Towards real-time in-situ monitoring of toxic algae

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Microbia Environnement

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France

Harmful Algal Blooms and Climate Change
Göteborg 19 -22 May 2015

Microbia Environnement

www.project-sms.eu

Introduction
Detection and monitoring system of harmful algae

Microscope observation

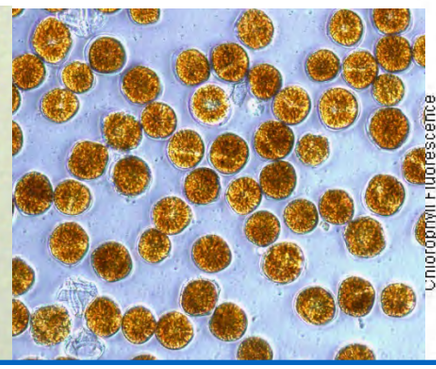
Molecular tools

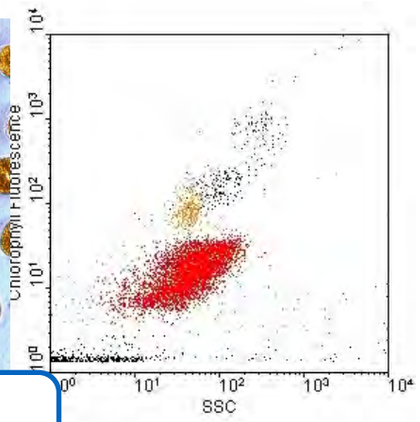
Flow cytometry

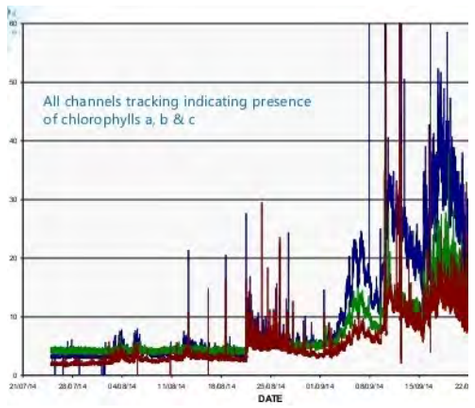
Modelling approaches

Fluorometry

Satellite remote sensing



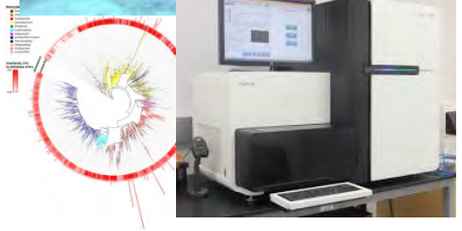




Early detection systems

Changes in species distribution with climate change

Introduction
Molecular methods for detection of harmful algae



Molecular tools

- **Quantitative PCR (qPCR)**

  Bowers et al. 2000; Popels et al. 20033; Galluzzi et al. 2004

- **Hybridization assays (fish hybridization, sandwich hybridization, microarray)**

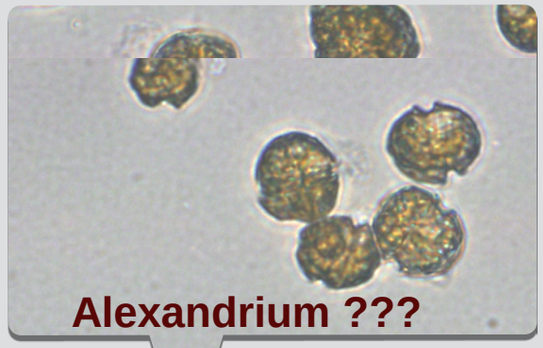
  Scholin et al., 1996;1999;

  Diercks et al., 2008: ALGADEC

  Greenfield et al.,2008; Scholin et al., 2009: ESP

- **Next generation Sequencing (NGS)**

Monitoring programs: Microscope observation

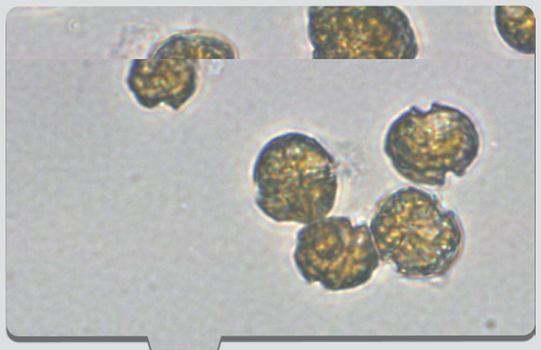


Introduction
Molecular methods for detection of harmful algae

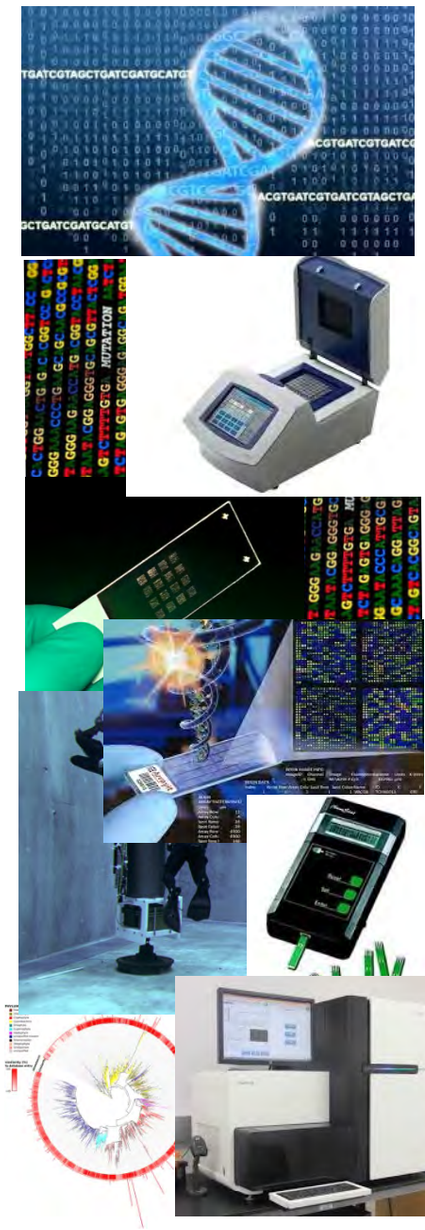
Molecular tools

- **Primers / probes available vs Number of toxic species**
- **Lab instrumentation and equipment**
- **Cost of materials and reagents**
- **Molecular protocols**
- **Identification of one or few species at a time**

Monitoring programs: Microscope observation



Introduction
Molecular methods for detection of harmful algae



Molecular tools

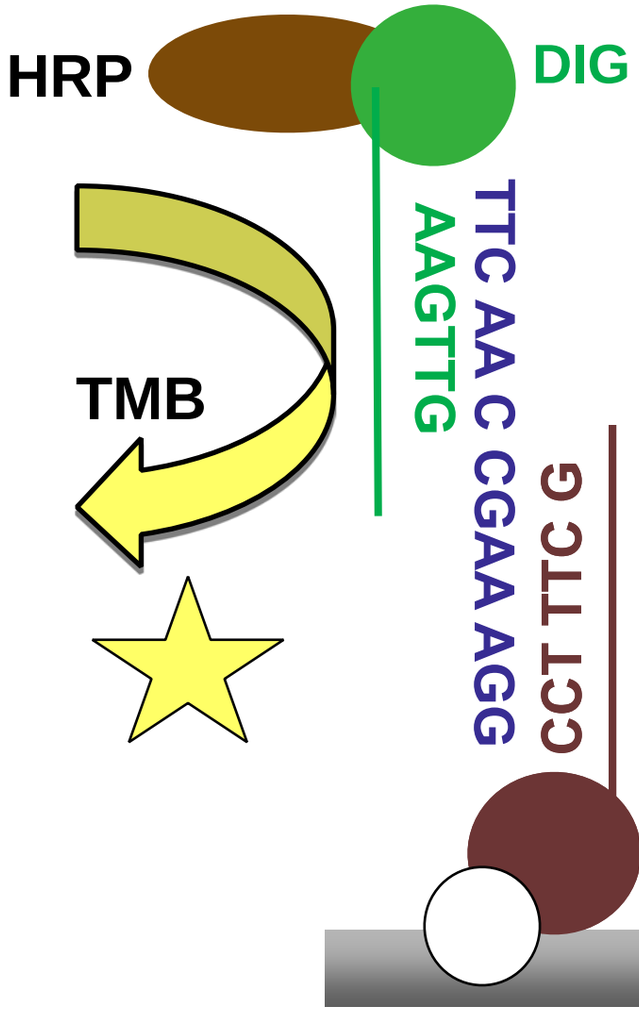
- Quantitative PCR (qPCR)
- Hybridization assays (fish hybridization, sandwich hybridization, mic
- Next generation Sequencing (NGS)

