Final publishable summary report

1. Executive summary

In EnviGuard a consortium of 11 SMEs and 7 research and development beneficiaries from 7 different countries combined forces to develop a biosensor technology for environmental monitoring and disease prevention in aquaculture.

The modular EnviGuard system is made up of three different biosensor units: one for toxic microalgae, one for pathogens, i.e. Betanodavirus & E. coli and one for Okadaic Acid, Saxitoxin and PCBs. They are connected to one common interface device, the EnviGuard Port. The Port saves, displays and sends the collected data to a server. The internet platform EnviGuard.NET gives the users the possibility to access real-time data from in situ measurements and facilitates remote control of the EnviGuard system that is located in the field e.g. at an aquaculture site.

EnviGuard achieved to detect and quantify

- ✓ relevant toxic **algae** in European waters (*Alexandrium minutum*, *Alexandrium tamarense*, *Alexandrium ostenfeldii* and *Pseudonitzschia sp*.),
- ✓ **pathogens** relevant to European aquaculture (specifically *Betanodavirus* and *E .coli*)
- ✓ emerging pollutants i.e. **toxins** (specifically Okadaic acid and Saxitoxin) and persistent, **man-made pollutants in the marine environment** (specifically PCB126 and PCB118).

In addition, an automated sample filtration and preparation device called AutoFiM was successfully tested and reached a commercial level.

EnviGuard also managed to deliver a platform for the integration of all three sensors into one device and accomplish real-time results for a period of one week without little maintenance in and a marine surrounding i.e. under multi-stressor conditions. It also includes a link to the so called FerryBox system that has been developed under the 5th framework program. This expands the systems capabilities by an array of sensors (chemical nutrient analyser, temperature, salinity, pH, turbidity, chlorophyll).

2. Summary description of project context and objectives

EnviGuard is a response to the growing need for accurate real time monitoring of the seas/ocean and the aquaculture industry's need for a reliable and cost-effective risk management tool.

Aquaculture operations are facing a multitude of challenges: Harmful algae blooms (HABs), which consist of microalgae able to synthesis toxins. HABs can have severe effects on fish and humans including mortalities; accumulation of enteric bacteria such as E.coli, which are related to water quality degradation. Food business operators must ensure that live bivalve molluscs placed on the market for human consumption must not exceed certain quantities of marine biotoxins or bacteria like E.coli. The appearance of diseases of farmed fish in aquaculture is a direct consequence of the intensification of the sector. Every year, the industry faces disease outbreaks caused by long-known and emerging pathogens. Some have the capacity to affect the sustainability of the business heavily, while others can chronically affect the stocks, reducing the efficiency of farming operations.

EnviGuard 's objective was to develop a highly specific, precise, quantitative and qualitative in situ measurement device for currently hard to measure man-made chemical contaminants and biohazards (toxic microalgae, viruses & bacteria, biotoxins & PCBs) that could be used as an early warning system in aquaculture and as an environmental monitor to assess the good environmental status of the sea in compliance with the Marine Strategy Framework Directive (MSFD). Being more cost-efficient than current monitoring devices led to a clear marketing advantage for the European analytical and research equipment industry.

The modular system consists of three different sensor units (microalgae/pathogens/ toxins & chemicals) integrated into a single, portable device, which saves, displays and sends the collected data to a server by means of mobile data transmission and internet. EnviGuard is able to accomplish this in real-time for a period of at least one week without maintenance in an offshore, marine surrounding. User of EnviGuard can access their data online any time they need to. Potential fields of use are marine environment pollution monitoring, marine research and quality control in seawater aquaculture, a sector in Europe highly occupied by SMEs. The biosensors developed in the project go far beyond the current state-of-the art in terms of accuracy, reliability and simplicity in operation by combining innovations in nanotechnology and molecular science leading to the development of cutting-edge sensor technology putting European research and highly innovative SMEs in the forefront of quickly developing markets.

EnviGuard started 01/12/2013 and lasted for 60 months. The project's objectives were:

- highly specific, precise and reliable in situ measurements of biohazards and chemical contaminants in seawater
- multi-class, multi-analyte method for the simultaneous determination of harmful microalgae species, *Betanodavirus*, *E*. *coli*, Okadaic acid, and Saxitoxin, PCB 126 and PCB 169
- quantitative and qualitative analysis through combination of nanotechnologies with bioreceptors
- automatic sampling for a period of at least one week in the marine environment
- real-time results early warning system for aquaculture industry and beach surveillance & national park services
- easy access to data through internet database allowing environmental status /risk assessment online
- durable design for offshore use under multi-stressor conditions
- modular system (of up to 3 sensors) integrated in a single, portable device
- easily maintainable, user friendly device
- compatible with the FerryBox
- more cost-efficient than current monitoring practices

3. Description of the main S&T results/foregrounds

At the beginning of the project, the system's requirements were determined through site visits, literature research and on-site monitoring/sampling specified. A **market survey** study on biosensors in finfish operations was performed to understand the demands of the main target group. The outcomes were published on major aquaculture conferences.

In parallel three sensor units were developed. An algae detection unit (ADU) for harmful microalgae, a pathogen detection unit (PDU) for E. coli and Betanodavirus and a chemical detection unit for PCBs and algae toxins (CDU).

The **algae detection unit** is able to identify numbers of algae that produce toxins and have been responsible for great environmental, economic and public health catastrophes. The specific recognition of the different poisonous strains required a distinction at molecular level in order to discriminate between harmful and inoffensive strains, which are, in some cases morphologically identical. The biosensor is calibrated for

- *Alexandrium minutum*
- *Alexandrium tamarense/fundyense*
- *Protoceratium reticulatum*
- *Dinophysis acuminata/acuta*
- *Pseudonitzschia sp.*

The detection limit is in the range of 1500 - 2000 cells.

