Deteção em tempo real de biotoxinas, será possível? Projectos LinguaTox e ImageTox

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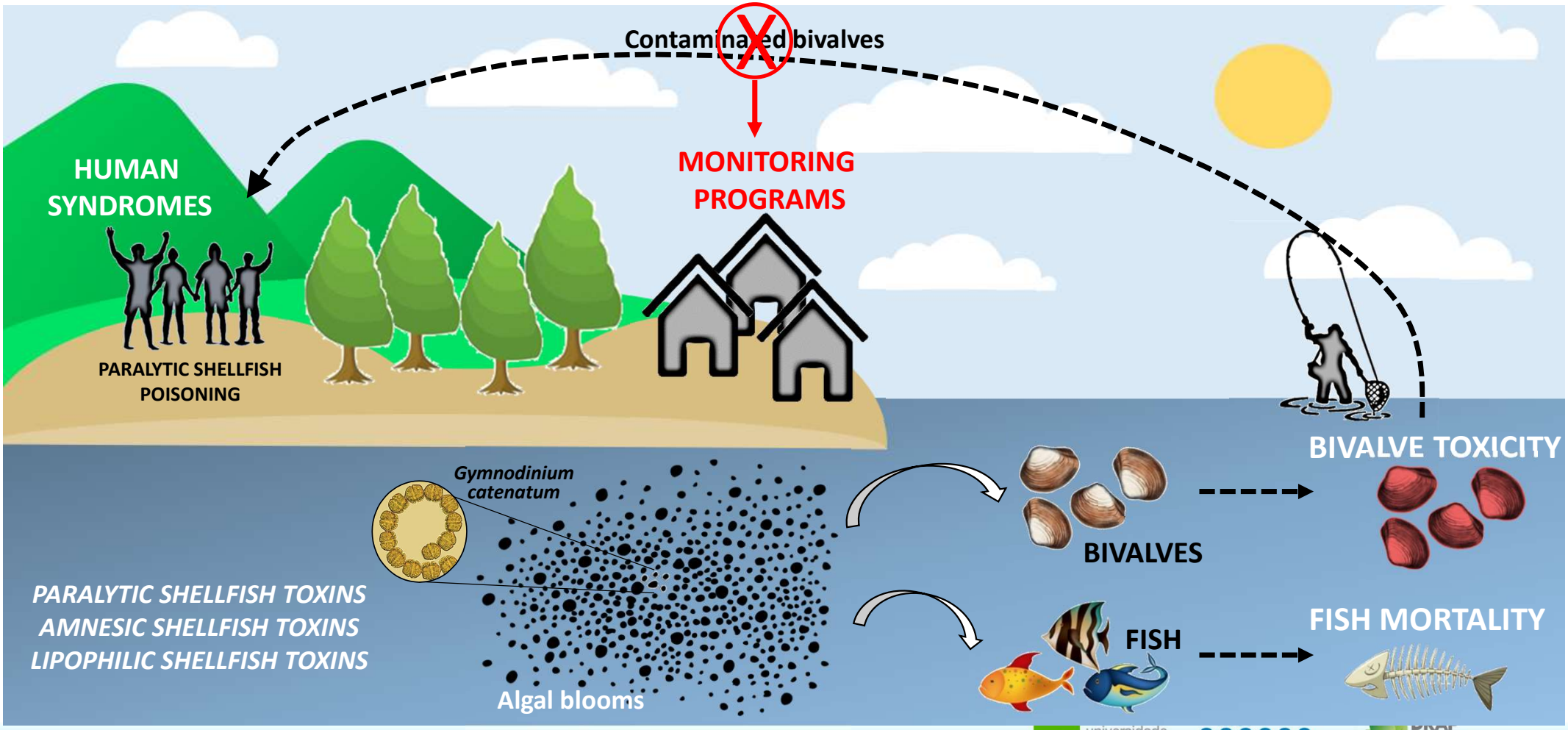