Microbia Environnement

- Development of easy and cost effective tools for the detection of toxic species (*microarray, colorimetric SHA, electrochemical biosensor*)
- Probe/primer design
- Consulting / Service delivery

Introduction
Sandwich hybridization

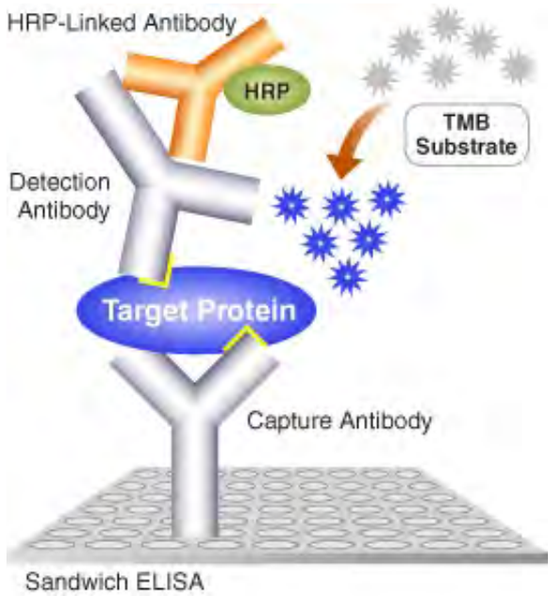
Sandwich hybridization assays in 96 wells microplate

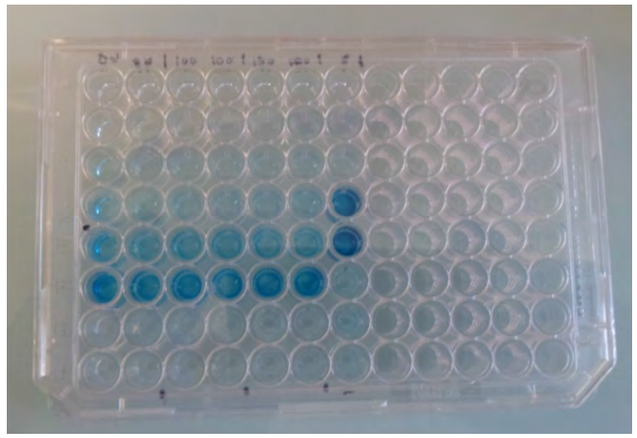


DIG- Labelled signal probe

Target sequence

Biotinylated capture probe

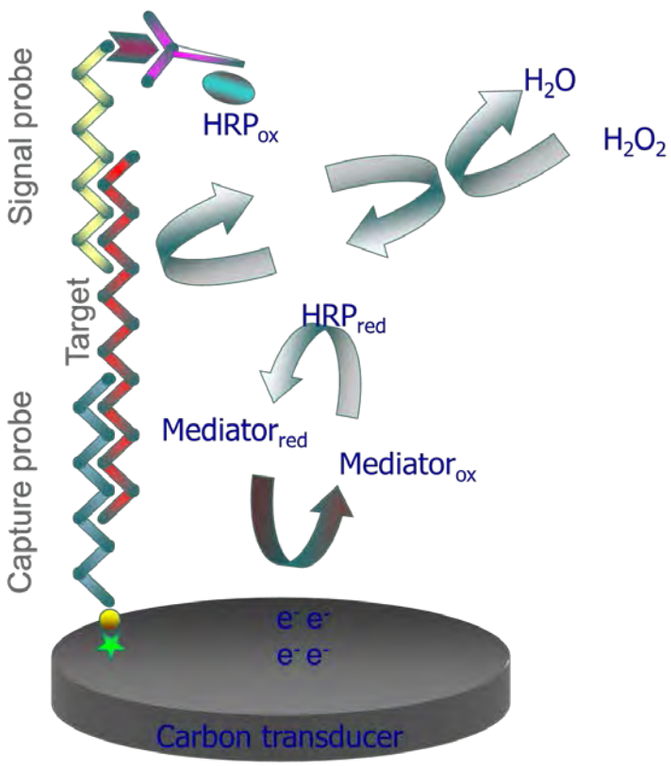


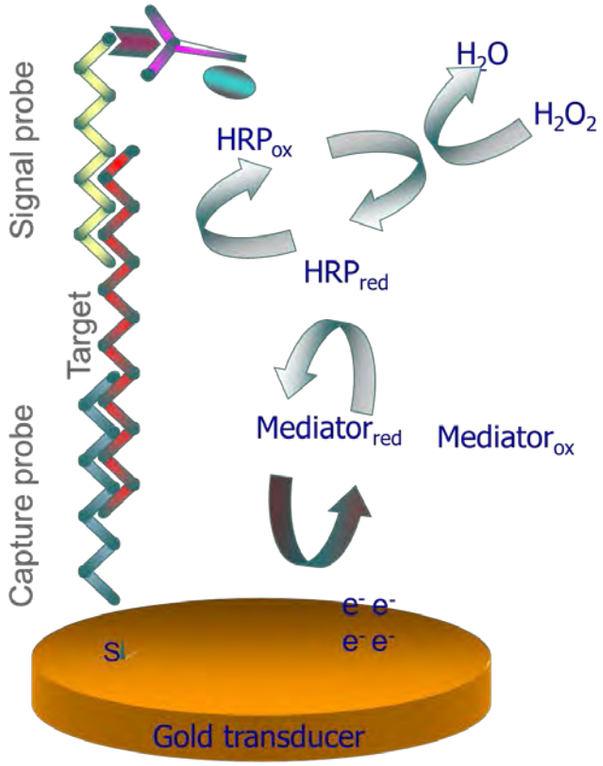


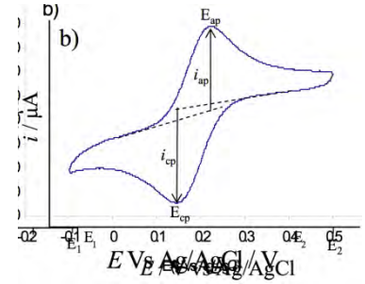
Introduction
Sandwich Hybridization

Sandwich hybridization assays in electrochemical format

Diercks et al., 2008, 2011. Orozco & Medlin, 2011







- ✓ Robustness, small size (versatility, portability)
- ✓ Integration in multisensor probes, miniaturised flow systems
- ✓ Useful information at real time scale (minimal sample handling requirements)
- ✓ Mass production, low cost.

Our goals

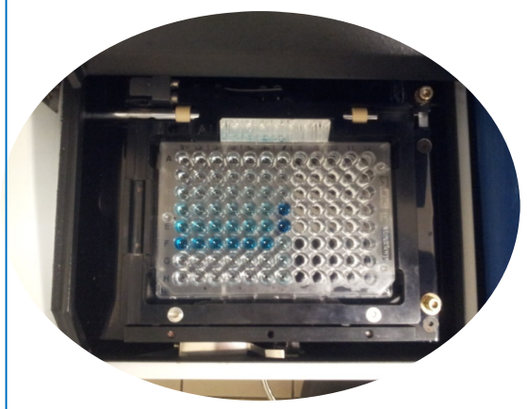
Sensing toxicants in Marine waters makes Sense using biosensors

SEVENTH FRAMEWORK PROGRAMME

Sandwhich Hybridization

1.Probes design

Development and validation of molecular probes for the identification of « Mediterranean » harmful algae



2.Probes test and validation

Development of fast/easy/cost effective protocols based on molecular tools for species identification

3.Automatization

Automatization and miniaturization: integration on a platform system

1. Probe design: Database creation

Creation of a Database of 172 capture probes and signal probes corresponding to around 80 species/complex of species

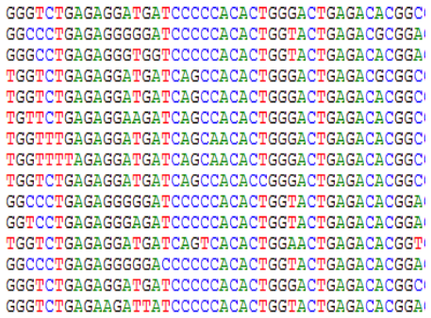
- all but 10 genera/species capture probes tested in a microarray format (EU FP7 project MIDTAL)
- all probes were tested in silico
- 14 species tested in microtiter plate format and with total RNA
- 14 species tested in biosensor format for the positive control

2. Probe validation

Test *in silico*

Computer

- Corresponding species (Genebank)
- Melting Temperature (Tm), % GC, potential hairpins (Oligocalc)
- Mismatches



Test *in vivo*

Lab

Positive controls

Cultures of target species

- **Specificity**
- **Sensitivity**
- **Target species spiked in natural sample**

Environmental samples

2. Probe validation

Test *in vivo*

Lab

Positive controls

Cultures of target species

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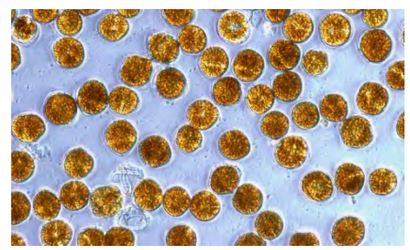
Environmental samples