The unit is remoted controlled, fully automated and able to monitor autonomously fixed at observation sites and onboard ships. It is composed of modules for sampling, sample preparation and target quantification (Figure 1) that can be either operated as an integrated autonomous system or if needed independently.

Fig. 1: *Overview of the individual modules of the ADU:* ***auto****mated* ***fi****ltration unit for* ***m****arine microbes (AutoFiM) installed on board RV Polarstern (1), sample preparation (2), and biosensor unit (3).*

The **automated filtration module** was a success by itself and was later on also used for the sample preparation of the chemical detection unit. It can also be marketed separately. During the testing phase of the filtration module, water samples with a volume of 1-2 liter were collected. One sampling cycle takes about 10-15 min, which is around 3-5 x quicker than filtration of a similar volume with a conventional table-top set up. Furthermore, samples can be collected automatically after fixed intervals of min. 15 min, which allows sampling with high temporal and spatial resolution.

The latter is of high relevance for marine plankton observation programs because marine plankton distribution is very patchy. Particulate organic matter collected on the filters is preserved with a lysis buffer that is used for detection of toxic algae with the biosensor system. Subsequent to isolation of DNA the integrity of the genomic DNA was very high, as no signs of degradation were visible after a quality assessment. An additional detailed assessment of the taxonomic composition of sequence assemblages obtained from samples collected manually with sequence assemblages collected via the automated filtration suggests no impact of automated filtration on the sequence composition. The overall positive evaluation of automated filtration for collecting marine microbial communities suggests a high market potential of the filtration unit in marine research, long-term observation, aquaculture, but also other applications e.g. drinking water security.

The design of the sample preparation of the algae detection unit involves that the cells collected on the filter are re-suspended in the lysis-buffer/hybridization-mix via a treatment with ultrasound. The heart of the sample preparation unit is a commercially available ultrasound unit (UP200St ultrasound device with the sonotrode S26d40 (Hielscher, Teltow, Germany)) that is operated subsequently to the filtration unit (Figure 1 (2)). Finally, the biosensor module enables a fully automated handling of the multichannel sensor chips together with the complete analysis process and measurement of the electrical signals from the rRNA-sensors. The results of a measurement is graphically displayed and stored by a PC-software (Figure 2).

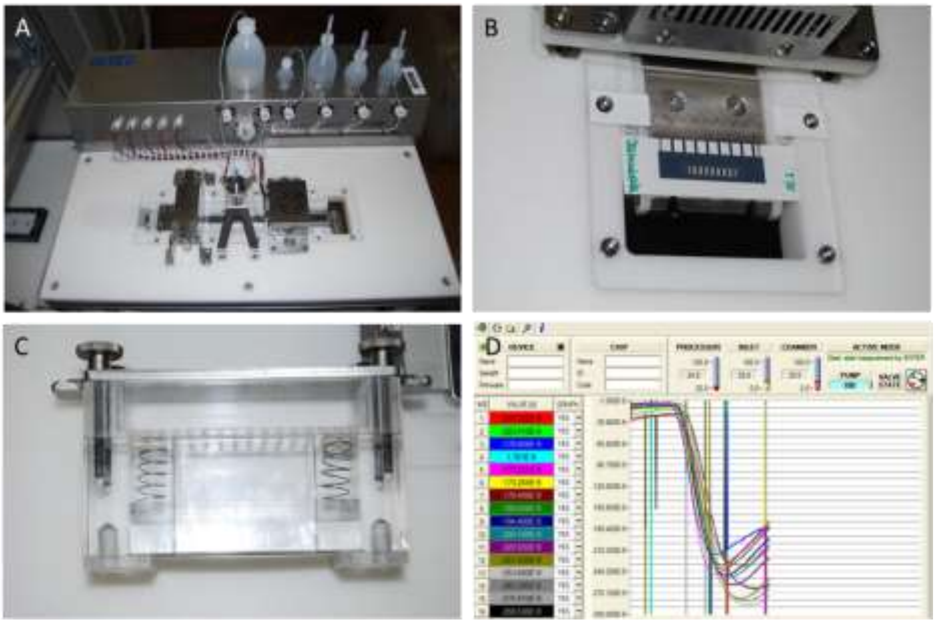


Fig. 2: *(A) Automated Biosensor System, (B) sensor chip after analysis in hybridization chamber (upper part of the picture), (C) archive for automated provision of sensor chips, (D) graphical display of sensor measurements*

The **pathogen detection unit** was developed to detect *E. coli* as an indicator for the contamination of aquaculture sites (e.g. by wastewater) and Betanodavirus as it poses a significant danger to the bass & bream industry in the Mediterranean. The unit was able to successfully quantify *E. coli* down to 10^5 cfu / ml and Betanodavirus particles down to the magnitude of 10^4 virus particles / ml. Both results for E. coli and Betanodavirus were achieved under laboratory conditions. Due to the technical difficulties experienced along the way, field tests had to be postponed until after the project's end. In addition, the sensitivity still has to be improved or sample concentration methods prior to analysis have to be integrated in order to reach a desirable range of 1-10 cfu /ml or virus particles / ml respectively.