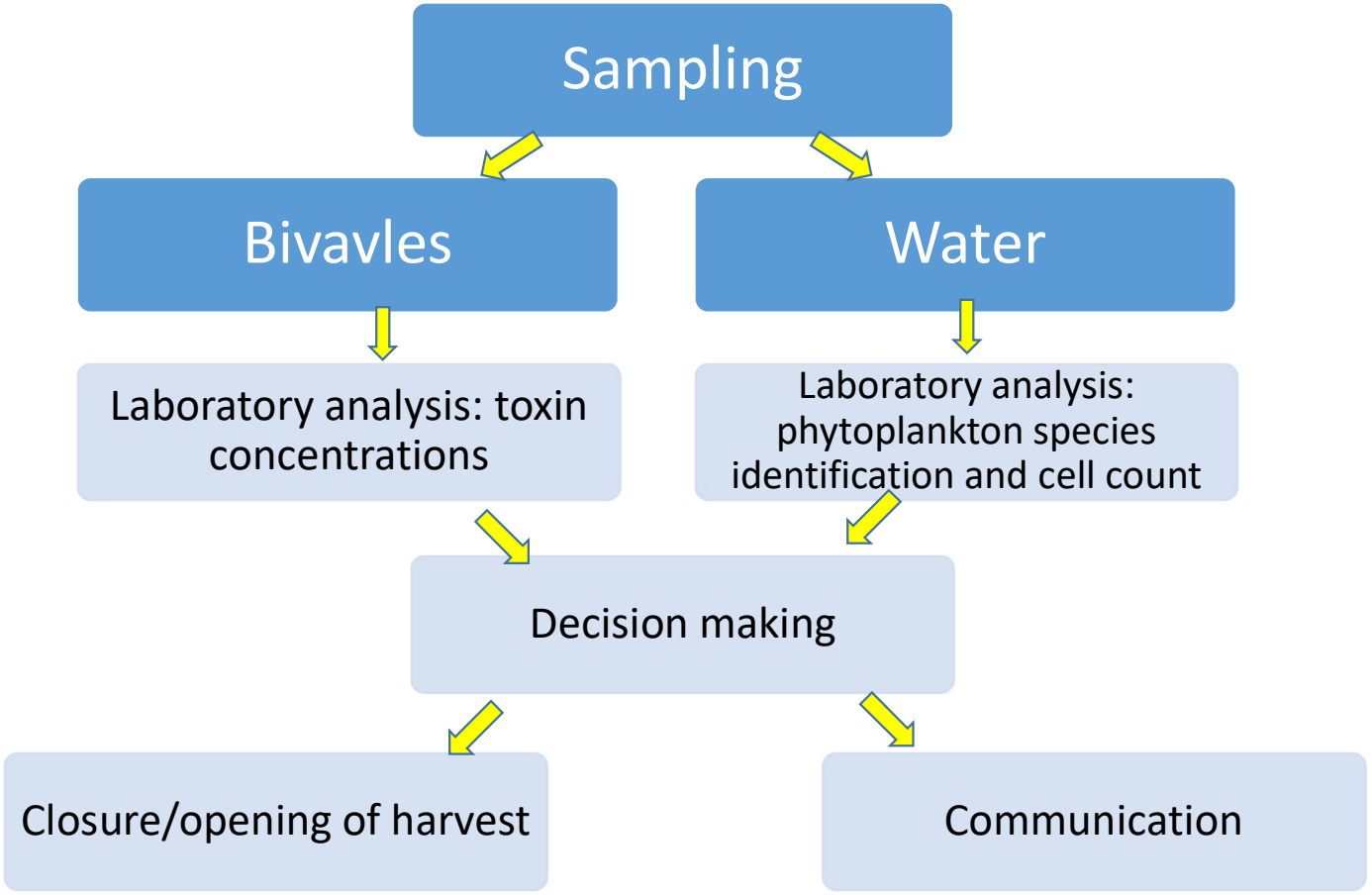
Seminário de divulgação de projetos da UNIVERSIDADE DE AVEIRO
apoiados pelo Mar 2020 | 30 SETEMBRO 2022 | ÍLHAVO ECOMARE