Methods

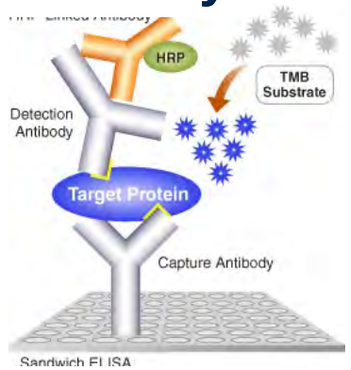
Algal cultures

Cell counting



RNA extraction

SH Assay



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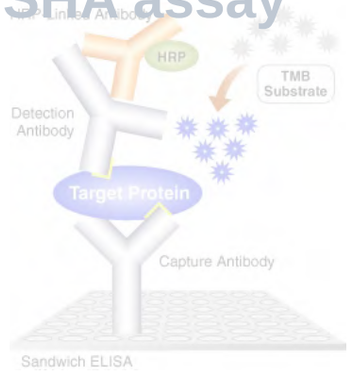
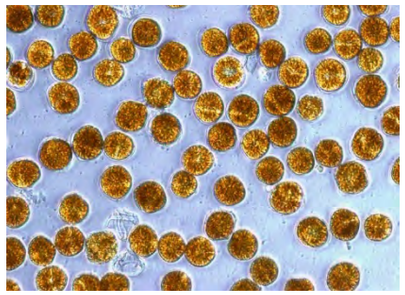


Species	Medium	Salinity (psu)	Temperature (°C)	Origin
Alexandrium minutum (AM205)	F/2; L1; K	38	20; 18	CSIC Barcelona
Alexandrium tamarense (VGO928)	F/2; L1; K	32	20; 18	IEO Vigo
Alexandrium ostenfeldii (VGO956)	F/2; L1; K	32	20	I
Chattonella subsalsa (CS0507 -BC)	F/2; L1	36	20	U
Chattonella subsalsa (CS0004-DE)	F/2; L1	36	20	U
Chattonella subsalsa (CS0507 -FL)	F/2; L1	36	20	U
Chattonella subsalsa (CS0704)	F/2; L1	36	20	U
Chattonella subsalsa (CS0607)	F/2; L1	36	20	U
Chattonella subsalsa (CS 0004 SS)	F/2; L1	36	20	U
Dinophysis acuminata (VGO1063)	L1/20	32	18	I
Dinophysis acuta (VGO1065)	L1/20	32	18	IEO Vigo
Gymnodinium catenatum (GC12V)	L1	32	20	IEO Vigo
Heterosigma hakashiwo (HA1V)	L1	32	20; 18	IEO Vigo
Karenia brevis (CCMP718)	L1	32	15	IE
Karenia mikimoitoi (Km0703)	F/2; L1; IMR	36	20	UN
Karenia mikimoitoi (Km0605-1)	F/2; L1; IMR	36	20	UN
Karenia mikimoitoi (KM0606-1)	F/2; L1; IMR	36	20	UN
Karenia mikimoitoi (Km0608-1)	F/2; L1; IMR	36	20	UN
Lingulodinium polyedrum	F/2	28	19	US
Mesodinium rubrum (AND-A0711)	L1/20	32	15	IE
Protoceratium reticulatum (GG1AM)	L1	32	20; 18	IEO Vigo
Teleaulax amphioxeia (AND-A0710)	L1	32	15	IEO Vigo

Methods

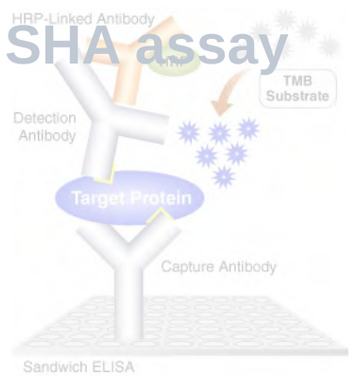
Algal cultures

Cell counting



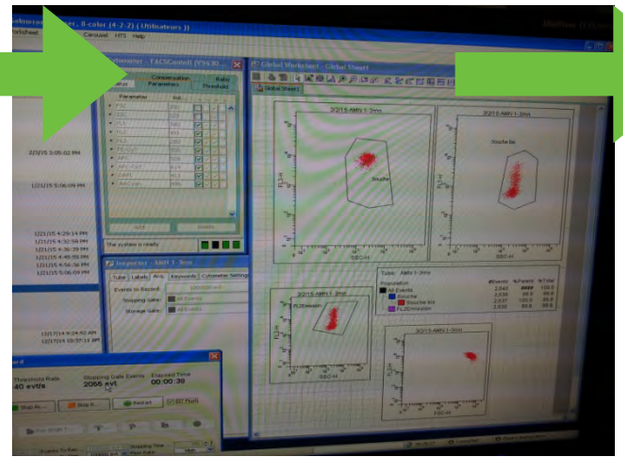
RNA extraction

SHA assay



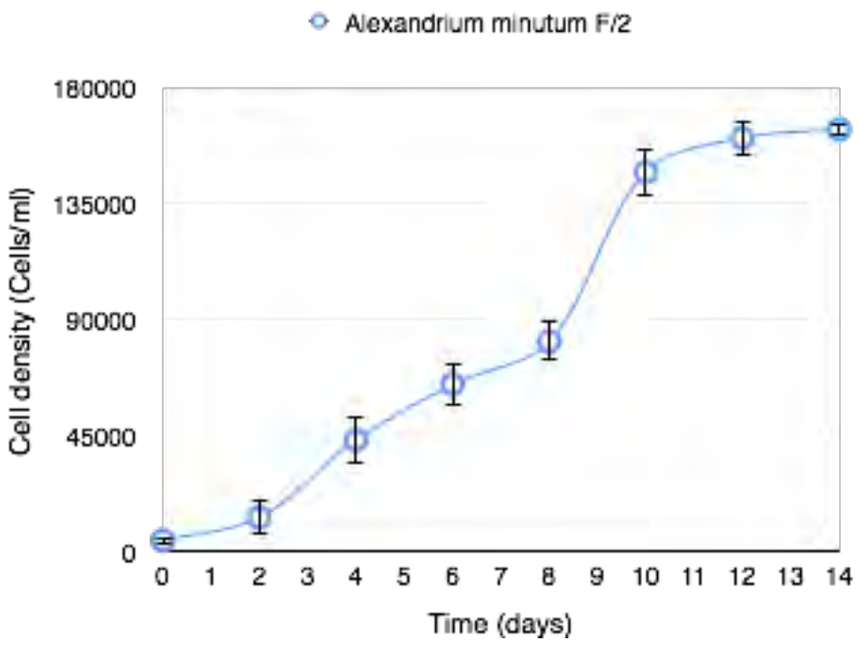
Microscope observation

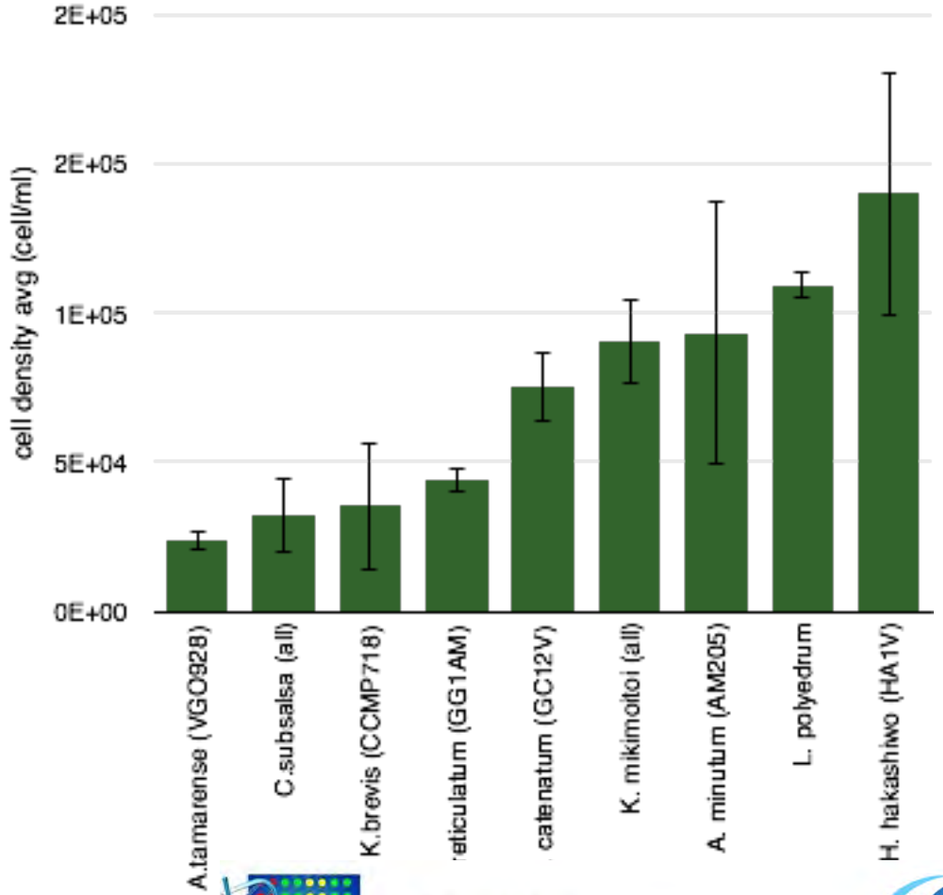
Cell counting (Flow cytometry)



