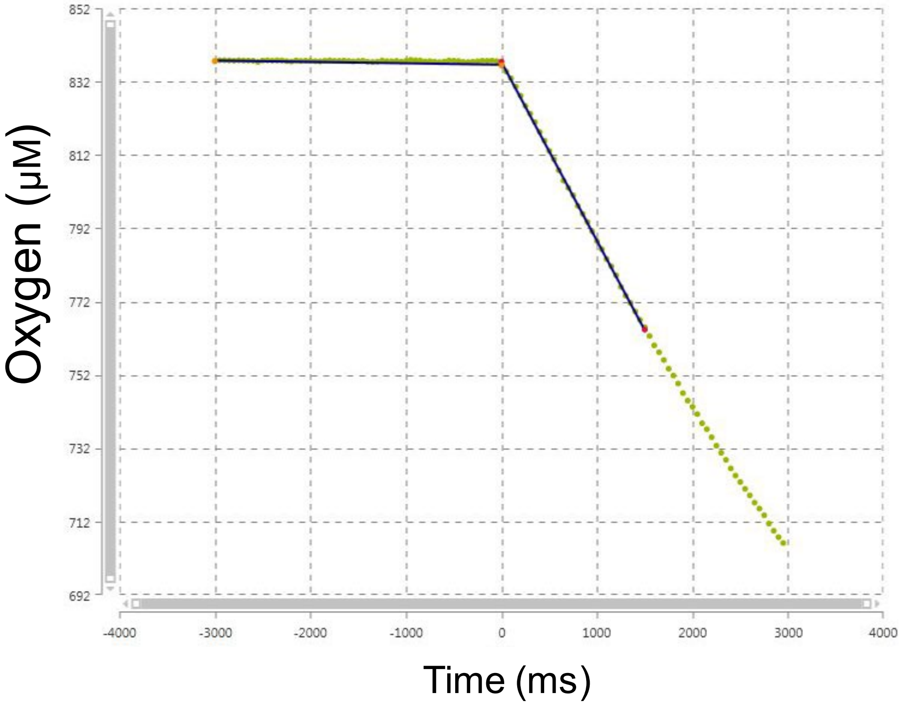
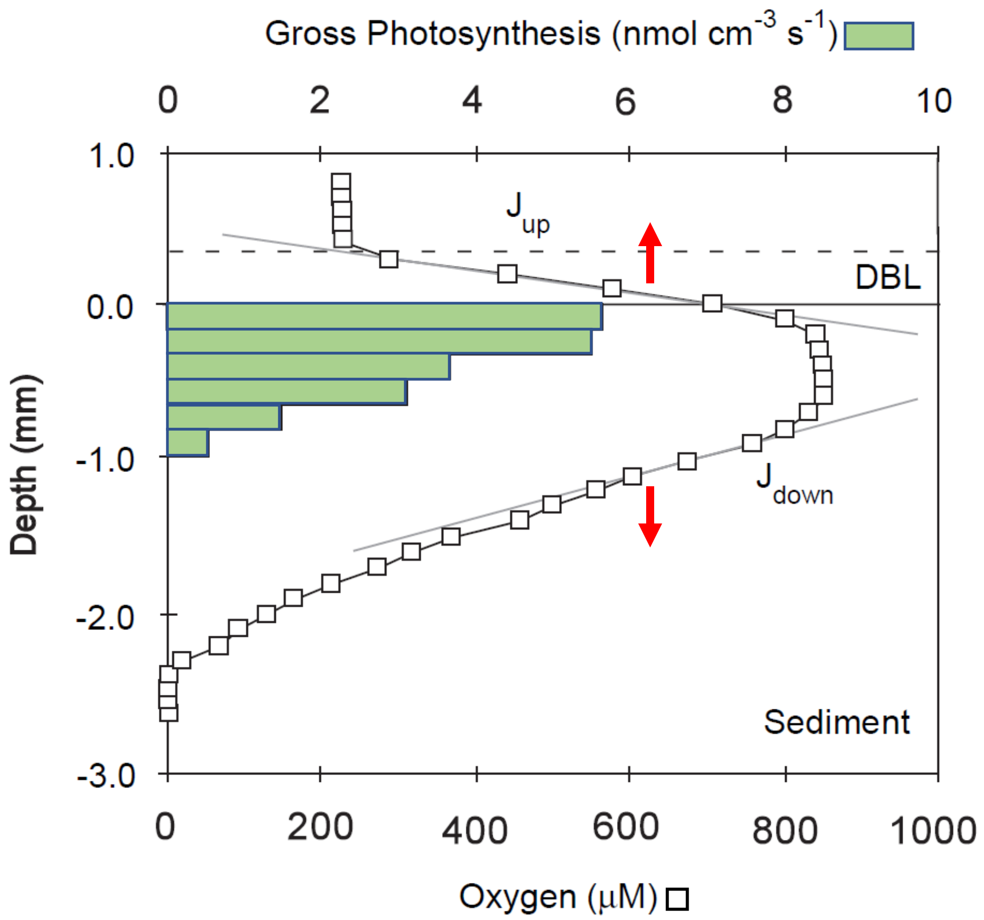
Photosynthesis - Light/dark shift technique







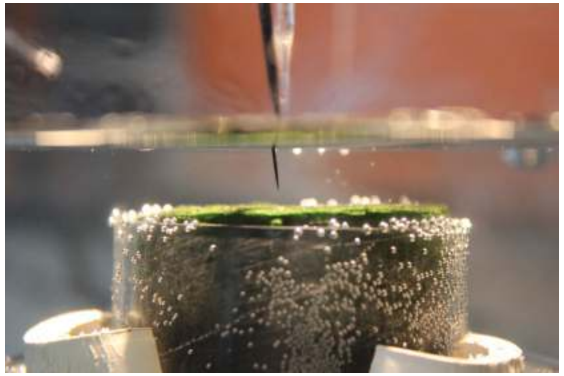
Photosynthesis - Light/dark shift technique



Glud, R. N. 2008. Oxygen dynamics of marine sediments. Mar. Biol. Res. **4**: 243–289.

Very strong tool

- Gross O_2 production
  - Directly measured (green bars)
- Net O_2 production: $J_{up} + J_{down}$
  - Ficks first law of diffusion (red arrows)
- Respiration in photic zone = Gross O_2 prod - Net O_2 prod