Harmful algal blooms and bivalve toxicity



Toxin monitoring programs - National Reference Laboratories



Toxin analysis

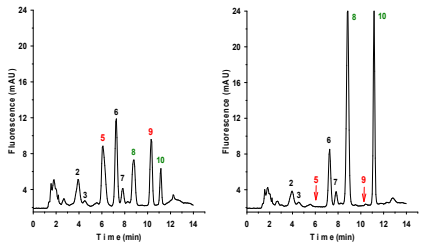
Tissue removal

Tissue homogenization

Toxin extraction

Sample preparation

Analysis by LC-FLD, LS-MS



Toxins' quantification and identification

Identification of toxic phytoplakton species e cell counting

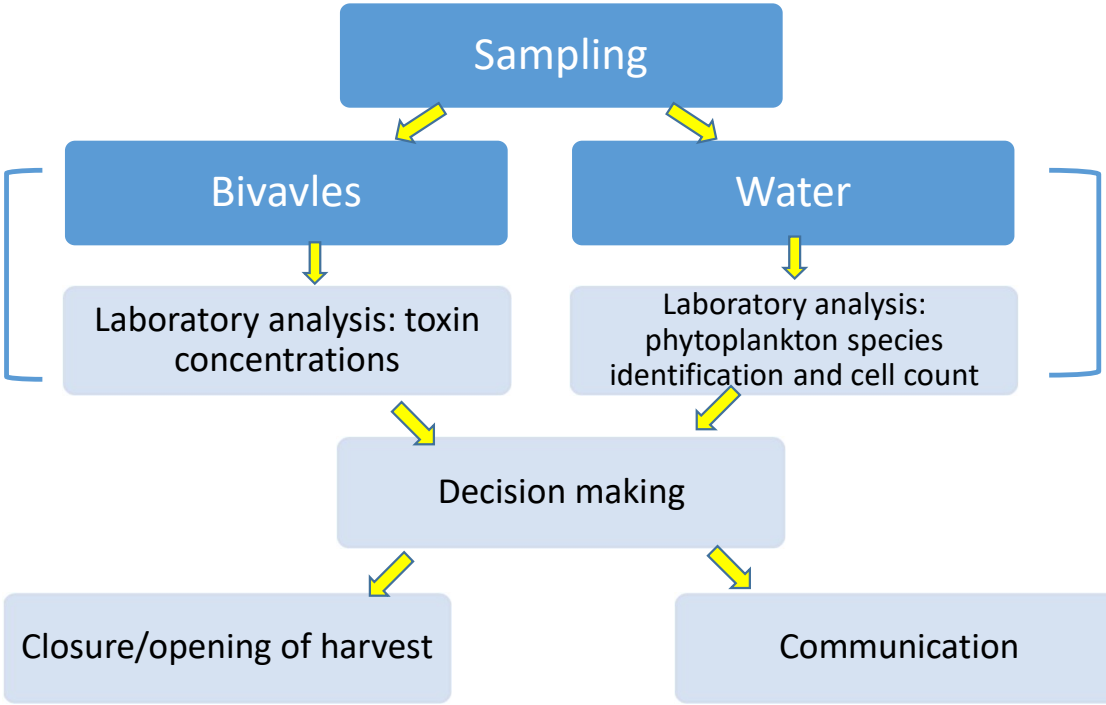
Water column sampling

Sedimentation of fixed samples (Utermöhl method)

Identification and enumeration using inverted light microscopy

Toxin monitoring programs

LínguaTox -
Língua bioelectrónica para a detecção de toxinas marinhas em bivalves
2014-2015
Funding 279 175 €



ImageTox –
Detecção automática de microalgas tóxicas através de imagens hiperespectrais
2018-2022
Funding 211 237 €

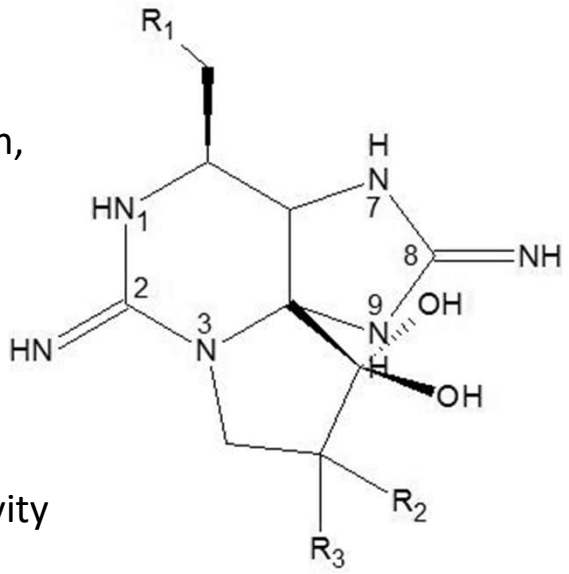
LinguaTox – Bioelectronic tongue for the detection of marine toxins in bivalves

Objective: development of the bioelectronic tongue based on chemical sensors and enzymatic biosensors for the rapid detection of Paralytic Shellfish Toxins (PSTs) detected on the Portuguese coast (decarbamoyl and N-sulfocarbamoyl).

Why PSTs?

High toxicity and severity of symptoms:

- Poisoning symptoms: tingling, burning, numbness, drowsiness, incoherent speech, respiratory paralysis.
- Severity: respiratory support within 12 hours of exposure results in complete recovery; mortality 8.5 - 9.5 %.

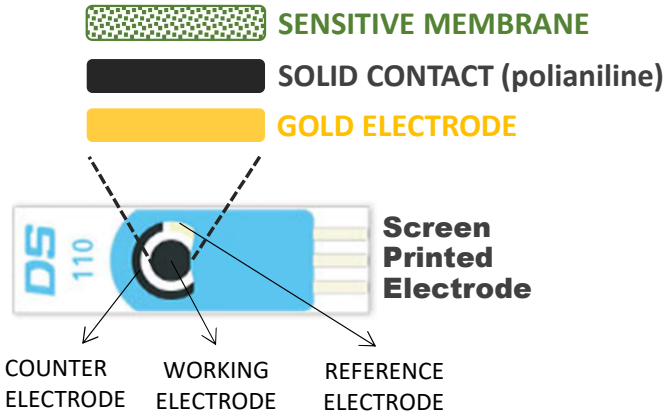


Challenge

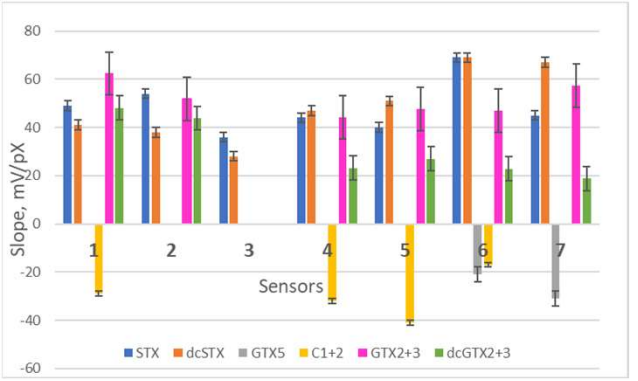
- More than 50 PSTs have been identified
- PST profiles in bivalves depend on toxin-producing species and bivavle enzymaric activity
- Existing sensors and assays not applicable to N-sulfocarbamoyl PSTs' detection