Centrifugation

Cell density over time





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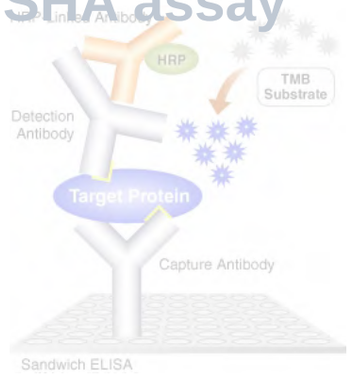
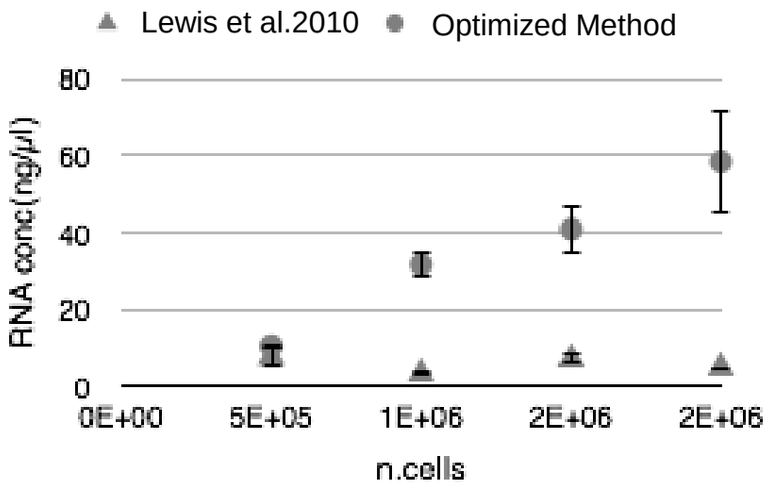
Cell counting