As detection molecules for the highly specific bioassay for the viruses aptamers were used and antibodies for the detection of the bacteria. A method using a Streptavidin coating and Biotin-labelled aptamers/antibodies was used to produce the microfluidic chips. A robust metal-polymer plug-and-play microfluidic flow cell was designed that is easily replaceable by non-specialist operators (Figure 3). The design overcomes the typical problems of microfluidic systems of fragility and requirements for highly skilled operators of microfluidic systems. The flow cell uses a highly reliable linear-style multi-region design allowing multiple target regions to be incorporated in a single channel.

Fig. 3. *Robust and simple to replace microfluidic flow cell.*

The newly developed, fully automated stand-alone pathogen detection unit (Figure 3) allows for easy transport. The systems can be operated by a single button, from priming, to assay, to clean-down. It also provides the capability for autonomous remote operation, monitoring and reporting with a reliable system operation for non-specialist operators.

Fig. 3. *Integrated design of autonomous Pathogen Detection System.*

The **chemical detection unit** represents a novel optical biosensing system for the in-situ and real time detection of toxins (*okadaic acid – OA, and saxitoxin – STX- produced by some species of microalgae and responsible of diarrheic and paralytic shellfish poisoning respectively due to contaminated shellfish consumption*) and man-made chemical pollutants (*polychlorinated biphenyls (PCBs)*) in oceanic water samples. It is a competitive analytical tool in comparison with other more expensive or complex techniques such as chromatography, usually used for the analysis of these targets. It was suitable to be working during one week autonomously (one sample a day). Table 1 shows the achieved measurement unit, range, alarm value and frequency for Okadaic acid and PCBs. The trials for Saxitoxin were not finished by the end of the project.

Tab.1. ***Measurement unit, range, alarm value and frequency for Okadaic acid and PCBs***

CHEMICAL DETECTION UNIT (CDU)

Parameter	Unit	Measurement range		Value from which the system should give an alarm	Measurement frequency	
		min	max		min	max
Okadaic Acid concentration	ng/ml	45	120	44-85 --> warning >85 --> alarm	Once a day	Twice a day
PCBs concentration	ng/ml	50	425	>48 --> alarm	Once a day	Twice a day
Temperature	°C	15	25	<18 or >25	same frequency than the conc. measurements	

The chemical detection unit uses a novel optical transducer based on nanostructured surfaces (using periodic arrays of resonant nanopillars (RNP) of SiO_2/Si_3N_4) and antibodies as specific bioreceptors (i.e. immunosensor). For the detection of specific targets, monoclonal antibodies were generated in mice. The biosensor comes with a newly build, fully automated fluidic handling system including a rotary valve, which enables a continuous flow and automated distribution of sample and reagents in a timed manner (Figure 4).

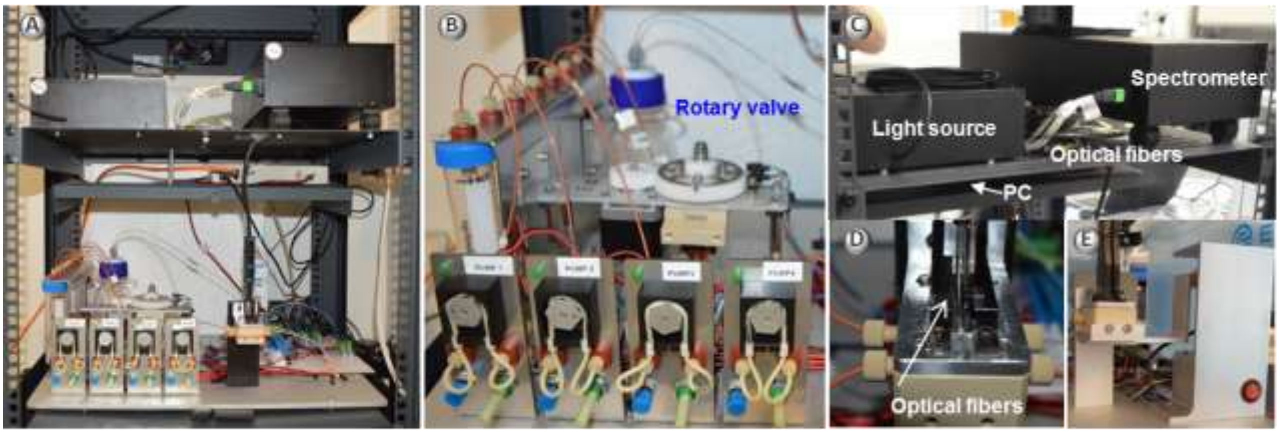


Fig. 4. *CDU and components. A) Integrated unit. B) Fluidic system: rotary valve, peristaltic pumps and reservoirs. C) Optical system: Light source, optical fibers, spectrometer and PC. D) Chip holder. E) Chip exchanger mechanism.*

For signal acquisition an optical fiber bundle probe, composed of 2x12 multimode fibers illuminated by LED as a light source coupled to a custom-developed 12-channels integral field spectrometer was developed. The system allows simultaneous multiplex detection. Furthermore, the software allows sensogram data processing, facilitating the calculation of targets concentration. The unit is protected with a proper housing and includes a chip exchanger mechanism in order to facilitate the access and the operation when sensor chip introduction in chip holder (Figure 4).

The algae, pathogen and chemical detection unit (CDU) together with the automatic filtration & sample preparation were integrated into a central unit, the **EnviGuard Port** (Figure 5).