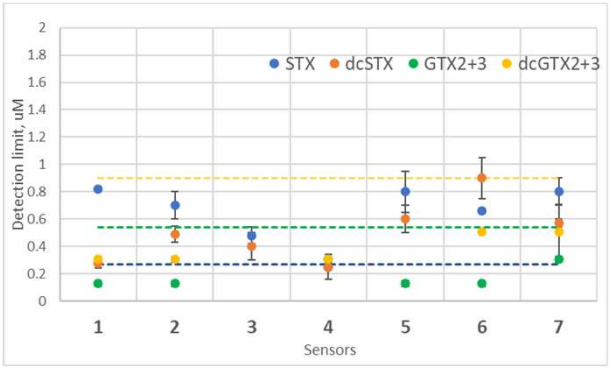
LinguaTox – chemical sensors' development

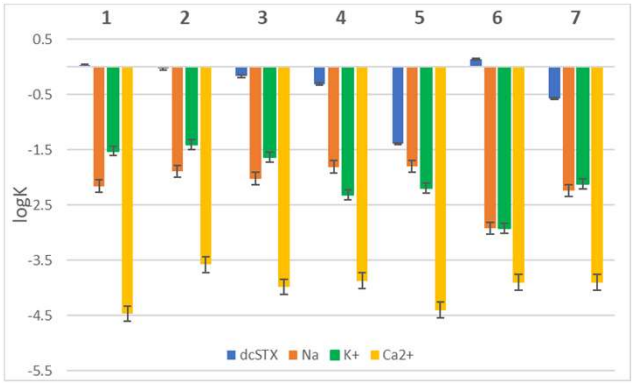
Potentiometric chemical sensors with PVC membranes



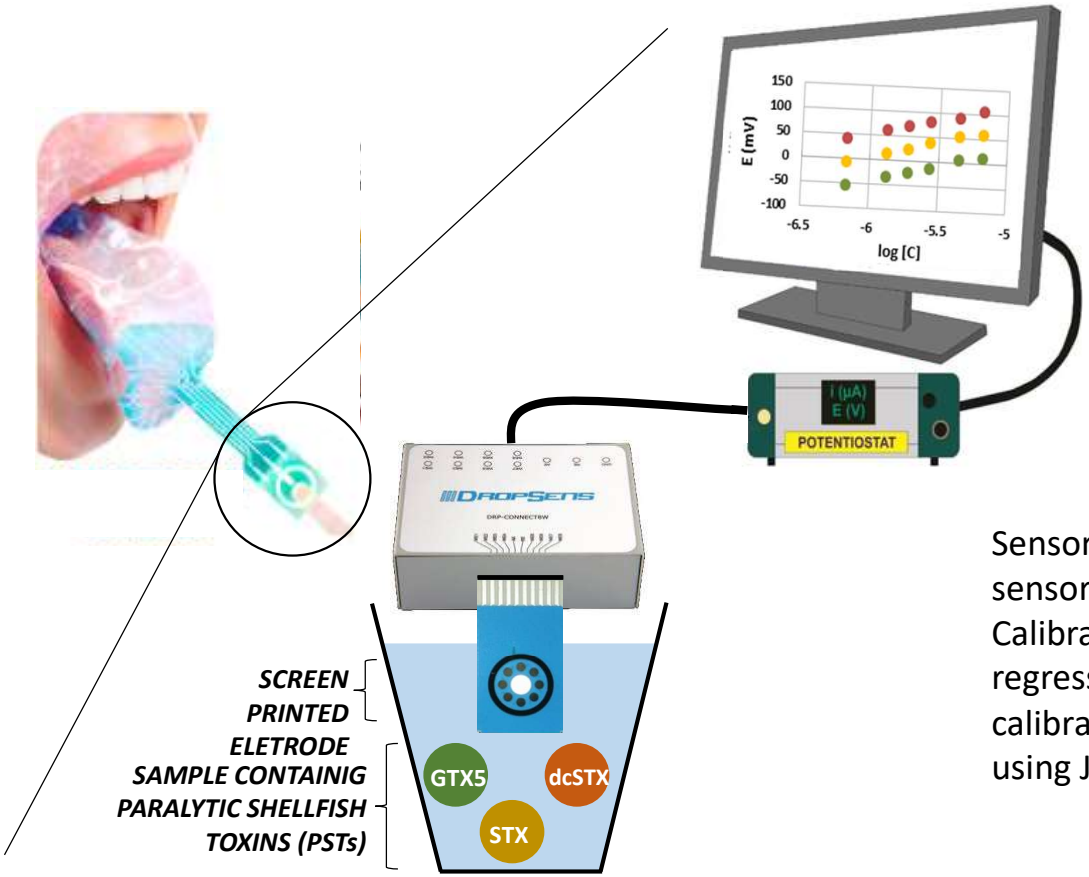
High sensitivity and selectivity to carbamoyl (STX, GTX2+3) and decarbamoyl (dcSTX, dcGTX2+3)
Low sensitivity to N-sulfocarbamoyl PSTs (GTX5, C1+2)