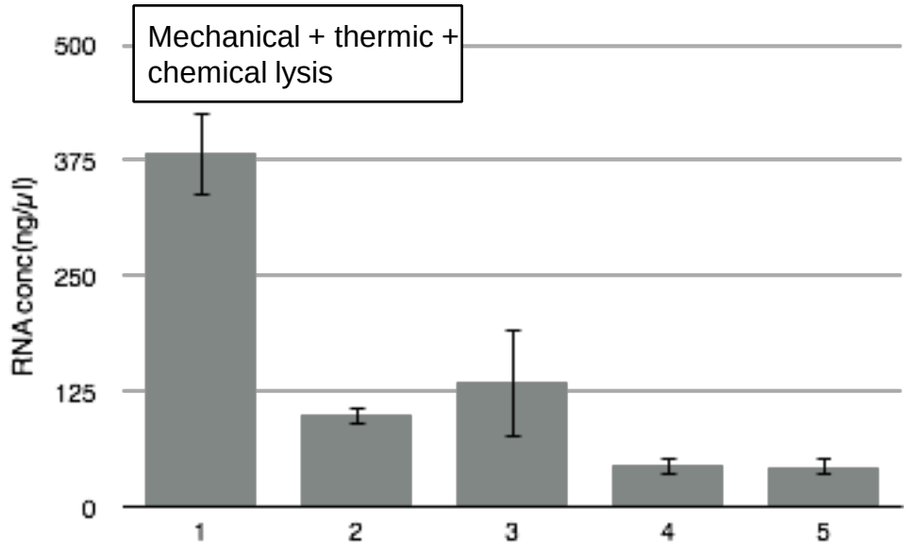
RNA extraction

SHA assay

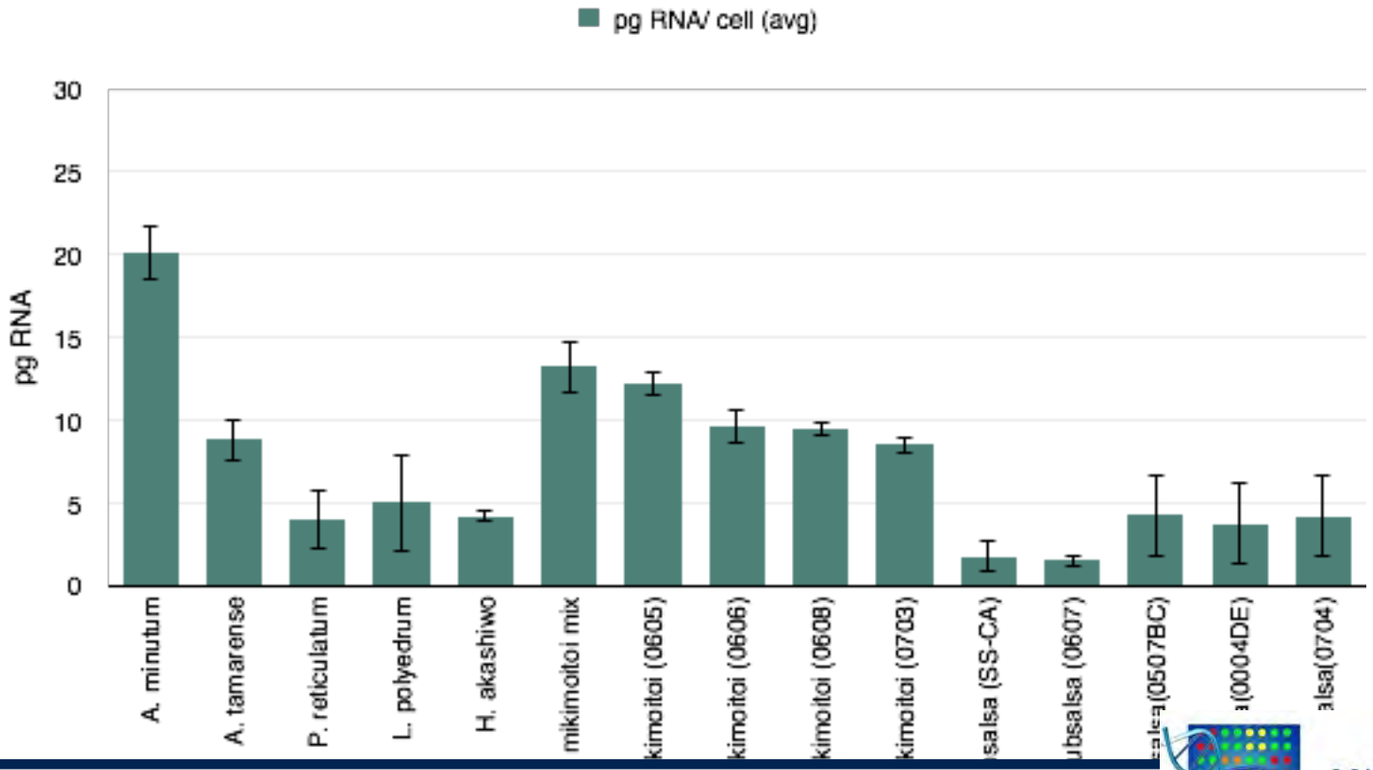


Optimization of Lab Method for RNA extraction





Realization of an RNA bank for probe testing

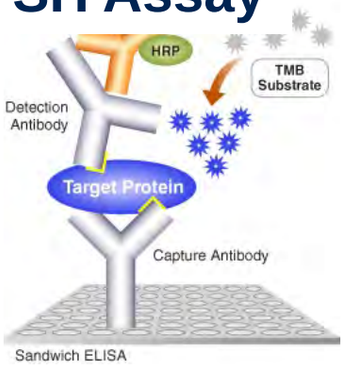


Methods

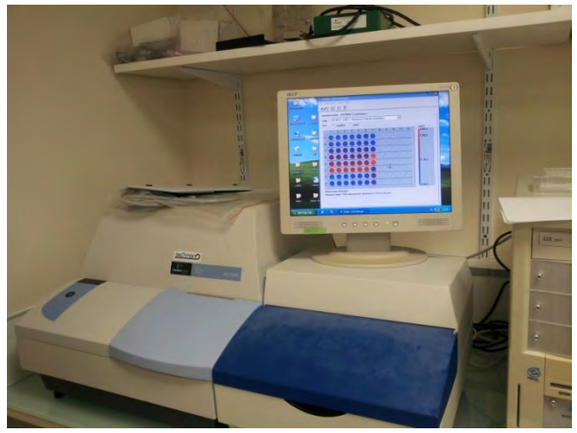
Cell counting

RNA extraction

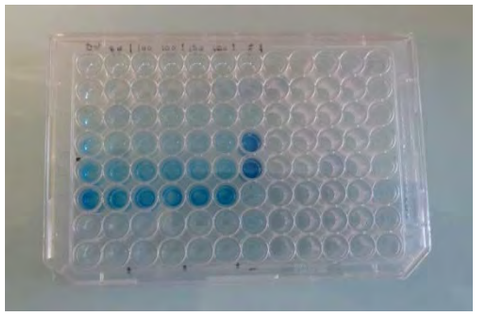
SH Assay

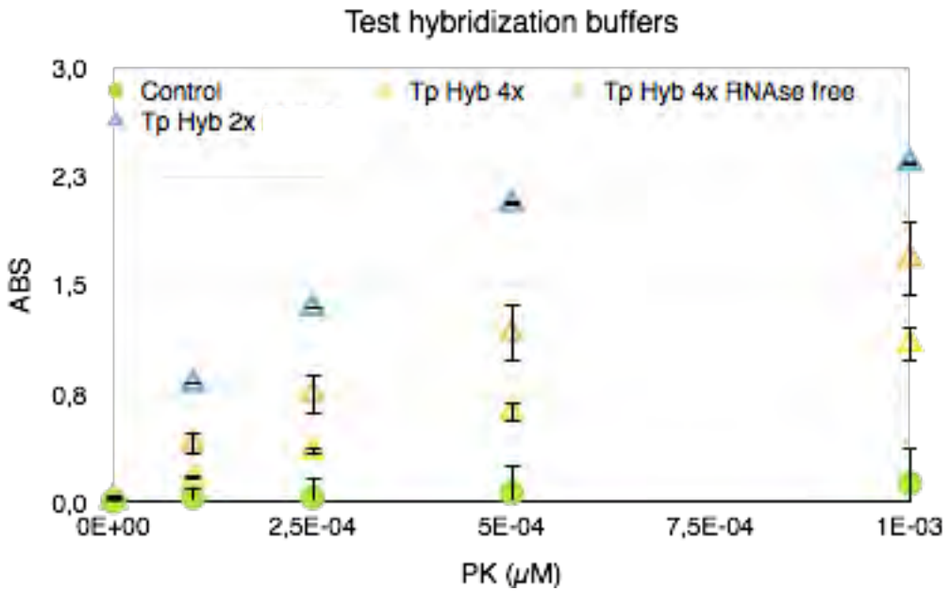


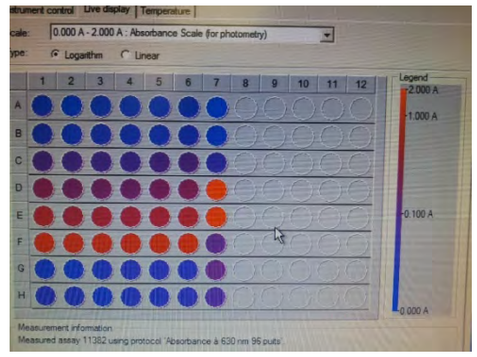
Development of a rapid and cost effective colorimetric detection using sandwich hybridization in a microtiter plate assay (ELISA TEST)



- **Optimization of incubation time, temperature and buffers**
- **Time reduction (<1h)**
- **Increase in sensitivity (1ng RNA)**





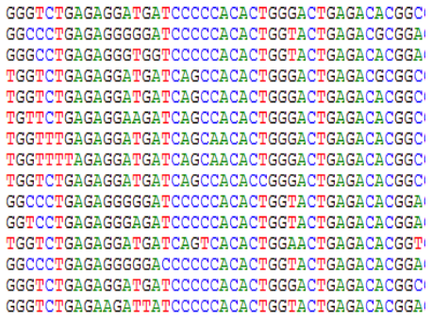


2. Probe validation

Test *in silico*

Computer

- Corresponding species (Genebank)
- Melting Temperature (Tm), % GC, potential hairpins (Oligocalc)
- Mismatches



Test *in vivo*

Lab

Positive controls

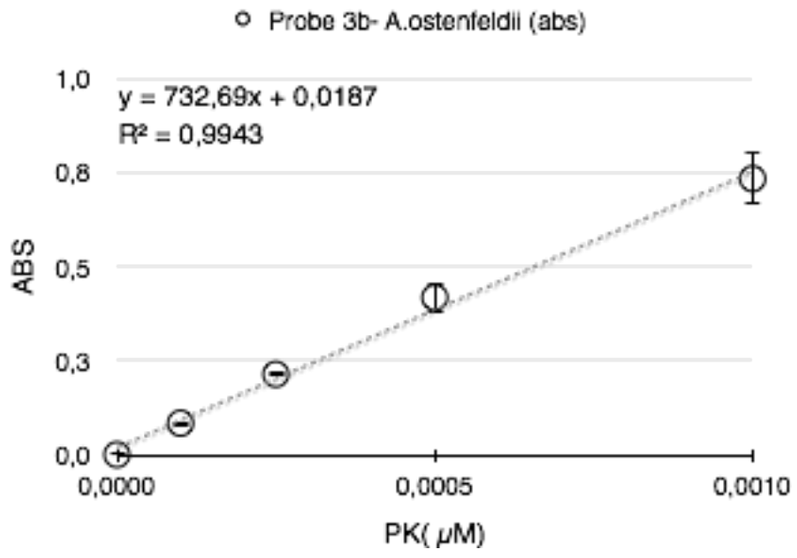
Cultures of target species

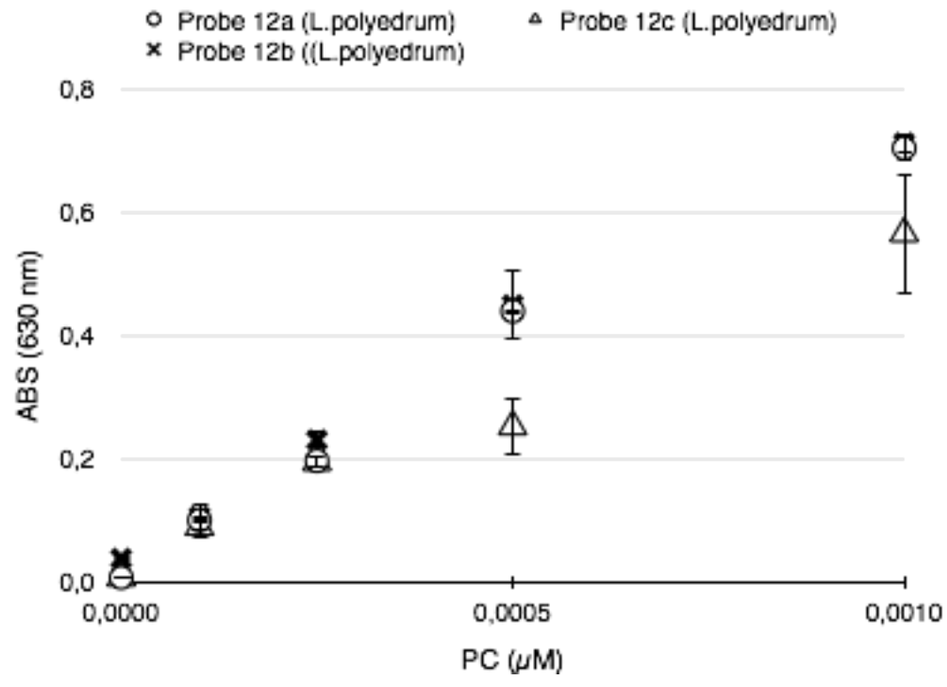
- **Specificity**
- **Sensitivity**
- **Target species spiked in natural sample**