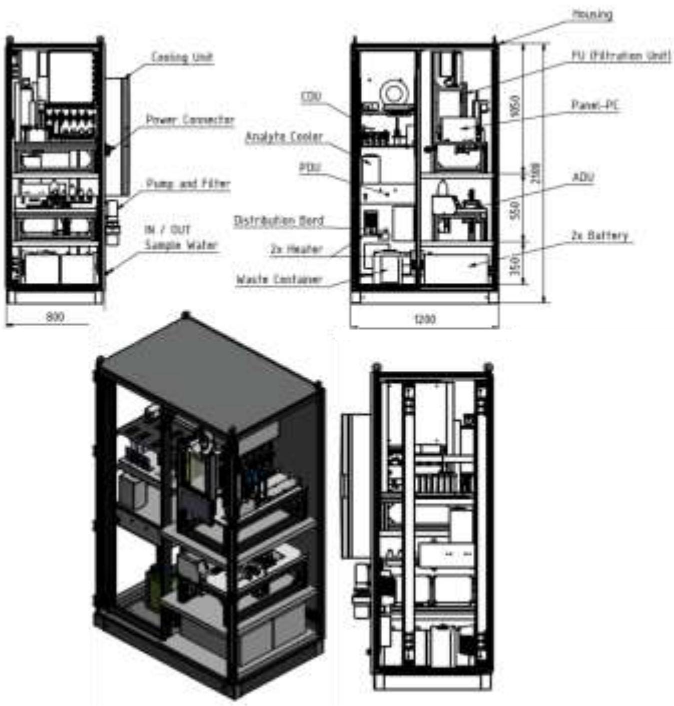


Fig. 5: *Drawing of EnviGuard Port prototype rack with filtration and sensor units*

The power supply of the EnviGuard Port is based on a rechargeable battery unit, which enables an autonomous operation, depending on the capacity of the installed batteries. With the use of batteries and the implemented charging device, the development goal for the EnviGuard Port to work in different environments (with net connection, off grid storage system and off grid with regenerative energies) has been archived. The main supply voltage of the system has been chosen with industrial standard 24V, which leads to moderate currents for power distribution. With secondary power supplies, additional voltages like 12V and 5V are generated to supply the different electronic components of the sensors. The temperature stabilisation with accuracy of 1K has been realized with an air cooler and air heater, that can be operated depending on the environmental temperature at deployment site. The internal as well as the external communication is based on Ethernet-TCP/IP and UDP, which enables connectivity to all devices via internet. For external communication to the WebTool and remote access the protocols have been extended with the OpenVPN, therefore safety aspects have been taking into account. For transmitting the data to the WebTool the HTTP(s) protocol (with the post and get method) was chosen. The same protocol was used to access the WEB-tool. A dynamic domain name service (dynDNS) has been established too. This service sends its actual IP-address to a server, which translates this address to a fix domain name.

In addition, EnviGuard.NET was developed to show the recorded data via an **online tool**.

During the in-situ tests on the Orkneys the automatic filtration and algae detection unit performed to expectations. The chemical detection unit suffered from problems with the sea-air environment on the circuit boards and for a commercial operation by none experienced users the loading of the fragile glass chips into the chip holder has to be improved. The pathogen detection unit was not tested in the field until the end of the project, as lab testing was more appropriate. The end-user expressed interest in taking forward some of the sensors for further testing and development after then end of the project.

Finally, the ***EnviGuard and Ferry Box system*** **were connected** and successfully formed one of the most complete and advanced monitoring system currently available. The results showed that the environmental, biotic and abiotic parameters measured by the established FerryBox system are one way to put the biosensor data into context a larger context. The biosensors developed within the EnviGuard project are a promising extension of already established systems like the FerryBox, as they fill in an existing gap. This is important for both basic scientific research as well as applied routine monitoring for ecosystem management and industrial purposes/aquaculture.

In summary, a variety of impressive technologies were developed that have reached different technology readiness levels (TRL) ranging from TRL 3 to 8. While in some cases, further work is required to apply the technology for environmental monitoring or early warning purposes in aquaculture (pathogen detection unit) others can be already used towards that direction (algae detection unit, chemical detection unit for Okadaic acid). The coupling of the EnviGuard system with a FerryBox can generate long thought after knowledge on the functionality of harmful algae blooms and take research in this area to a new level.

In order to use the sensors a **training handbook**, 60 pages report in English, including general information of the EnviGuard project, a description of each unit, and a description of the EnviGuard.NET (webtool) was developed and is available through the homepage. The **presentations** of all four workshops and the final conference are also available for download.

Two project videos were produced in the frame of the project in order to help disseminate the project outcomes and to support their exploitation. Both are available at the EnviGuard project website and in the following links:

- 1st video (https://www.youtube.com/watch?v=OU91wK0gPnA9
- 2nd video (https://www.youtube.com/watch?v=cd4ZkqpOz1Q)

- ## Potential impact and the main dissemination activities and exploitation of results

Potential impact WP1: WP1 determined the system requirements and evaluation criteria for the EnviGuard system. For achieving this goal, physical, biological and economic parameters as well as end user comments were collected and evaluated. Collected data was essential for the design and to establish a working and user-friendly prototype. Targeting real problems encountered by marine-culture industry was very important to design and construct a monitoring technology, which would be desired by the industry.

