OoT - Ocean of Tomorrow

NEWSLETTER Issue 2 June 2017

Nine European projects to tackle the marine environmental problems through biosensing

OCEANS '17 MTS/IEEE ABERDEEN
19th to 22nd JUNE 2017

The Ocean of Tomorrow Projects will be present at the
IEEE OCEANS 2017 in ABERDEEN
Workshop
"Sensor and System Innovations for the Oceans of Tomorrow"

http://www.oceans17mtsieeeaberdeen.org

SEVENTH FRAMEWORK PROGRAMME

Oot Facts:
71,6M€ EU funding
4 topics
12 projects

This newsletter is result of a joint effort of the projects that comprises the Topic 1 and Topic 2 of the FP7-OCEAN-2013 call

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The first MariaBox device prototype has been recently prepared and is currently being validated in the lab. The prototype is about 1m high, 1m long and 0.5m wide and includes castor wheels for easy transportation. As earlier mentioned, the modular design concept has been followed. In terms of device operation, the following main modules are combined on the MariaBox device:

- Sample preparation module
- Biosensors storage and replacement module
- Analysis module

The sample preparation module is responsible for preparing the water sample to be analysed by the sensors of the device. It also includes a smart, automatic filter replacement module that automatically replaces all device filters, before each measurement. The same module is also responsible for washing all device pipes with deionised water before each measurement, in order to avoid cross-contamination. The biosensors storage and replacement module includes a temperature controlled chamber in which the biosensors (in the form of discs) are stored when not used. The same modules includes a mechanism for transferring the biosensor discs to the analysis module, whenever an analysis needs to take place. When a biosensor disc is fully used, then it is positioned in a dedicated position inside the storage chamber and a new disc is automatically loaded. The analysis module is responsible for reading the biosensors. The discs containing the biosensors have to be spun following a specific protocol that allows the necessary binding to take place. Afterwards, optical analysis of the disc is performed using an LED and a photodetector, working at predefined wavelengths, using also the appropriate optical filters. The output of this module are the concentrations of the four man-made chemicals and the four categories of micro-algae toxins targeted by MariaBox. Additionally, the routine-POD sensor measurements are also collected. Those are off-the-shelf sensors. Data collected by the MariaBox device are wirelessly transmitted in user defined intervals to a dedicated, web-based platform. Data collection is compatible with the INSIRE directive, the Copernicus European Earth observation programme, the GOOS observing system, the SeaDataNet and follows the SOS standard. Transmission can be achieved using mobile networks, satellite connection or even Wi-Fi. The device includes its own Power module, which generates the necessary voltage levels for the MariaBox device operation. The Power module's batteries can be recharged by either PV panes or wind turbines. There is also the possibility to power the device from a 220V-AC supply.

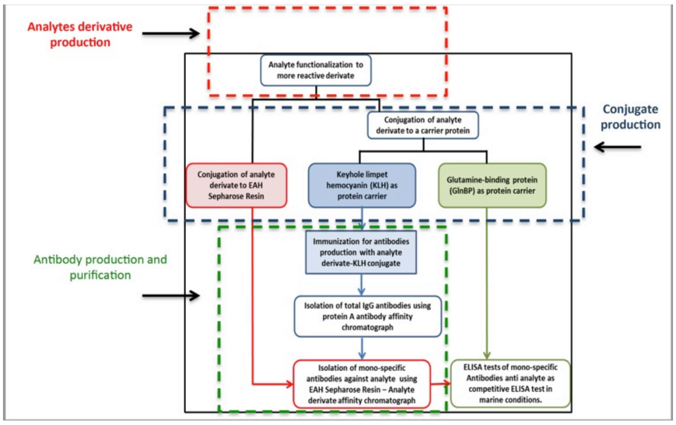
The following figure presents the 3D rendering of the first MariaBox prototype.

3D rendering of the first MariaBox prototype

BIOSENSORS

Biosensors are defined as compact analytical devices composed by molecular recognition element (MRE), integrated within or intimately associated with a physic-chemical transducer. In frame with the Mariabox project, biosensors able to detect in the in real-time and with high specificity chemical pollutants and microalga toxins molecules were been developed. Chemical pollutants Naphthalene, Perfluorooctaic acid, Camphechlor and as microalga toxins Saxitoxin (and its derivatives), Microcystin (different structural variants), Azaspiracid and Domoic acid were identified as target analytes and in the biosensor development, we choice as MREs, polyclonal antibodies due to their high selectivity and stability in different operative condition. No commercial antibodies are available for each target analytes selected, due to their size that does not allow to elicit immunological response. In this project we develop antibodies for these compounds and the adopted strategy was been to chemically modify their structure with a bi-functional chemical linker in order to obtain an amino reactive and/or carboxyl reactive derivative. This strategy relies on the production of functionalized analytes, on the production in mouse and/or rabbits of polyclonal antibodies against them and on the affinity purification of analyse-specific antibodies to be tested for binding efficiency and specificity.

The Figure summarize strategy needs with the three main steps i) analytes derivative production, ii) conjugate production and iii) antibodies production and purification.

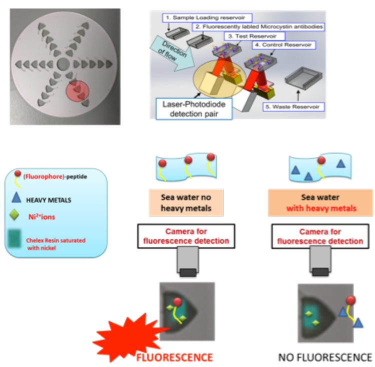


Flowchart of strategy for Abs production, purification and quantification

The mono-specific antibodies against the different compounds were purified and characterized for their binding capability in marine condition (seawater, 460 mM NaCl) and in the operative condition of Maria box device (22 °C) through the ELISA test. Then were labelled with commercial dyes, with spectral characteristics (excitation and emission wavelength) in the visible region of the light spectrum. In particular for this purpose the fluorescent probes Alexa Fluor 430 nm was used. The labelled antibodies were used for the competitive binding assay on the disc surface in the MariaBox device.

Heavy metal biosensor

A different approach was proposed for MREs for Heavy Metals, that are too small and not highly immunogenic to induce the production of specific antibodies. Fluorescent metal-binding peptides were chosen as MREs and the procedure indicated in figure 3 was established for the detection of heavy metals in sea water. Briefly, when the sea water sample delivered to the analysis disc does not contain heavy metals, the fluorescent peptide also present in the chamber will be free to interact with the Nickel that is bound in the biosensor chamber, producing a light signal. On the contrary, when heavy metals are present in the sea water sample, the labelled peptides will bind to them and will not be free to interact with the biosensor disc. In this case the labelled MRE will be washed away and a light signal will not be produced and detected (figure 4). A bioinformatics analysis of the amino acid sequences of protein known to efficiently bind heavy metals was performed to identify eight-amino acids peptides as MRE. The peptides were produced, labelled by the addition of AlexaFluor 430 and tested for heavy metal binding by Resonance Energy Transfer analysis. Heavy metals tested were Cu^{2+}, Pb^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+} and Zn^{2+}. Chelex resin was proposed to immobilize Nickel on the analysis disk to detect heavy metals by the MARIABOX biosensor. Several experiments in simulated seawater were performed to verify the efficiency of the system .