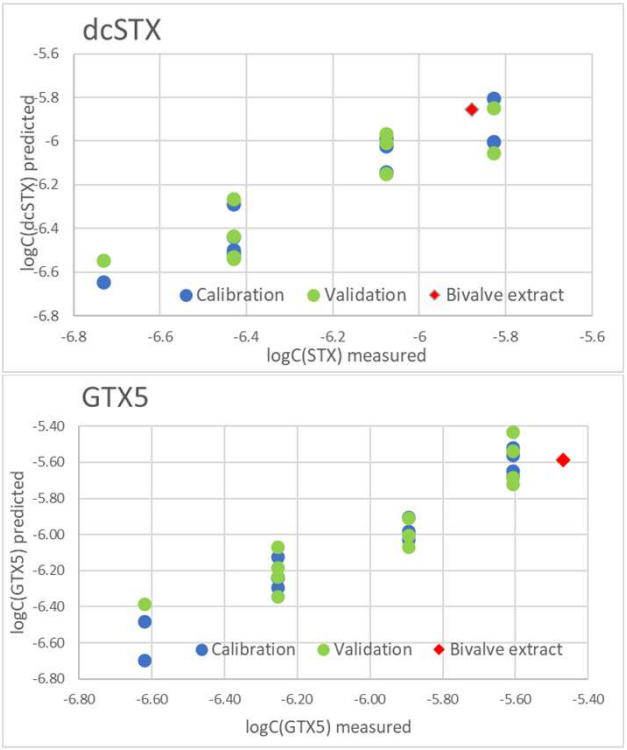


LinguaTox – electronic tongue development



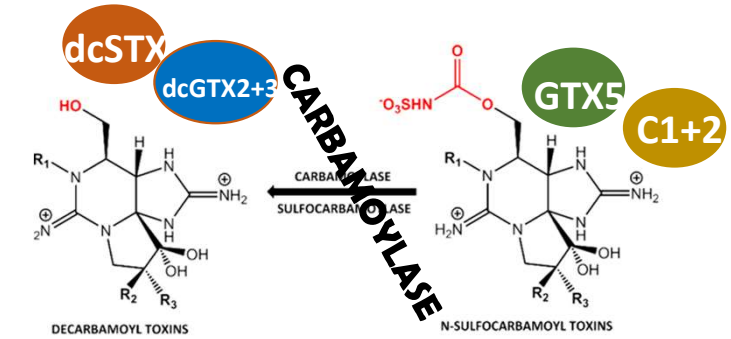
Sensor array – 6 sensors
Calibration – PLS-regression + calibration update using Joint Y-PLS

Quantification of STX, dcSTX and GTX5 in mussel extracts



LinguaTox - Enzymatic biosensors for detection of the N-sulfocarbamoyl PSTs

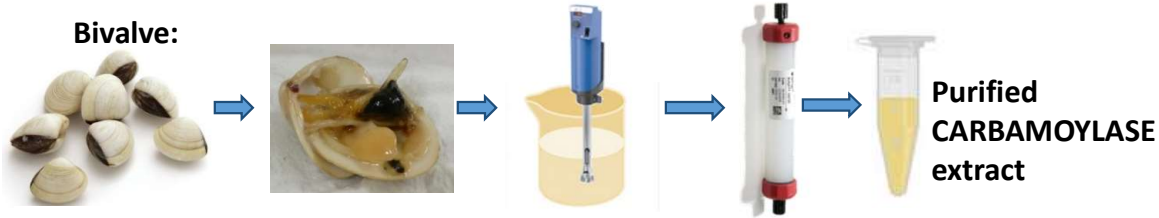
PST transforming enzymes



Decarbamoyl toxins **N-sulfocarbamoyl toxins**

Carbamoylase extraction

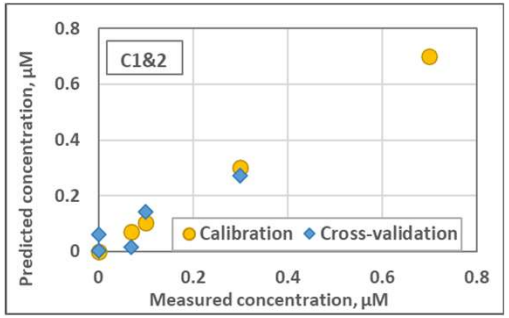
Bivalve:



