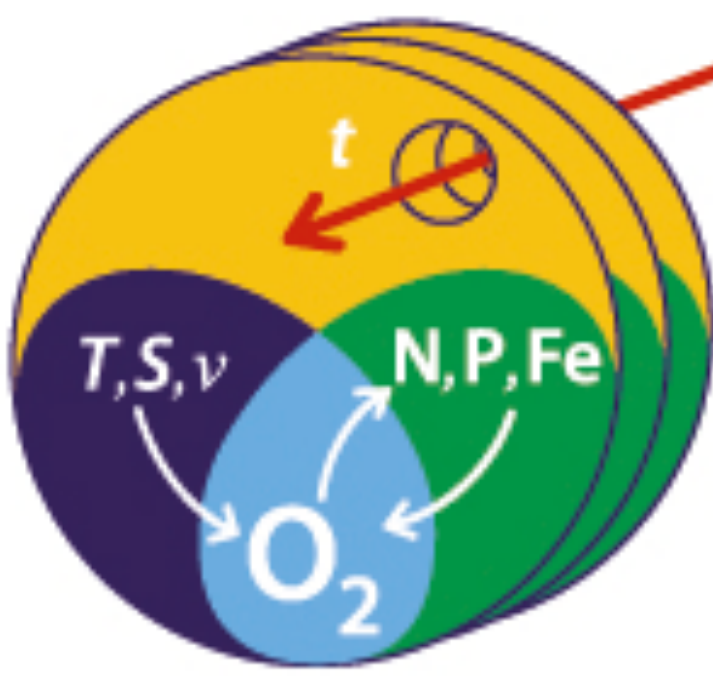




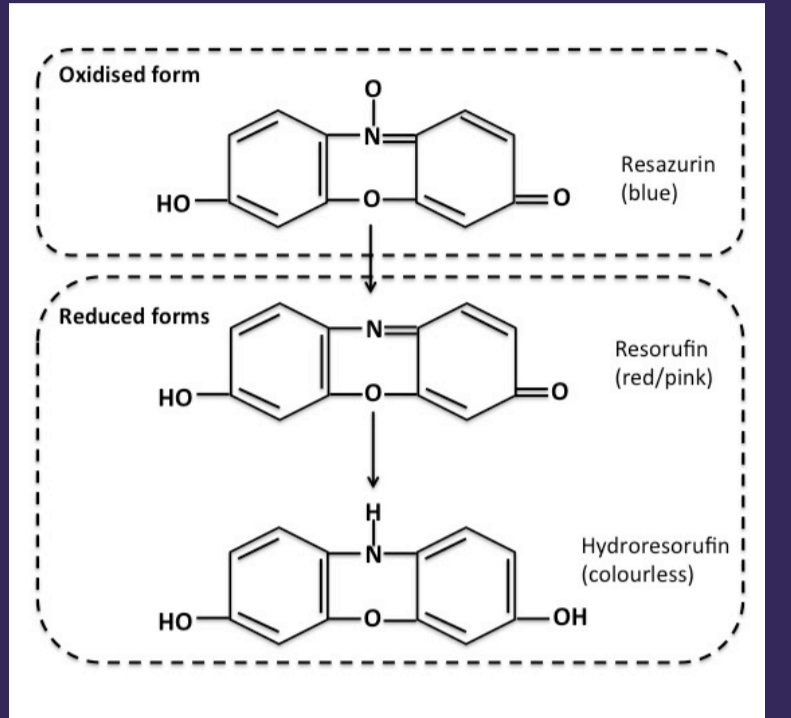
# The Resazurin-Agar Method: A Quick Test to Determine Water Quality

Jonas Huckfeldt\*, Lisa Claußen and Bjarne Westphal  
Humboldt- Schule Kiel  
GEOMAR Helmholtz Centre for Ocean Research Kiel, Kiel, Germany



SFB 754

Resazurin is a blue redox-indicator which turns irreversibly to the pink coloured resorufin when reduced by bacteria. Thus the degree and speed of colour change is an indication of microbial activity and bacterial numbers. We tested the suitability of resazurin agar plates to test the microbial activity in small volumes of different water samples.