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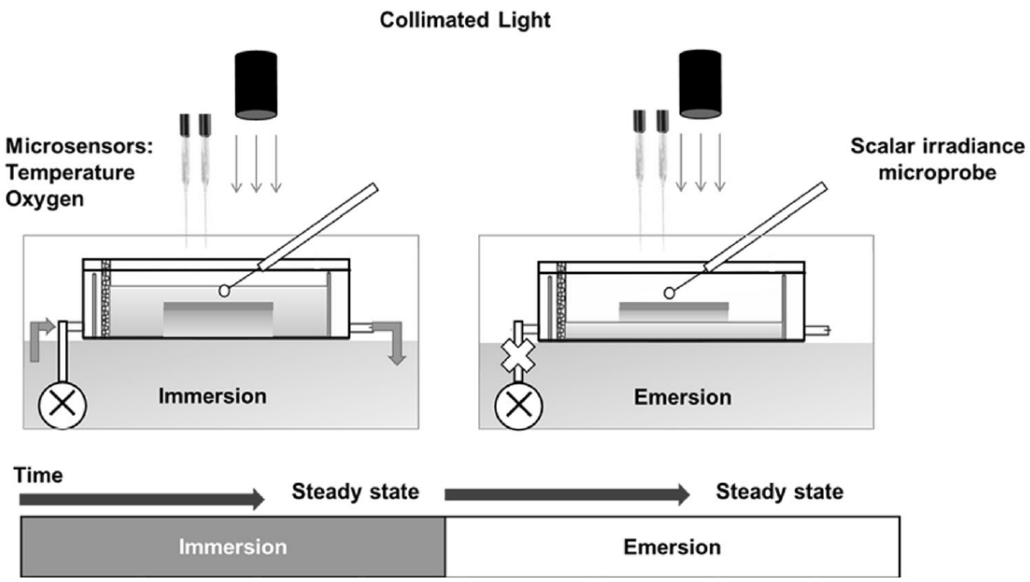
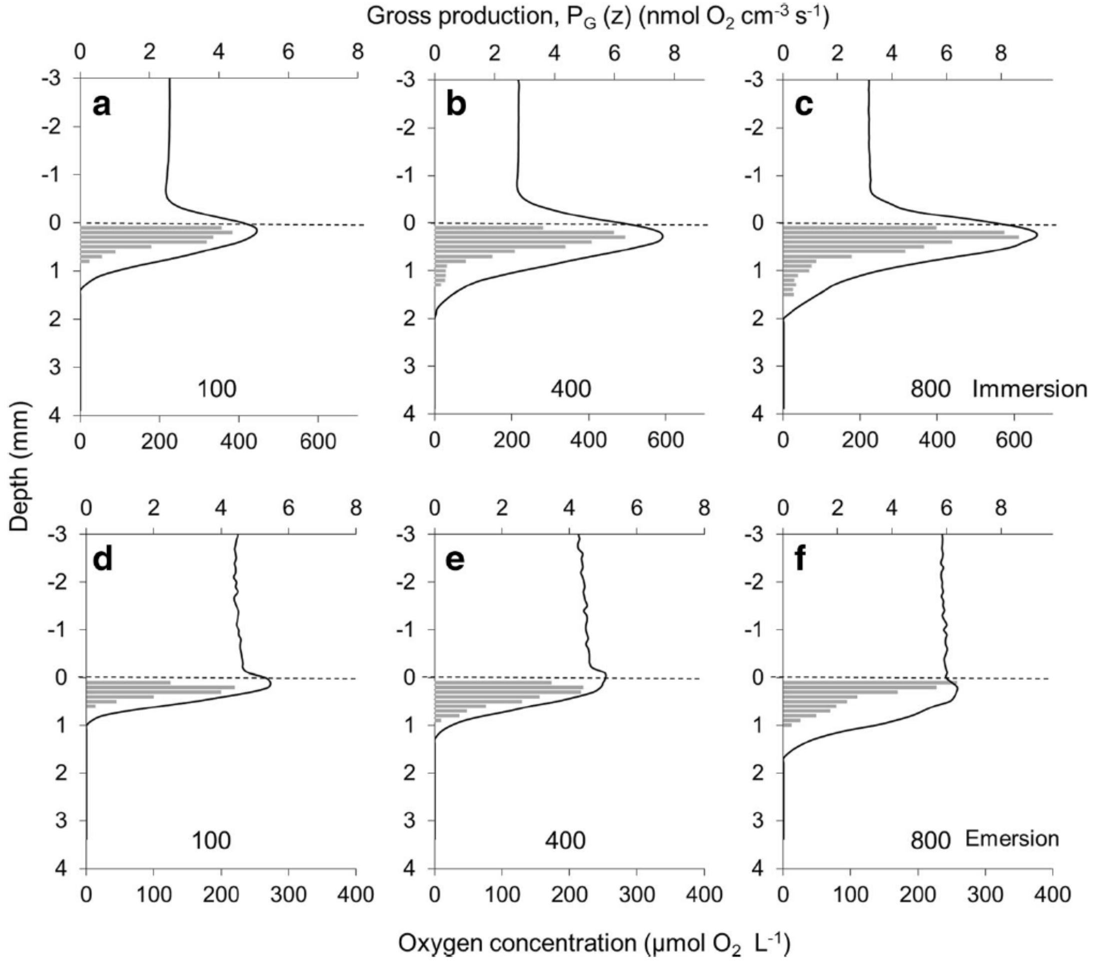
Photosynthesis - Light/dark shift technique

Effect of tidal conditions on photosynthesis in a microbial mat.





Haro, S., K. E. Brodersen, J. Bohórquez, S. Papaspyrou, A. Corzo, and M. Kühl. 2019. Radiative Energy Budgets in a Microbial Mat Under Different Irradiance and Tidal Conditions. Microb. Ecol. doi:10.1007/s00248-019-01350-6