Spisula Solida ***(Crystalline style)*** **Homogenization** **Purification**

Purified CARBAMOYLASE extract

N-sulfocarbamoyl PSTs detection



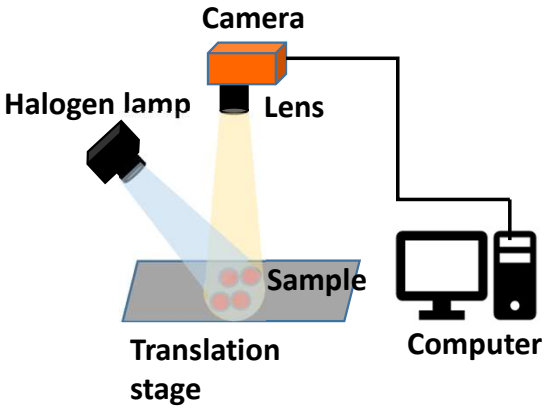
Potentiometric detection of decarbamoyl PSTs

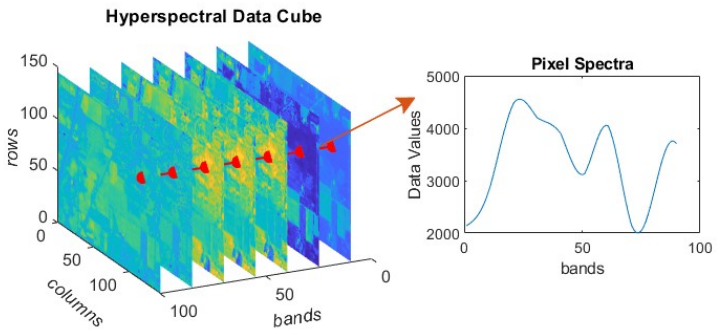
Impedimetric detection of enzymatic activity

ImageTox – Automatic detection of toxic microalgae using hyperspectral imaging

Objective: development an inexpensive, reagentless and easily automatable tool for the early detection and identification of microalgae species responsible for harmful algal blooms (HABs) based on the statistical analysis of the hyperspectral infrared images of phytoplankton.

Hyperspectral imaging





Spectral range: visible – far LWIR (400 nm – 5.3 µm)

Applications:

Industry
- Food
- Pharmaceutical
- Plastic sorting
- Packaging
- Mineral exploration

Agriculture

Research

Operational environment:

Airborne

Field

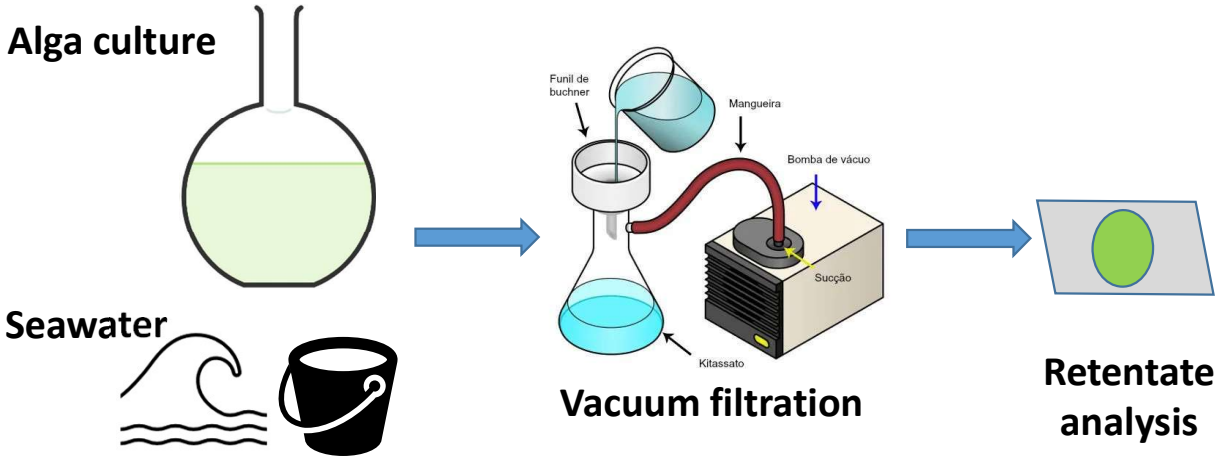
Plant

Lab

ImageTox - samples

Identification and quantification of toxic microalga in water samples in the presence of a mixed phytoplankton assemblage and its discrimination from nontoxic species with similar morphology using NIR hyperspectral imaging.
Comparison of NIR hyperspectral imaging with well-established spectroscopic techniques - NIR and FT-MIR spectroscopy.
Species selected for the study: *Gymnodinium:* G. catenatum and *G. nolleri; Pseudo-Nitzschias: P. australis and P. fraudulenta; Prorocentrum lima.*

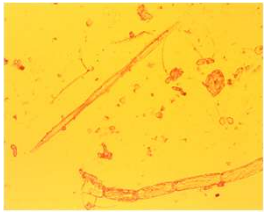
Sample preparation



- Cultures of *G. catenatum* (toxic) and *G. nolleri* (nontoxic);
- Natural seawater collected at the Aveiro lagoon;
- Mixtures of seawater and algal cultures at cell concentrations 200, 500, 1000, 1500, 2000 and 5000 cells/L, selected to encompass the warning (500 cells/L) and closure (1500 cells/L) thresholds established in EU regulation for *G. catenatum*.


