



EMERTOx: New EU project for the detection of emergent toxins and the species that produce them

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EMERTOx is a new EU project in the Horizon 2020 RISE program that aims to map the actual situation in emergent marine toxins and the producing organisms, develop new approaches to assess their occurrence and predict the possible future scenarios in the framework of global warming. Our task in this new project will be to develop probes for emerging species using an electrochemical detection method with a new detection system, apply these first in manual set up in an electrochemical microtiter plate and later incorporate them into an automated device with multiplexed electrode array of 164 electrodes units.

WP Objectives:

- to design oligonucleotide probes for emergent SE Atlantic and Mediterranean toxic algae: *Gambierdiscus*, *Ostreopsis*, *Coolia*, *Vulcanodinium*, and clades of *Prorocentrum* spp. sister to *P. lima*
- to test and validate the probes designed with pure cultures using an electrochemical ELISA plate with magnets to regenerate the probes and then in field test using on natural water samples
- to adapt the ELISA plate to a multiplex electrode array with 164 electrodes
- to field test the probes with field samples from Cape Verde Islands and Canary Islands
- to reconstruct the historical biogeographic distribution of these species using field samples from the Hardy Plankton Recorder from the SE Atlantic
- to use the reverse transcriptase microarray developed in the EU μ Aqua, we will extend ITS barcodes for *Pseudo-nitzschia* directly in the electrochemical ELISA plate



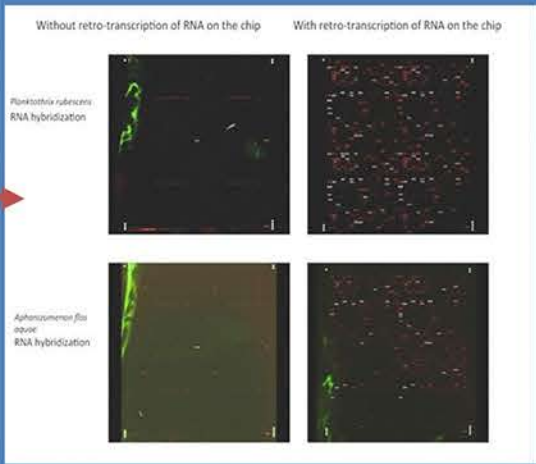
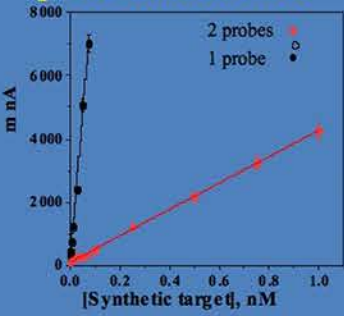
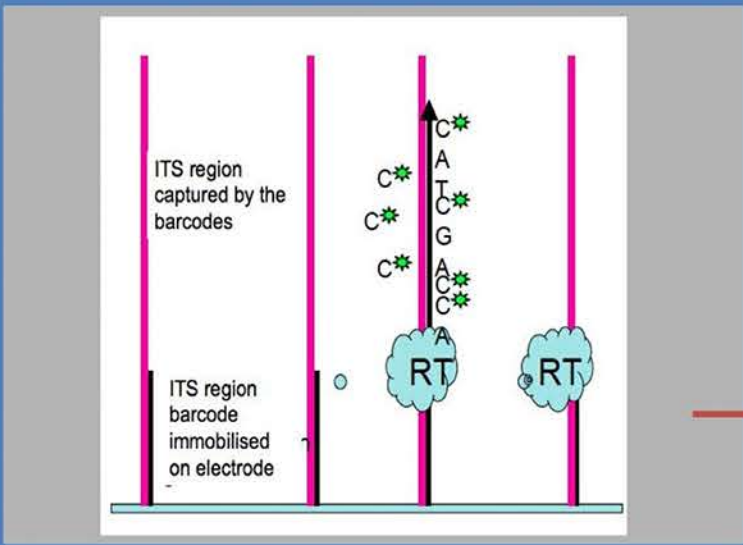
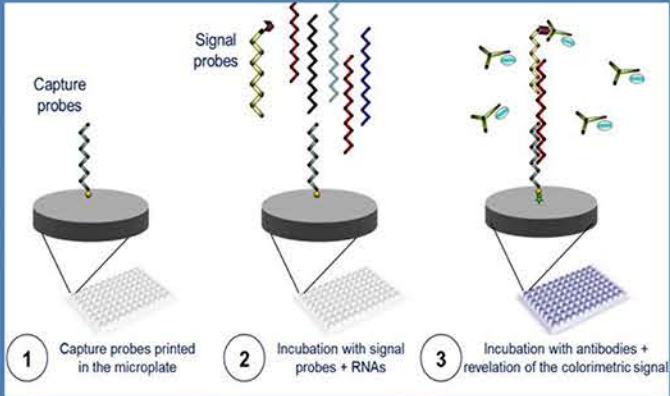
Comparison of mapping of *Coolia* by David et al. 2014 Phycologia (left) with current SAFS plankton recorder routes in the same area (right) where samples back to 1964 are available for analysis