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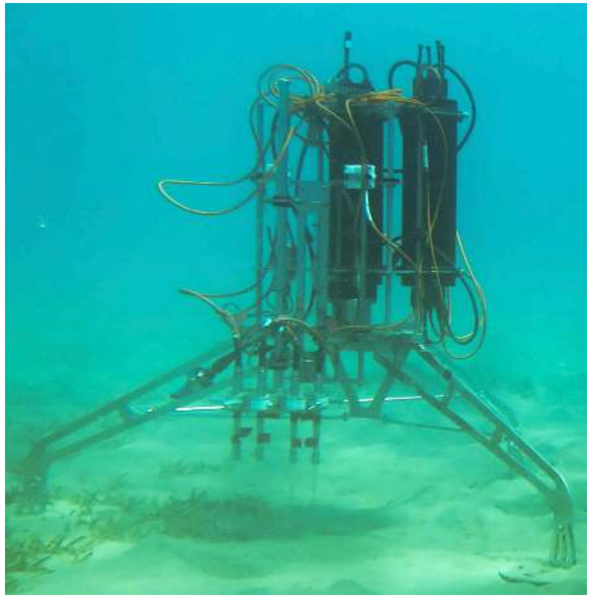
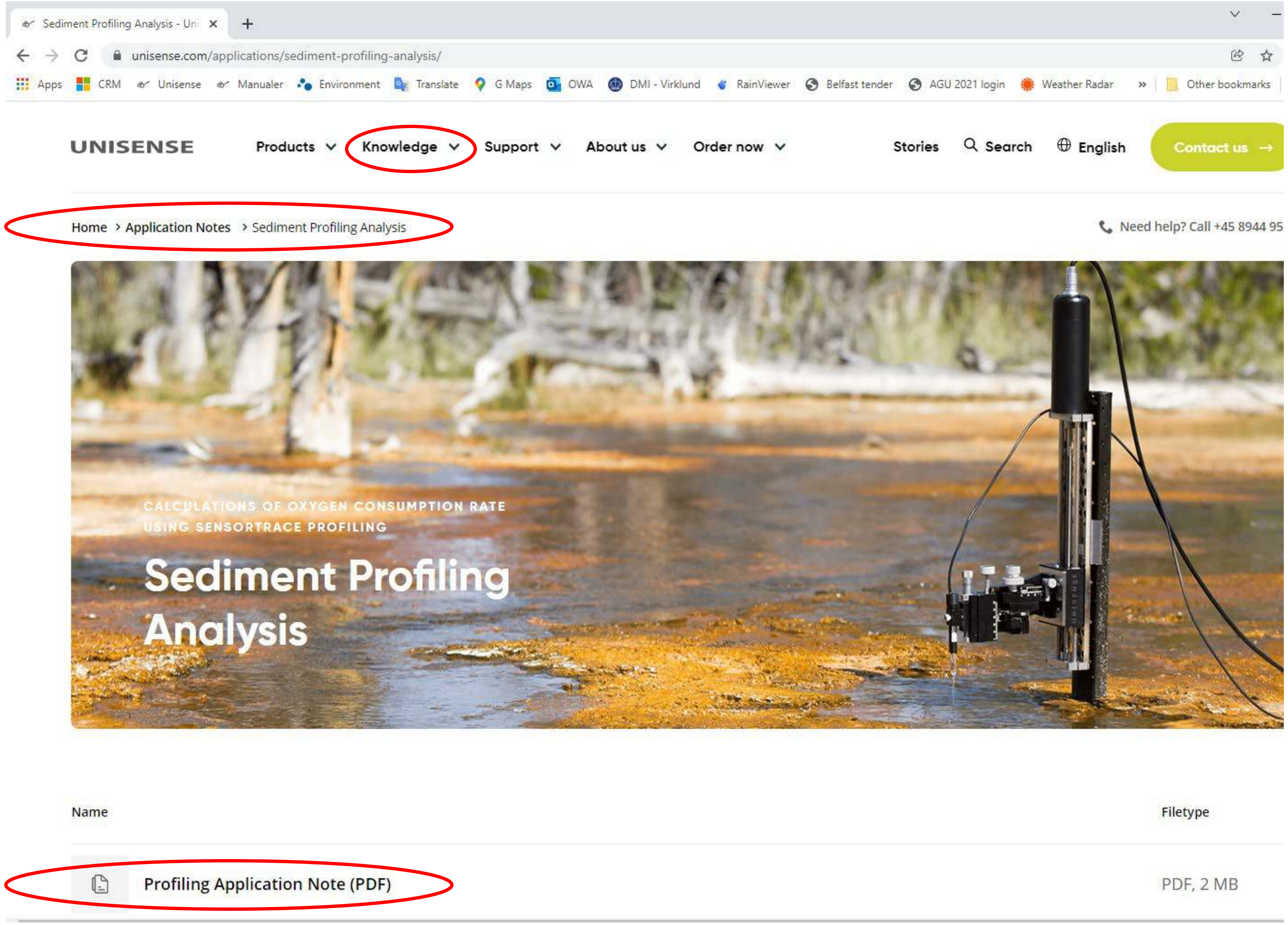
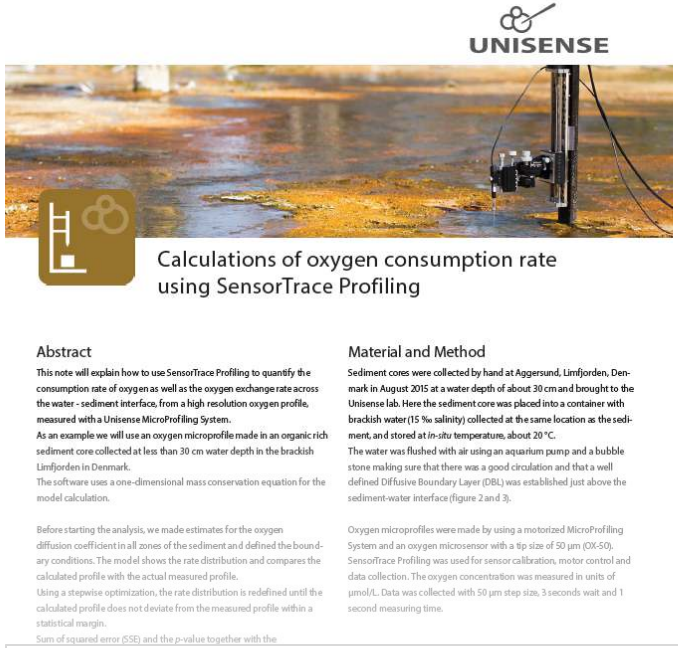
SensorTrace – Profiling



Calculations of oxygen consumption rate using SensorTrace Profiling

SensorTrace – Profiling





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Calculations of oxygen consumption rate using SensorTrace Profiling

Abstract

This note will explain how to use SensorTrace Profiling to quantify the consumption rate of oxygen as well as the oxygen exchange rate across the water - sediment interface, from a high resolution oxygen profile, measured with a Unisense MicroProfiling System.
As an example we will use an oxygen microprofile made in an organic rich sediment core collected at less than 30 cm water depth in the brackish Limfjorden in Denmark.
The software uses a one-dimensional mass conservation equation for the model calculation.

Before starting the analysis, we made estimates for the oxygen diffusion coefficient in all zones of the sediment and defined the boundary conditions. The model shows the rate distribution and compares the calculated profile with the actual measured profile.
Using a stepwise optimization, the rate distribution is redefined until the calculated profile does not deviate from the measured profile within a statistical margin.
Sum of squared error (SSE) and the *p*-value together with the

Material and Method

Sediment cores were collected by hand at Aggersund, Limfjorden, Denmark in August 2015 at a water depth of about 30 cm and brought to the Unisense lab. Here the sediment core was placed into a container with brackish water (15 ‰ salinity) collected at the same location as the sediment, and stored at *in-situ* temperature, about 20 °C.
The water was flushed with air using an aquarium pump and a bubble stone making sure that there was a good circulation and that a well defined Diffusive Boundary Layer (DBL) was established just above the sediment-water interface (figure 2 and 3).

Oxygen microprofiles were made by using a motorized MicroProfiling System and an oxygen microsensor with a tip size of 50 µm (OX-50). SensorTrace Profiling was used for sensor calibration, motor control and data collection. The oxygen concentration was measured in units of µmol/L. Data was collected with 50 µm step size, 3 seconds wait and 1 second measuring time.

Thursday 24 February 16:45-17:30 CET
Demonstration of Activity Calculation - Software