The Analytical assay-on-disc breakdown

Fluorescence titration experiments demonstrated that the detection limits (LOD) for heavy metals of the MariaBox biosensor are lower than those established by the Directive of the European Commission. Moreover, the reproducibility of the system and reliability in analytical data were confirmed by a relative standard deviation value lower than 5% in triplicate experiments, indicating the validity of the Biosensor

The team:

EnviGuard started in December 2013 with the aim to provide the marine aquaculture industry with a highly specific and precise in situ measurement device for monitoring chemical contaminants and biohazards in seawater. The EnviGuard system combines technologies from the field of nanotechnologies, genomics, molecular science, bio-receptors as well as material science and data processing. It is made up of three different sensor modules one for detecting toxic microalgae, one for pathogens and one for toxins and PCBs. They are connected to the EnviGuard Port, a common interface device and platform, which is responsible for continuous sampling, filtration and sample preparation. It also records and sends the data to a server. Users can access their information online.

The algae detection unit (ADU) and chemical detection unit (CDU) were completed in 2016 and used for the analysis of different water samples. The ADU is based upon the detection of nucleic acids via sandwich-hybridisation. A target specific capture probe is deposited on an electrode. If the target is bound, detection takes place via the hybridization of a second probe. Attached to the second probe are components, which allow for an electro-chemical signal to be measured. The biosensor is fully automated using the AUTOFIM filtration unit for sample preparation. Readings from real water samples were compared to the results from classic microscopy. The CDU is an optical immuno-sensor based on photonic nanostructured surfaces called Bicells. The Bicells are integrated in a reusable multiplexed chip within an autonomous fluidic system which allows simultaneous multi-analyte detection. Monoclonal antibodies and a competitive format have been applied for specific detection of toxins and PCBs. Solutions with increasing concentrations of target analytes were used to obtain calibration curves and assess system detectability. Spiked water samples were used to evaluate the system's capacity. The ADU has been used to measure the occurrence of two different Dinophysis species in field samples. The biosensor gave positive results for their occurrence. The fully automated system needs two hours per sample. The results of the ADU were confirmed by microscope counting of the samples.

Regarding the CDU, successful protocols for label-free and real-time detection of PCB (126&169) and Okadaic Acid (OA) have been developed and implemented in the integrated unit. Results show a detection limit close to 25 ng/ml for OA and 22 ng/ml for PCBs revealing the sensitivity of the novel sensor. The sensor is able to analyze one sample per hour. Monitoring of toxic microalgae is usually done by microscopy. This method is time consuming and requires trained personal. Molecular methods have a higher resolution and distinguish better between species. The ADU allows for both quantitative and qualitative measurements. The reaction only takes place when a certain species or group is occurring and the strength of the signal allows for measuring the amount of cells in the sample. This new method is faster - a single measurement takes about 2 hours after filtration - and the device can be used with little training

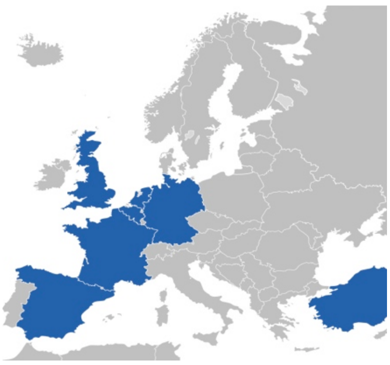
EnviGuard partners have presented the project in several international conferences and trade fairs as:

· International Innovation Workshop Aquaculture · NanoSpain Conference ·
· SEA-on-a-chip Project Workshop · Hannover Messe ·

The main operation of the device can be done remotely, as well as the analysis of the results. Consumables such as different solutions and a new pair of bio-chips is needed once a week. The results of the CDU constitute the developed bio-sensing technology as a competitive analytical tool, since usually PCBs and OA are determined by more complex and expensive methodologies such as GC/MS or LC/MS, which require sample shipment to analysis laboratory.

Due to these advantages, the two new biosensors have the potential to replace standard procedures and diagnostic tests. The degree of automation allows remote operation in the marine environment. Only basic personal is needed for quantitative analysis. Thus, EnviGuard can serve as an early warning system for aquaculture enterprises as well as regional authorities. The modularity of the platform allows users to purchase a customized product according to their needs and the addition of new biosensor modules in the future.

The EnviGuard consortium is composed of 18 partners from 6 different countries across Europe plus Turkey, grouping the companies (ISI, BIOD, MT, LX, ABT, NBS, VFF, AET, BIOAZUL, ILK), research institutes (TTZ, AWI, HZG, CNT,



Technology Innovation in Emerging Pollutants Water Quality Monitoring using Biosensors

The SMS concept is based on a novel automated networked system, which includes a multimodular apparatus that hosts in a single unit—the Main Box—a Sampling Module and an Analysis Module. The former contains sample collection, filtering and preconcentration and treatment components, whereas the latter includes five automated sub-modules that enable unattended measurement of (i) toxic algal species, (ii) their associated toxins, (iii) hazardous compounds, namely glyphosate and pentaBDPE, (iv) sulphonamides and a (v) series of standard water quality parameters, including nutrients. The water monitoring system is equipped with a communication module for real-time wireless data transfer to a remote control centre, where data processing takes place, enabling alarm functionality of early warning system. All research work culminates in demonstrating the project's results in three sites: in La Spezia (Italy) and in Piran (Slovenia) field tests will be performed in June-August 2017, while in the inner Pagasitikos Gulf in Greece water samples will be collected and measured in laboratory; the marine Alonissos marine park (Greece) will be used as a reference pristine site. These sites were chosen because they range (in terms of anthropogenic and chemical contamination) from pristine to polluted conditions, in order to offer a wide range of test conditions for monitoring. The innovative analytical concept allows its adaptation to detect other similar biological targets such as cyanobacteria and their toxins for freshwater monitoring purposes.

<u>Our innovative sensors</u>

- Toxic algal species (*Alexandrium minutum, Pseudo-nitzschia, Dinophysis acuta, Dinophysis acuminata*)
- Algal toxins (Saxitoxin, Okadaic acid & Domoic acid)
- Flame retardants (PBDE)
- Herbicides (Glyphosate)
- Pharmaceuticals (Sulphonamides)

<u>Our Reference Sensors</u>

- Temperature
- pH
- Salinity
- Dissolved Oxygen
- Turbidity
- Chlorophyll a
- Ammonia
- Nitrate
- Nitrite
- Orthophosphate

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under grant agreement no 613844

Sensing toxicants in **M**arine waters makes **S**ense using biosensors

Our instrumental prototypes

µMac-Smart
Key features: portable analyser completely automating complex analytical methods, with the capability to be used on board of floating platforms and coastal buoys for unattended measurements.

WIZ Probe

Key features: in-situ field deployable probe automating complex analytical methods for long-term unattended measurements.

- **TOXIC ALGAE DETECTION:**

A µMac-Smart module, based on Enzyme Linked Immuno- Magnetic Optical (ELIMO) assays, is operative to detect algae from genera *Alexandrium, Pseudonitzschia* and *Dinophysis.* The water sample is automatically pretreated by a separated automated module performing water sampling, cells preconcentration and lysis.

- **ALGAL TOXINS DETECTION:**

A µMac-Smart module was developed and tested in laboratory for the quantitative measurement of:

⇒ Okadaic acid
⇒ Domoic acid
⇒ Saxitoxin

The automated module is capable to measure, unattended, up to 50 water samples and the measurement ranges between 0.01 and 1 µg/L.