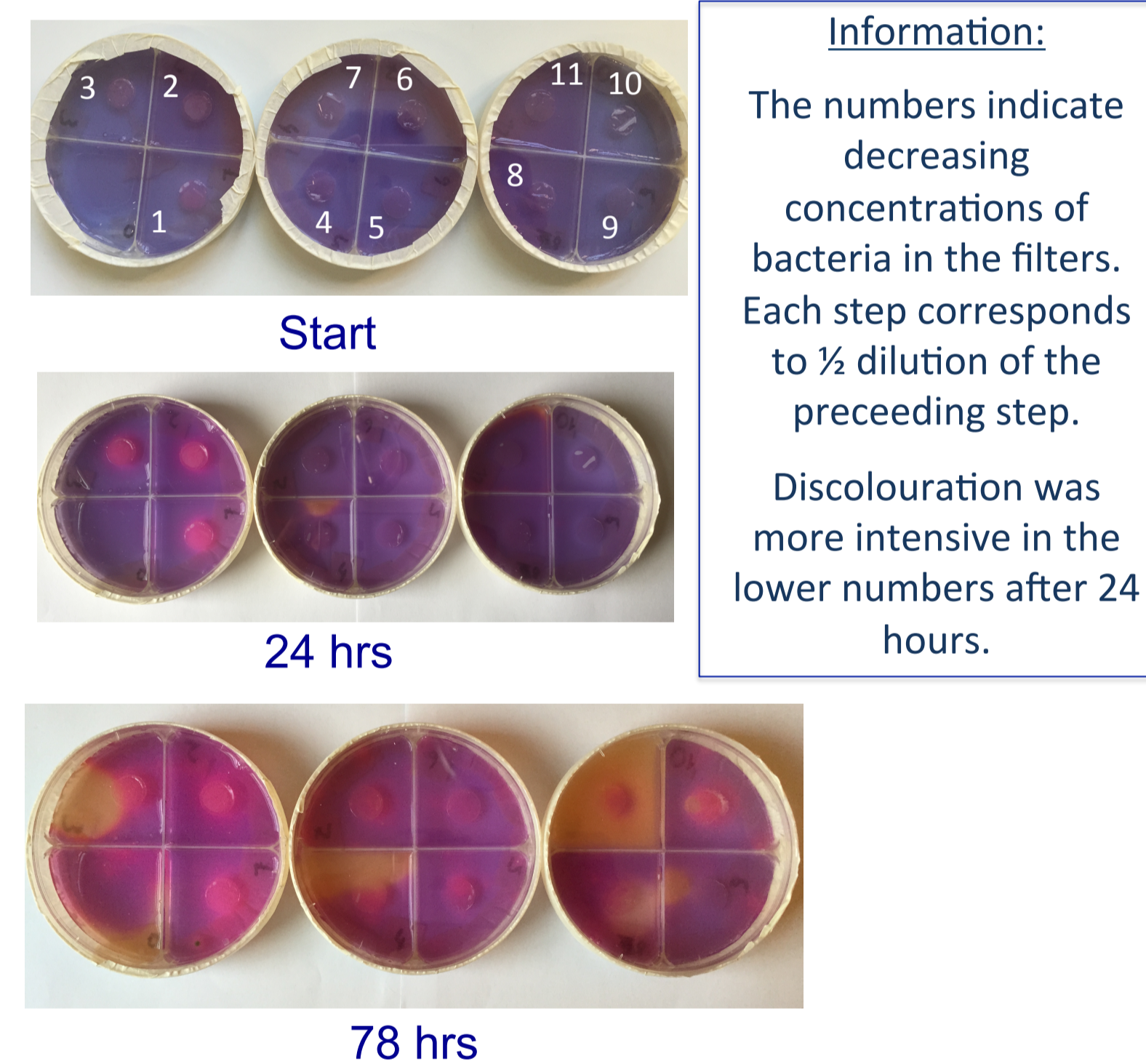
Structure of Resazurin

## Laboratory Tests

Preliminary experiment using a stock suspension of yogurt bacteria was prepared. Different dilutions of the suspension were placed onto filters and the filters were placed face down on the resazurin agar plates.