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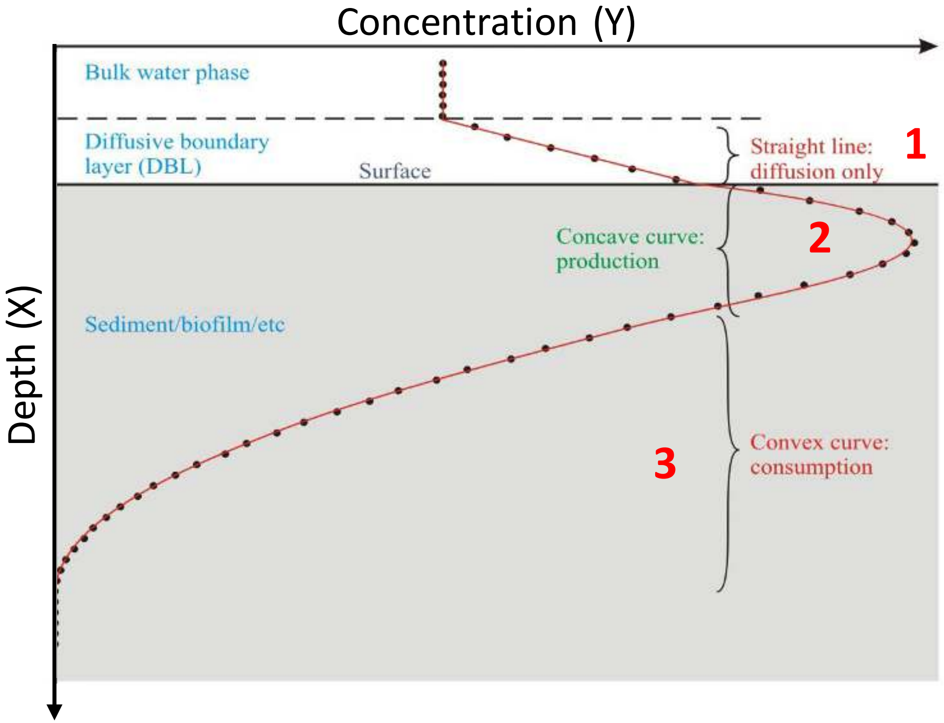
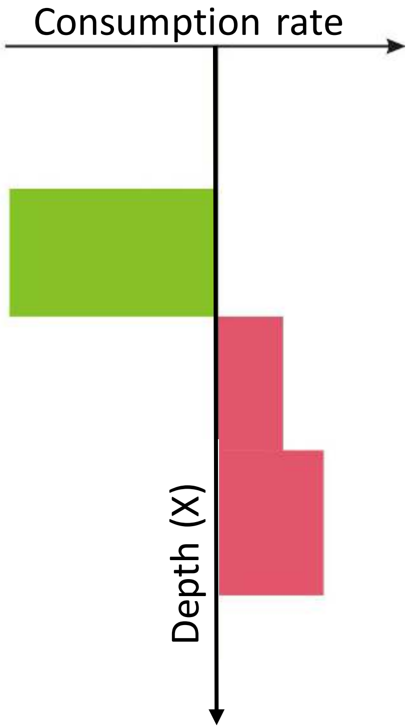
Microprofiles - Theory

Example:
Sediment or biofilm with O_2 production in top layer, O_2 consumption below





Qualitative information from profiles

1. Straight line: No net consumption or production – only diffusional transport.
2. Concave: Net production (e.g. O_2 production by photosynthesis, H_2S production by sulphate reduction)
3. Convex: Net consumption (e.g. Respiration, oxidation of reduced compounds)

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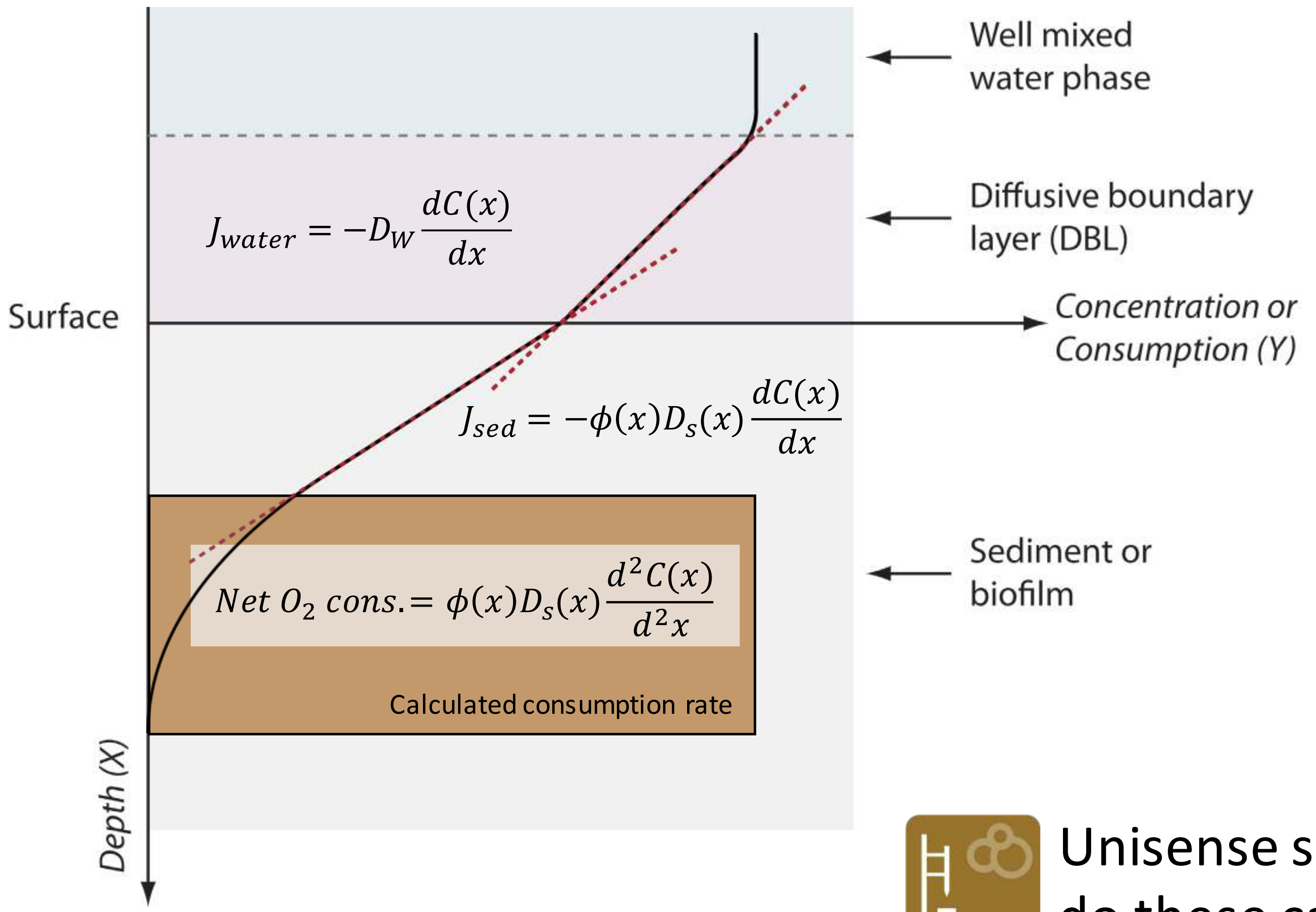
Microprofiles - Theory

1-dimensional system – Steady state



Quantitative Information from microprofiles

1. **C(x) - Concentration**: Penetration depth, overlapping zones
2. **dC(x)/dx – Flux:** Into sediment, within sediment
3. **$d^2C(x)/dx^2$ – Production and consumption:** Activity distribution within the sediment

D_w = Diffusion coefficient in water
$\phi(x)$ = Porosity
$D_s(x)$ = Diffusivity
$D_s(x)$ = $D_w \times \phi(x)$ (simplest form)