G. catenatum

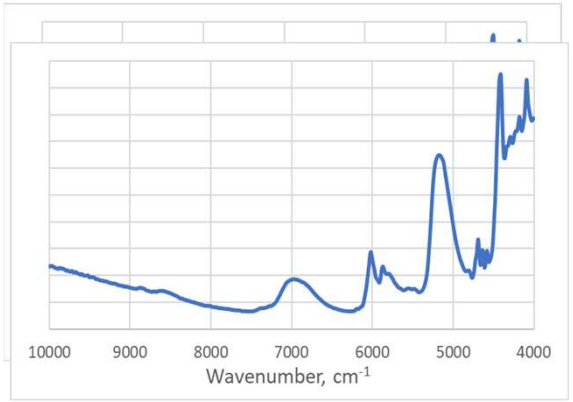
G. nolleri


Seawater

ImageTox – measurements and experimental set-up

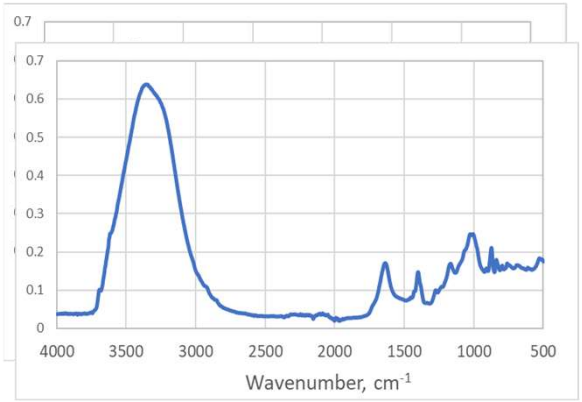
NIR spectroscopy

Perkin Elmer Spectrum 100N
10000-4000 cm^{-1}



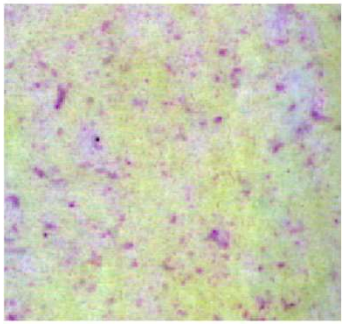
FT-MIR spectroscopy

Bruker Alpha Platinum with ATR accessory
4000-600 cm^{-1}



NIR HSI

LabSCANNER 40x20
FX17 spectral camera,
900 – 1700 nm
Macro lens OLES
MACRO (SPECIM, Ltd).
KL 1500 HAL Halogen;
Annular fiber optic
Darkfield ringlight
(SCHOTT AG)



ImageTox - Data processing

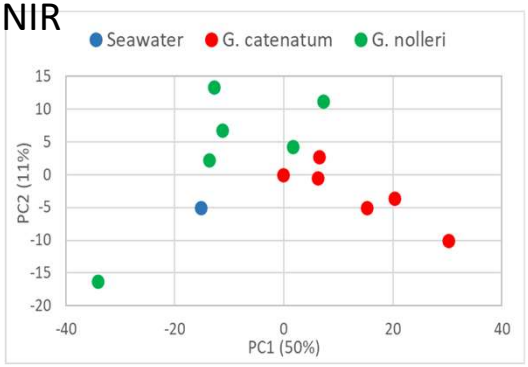
	NIR spectra	FT-MIR spectra	NIR imaging
Data pre-processing	• Smoothing (Savitsky-Golay) • SNV normalization • Scaling	• Smoothing (Savitsky-Golay) • SNV normalization • Calculation of 2^d derivative • Scaling	• Smoothing in the spectral mode (Savitzky-Golay) • SNV normalization in the spectral mode • Endmember signature extraction • Abundance maps of endmember signature corresponding to G. catenatum
Data exploration		PCA	Abundance maps w.r.t. G. catenatum endmember signature
Classification		PLS-DA	NPLS-DA

ImageTox

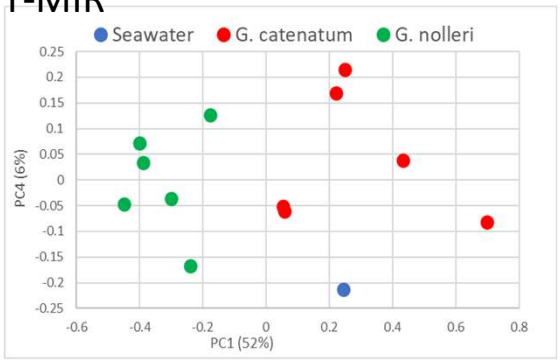
Case study *G.catenatum* and *G. nollery*