- **PBDE & GLYPHOSATE DETECTION:**

We've chosen commercially available Enzyme Linked Immuno- Magnetic Optical

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(ELIMO) assays for PBDE and Glyphosate detection due to their low limit of detection (0.017 ppb and 0.05 ppb respectively) as well as full compatibility with real samples, such as seawater. These methods are promising for further implementation in µMac-Smart module.

Moreover, we have developed a novel microfluidic platform for electrochemical detection and graphene-based removal of PBDE. The working concentration range of this approach is 0.025-1.0 ppb and LOD equal to 0.018 ppb.

- **<u>SULPHONAMIDE DETECTION:</u>**

A µMac-Smart module was developed and full validated in laboratory to measure sulfonammides down to 1.5 µg/L using a spectrophotometric method; the same method was improved in a WIZ in-situ probe too.
An electrochemical carbonic anhydrase based biosensor for inhibitive determination of sulfanilamide was elaborated for the first time. The calibration curve showed a linear range from 0.5 to 5 µM SAD concentration, and estimated LOD equal to 0.4 µM.

<u>Field tests:</u>

A large coastal water buoy is already in operation in Piran (Slovenia) and is going to host the equipment to measure the following parameters:

- Toxic algae species
- Dissolved nutrients (NH_3, NO_3, NO_2, PO_4) by WIZ in-situ probe + 0.1 µm cut-off filtration & autocleaning
- Standard water quality parameters (Temperature, EC, pH, DO, Turbidity, Chlorophyll).

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A relocatable floating platform is going to be deployed in the bay of La Spezia (Italy), for field monitoring of the following dissolved compounds (after 0.1 µm cut-off filtration & autocleaning):

- Domoic acid
- Okadaic acidSaxitoxin
- PBDE
- Standard water quality parameters (Temperature, EC, pH, DO, Turbidity, Chlorophyll)

<u>For all the field tests, monitoring data will be available on a remote Web server.</u>

Further information available on request

About SMS's partners:

University of Rome "Tor Vergata", Italy – Coordinator
Italian National Agency for New Technologies, Energy and Sustainable Economic Development, Italy
Catalan Institute of Nanoscience and Nanotechnology, Spain
Acromed, Sweden
National Institute of Biology, Slovenia
University of Thessaly, Greece
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Sensing toxicants in Marine waters makes Sense using biosensors

Summary of main achievements of the BRAAVOO project.

1. Eight-channel bimodal waveguide (biMW) and three-channel asymmetric Mach-Zehnder Interpherometric (aMZI) nanophotonic platforms fabricated. Leak-free fluidic connectors for independent sample delivery on the nanophotonic platforms. Fabricated laser light incouplers to both platforms. Biofunctionalization protocols of both biMW and aMZI surfaces. Complete detection assay procedures for Irgarol, Tetracycline and Okadoic Acid with method of detection limits of 0.04-0.2 µg/L. Less efficient detection of Ampicillin and Domoic Acid (10-20 µg/L).
2. Coherent set of *Escherichia coli* bioreporter strains producing bioluminescence in response to organohalogens, antibiotics, marine toxins, oil (alkanes), oil (monoaromatics), oil (polycyclic aromatics), heavy metals (Hg, Cd, As, Zn), DNA damage, oxidative radical damage. Single-use functional multi-target cartridges (10-wells, 10×36 mm) with lyophilised bioreporters. Continuous-use single-target bioreporter chip fabricated: 1 week life-time, demonstrated for arsenic detection at 10 and 50 µg/L.
3. Marine water resistant algal photosystem II fluorescence sensor based on *Chlorella vulgaris* in symbiosis with *Tetrahymena pyriformis.* Demonstrated detection of herbicides at 0.5 µg/L. Specific six-well continuous flow-through cartridge with immobilize algae produced. Life-time several months at 4–20°C.
4. Standalone instrument implementing the aMZI nanophotonic platform, including optical sources and detectors, including automated sample delivery as well as reagent deliveries. Standalone fluorimeter implementing the *Chlorella vulgaris* algal sensor and cartridge, and controlling algal maintenance. Standalone bacterial bioreporter unit implementing the 10-well cartridge with lyophilized bioreporters, and reading out bioluminescence kinetics. Design and fabrication of specific microfluidic rotary valves for the standalone instruments.
5. Automated biosensor instruments in watertight cases for marine deployment with sample and reagent delivery. Bacterial biosensor: implementing 30 cartridges with automated displacement allowing 1 month operating time. Optimized measurement protocol including standards, negative control and spiking control. Nanophotonic aMZI sensor: three parallel line chip, with automated sample and reagent delivery, regeneration protocol tested. Algal sensor: six-parallel line format for continuous measurement, with automated sample and reagent delivery.
6. Marine monitoring buoy and autonomous operating trimaran vessel built. All biosensors implemented and tested. Data acquisition and telemetry systems installed. Guidance, navigation and control systems implemented. Sampling system fabricated and tested; connections to biosensor fluidics built.
7. Biosensors first benchmarked on blind ring test with unknown marine samples. Second benchmarking in mesocosm facilities on Sicily. Third benchmarking in harbor in Ireland.

The COMMON SENSE project has successfully finished after 40 months of in-depth research and continual development of marine sensors and systems. The EU-funded project started in November 2013 and ran until February 2017 with a focus on developing specific sensors in direct response to current marine monitoring challenges, and the requirement of EU Member States in meeting their Marine Strategy Framework Directive (MSFD) requirements and achieving Good Environmental Status (GES) of their marine territories.

The COMMON SENSE project was a great success, with significant progress made to improve marine data acquisition using sensors to contribute towards increasing the availability of standardised data on: eutrophication; concentrations of heavy metals; microplastic fraction within marine litter; underwater noise; and other parameters such as temperature, pH, pCO_2 and pressure.

The progress achieved by the project partners is impressive, with the majority of the sensors moving from a technology readiness level (TRL) of 2-3 up to 6-7, with one sensor now at TRL 8 – the Mini Sea Sampling System. While commercialisation of these sensors is beyond the scope of the project as it has finished, interested stakeholders are welcomed, and encouraged to engage with COMMON SENSE partners to ensure the sensors are brought to market. In many cases, partners have committed to continuing the work in COMMON SENSE in order to do so.

To optimise the exploitation potential of the COMMON SENSE project's generated knowledge, the partners incorporated an in-depth communication, dissemination and knowledge transfer strategy from the very beginning of the project. Several different resources are available to stakeholders, which will allow them to understand exactly what the knowledge is, and how it could be applicable to them. From an industrial point of view, sensor profiles were developed as technical briefs, outlining the technical specifications and highlights of each sensor. These are available to download from the COMMON SENSE website's media section. Also, the project carried out a feasibility analysis and have outlined manufacturing procedures for each sensor, providing in-depth information on how the sensors can be reproduced and brought to market.

The COMMON SENSE outreach in general was enthusiastically taken on board by all partners from an early stage, in recognition of the need to raise awareness of progress and results of the project on an ongoing basis. To this end, regular factsheets were developed, published and widely disseminated. As well as an introductory factsheet developed at the start of the project to introduce stakeholders to the COMMON SENSE project, its objectives, methodology and expected impacts, three other factsheets provided information on important aspects of the project, such as: how COMMON SENSE sensors will contribute to improving marine monitoring and marine data management including an infographic that shows the project development timeline alongside a timeline for MSFD implementation; introductory detail on each of the innovative sensors under development by COMMON SENSE including the description of how the sensors could work together on one platform through the smart sensor unit and common sensor platform whose goal was to collect data from multiple sensors; detail on the deployment and testing activities carried out by partners to ensure developed sensors were fit for purpose and to identify areas which required further modification. Significant effort was expended in these activities, with all sensors being tested a multiple of times at different locations and using different platforms.