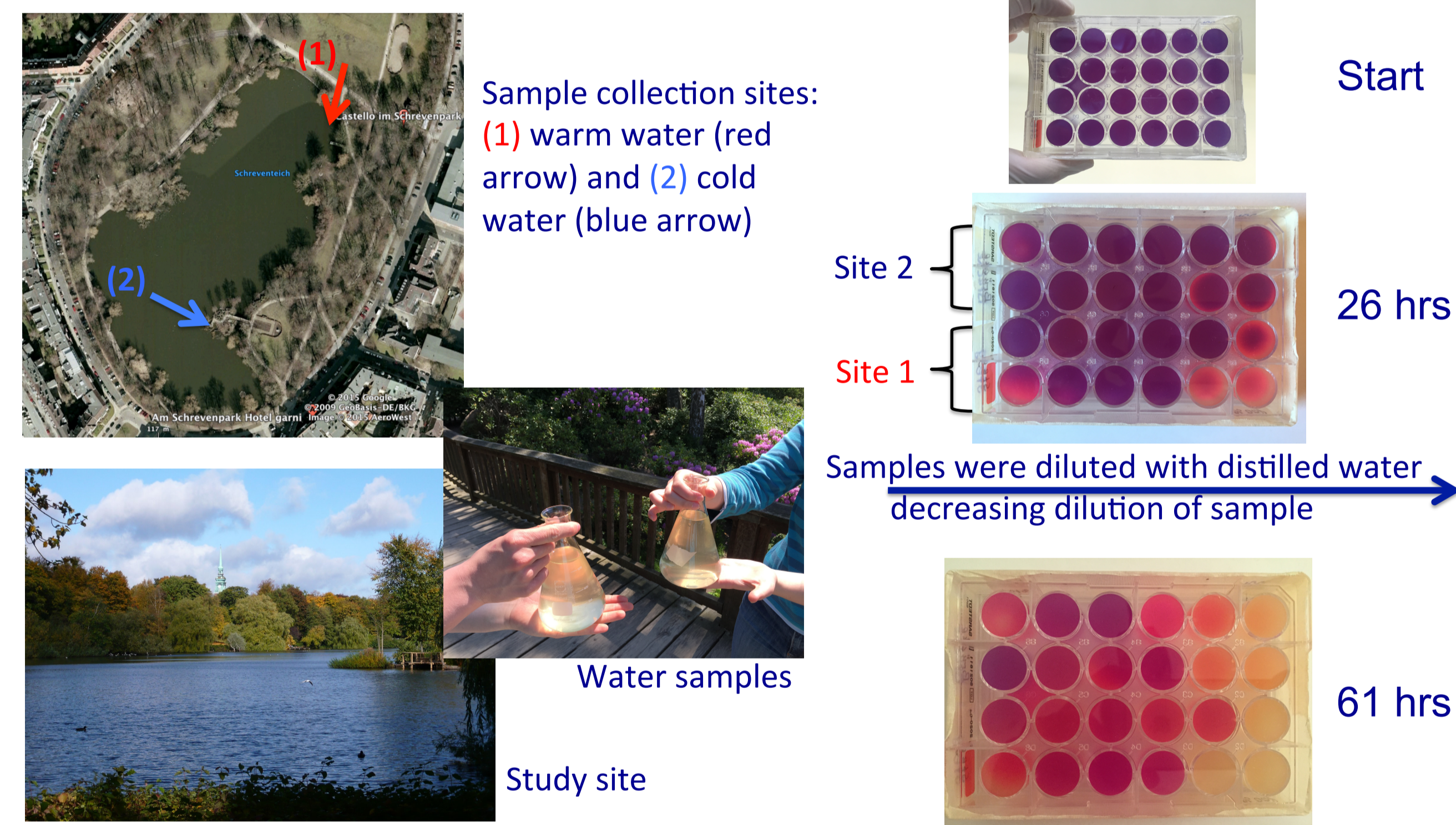
Materials used for making the agar plates



- The resazurin was reduced in the presence of yogurt bacteria in the filters.
- A discolouration gradient with regard to the concentration of bacteria was recognizable.
- A strong contamination by foreign bacteria caused reduction of resazurin and resorufin in the plates after 78 hrs.