Environmental samples

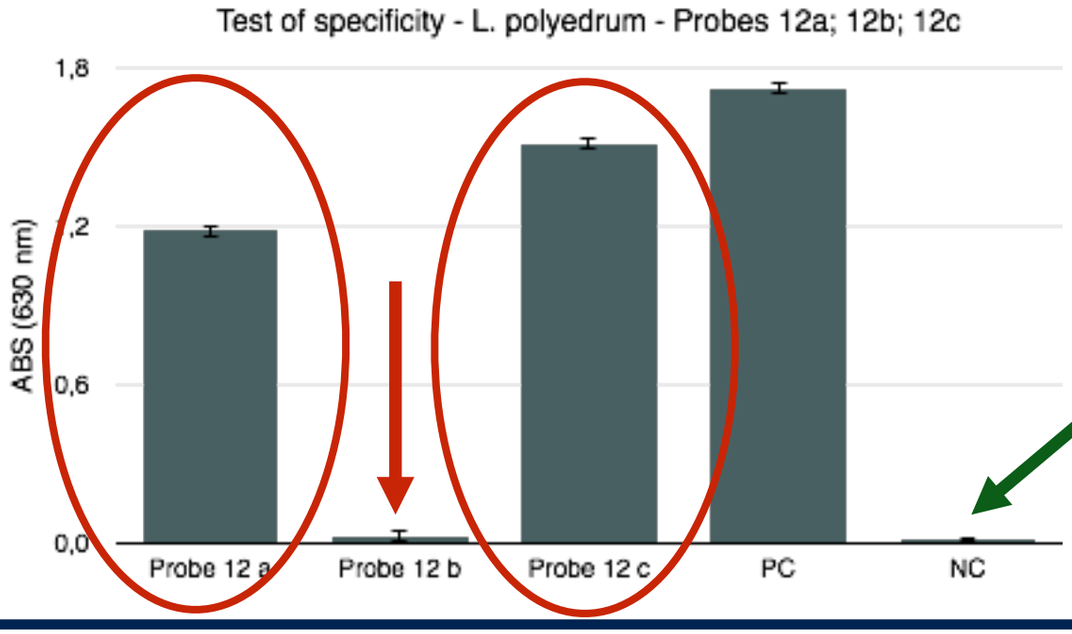
2. Probe validation

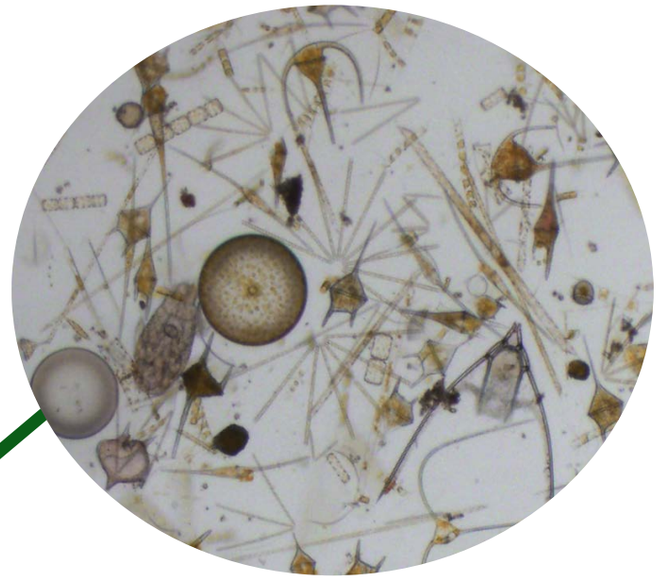
Test of probes for positive control





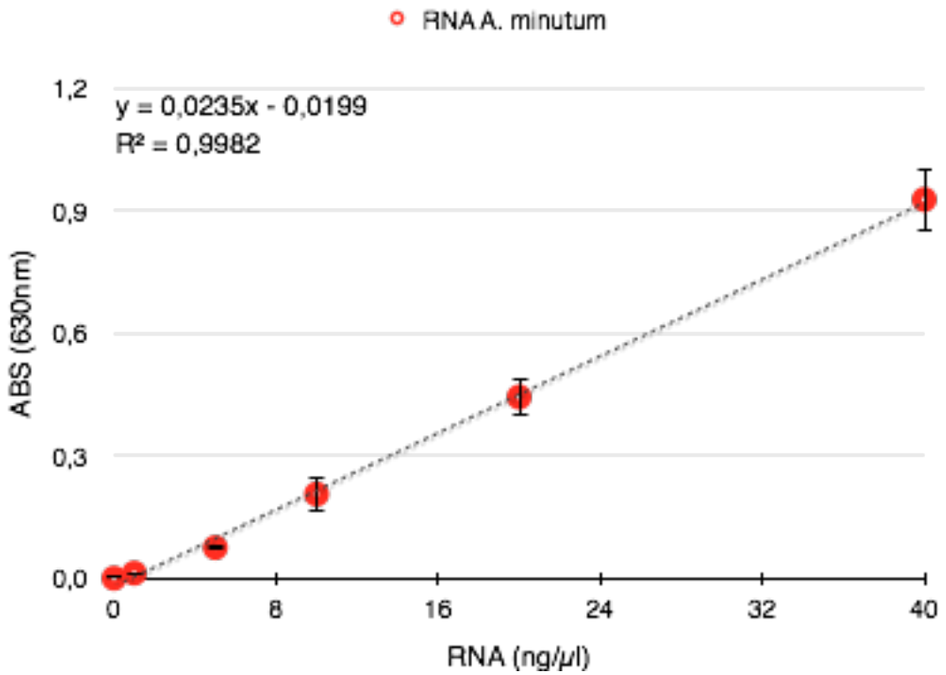
Test of probes for specificity



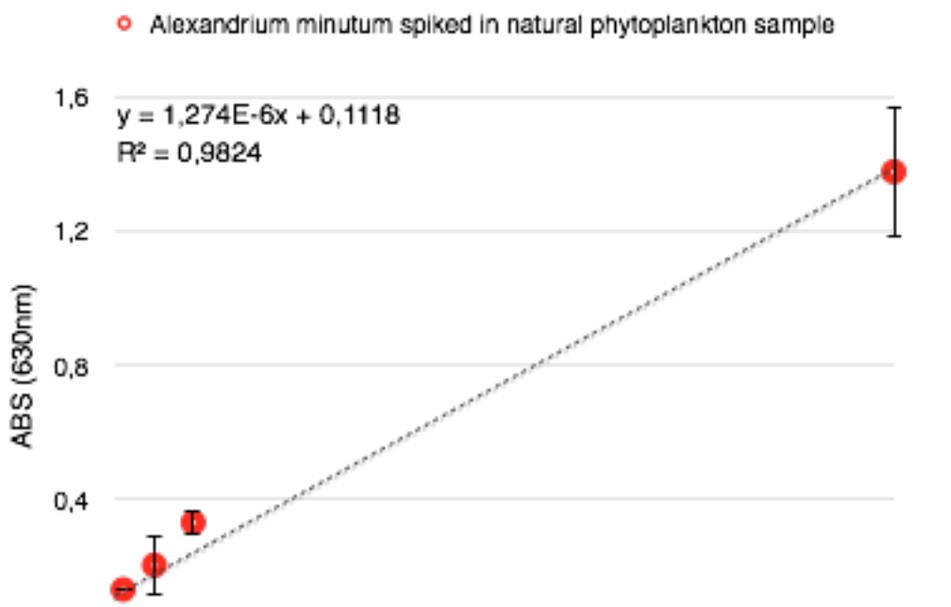


2. Probe validation

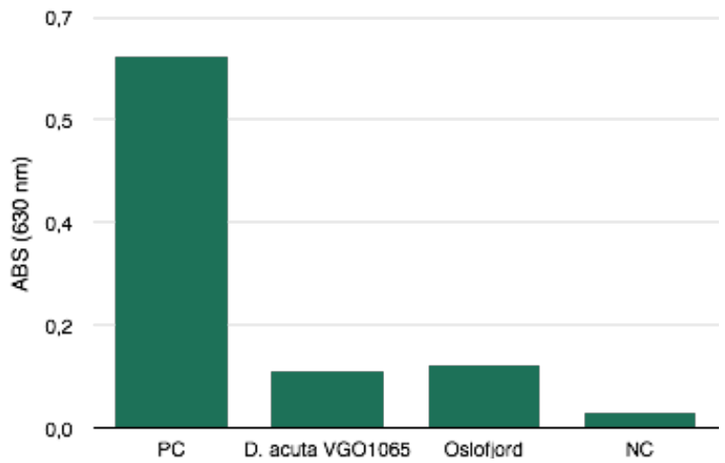
Calibration curves with RNA extracted from cultures