A project video was also created, which quickly explains the project and its relevance to marine monitoring policies across Europe, using a mixture of real footage and animations. The video is available to view online at https://vimeo.com/201643243 or through the COMMON SENSE website.

The COMMON SENSE project closed with a final partner meeting and demonstration event in Barcelona at the end of January 2017. The Coordinator, Sergio Martinez of LEITAT, expressed his appreciation for the efforts of each partner and congratulated the consortium on their achievements, saying:

"Tomorrow has arrived, now at the project end the proposal vision has come true, our objectives have materialised and the results are widely visible; we looked at the requirements of next generation sensors, including measuring new pollutants and increasing performance and compared them to existing sensors, to develop cost-effective solutions, transferring acquired data to an interoperable web platform. We tested and deployed our marine sensors and systems with exciting and hopeful results. Some sensors are now advanced prototypes, others require further validation. A mission for COMMON SENSE partners now is to continue with the legacy of the project, to keep working on these innovative marine solutions, reach the market, and help society by contributing to the realisation and maintenance of good environmental status in all EU Member States".

The results of the COMMON SENSE project can be used to increase knowledge of the marine environment and access to related data, allowing strategic decisions to be taken in marine protection and conservation. It will also help to support EU policies (MSFD / CFP) by providing multifunctional, innovative and cost-effective sensors that are easy to use across a range of platforms to detect reliable measurements on key parameters by means of methodological standards that interoperate with, existing or new, international observing services. All COMMON SENSE resources are available to download from the COMMON SENSE website. www.commonsenseproejct.eu, or by contacting WP10 Leader Cliona Ní Cheallachain of AquaTT (www.aquatt.ie), or the project coordinator Sergio Martinez of Leitat (smartineznavas@leitat.org).

COMMON SENSE came to a close at the end of February 2017.

The project received a total of €4.66 million of EU funding under the OCEAN 2013.2 area of the Seventh Framework Programme (FP7).

COMMON SENSE was coordinated by Spain's LEITAT Technological Center,

COMMON SENSE
MARINE SENSORS - MARINE MONITORING

2017 PROGRESS UPDATE

COMMON SENSE AT A GLANCE

PROJECT TITLE:
Cost-effective sensors, interoperable with existing international ocean observing systems, to meet EU policies requirements

FUNDING PROGRAMME:
FP7 Environment, Ocean 2013.2

INSTRUMENT:
Collaborative project

TOTAL BUDGET:
€6,074,497

EC CONTRIBUTION:
€4,664,072

DURATION:
40 Months (Nov 2013 - Feb 2017)

COORDINATOR:
Sergio Martinez (smartineznavas@leitat.org)
LEITAT Technological Center (LEITAT), Barcelona, Spain

CONSORTIUM:
15 partners from seven different countries (the **COMMON SENSE** consortium comprises six SMEs, five research development institutes, three universities and one foundation)

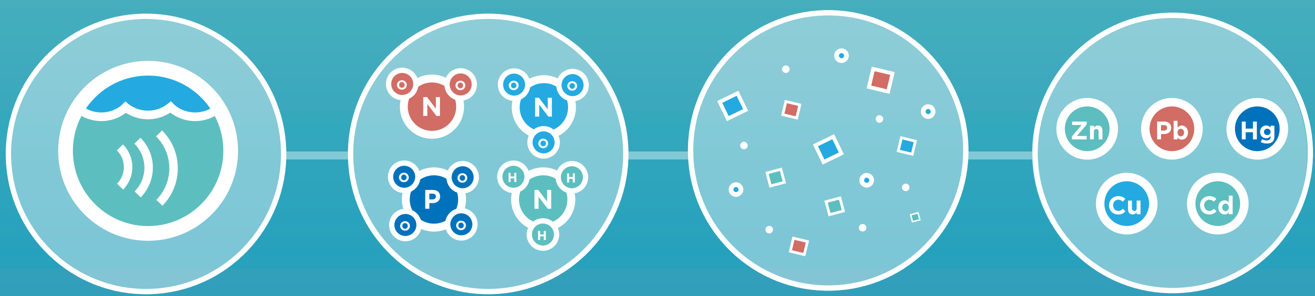
For more information please visit the **COMMON SENSE** website:

WWW.COMMONSENSEPROJECT.EU

INNOVATIVE SENSOR DEVELOPMENT

COMMON SENSE has developed prototypes of in situ next generation marine monitoring sensors that will increase the availability of standardised data on eutrophication, concentrations of heavy metals, microplastics, underwater noise and other parameters. These cost-effective sensors directly respond to current marine monitoring challenges and provide new ways to assess the overall health of marine environments.

DEDICATED SENSORS



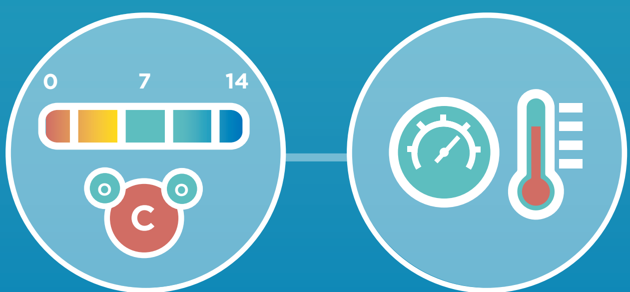
Sensors for monitoring underwater noise

Sensors for monitoring eutrophication

Sensors for monitoring microplastics

Sensors for monitoring heavy metals

REFERENCE SENSORS



Nanosensors for autonomous pH and pCO_2

Innovative temperature and pressure sensors

DEPLOYMENT AND TESTING

To test the operability of the **COMMON SENSE** sensors, the team deployed and tested the sensors in a range of environments. The objectives were

- To optimise the design and performance of the precompetitive prototypes
- To test whether the deployed sensors interfered with everyday professional activities and adjust their compatibility accordingly
- To ensure the correct and timely transmission of data

TURN PAGE TO VIEW DETAILS OF COMMON SENSE DEPLOYMENT AND TESTING ACTIVITIES

DEPLOYMENT AND TESTING

The **COMMON SENSE** project committed to rigorously test all hardware developed to ensure that the sensors' performance is not inhibited by even the most changeable and challenging conditions.
COMMON SENSE sensors underwent field testing in the Mediterranean, North, Norwegian, Baltic and Arctic seas.

The team wanted to prove that the sensors could be integrated into a variety of vessels and platforms, a key part of the project. The tests took place in all sorts of locations, including vessels, oil platforms, buoys and even racing yachts!

SENSOR

PLATFORM

	Temperature	pH (Res.)	pCO_2	pH (Volt.)	Eutrophication	Microplastics	Microplastics Analyzer	Microplastics and MISS System & Analyzer	Heavy metals	Underwater noise	MISS
OCEANIA VESSEL	✓						✓			✓	✓
MINERVA UNO VESSEL					✓	✓			✓		
LOCAL HARBOUR - ORISTANO	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
CNR ARCTIC BASE				✓	✓				✓		
HAWAII FLOATING PONTOON					✓						
RACING YACHT						✓	✓				
MOTOR BOAT										✓	

All photos © Alberto Ribotti, CNR

This project has received funding from the European Union's Seventh Programme for research, technological development and demonstration (OCEAN 2013.2) under grant agreement No 614155.
This publication reflects the views only of the author, and the European Union cannot be held responsible for any use which maybe made of the information contained therein.