Unisense software (Profile) can do these calculations

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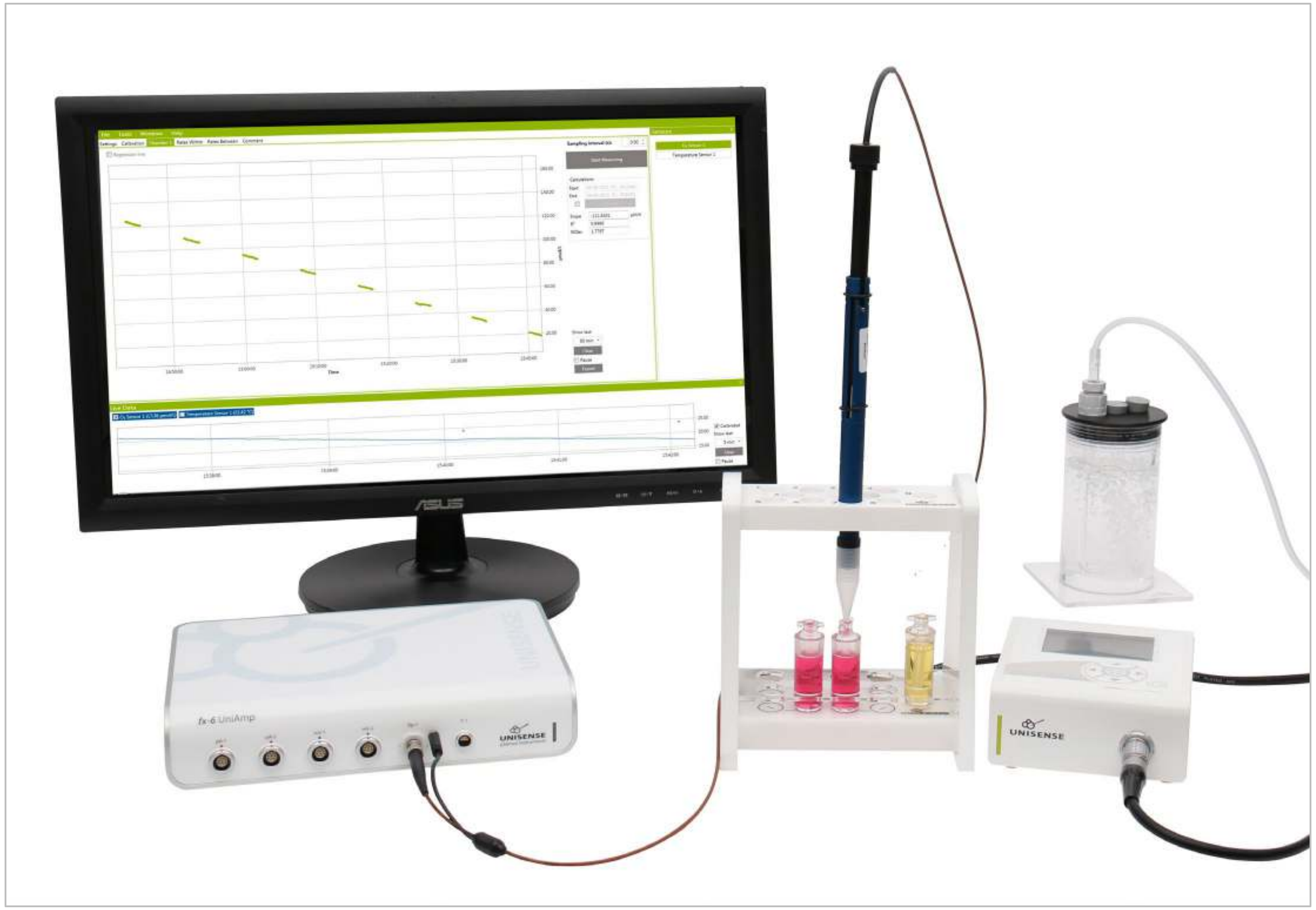
MicroRespiration system

Microrespiration: Quantify metabolic activity of small organisms.



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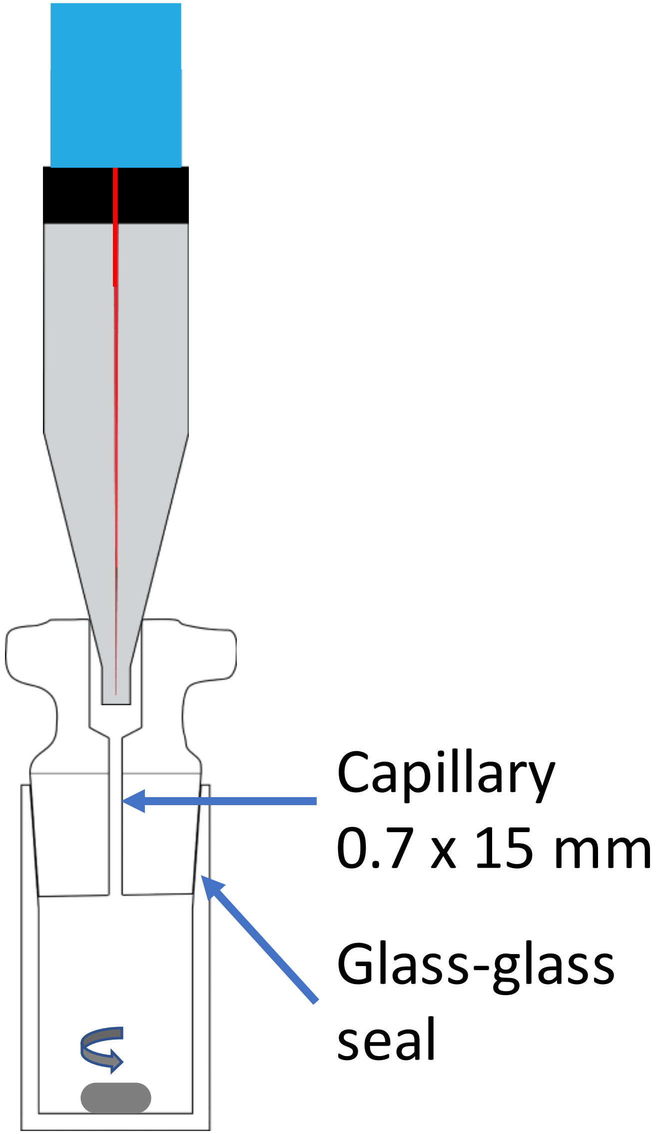
MicroRespiration system



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MicroRespiration system

Changes in small volumes

- O_2, pH, H_2, H_2S, N_2O, and NO
- Complete system
  - Glass chambers, Magnetic stirring, Rack, Sensors
- SensorTrace Rate Software
  - Real-time data
  - Data for each chamber individually
  - Graphs for each chamber

Chambers: Small (400-4000 µL)
Medium (20, 40 and 50 mL)
Large (200-400 mL)