Aims and results of this WP was published in several conferences and a scientific journal for the dissemination and exploitation of the results:

- EnviGuard: Development of a biosensor technology for environmental monitoring and disease prevention in aquaculture ensuring food safety, June 2 – 4, 2016, Middle East and Central Asia Aquaculture Conference, June 2-4 2016. Izmır/Turkey – Oral Presentation.
- Market Study for EnviGuard – A biosensor technology for environmental monitoring and disease mitigation in aquaculture, 21 – 23 September 2016. Aquaculture Europe 2016, Edinburgh, Scotland – Poster Presentation.
- Tosun, D , Özden, Ö , Çağıltay, F , Bardócz, T , Mantas, G , Hunter, S . (2018). A Survey On Monitoring System Requirements Of Turkish And Greek Mariculture Industry With Assessment Of Production Complications. Aquatic Research, 1 (4), 162-170. DOI: 10.3153/AR18018, 2018 – 10 – 01, Aquatic Research e-ISSN 2618-6365.

Potential impact WP2: the modular design of the ADU composed of three separate modules for automated filtration, sample preparation and target detection was supposed to maximize the socio-economic impact of the project results. The automated filtration unit AutoFiM and the automated biosensor system can be operated independently according to user requirements. The results of WP2 demonstrate that regular automated or remote-controlled quantification of toxic algae is feasible in the field. If the fully automated ADU would become a marketable product aquaculture companies would have to possibility to use the ADU as an early warning system for toxic algae blooms to avoid financial loss. However, observation and enumeration of microbes in aquatic environments is not only relevant in aquaculture, but also an important task in understanding consequences of environmental change for marine ecosystems, their functionality and services. The latter requires information on microbial biodiversity and species occurrences with adequate temporal and taxonomic resolution in process studies and long-term time series observations. Sampling automation and molecular analyses methods could serve these needs by improving the resolution and accuracy of current conventional marine time series observations. Furthermore, it would be very much feasible to adjust the automated biosensor system for other microbial target species in diagnostics or environmental monitoring providing a wealth of opportunities to facilitate specific detection of microbes in different environments.

Potential impact WP3: So far, the results achieved within WP3 were under lab conditions. Given that field test proves the applicability of the Pathogen Detection Unit (PDU) in an operational environment and that the sensitivity can be increased, the PDU can potentially help fish farmers in the Mediterranean and shellfish farmers to protect their stocks from infections by pathogens by taking adequate actions prior to a wide spread infection of all animals. This offers direct economic benefits to the user in terms of less danger for losing stocks and better insurance premiums by applying the technology On a social level this can increase the job safety for the users and in general will provide safer seafood for consumers. The PDU could also be used to identify less optimal bathing waters or track down the origin of unknown E. coli rich waste water streams so it can be a tool for the assessment of the good environmental status of European waters.

The work and results of WP3 were widely disseminated on various conferences, trade fairs and meetings such as Aquaculture Europe 2014 to 2018, International Ocean Research Conference in

Barcelona (November 2014), Oceanology International in London (March 2016), the EuroScience Open Forum in Manchester (July 2016), a meeting at the Centre for Environment, Fisheries and Aquaculture Science (CEFAS) Meeting in Weymoth (July 2018) and of course during the four EnviGuard workshops and final conference. The means of communication have mainly been poster, oral presentations, videos and flyers.

The knowledge generated in WP3 is already being exploited. The Optical Detection Unit developed by UH, will be used by TTZ to conduct research into development of bioassays for MRSA in a national funded project. The application for funding has been submitted and is pending review. In addition, TTZ plans to use the knowledge generated on working with aptamers on a European proposal in the aquaculture sector. UH's Optical Level Sensor is scheduled for use within future prototype autonomous research systems developed for biodetection applications by UH. In addition, UH and CEFAS have already submitted a joint application for improving detection in aquaculture to the UKs Natural Environment Research Council (NERC) & Biotechnology & Biological Sciences Research Council (BBSRC).

Potential impact WP4: Several developments within WP4 are considered to have a potential impact and are summarized as follows:

- Biosensing system for simultaneous and multiplexed optical interrogation: the current biosensing system could be adapted and validated into a precompetitive prototype (TRL 6) for relevant biological/chemical parameters in a specific field to be defined (health, agro-food, aquaculture among others). This technology could be offered to a selected field industry as disruptive and affordable photonic technology, able to perform automatic and standalone bioassays of relevant bioapplications.
- Macro/Microfluidic management system, including hybridly integrated selection valve: The fluidic handling system will enable the development of biochemistry on PIC based sensors of LX. This enables a market introduction of PIC based disposable sensors for consumer applications also. LX already made a leaflet on the rotary valve. Their main target audience is customers on their sensing platform, which need a fluidics solution for that sensing platform.
- The two technologies developed by MT, (i) the 12 channel spectrometer with embedded signal processing of the spectra and of the sensograms, and (ii) an optical fibre bundle probe to make the interface with the RNP sensor chip, will be exploited by MT as trade secrets. They can be applied to a versatility of sensor technologies (RNP, surface plasmon resonance, ring resonators, Raman, fluorescence, etc.) or general light source characterization by custom adaptation of the wavelength range/resolution and probe design layout in the visible and NIR range.
- Resonant nanopillars (RNP): UPM owns the patent of the RNPs and is licensed to BIOD for commercial exploitation. UPM will continue researching and developing novel RNPs.
- RNP technology: BIOD will adapt and implement this technology to work with its developed optical biosensing technology. Both technologies are intellectual property of UPM and are exclusively licensed to BIOD. This will permit BIOD to develop other products and offer new solutions to our clients.
- Fabrication process of high aspect ratio nanostructures: CTN will offer the developed fabrication process to new research projects and interested customers independently of its application. The process can be used for the fabrication of multiple nanostructured surfaces made of different materials, for their use in different applications such as wettability, antireflection, optical biosensing or other optical purposes.
- Protocol for covalent and stable biofunctionalization of SiO_2 and Si_3N_4 nanostructures for biosensor development: The protocol will be exploited in further R&D projects and offered to interested customers or partners. Biofunctionalizated surfaces can be integrated in biosensing devices and allow the detection of different compounds in the fields of agriculture, aquaculture, defence, agriculture, food safety, health, etc.