Designed and developed by **AquaTT**

1 Passive Acoustic sensors

For ocean passive acoustic monitoring, two devices have been developed in NeXOS, named A1 and A2. Four A1 (Fig. 1) and one A2 (Fig.2) were manufactured for demonstrations in the project.

Table 1 – NeXOS Passive accoustic sensors and capabilities

Ocean Passive Acoustics Sensor	Description		Applicationss
A1	Digital hydrophone compact, low power	Noise and bioacoustics embedded processing	Mobile platforms, surface/deep waters
A2	4 digital hydrophone array + Master Unit	4 synchronized channels for TDOA measurement	Fixed platforms (incl. deep water)

The NeXOS acoustic sensor innovations include:

- Broad dynamic range and frequency spectrum
- Compactness
- Cost-efficiency
- Low power consumption allowing for small integration into mobile and fixed platform (30 mW in sleep mode and 900mW in operation)
- Dynamic range adjustable to cover 50 to 180dB re 1µPa, manual or automated
- Programmable gain amplifier stage to configure the analogue signal conditioning stage for both low and high sound level monitoring
- Web-enabled pre-processed marine acoustic data
- Plug and play OGC-PUCK Enabled
- OGC SWE web-enabled pre-processed marine acoustic dataStream / store raw and spectral data
- Streaming / storing (up to 32/128 GB) of raw and processed data

Figure 1 NeXOS A1 sensor

- Open source for add-on programming
- Depth rated down to 3600m
- Supply voltage: 4.6 to 42 Vdc
- Data storage internal memory 128GB

Figure 2 NeXOS A2 sensor

A1 sensor details

A1 is a compact, low power consumption digital hydrophone (see Figure 1) with embedded pre-processing of acoustic data, using the Open geospatial Consortium (OGC) Programmable

Underwater Connector with knowledge (PUCK) and Sensor Web Enablement (SWE) interoperability standards. A1 enables acoustic measurements and characterization of underwater noise, bio-acoustic sources and several soundscape sources. A1 consists of one transducer and two A/D converters, simultaneously sampled, with different gain, to measure and detect a broad ranged of acoustic source levels from 50 dB to 180 dB with reference to 1µPa from selectable analog gain settings or traceable automated gain control, in the frequency range from 1Hz to 50kHz. Three types (model and brand) of transducers suitable for A1 have been identified. Differences consist in sensitivity, shape and maximum operating depth Within NeXOS, a prototype of A1 for each of these transducers was manufactured.. The architecture of A1 sensor is composed of:

- Transducers (HYD) of different types depending on depth requirements and cost: Neptune Sonar D/70, Technology Limited SQ26-01, JS-B100-C4DP
- Signal Conditioning Unit (SCU): 2 channels of amplifier stage including selectable whitening equalizer, anti-aliasing filter, selectable gain, sampling frequency
- Micro-power SAR Analog to Digital Converters 16 bit ADS8867
- MicroController Unit (MCU) LPC4370
- Underwater connector 12-pin male MCBH12M

	Hydrophone Type		
	Neptune Sonar mod. D/70	***Technology Limited mod. SQ26-01***	***JS-B100-C4DP Acoustic Sensor***
Sensitivity CHA	-138/-158 dB re 1µPa	-133.5/-153.5 dB re 1µPa	-141/-161 dB re 1µPa
Sensitivity CHB	-178 dB re 1µPa	-173 dB re 1µPa	-181 dB re 1µPa
Frequency range (±1.5dB)	From 1 Hz to 50 kHz	From .151 Hz to 28 kHz	From 1 Hz to 50 kHz
Sea noise equalizer	HP filter one pole 3.2 kHz	HP filter one pole 3.2 kHz	HP filter one pole 3.2 kHz
Beam pattern	Omni-directional	Omni-directional	Omni-directional
Input equivalent noise (@5kHz G=60dB	27 dB re 1µPa/√Hz	22.5 dB re 1µPa/√Hz	30 dB re 1µPa/√Hz
Working depth	Up to 1500 m	Up to 2000 m	Up to 3600 m
Weight	333 g	317 g	480 g
Size	Φ 34 x 255 mm	Φ 34 x 255 mm	Φ 34 x 255 mm

Figure 3 A1 sensor characteristics as a function of hydrophone

A2 system

A2 is a compact volumetric hydrophone system, enabling real-time measurement of underwater noise and of several soundscape sources. It consists of an array of four digital hydrophones (A2hyd) with Ethernet interface and one Master Unit for data processing. A2hyd sensors have the same characteristics as the A1 sensor regarding the Signal Conditioning Unit (SCU), the A/D Converter (ADC) and the Micro Controller Unit (MCU), with the difference of a smaller internal memory (32 GB) and no internal battery. A JS-B100 hydrophone transducer has been selected for deep underwater application.

Each of the four digital A2hyd hydrophones transmits the digitised acoustic data to the Master Unit through Ethernet. The Master Unit manages the timing synchronization of the four A2hyd sensor signals for simultaneous sampling. The time synchronization of the Master Unit and the slave units (A2hyd) is performed using the IEEE1588 Precision Time Protocol (PTP) standard. The Master Unit processes the acoustic data.

The signals from the four A2hyd are transferred to the Master Unit through four underwater cables, 10 m long, providing diverse geometric configurations, connected to the cap of an underwater housing containing the Master Unit. Another underwater cable connects the housing to the user interface for transmitting the data processed by the Master Unit. The Master Unit includes:

- DC/DC converter used to supply power to the Master Unit: it converts the 24~36Vdc input into 5Vdc, required by the embedded Single Board Computer (SBC).
- SBC model Odroid C2 is a 64 bit quad-core SBC with a linux operating system preinstalled where a python compiler can be installed for detection and tracking algorithms. The Odroid C2 is powered by the 5Vdc from the DC/DC converter. This SBC is one of the most cost-effective 64 bit development boards available in the ARM world.
- A2 system is targeted primarily for fixed platforms with sufficient power autonomy and/or communication capability (e.g. fixed observatories).

Considering that the precision and range detection of a sound source depend on both the sampling frequency and the array dimensions (hydrophone distance), and fixing the sampling frequency at the maximum value, A2 innovation consists in the possibility to increase array dimensions, depending on the applications. In particular, the hydrophone maximum working depth of 3600m allows to use A2 system in deep water applications.

2 Optical Sensors

The NeXOS project is developing three types optical sensors: O1 sensors are based on the use of fluorescence, O2 sensors on absorption, and O3 sensors on carbon sensing.

NeXOS-O1 - Fluorescence sensors

Figure 4 - Matrix_Flu UV and VIS sensors

Three new compact low-power multifunctional optical sensor systems based on multi-wavelength fluorescent technology were developed. Their aim is to provide detailed information on both water constituents and relevant contaminants that are optically active in the respective spectral region. Two of them are based on a similar system design with slightly but decisively different inner optical designs: MatrixFlu-UV and MatrixFlu-VIS (Figure 4). Both use different combinations of three or four narrow band excitation and emission wavelengths. The third, the MiniFluo (Figure 5), consists of two separate single channel fluorescence detectors within a single housing.