PCA score plot of seawater and its mixtures with cultures of two Gymnodinium species

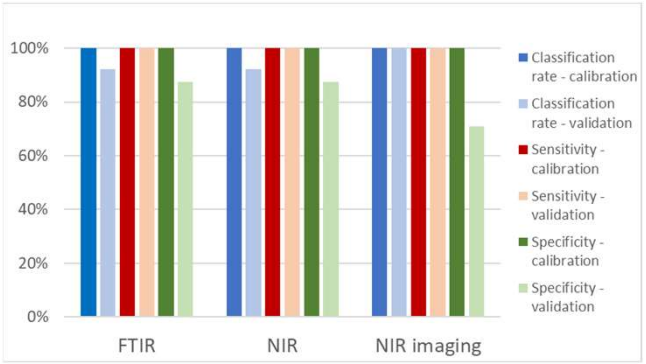
NIR



FT-MIR

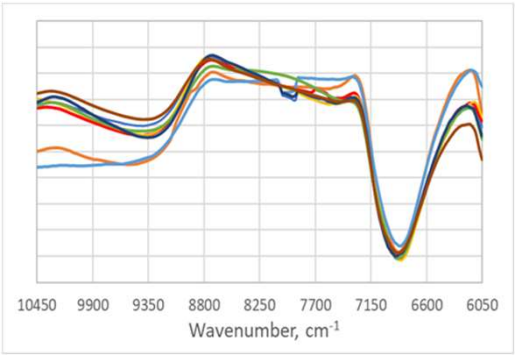


Classification results

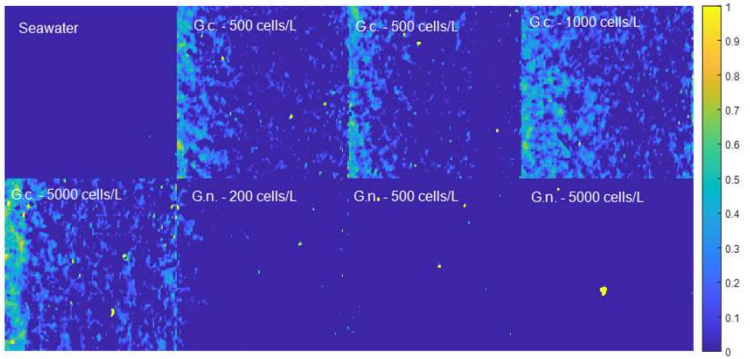


NIR Imaging

Endmembers signatures

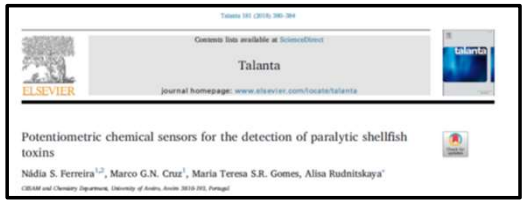


Abundance maps of endmember signature corresponding to G. catenatum



LinguaTox

Articles:


Talanta

Potentiometric chemical sensors for the detection of paralytic shellfish toxins

Nádia S. Ferreira, Marco G.N. Cruz, Maria Teresa S.R. Gomes, Alisa Rudnitskaya


Sensors and Actuators B: Chemical

Determination of paralytic shellfish toxins using potentiometric electronic tongue

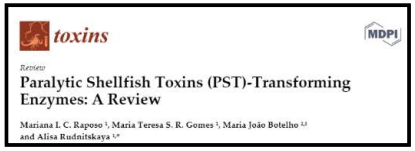
Marco G.N. Cruz, Nádia S. Ferreira, Maria Teresa S.R. Gomes, Maria João Botelho, Sara T. Costa, Carlos Vale, Alisa Rudnitskaya


sensors

Article

A Carbamoylase-Based Bioassay for the Detection of Paralytic Shellfish Poisoning Toxins

Mariana Raposo, Maria João Botelho, Sara T. Costa, Maria Teresa S. R. Gomes and Alisa Rudnitskaya


toxins

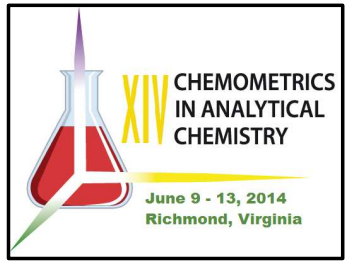
Review

Paralytic Shellfish Toxins (PST)-Transforming Enzymes: A Review

Mariana I. C. Raposo, Maria Teresa S. R. Gomes, Maria João Botelho and Alisa Rudnitskaya

Oral Presentations:

Poster:



ImageTox

Oral Presentations:

Poster:

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Thank you for your attention!