200 ml

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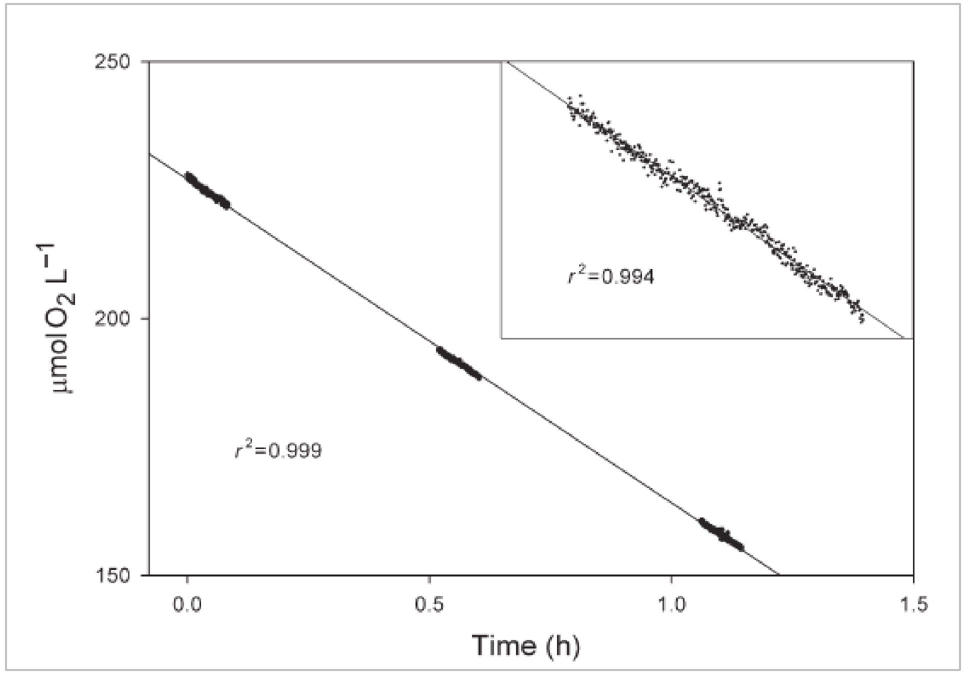
MicroRespiration system

Rate

MicroRespiration system

Respiration rate of estuarine hydromedusa *N. bachei*.

Nemopsis bachei



Organism: 3 – 5 mm dia.
Chamber: 2 ml

Marshalonis, D., and J. L. Pinckney. 2007. Respiration rates of dominant hydromedusae in the North Inlet tidal estuary during winter and summer. J. Plankton Res. 29: 1031–1040.

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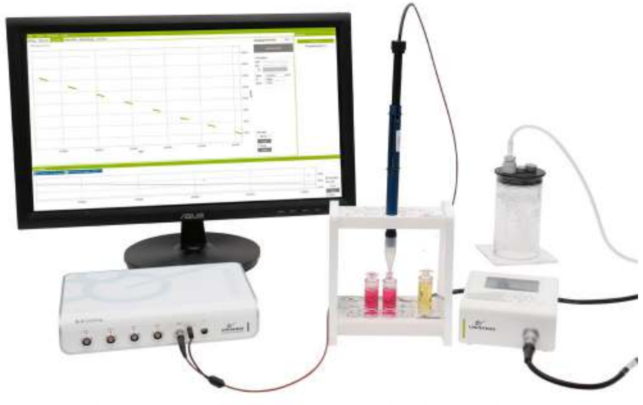
MicroRespiration system

nature Vol 461|15 October 2009|doi:10.1038/nature08465

LETTERS

Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria

Willm Martens-Habbena[1], Paul M. Berube[1]†, Hidetoshi Urakawa[1], José R. de la Torre[1]† & David A. Stahl[1]



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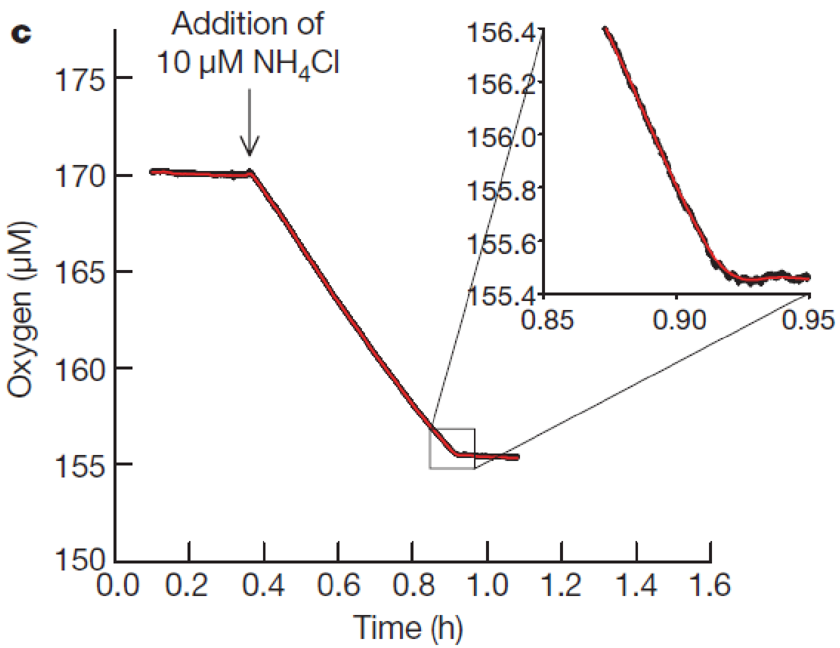
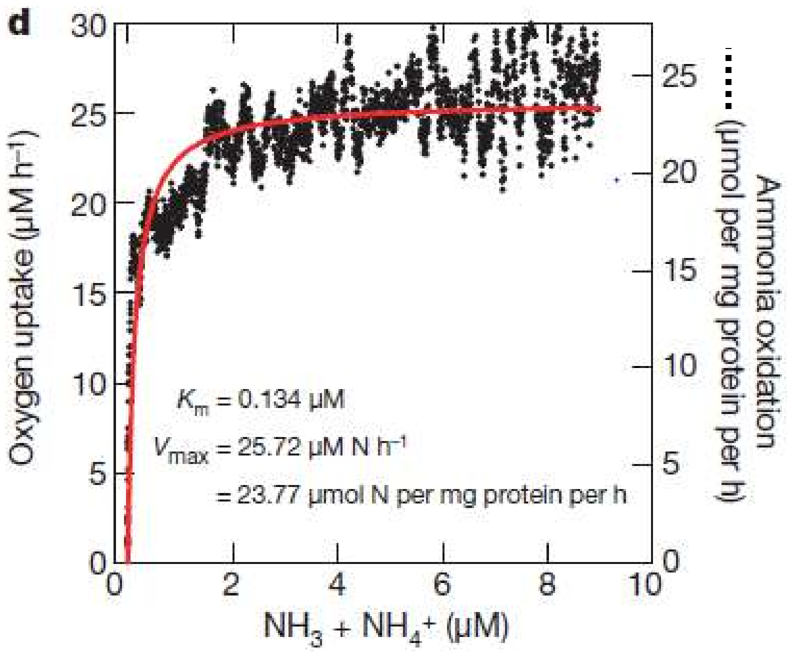
MicroRespiration system