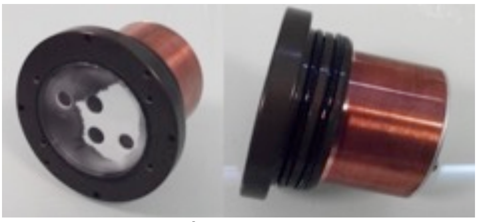


Figure 5 MatrixFlu sensors

This way it detects two distinct parameters in the water column, precisely dedicated to wavelength combination of fluorescence signals from hydrocarbons in the water.

NeXOS O2 Absorbtion sensors

Two devices for continuous flow-through measurements of water absorption coefficients have been developed, the OSCAR-G2 (Figure 6) and the Hyperspectral Absorption Sensor (HyAbS - Figure 7). Both take advantage of the use of an integrating cavity, which avoids errors introduced by light scattering on particles present in the sample and it increases the sensitivity of the measurements. From the hyperspectral (2 nm resolution) absorption coefficient data, which is the primary output of the sensors, phytoplankton-related information can be derived. Certain coefficients can be used as optical proxies for important bulk parameters like chlorophyll-a and total suspended matter, which are a quantitative measure of phytoplankton in the water. Furthermore, the shape of the spectrum can be evaluated with respect to taxonomical information, since it is determined by the presence of accessory pigments, which are often specific for certain types of phytoplankton. This phytoplankton identification is done by comparison of the measured spectrum to a database of spectra with known phytoplankton compositions. The algorithms have also been developed in the course of the project.

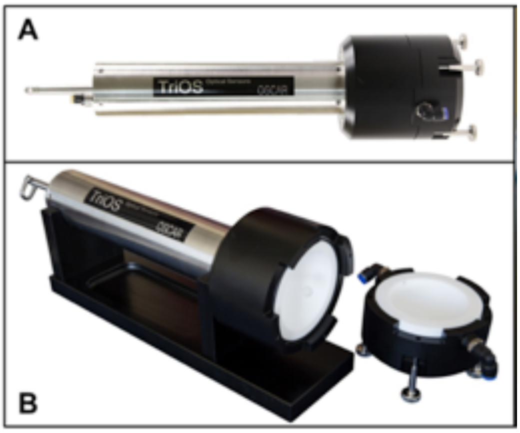


Figure 6 Oscar G2 Commercialiy available from Trios, Germany

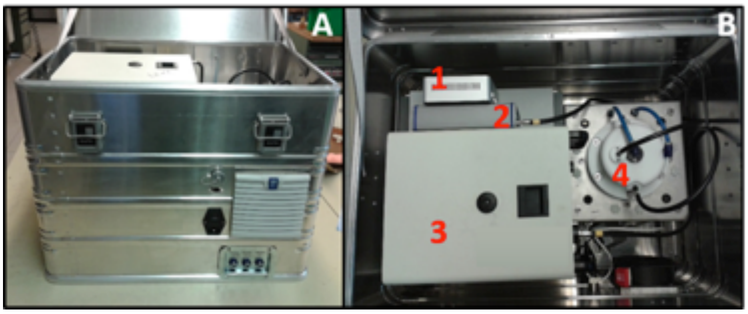
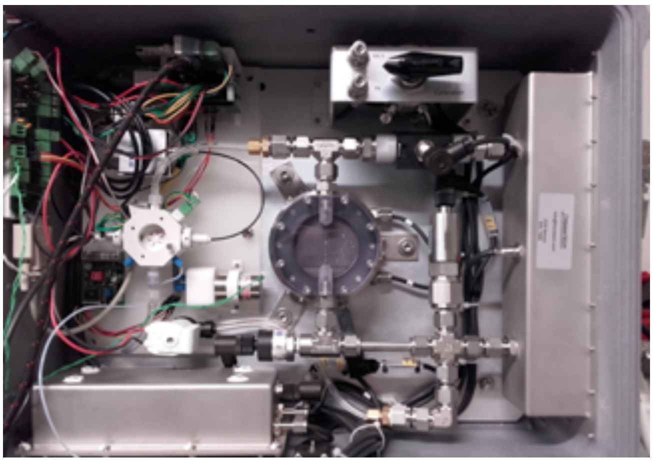


Figure 7 (A) Hyperspectral Absorbtion sensor (HyAbs);(B) Single Components of the HyAbs, 1: PC; 2: spectrometer; 3: light source; 4: integrating cavity

Nexos O3 Carbon sensor

The ultimate goal of the O3 sensor, following the global approach for ocean acidification monitoring, is achieving an estimate of the calcium carbonate saturation state with enough accuracy to provide trends and early warning of major threats to some marine organisms and their role in the ecosystem. According to McLaughlin et al., 2015, "a maximum uncertainty of ±0.2 in the calculation of Ω_{arag} is required to adequately link changes in ocean chemistry to changes in ecosystem function". Fully understanding the carbonate system demands not only measuring temperature and salinity, but also knowledge of at least two of four measurable carbonate chemistry parameters: pH, dissolved inorganic carbon (DIC) which is the sum of all inorganic carbon sources (carbon dioxide (CO_2), carbonic acid (H_2CO_3), bicarbonate ions (HCO_3^-) and carbonate ions (CO_3^{--}) in solution), total alkalinity (TA, the buffering capacity of seawater), and pCO_2. If two of these parameters are known, then the calcium carbonate saturation state can be estimated.



pH measurements require a thermal bath for the sample, flow injection analysis or discrete additions of a suitable dye and a high resolution spectrophotometer to detect protonation of the dye. Achievable precision with some minutes of operation is as low as 0.0005 and accuracy limited to the characterization of the dye being used and the temperature stability of the sample (0.003). Such measurements require attendance and some minutes for complete analysis and portability is not among the advantages.

pCO2 is based on the shower head equilibrator scheme where a volume of seawater, collected from a continuous flow, is equilibrated with its headspace, gas is dried and CO2 measured with non-dispersive infrared spectroscopy. This arrangement is suitable for fixed ship-based operations and inside a laboratory onboard a research vessel. Accuracy is limited by drifts of the infrared spectrometer (2µatm), while precision is kept at level of 0.1 µatm.

Total alkalinity (TA) and inorganic carbon are measured from discrete samples only by a bench-top system

ure 8 Cbon2 Carbon Sensor

that is a compact chemical laboratory for sample handling and titration with standard methods. The system requires strict attendance, even if all operations are carried out automatically following a list of instructions. Samples need to be loaded by the operator. The equipment includes a thermal bath, thermal loop for conditioning the samples, a coulometer, proprietary electrochemical solutions, ultrapure nitrogen as carrier gas for CO2 stripping, ultrapure CO2 for DIC calibration, certified reference material for DIC and TA measurement referencing. A complete TA analysis takes up to 25 minutes. The system is suitable for laboratory analysis and onboard research vessels.