- Measurement protocol for immuno-detection of Okadaic acid and PCBs on optical biosensor: CTN will offer this development to other R&D projects and potential customers/partners. These protocols could be used in integrated units to be applied for the detection of these analytes in oceanic water, sediments, shellfish tissue or other matrices of interest useful in aquaculture (warning system) or environmental monitoring.

Multiple dissemination activities have been developed by WP4 partners: 5 scientific publications in international journals. Currently, another paper is under revision and further papers are foreseen; More than 10 presentations (oral or poster) in several International and Spanish conferences on RNP technology, rotary valve and fluidic system, 12-channel spectrometer and optical data processing, biofunctionalization process and measurement protocol for specific targets; and 3 Thesis have been developed under the frame of the project.

Potential impact WP5: From the work package no direct impact in socio economic issues or social implications are expected. The work package dealt with technical aspects. Impacts regarding the competitiveness of the participants of this work package are expected. Every participant broadened his technical knowledge.

The full EnviGuard system cannot be commercialized right away by the project's end. But the results are promising and the interest by the fish farming industry for a system like EnviGuard is very big. The developed system seems to be a good solution but the development has not been completed yet. Further investigations particularly regarding cost reduction, miniaturisation and simplified operation are necessary.

The automatic filtration and sample preparation unit (AutoFiM) and the algae detection unit (ADU) as part of the system are already ready for the market. Through the work done within the project the units have been finally developed and the commercialisation has already been started. Especially the AutoFiM device has a high market potential because of wide range of application fields. It has been sold already to a local scientific institution.

The dissemination activities in the project helped to get in contact with potential customers from the fish farming industries and from other scientific and environmental monitoring organisations. The company iSiTEC is looking forward that both products can bring a fundamental contribution to their future business activities.

Potential impact WP6: Globally, aquaculture has been identified as the world´s most promising source of fish protein and this notion is reflected by the vast industry investment and growth in the sector. Aquaculture is also the fastest growing food production sector in the world, with average annual growth rates since 2000 of about 6%-8% (FAO, 2016). This is due to the combined forces of a rapidly increasing seafood demand and the absent growth potential of global capture fisheries. Different reports estimate the required growth in aquaculture by 2030 between 30 (WorldBank 2013) and 100 million metric tonnes (Subasinghe 2014; www.fishingfuture.org) above the current levels of about 70 million MT. The EU self-sufficiency in seafood production is low, with nearly 70% of the seafood consumed in EU being imported. Yet, in spite of this huge market demand, the European aquaculture industry is growing at a much slower pace (2-3%) than global levels because of its specific barriers, especially its competitiveness. To turn this situation around, EU member states have prepared national strategies, which opt for an ambitious growth in aquaculture of 300,000 tonnes (125%) by 2020 from the current total of more than 1.5 million tonnes. Obviously, this increase in the production volume has to be sustainable and carried out in line with the strategic guidelines of the European Commission.

The strong expansion of the aquaculture sector worldwide created new environmental challenges and will emerge new problems, as the production grows further. The experience in other parts of the world shows that accelerated growth of fish farms may lead to important socio-environmental conflicts that decrease, or even in some cases stop the expected growth in finfish aquaculture. The increased number of farms and enlarged production volumes will result in higher risks in Europe too in terms of environmental interactions such as:

- Increased risk of nutrient and pathogen emission from fish farms creating favourable conditions for harmful algal blooms and disease outbreaks.
- The lack of available space for fish farms in protected and clean coastal areas will force the future fish farmers to move closer to industrialised and polluted areas. This will increase the hazard of man-made pollutant caused problems on the production sites.
- Off-shore aquaculture is considered as a possibility to reduce the environmental impact of cage aquaculture, but these systems are still very much exposed to harmful algae and disease outbreaks, because any kind of intervention is more difficult than in the on-shore aquaculture facilities.

However, fish farmers already have to face these problems. Real-time early warning systems integrating environmental monitoring and disease prevention are not widely used in the sector so far. Marine sensors and monitoring systems also can be used for evaluation of new or proposed aquaculture sites before the establishment (Schmidt et al., 2018). This would lead to a reduction of risks concerning environmental conditions that affect the future aquaculture in that area.

WP6 showed the need for a system like EnviGuard. If it is possible to finance further development of the prototype into a system that can be operated easily by non-scientific staff, as scientists will not be the main users of the sensor, but fish farmers and their employees, EnviGuard can have a great impact on the sector. Obviously, the system needs to deliver highly accurate data (Schmidt et al., 2018). If this would be not the case, severe problems could arise, as wrong management decision could be taken. This could directly lead to economic losses in aquaculture. Another important issue that need to be considered are the costs. It is essential that the EnviGuard sensor is affordable, so it is suitable to buy even for small fish farms (Schmidt et al., 2018). The study of Schmidt et al. (2018) suggests that the design, building and deployment of such a buoy could be below £ 5000.

Potential impact WP7: The work performed in the frame of the project has produced very important results at scientific and technical level, and potential commercial applications are wide and relevant for the sector.

These results have been widely disseminated by different means: peer reviewed publications, thesis, presentations at conferences, workshops and other scientific events, organisation of project events and production of project specific dissemination material.