## Lake Water Samples

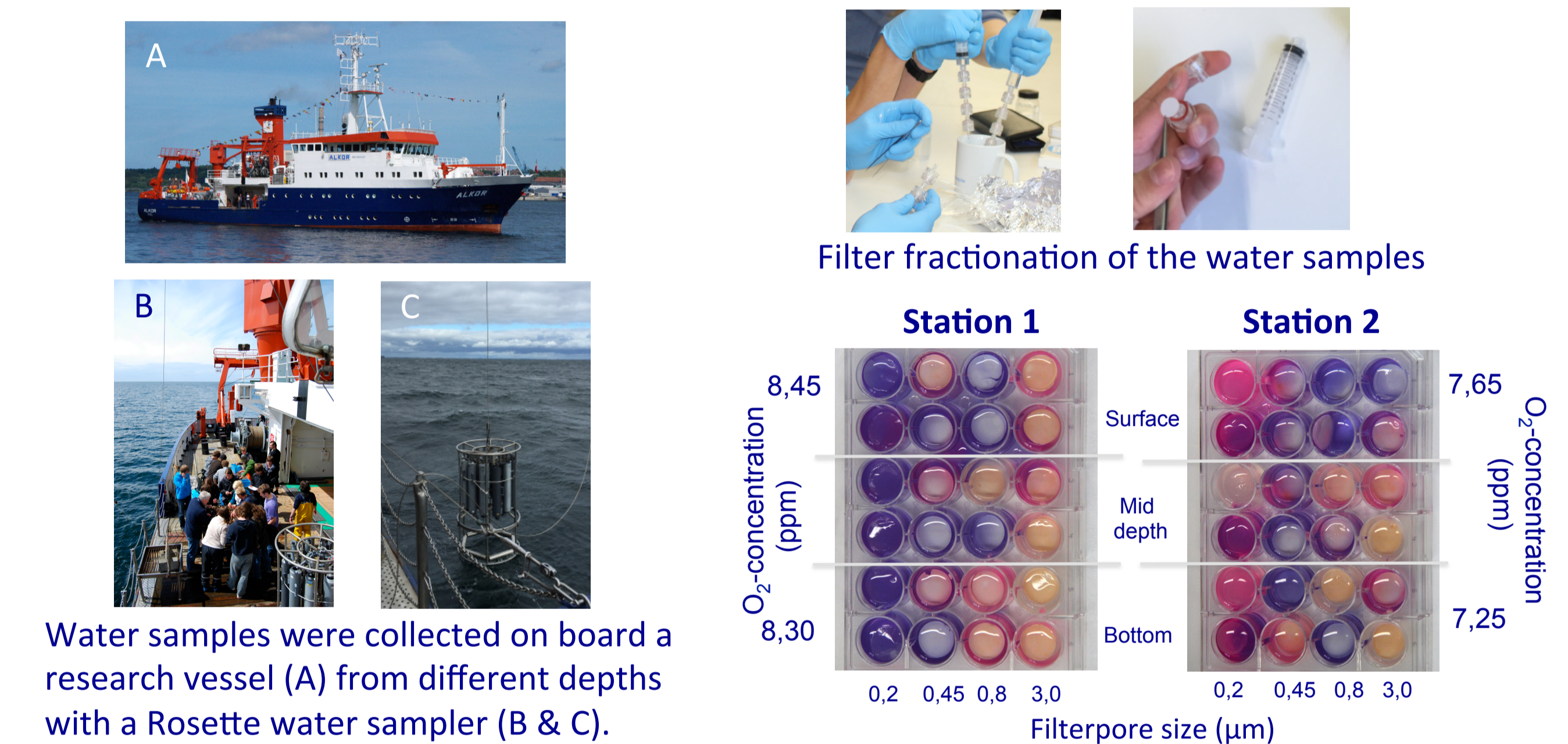
The method was verified using bacteria collected from 2 different sources in a pond. One source was near a sub-surface pipe which drains warm water into the pond. The second source was on the opposite side of the pond, where the water is colder.



- More reduced resazurin was observed in the sample from the site where the water was warmer. The higher temperatures there might have caused faster multiplication of the bacteria leading to higher numbers of microbes.
- Cell wells were found to be practical for this kind of test, enabling multiple assays with different water samples. This also makes comparison of water samples easier.

## Seawater Samples

Water samples from different depths at two stations in the Baltic Sea were collected and filter fractionated to separate different sizes of bacteria. The oxygen concentrations at the stations were measured separately using the Winkler method.



Water samples were collected on board a research vessel (A) from different depths with a Rosette water sampler (B & C).

- In water samples collected from the station with lower oxygen concentrations a higher resazurin reduction was observed. High microbial activity may have contributed to the low oxygen levels at the station.
- Water samples collected lower in the water column showed higher microbial activity.
- Particles collected on the 3.0 µm filter showed higher resazurin reduction probably due to bacteria attached to sinking organic matter.

The Resazurin- Agar- Method for estimating microbial activity and thus water quality has several advantages :

- The test is quick. Results are obtainable within 24 hours as compared to other screening tests like BOD (Biological Oxygen Demand), which need several days before results can be evaluated.
- It is simple and can be performed with a minimum of effort and expense, with several screenings costing less than \$1.
- Using cell well plates, multiple assays can be done simultaneously lessening the time for processing numerous samples.
- The test can be used for freshwater and marine environmental samples.
- The method is very simple and can thus be adopted for microbiology and ecology lessons in schools.