Table 3 – NeXOS Optical Sensors

Ocean Optical Sensors	**Description**	**Characteristics**	**Applications**
O1- MatrixFlu	Based on multi-wavelength Fluorescence technology	MatrixFlu-UV & MatrixFlu VIS have the same design; different inner optics	Measurements of water constituents and other contaminants from mobile platform
O1-Mini-Fluo	Based on multi-wave-length fluorescence technology	Two separate single channel detectors in single housing	Measurement of CDOM from mobile platform `
O2 – OSCAR-G2	Based on continuous flow-through measurements of water absorption coefficients	Integrating cavity	Compact, submersible, and commercially available; bench-top or profiling instrument; for measurement of phytoplankton information
O2 - Hy-AbS	Based on continuous flow-through measurements of water absorption coefficients	Integrating cavity	Completely automated absorption sensor dedicated for long-term usage in locations with no restrictions regarding power consumption.; for measurement of phytoplankton
O3 – Cbon-2fb	Automated Flow-through Embedded Spectrophotometry (AFtES) unit based on a flow-through arrangement and absorbance detection	pCO2 is measured by extraction from liquid phase in a continuous flow, followed by detection in the gas phase by an electrolyte sensor.	Measurement performed on a ferry vessel
03 - Cbon-2sv	See 03-Cbon-2fb	In the Cbon2-sv version extraction of gas phase from water phase is by a semipermeable membrane supported by a titanium disc. The CO2 detection in the gas phase is then performed by miniature near-infrared spectroscopy	Measurement performed on a sail buoy vessel
O3 – Cbob3	Automated Flow-through Embedded Spectrophotometry unit based on a miniaturized flow-through arrangement and absorbance detection	Basically a Cbon2-fb coupled with a total alkalinity analyzer based on flow injection analysis	Ferrybox application

3 Sensor system for an Ecosystem Approach to Fisheries (EAF)

Figure 9 - Detail of sensor on net support

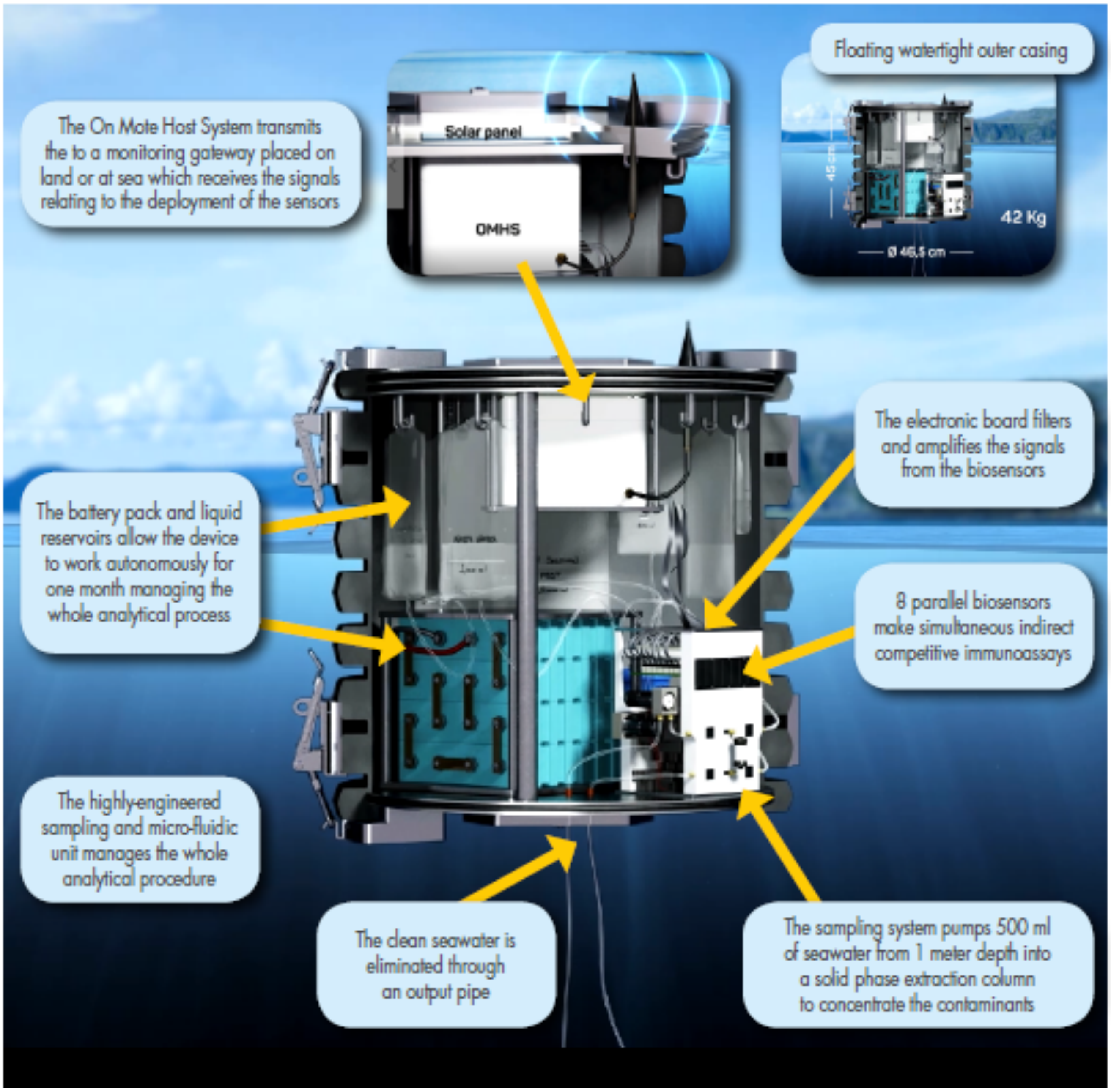
The EAF multi-functional sensor system builds upon the RECOPESCA concept and technologies, by adding new parameters relevant to fisheries management. The NeXOS challenge in extending RECPOPESCA was to develop cost efficient sensors that do not require efforts by the fishermen, are tough enough to be placed on fishing gear, and are self-powered and autonomous. Insofar as the selection of targeted vessels is representative of the fishing fleets, the sensors must be modular and scalable to collect new data. RECOPESCA already measures pressure, temperature, salinity and turbidity. It includes a hauler counter, specifically based on the ship weighting scale. A data concentrator is used to store and transmit the data to a shore management center in pseudo-realtime.

For the EAF application, NeXOS created two additional sensors. Those selected were oxygen and fluorescence sensors (as a proxy for chlorophyll) both for their applications to fish population assessments and because they are reported as Essential Ocean Variables by the operational oceanographic community. To have a low cost sensor, existing sensor components were evaluated and inexpensive components offering adequate accuracy were selected. The robustness of sensors was improved by mechanical modifications, and calibration and processing improvements were implemented to assure good quality measurements. A prototype for each parameter (oxygen and fluorescence) has been developed, built and is being tested on fishing vessels in Italy and Norway. A photograph of the installation is given in Figures 9.

SEA-on-a-CHIP aims to develop a miniaturized, autonomous, remote and flexible immuno-sensor platform based on a fully integrated array of micro/nano-electrodes and a microfluidic system in a lab-on-a-chip configuration combined with electrochemical detection (amperometric measurements) for real time analysis of marine waters in multi-stressor conditions.
To have more information please visit www.sea-on-a-chip.eu

How is the final device?

The SEA-on-a-CHIP device is an autonomous, miniaturized on site analytical system for real-time monitoring of harmful contaminants in seawater, developed to be used as an early warning system in aquaculture facilities. The third and last prototype affords at least two daily measurements of 7 pollutants, and one control.



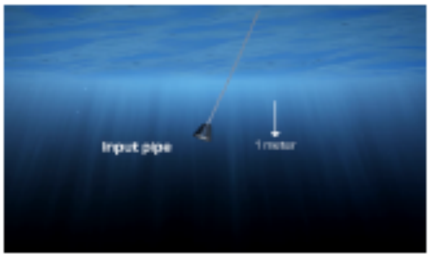
Does the device work properly?

The SEA-on-a-CHIP partners responsible for the validation of the device worked hard to thoroughly test and validate the performance of the SEA-on-a-CHIP biosensor system. It was tested for the analysis of 7 compounds against conventional analytical chemistry under laboratory and simulated mesocosm conditions, and finally within aquaculture facilities.