Injection lid

Nitrification

$2NH_4^+ + 3O_2 \longrightarrow 2NO_2^- + 2H_2O + 4H^+$

$NH_3 + O_2 \longrightarrow NO_2^- + 4H^+ + 2e^-$

Ammonium/oxygen consumption = 1:1.52

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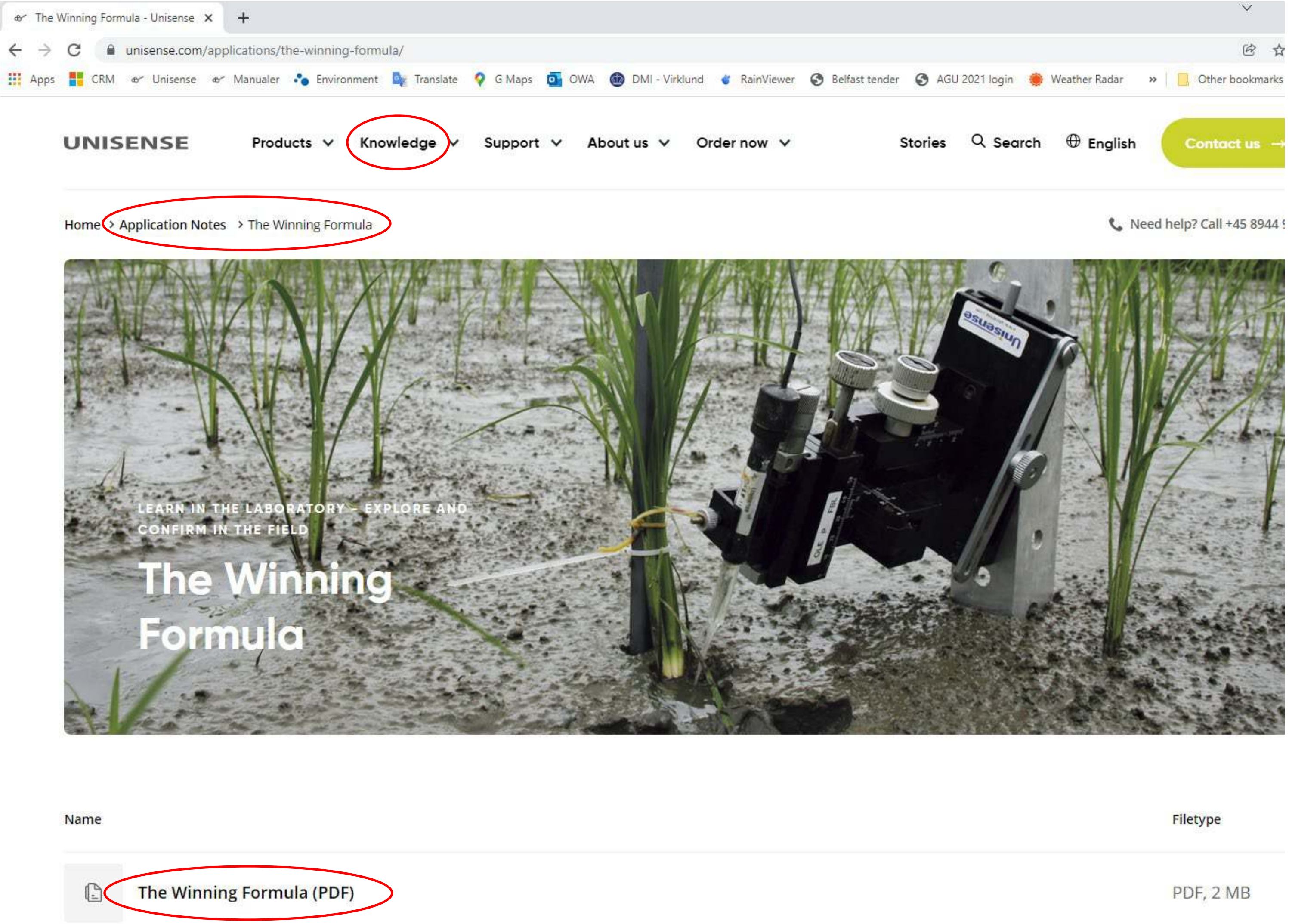
In situ and lab

Learn in the laboratory - explore and confirm in the field!



Photos: Ole Pedersen

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SOLUTIONS FOR MICROSCALE MEASUREMENTS

The Winning Formula

Learn in the laboratory - explore and confirm in the field!

Ole's important take-home messages:

- Include *in situ* measurements - it is a winning formula
- Learn your system in the laboratory before going *in situ*
- Choose the right size of sensor to answer your question
- Avoid breaking sensors in a wobbly experimental setup

Professor Ole Pedersen at University of Copenhagen is a very experienced microsensor user with a long track record of published articles where Unisense microsensors have been a key technology. Ole has travelled the world with microsensors and applied them in seagrass beds in the Caribbean Sea, rice paddies in the Philippines and peat swamps in Australia just to mention a few exotic research locations. A number of Ole's publications are based on initiating microsensor studies in the laboratory and subsequently taking the measurements to the field, a strategy that he calls "the winning formula". Unisense invited Ole for a talk about microsensor research and the so-called winning formula.

What are the typical challenges you meet when setting up a microsensor experiment?

It's all about stability and ease of access! Stability – no unsteady tables or shaky tanks and containers – is the main thing in any microsonsor set-up. I use a lot of time to ensure that the pots, the cores or the trays where I have my specimens are well fixed, i.e. to the table or the bottom of the tank. Then, I fix all cables, and also the leaves if working with plants, to avoid that I break a sensor already in the process of mounting it in the micromanipulator… My laboratory set-up's look very "clean"!

What were your initial reasons for going into the field?

Curiosity! I started with laboratory measurements in seagrasses and got fantastic data. But the uncertainty of whether the observed phenomena also occurred in the field situation was there – so I had to go and see.

> *"…the uncertainty of whether the observed phenomena also occurred in the field situation was there – so I had to go and see.*
>
> *- Prof. Ole Pedersen*

What would you state as the greatest advantages of starting the experiment in the laboratory and then proceed into the field?

You know exactly what you are looking for! I strongly prefer to identify a mechanism in the lab where I can control all the environmental parameters such as temperature, light, flow – and stability of the set-up. The interesting part for me and the readers of the scientific papers is then to go and show that this mecha-

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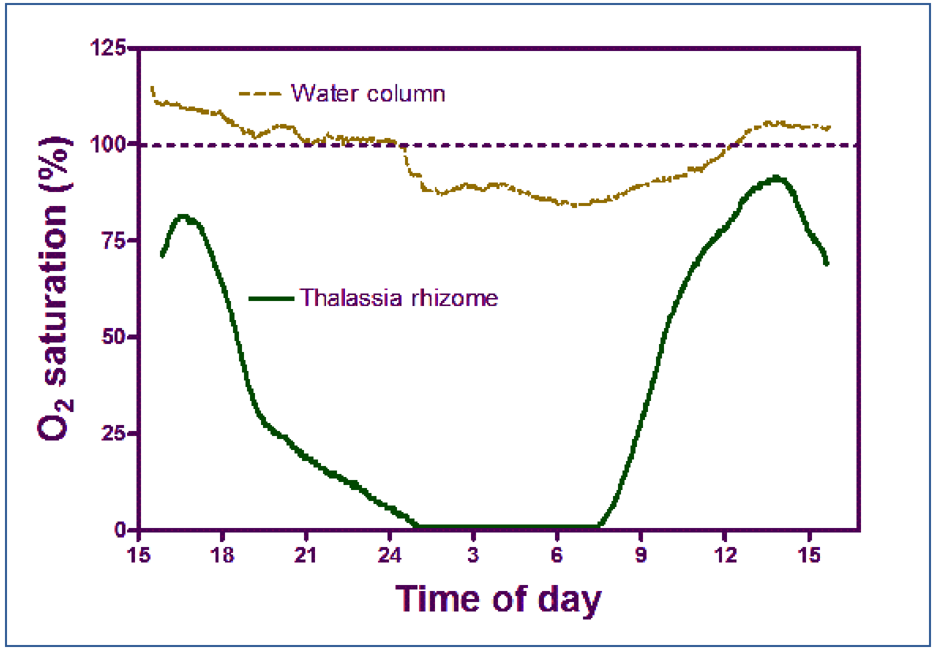
Monitoring O_2 in rhizosphere