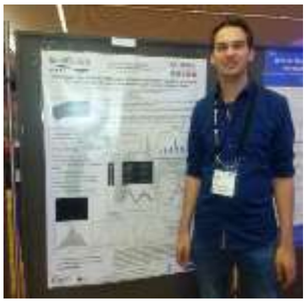
- Peer reviewed publications: nine publications have been produced in the frame of the project on different fields such as Optical, Aquatic and Sensors research, published by Elsevier, Optical Society of America, European Geoscience Union and Scientific Web Journals. Main authors have been research centres within the consortium, but it is relevant to mentioned that also EnviGuard SMEs BIOD and ABT have contributed to the publications.

Title	Author(s)	Title of the periodical or the series	Number, date or frequency	Publisher	Place of publication	Date of publication	Relevant pages	Open access is/will be provided to this publication
Optical sensor based on periodic array of resonant nanopillars for real time monitoring	Fátima Fernández , Paula Caurriz , Iñaki Cornago , R. Casquel , Ana L. Hernandez , F.J. Sanza , M.F. Laguna , M. Holgado	Sensors and Actuators, B: Chemical	Vol. 244	Elsevier	Netherlands	01/06/2017	323-329	No
Bulk sensing performance comparison between silicon dioxide and resonant high aspect ratio nanopillars arrays fabricated by means of interference lithography	I. Cornago , A. L. Hernández , R. Casquel , M. Holgado , M. F. Laguna , F. J. Sanza , J. Bravo	Optical Materials Express	Vol. 6/Issue 7	Optical Society of America	United States	14/06/2016	2264-2272	Yes
A SURVEY ON MONITORING SYSTEM REQUIREMENTS OF TURKISH AND GREEK MARICULTURE INDUSTRY WITH ASSESSMENT OF PRODUCTION COMPLICATIONS	Deniz D. Tosun, Özkan Özden, Ferhat Çağıltay, Tamás Bardócz, George Mantas, Shane A. Hunter	Aquatic Research	Vol. 1/Issue 4	ScientificWebJournals		20/09/2018	162-170	Yes
Arrays of resonant nanopillars for biochemical sensing	A. L. Hernández, R. Casquel, M. Holgado, I. Cornago, F. J. Sanza, B. Santamaría, M. Maigler, F. Fernández, A. Lavín and M. F. Laguna	Optics Letters	Vol. 40, No. 10	Optical Society of America		15/05/2015	2370-2372	No
Resonant nano-pillars arrays as optical biosensors	A. L. Hernández, R. Casquel, M. Holgado, I. Cornago , F.J. Sanza, B. Santamaría, M. Maigler, F. Fernández, A. Lavín, M.F. Laguna, F.Pozo.	Optics Letters	Vol. 41, No. 23	Optical Society of America		01/12/2016	323-326	No
The Coastal Observing System for Northern and Arctic Seas (COSYNA)	B. Baschek , F. Schroeder , H. Brix , R. Riethmüller , T. H. Badewien , G. Breitbach , B. Brügge , F. Colijn , R. Doerffer , C. Eschenbach , J. Friedrich , P. Fischer , S. Garthe , J. Horstmann , H. Krasemann , K. Metfies , N. Ohle , W. Petersen , D. Pröfrock , R. Röttgers , M. Schlüter , J. Schulz , J. Schulz-Stellenfleth , E. Stanev , C. Winter , K. Wirtz , J. Wollschläger , O. Zielinski , F. Ziemer	Ocean Science Discussions	13	European Geosciences Union	Germany	15/05/2017	1-73	No
How the surrounding environment affects the biosensing performance of Resonant 12Nanopillars arra13ys: under dry con14ditions or immersed in fluid	Hernández, A.L; Casquel, R; Holgado, M; Cornago, I; Fernández, F; Caurriz, P; Sanza, FJ; Santamaría, B; Maigler, M; Quintero, S; Laguna, MF	Sensors and Actuators, B: Chemical	259	Elsevier		15/04/2018	956-962	No
Fabrication of Si3N4/SiO2 tiered resonant nanopillars with nickel caps arrays: application for optochemical sensing	V Canalejas-Tejero, AL Hernández, R Casquel, SA Quintero, MF Laguna, M Holgado	Optical Materials Express	Vol. 8, issue 4	Optical Society of America		01/04/2017	1082-1090	No
A SURVEY ON MONITORING SYSTEM REQUIREMENTS OF TURKISH AND GREEK MARICULTURE INDUSTRY WITH ASSESSMENT OF PRODUCTION COMPLICATIONS	Deniz D. Tosun , Özkan Özden , Ferhat Çağıltay	Aquatic Research	4	ScientificWebJournals	Turkey	01/01/2018	162-170	No

- PhD Thesis: two PhD thesis have been produced based on the work performed within WP4. Two more are expected to be finished in the frame of WP2 and WP4.
- Presentations at conferences, workshops and other scientific and trade events: EnviGuard consortium has presented EnviGuard project and its results in almost 60 events at national and international level through posters, oral presentations, exhibitions, etc. The target audience of the events included research community, public administration and private companies (farmers, technology developers/suppliers). Some examples of the events in which EnviGuard has been disseminated are given below:
  - Aquaculture Europe 2014 (14-17/10/2014, San Sebastián, Spain): presentation and poster by TTZ. ABT at their own booth at the trade fair that was accompanying the conference.