Calibration curves with cultures spiked in natural sample



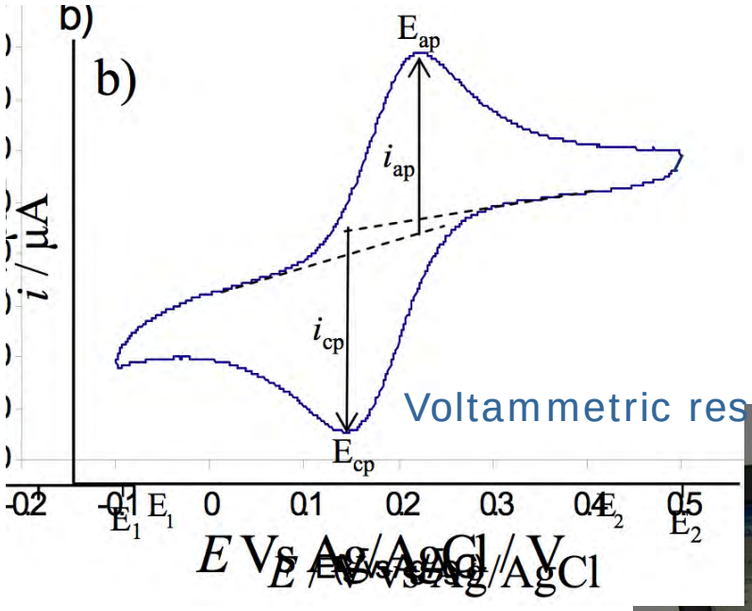
Environmental samples
Preliminary test



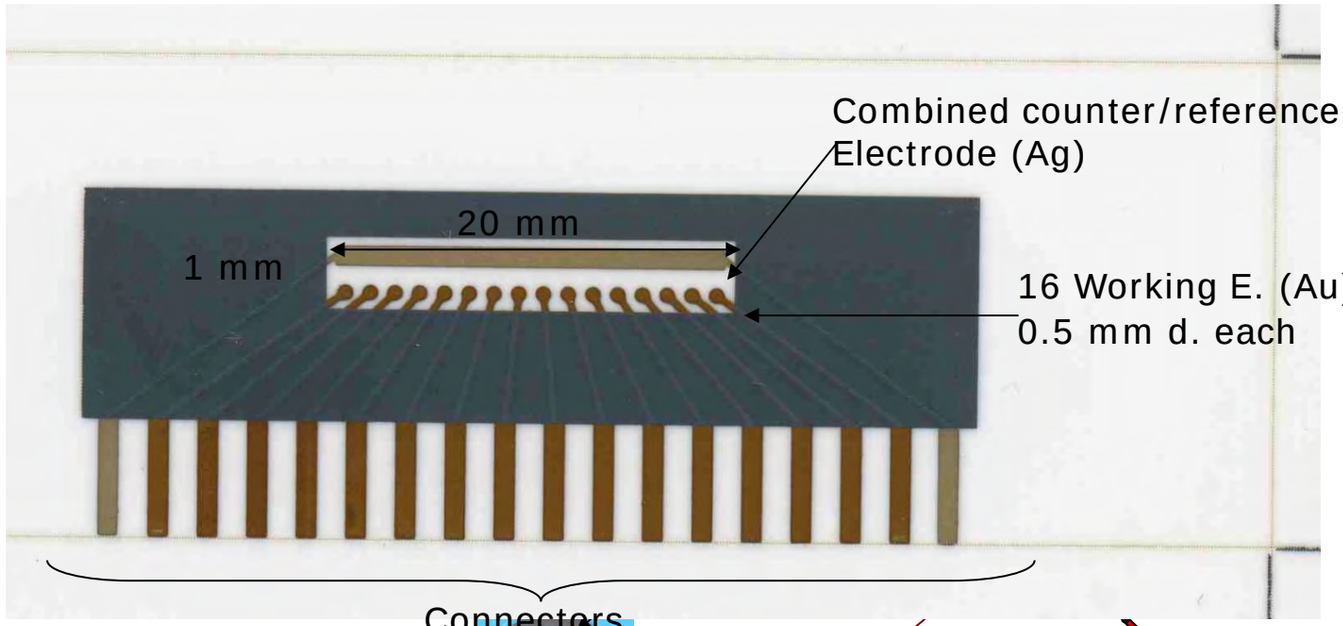
Next:

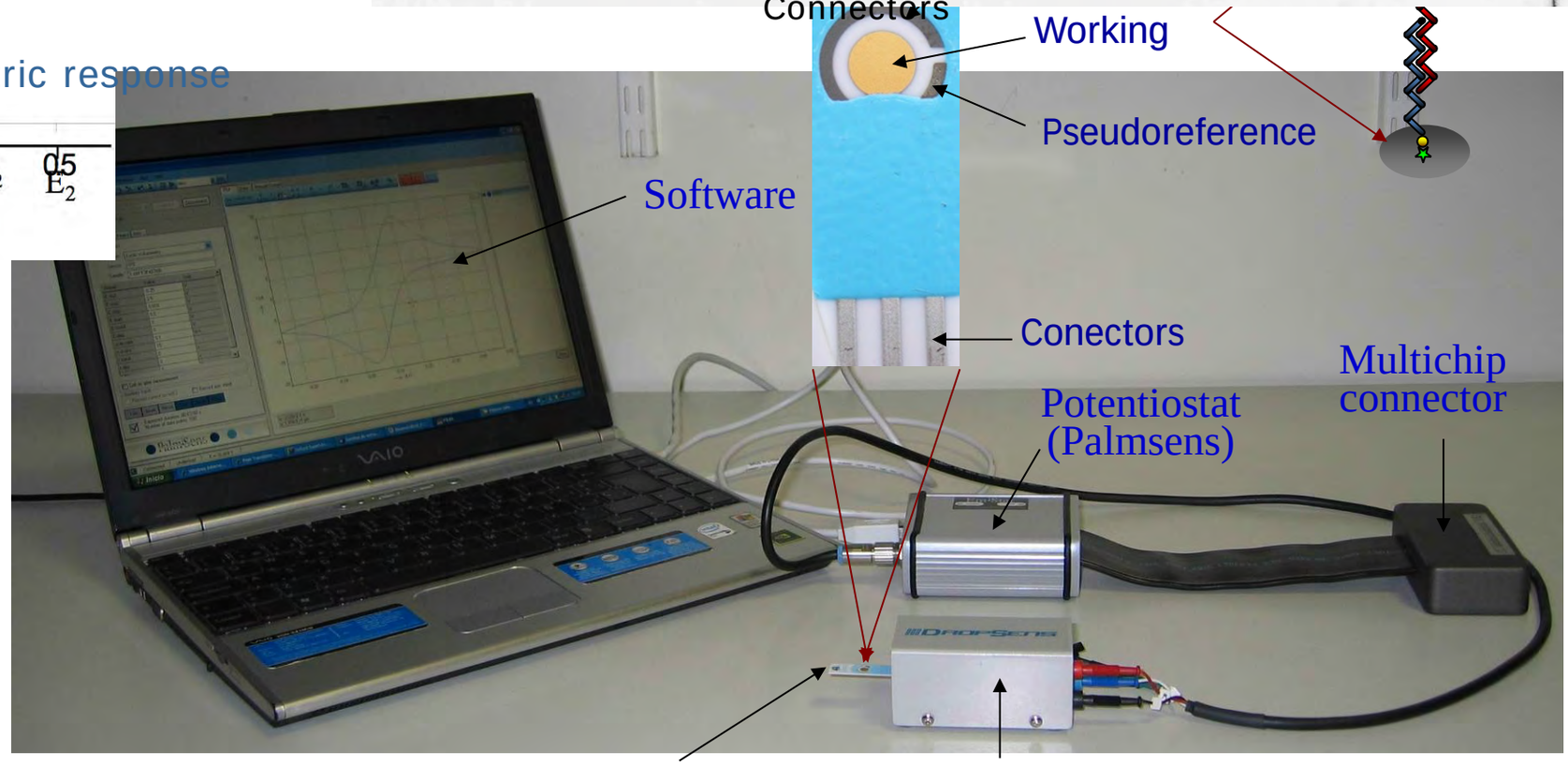
- Validation with Environmental samples
- Test of specificity for different strains/geographic region