In-situ measurements of oxygen dynamics in sea-grass beds

Diurnal variation in the oxygen level within the rhizome of seagrass and in the water phase



Ole Pedersen, Univ. Copenhagen, DK,
unpublished

Underwater meter

UNISENSE

Bioturbation by Fiddler Crabs

- Thuwal, Red Sea
- Field Microprofiling System
- Splash proof meter
- Water proof motor
- Battery power
- Stand alone or with PC

Booth, J. M., M. Fusi, R. Marasco, T. Mbobo, and D. Daffonchio. 2019. Fiddler crab bioturbation determines consistent changes in bacterial communities across contrasting environmental conditions. Sci. Rep. **9**: 3749. doi:10.1038/s41598-019-40315-0

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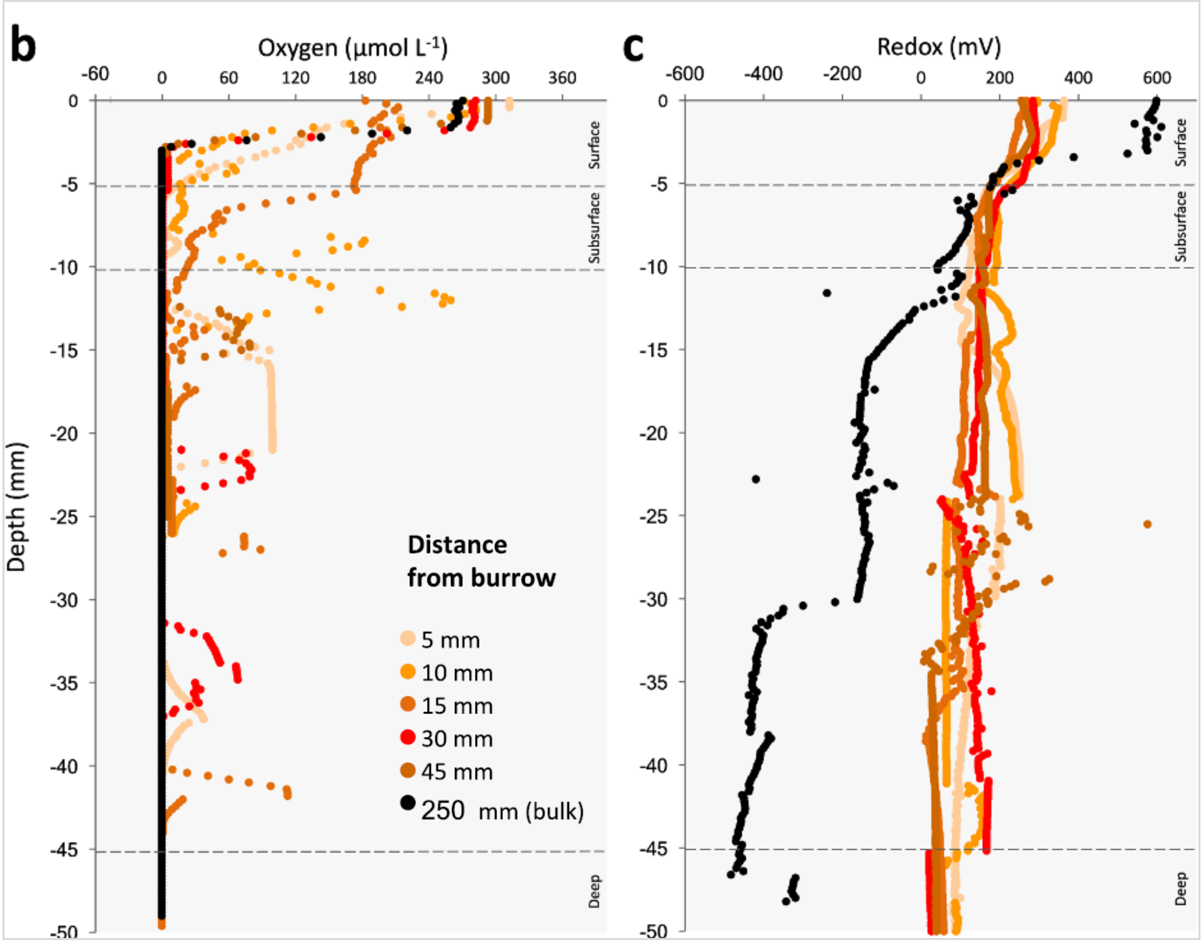
Bioturbation by Fiddler Crabs



Booth, J. M., M. Fusi, R. Marasco, T. Mbobo, and D. Daffonchio. 2019. Fiddler crab bioturbation determines consistent changes in bacterial communities across contrasting environmental conditions. Sci. Rep. **9**: 3749. doi:10.1038/s41598-019-40315-0

UNISENSE

Field MicroProfiling System

Lab system - PC operated

Field system - Stand-alone

2-D System

Sensor holder or Micromanipulator

UNISENSE

Field MicroProfiling System

- Operated from keypad or PC with SensorTrace Suite
- Splash proof 8 channel amplifier
- Battery (20 hours)
- Field motor, waterproof (min 10 µm step size)
- Field stand for lab and field mounting
- 5 m sensor cables (optional 10 m)

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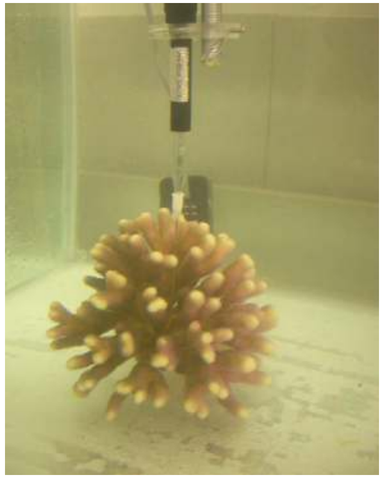
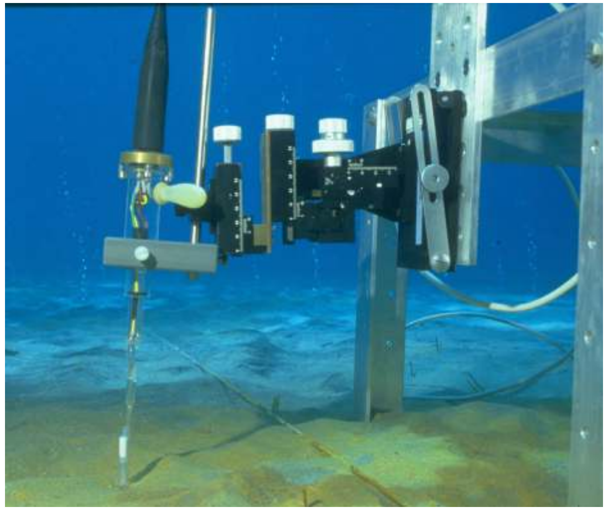
Laboratory vs. Field measurements





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

UNISENSE

Time for questions !

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

Contact us: sales@unisense.com