On-site biosensor measurements reduce the sample handling and time constraints associated with conventional analytical chemistry.

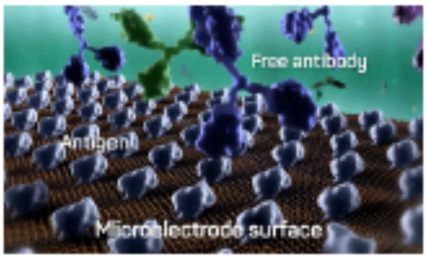
SAMPLING

Water is pumped into the device from 1 meter depth through a mesh to prevent debris uptake.



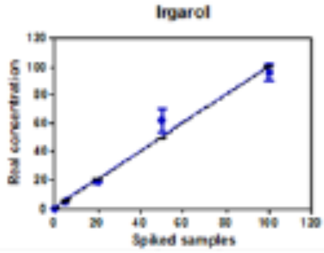
PRE-CONCENTRATION STEP

Pre-concentration of the pollutants onto a sorbant allows the monitoring of environmentally realistic concentrations. The sorbant was chosen and optimised to afford reproducible recovery of the chosen analytes.

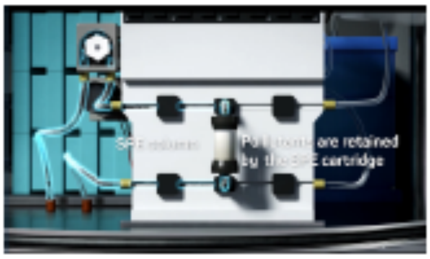


IMMUNOASSAY

The linearity of the immunoassay response was calibrated and validated over a large range of concentrations.



	Real concentration	Theoretical concentration
Slope	[illegible]	1.000 ± 0.0
	Real concentration	Theoretical concentration
R square	[illegible]	1.000

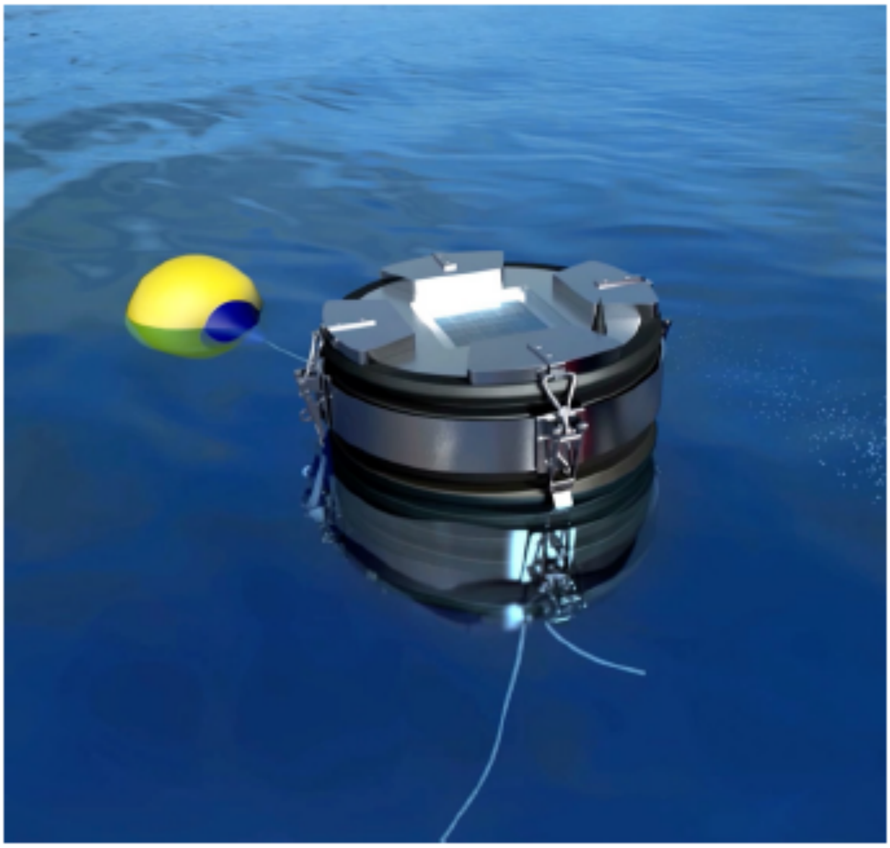


TARGET COMPOUNDS

The contaminants were chosen to be representative of potential threats to aquaculture production. They include antifoulants, endocrine disruptors, persistent organic pollutants (POPs), biotoxins, and pesticides and antibiotics used in aquaculture treatments.

Target compounds for the 3rd prototype:

- Irgarol
- Estradiol
- Sulfapyridine
- PBDE 47
- Domoic acid
- Deltamethrin
- Chloramphenicol



What information does the user have access to?

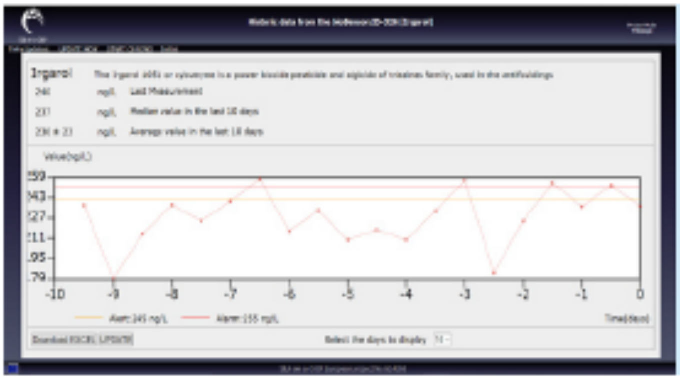
The aquaculture manager can visualize and manage the data from the deployment of devices in real time from his/her computer through a user interface. The device sends several types of data: measurements of the contaminants level from the electrodes, internal temperature, humidity and other information about the status of the system.

The heart of the system is in cyberspace. A process, called listener, picks up data from each device. This process runs autonomously. When the data arrive, the system puts the data in a database, makes the proper calculations and if there is any anomaly in the device, for instance the temperature is too high, an alert e-mail and text is sent to the manager.

This is the general overview screen. The main aim of this screen is to show to the user where the problems are arising. Here you can see all the connected devices, both in a map (geographic location) and in a list showing their status. A coloured circle represents every device: the blue colour indicates the device has never connected, the brown colour shows the device is in stand-by status, the yellow or red colours indicate that at least one parameter measured by the device results within the pre-alarm or alarm range of values. When the device is properly working, the circle is green. Clicking on one circle you can access to a detailed view of the data associated to that device.

This screen gives specific information about the measured parameters for one device. The colours code and the shape points out the situation about the parameter. Furthermore, the map shows the geographic location of the other sensors of the same site.

Each parameter can be in one of the following statuses: the desired status (green zones), the pre-alarm status which is telling the user that the value is going on the wrong direction but it is still OK (yellow colour), and in the end, the alarm status when the value is outside the limits (red colour).



The screen shows -in a graphical way- the evolution of a value of one parameter during the previous days.

The function of this screen is to show the history of the values that brought the parameter to the current status.

The system allows to set the pre-alarm and alarm limits on the graph and to modify the number of points to be displayed. The user can also download the data in an EXCEL sheet. In addition to this screen, the system has a query system on the database in order to do a more sophisticated data analysis.