SHA and Single probes:

- designed using the ARB software (<http://www.arb-home.de>)
- signal probe with new label (NL) and new mediator (proprietary information)
- RNA target captured and detected with only one probe (proprietary information)

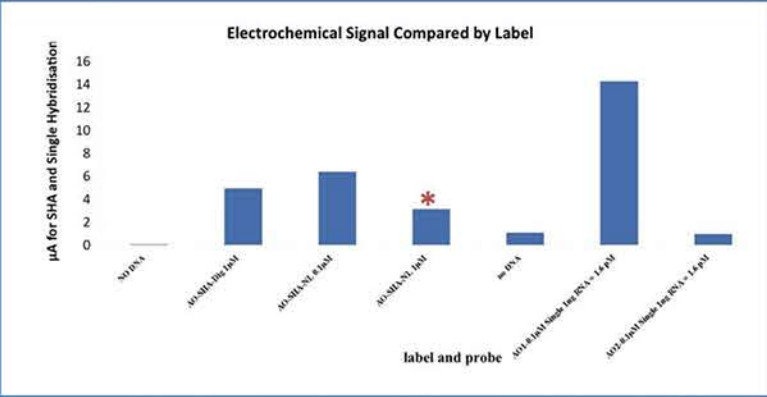


Methods



Alexandrium ostenfeldii

Comparison of DIG labelled signal probes with new label (NL) SHA signal probes with new mediator with synthetic DNA and two different single probes with total RNA



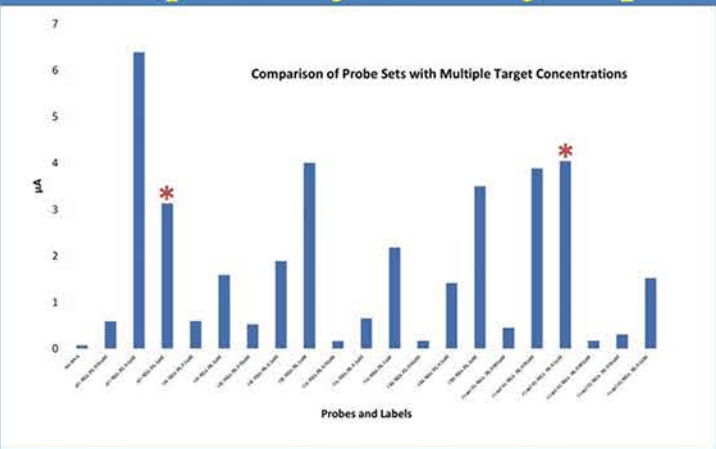
Results

- 46 SHA probe sets designed (15 are emergent toxic algae)
- Seven tested with synthetic DNA. One tested with RNA
- Magnetic beads with 1 μ M signal probe for *Alexandrium ostenfeldii* is saturated (*). Magnetic beads 0.1 μ M signal probe with new label is 50% stronger than 1 μ M signal probe DIG-labelled SHA probes).
- Single probe hybridisation signal for *Alexandrium ostenfeldii* is 2.25 fold stronger than SHA hybridisation with two probes, 1 ng of RNA = 4000 cells of *A. ostenfeldii* (see Metfies et al. 2005, Bios. Bioelec)
- Electrodes are saturated with 1000 pM target (see *), have signal for target down to 1 pM

Outlook

- Complete tests for probe specificity
- Make calibration curves from total RNA
- Adapt Single Probe electrode to Multiplex electrode chip
- Multiplex electrode chip to be tested with archived SAFOS samples and field samples from EMERTOx, estimated cost per chip of 4€

Comparison of SHA NL for 7 probe sets



Alexandrium ostenfeldii
Gambierdiscus australis
Gambierdiscus silvae
Gambierdiscus excentricus
Coolia monotis
Coolia canaryensis Clade1
Coolia canaryensis Clade2
* = saturated magnetic beads

Acknowledgements

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