3. Towards automatization



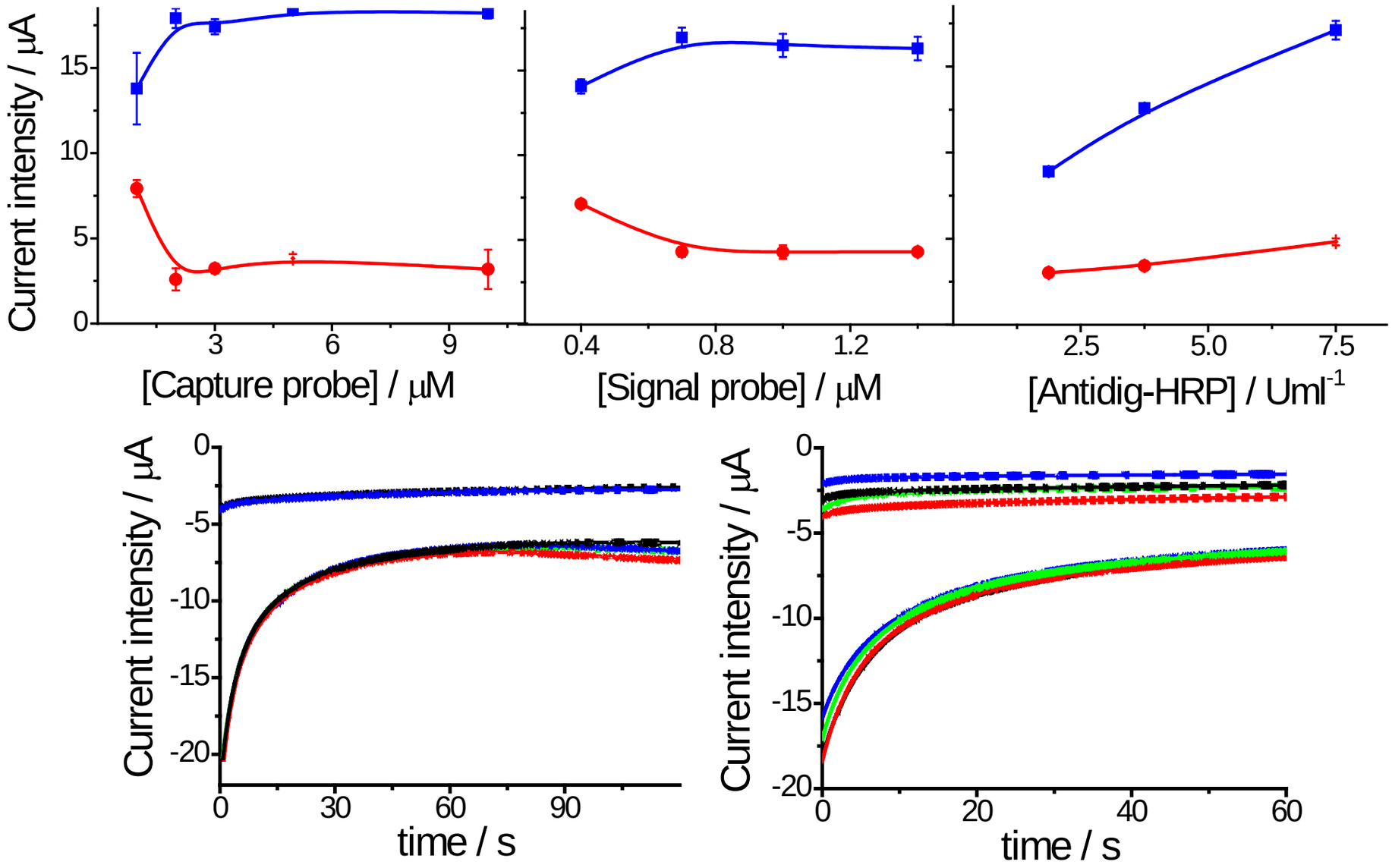
Voltammetric response





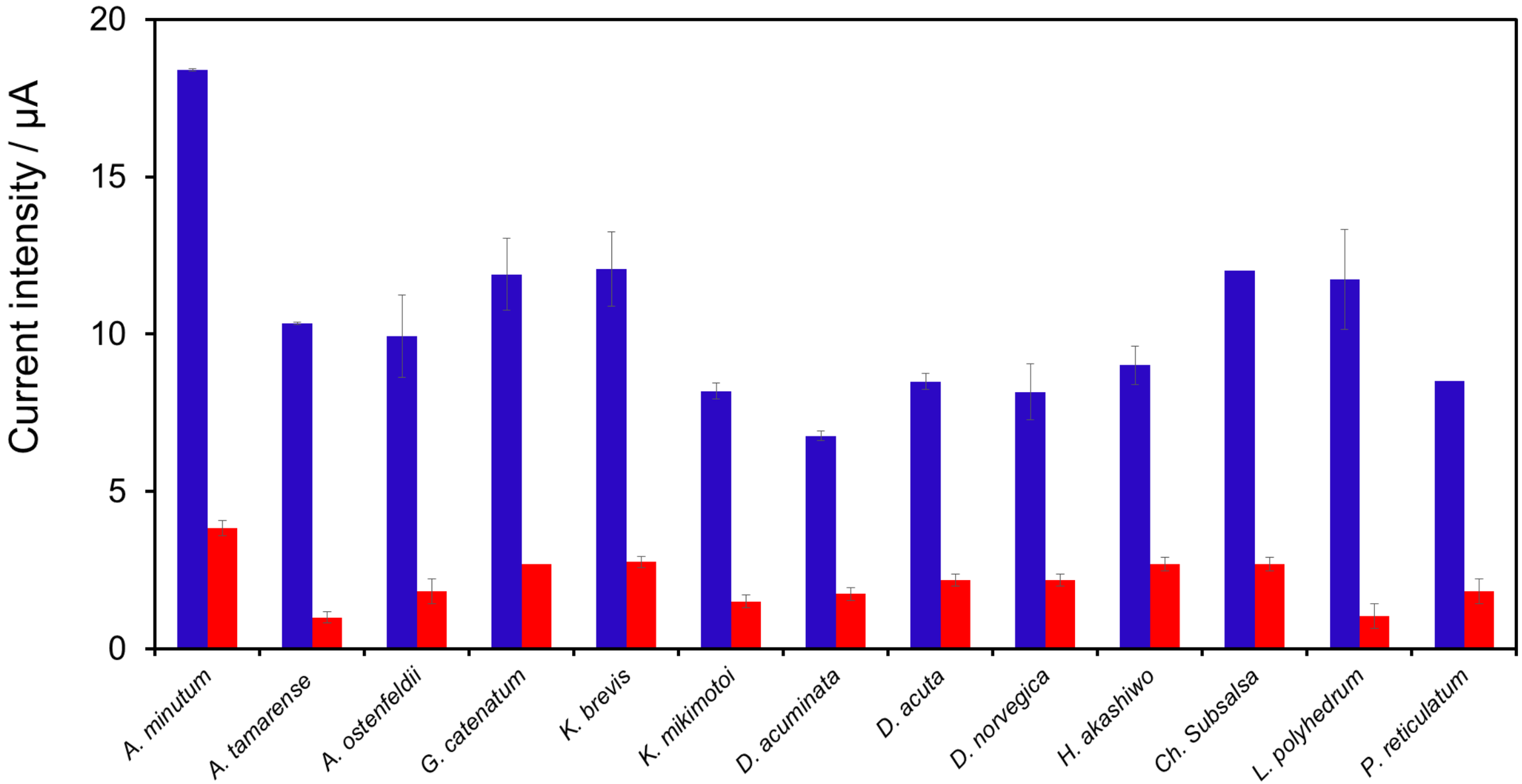
3. Towards automatization

Optimization of sandwich hybridization in electrochemical format



Orozco et al., in prep.

3. Towards automatization

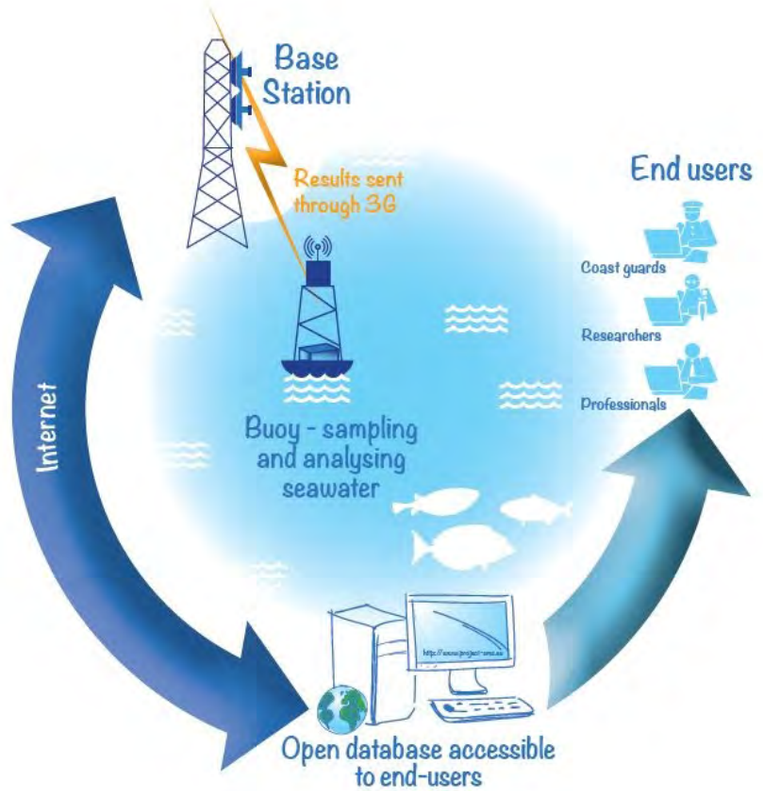


Orozco et al., in prep.

EU FP7 PROJECT « SMS »

Sensing toxicants in *Marine* waters makes *Sense* using biosensors

SMS project aims to deliver a **novel automated networked system** that will enable **real-time in-situ monitoring of marine water** status in coastal areas by the detection of a series of contaminants and **TOXIC ALGAE**



PROJECT PARTNERS

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Conclusions

Towards real-time in-situ monitoring of toxic algae

Ecology

Physiology

Taxonomy

Molecular biology

Probes validation

Electrochemistry

Nanomaterials

Microfluidics

Electronics

Early detection systems

Automatization

Changes in species distribution with climate change

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Thanks for your attention!

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