  - World of Photonics 2015 (22-25/06/2015, Munich, Germany): EnviGuard poster was presented by MT.
  - OPTOEL (13-15/07/2015, Salamanca, Spain): Two posters and two communications on “Resonant nano-pillars arrays as optical biosensors” and “Reflectometry at profile level for label-free biosensing” were presented by UPM, CTN and BIOD.
  - Aquaculture Europe 2015 (20-23/10/2015, Rotterdam, Netherlands): a presentation of the “Market study for EnviGuard – A biosensor technology for environmental monitoring and disease mitigation in aquaculture ensuring food safety” was performed by ABT and one on “Development of an aptamer-based detection system for pathogens in marine aquaculture” by TTZ presented their respective findings in oral/poster presentations.
  - Oceanology International 2016 (15-17/03/2016, London, UK). (OI2016): the world’s largest marine science and ocean technology exhibition and conference. TTZ presented a poster and attended several meetings with the coordinators and partners of the other Ocean of Tomorrow projects to discuss fields for collaboration and disseminated the first results of the EnviGuard project.

- EuroScience Open Forum (ESOF) (July 2016, Manchester, UK): UH presented the EnviGuard project.
- 1st Blue Economy Business and Science Forum (12-13/09/2016, Hamburg, Germany): The Hamburg Summit gathered together around 200 Blue Economy stakeholders representing industry, science, clusters, public authorities, and finance sectors from 32 different countries from around the World. TTZ) presented and illustrated the results of the EnviGuard project during the thematic session "Showcase of the results of the eu innovative blue economy projects, with particular focus on SMES" chaired by S. Gruber, Head of Unit at European Commission DG RTD.
- Aquaculture Europe 2016 (20-23/09/2016, Edinburgh, UK): ABT, TTZ, NBS and VFF disseminated EnviGuard using roll-ups and flyers.
- Aquaculture Europe 2017 (17-20/10/2017, Dubrovnik, Croatia). AE2017 attracted a total participation of 1.688 from 62 countries. Of the full conference delegates (1294), 255 were students. The trade show presented products and services from 92 exhibitors and had 394 visitors. A total of 437 oral and 356 poster presentations were given during the conference. TTZ presented EnviGuard 's latest findings from the Algae and Chemical Detection unit.
- Laser World of Photonics (June 2017, Munich, Germany): With 1.293 exhibitors and more than 32.000 visitors from 90 countries, it is the world's leading photonic trade fair.. In parallel to the trade fair, the World of Photonic Congress took place with about 3.500 attendees. Part of this congress was the **CLEO/Europe-EQEC conference**, where MULTITEL presented a **poster showing results of the EnviGuard project**.



- HANNOVER MESSE (23-27/04/2017 Hanover, Germany): MT provided flyers and information about the EnviGuard project to several visitors at MT booth. Further, they showed a **demonstrator of the multichannel spectrometer** developed within WP4.
- International Innovations Workshop Aquaculture (14/03/2017Berlin, Germany): **EnviGuard** was represented by TTZ. The results were discussed in the session **"New approaches to unlock the potential of off-shore aquaculture"**. A dedicated session of biosensors in Aquaculture was proposed to the organizers of the Aquaculture Europe 2019 in Berlin, Germany.

- Project events: one international conference and four workshops have been organised in the frame of the project. More than 130 people participated in the project events from companies, research centres, universities and public authorities. Their feedback was very positive on the results presented, and they showed high interest on EnviGuard results application. The attended comments on the system will be taken into account for designing the next steps needed to achieve a competitive EnviGuard system ready for its commercialisation.
  - International EnviGuard Conference, the main dissemination event of the project, took place in Bremerhaven (Germany) the 7th of November 2018 at the at the German Immigration Centre in Bremerhaven (Germany). The final number of attendees was 53 coming from private companies, public administrations, universities and research centres. It was organised by BAZ and TTZ with the support of project partners. Main issues presented and later discussed in the networking sessions were related to:
    - the political perspective on environmental monitoring,
    - the results of the latest European projects on the detection of known and emerging pollutants and pathogens,
    - the advancements of the industry to supply reliable bio-/sensors and
    - the end-user perspective.

- Workshop 1 took place the 18th October 2018 in Orkney (Scotland), hosted by NBS. and was aimed at presenting the EnviGuard prototype and its trials goals. The event was attended by 15 people who met the EnviGuard team and knew a general overview about the EnviGuard port. After this, the attendees went to Lamb Holm to see the prototype and know how it works. In the port they did a trial run of prototype, a practical examination and discovered the functionality of the units.
- Workshop 2 took place the 8th November 2018 in Bremerhaven (Germany), hosted by TTZ and organised by BAZ, TTZ and AWI. 26 people from 10 different nations participated representing companies from the UK, Brazil, Colombia, Germany, Spain, Malta and Italy, as well as researchers from Spain, Germany, UK and Vietnam. Besides a presentation of the technical functionality of the EnviGuard system and the ferry box, a practical hands-on examination of both was done. In addition, the Centre for Aquaculture Research was visited to give an inside into possible applications of the technology.
- Workshop 3 took place the 19th November 2018 in Madrid **(Spain),** hosted by UM and organised by UPM, BIOAZUL, BIOD and CTN. The event was attended by 29 people who knew the latest news of EnviGuard project, the development of a multiplexed biosensor for the simultaneous detection of toxins and PBS in marine waters or the technological evolution in EnviGuard. In addition, a panel of related H2020 projects was presented, including: MedAID, TAPAS, AQUAEXCEL2020 and VIVALDI.
- Workshop 4 took place the 30th November 2018 in Malta, hosted by ABT. it was celebrated the fourth EnviGuard workshop in the island of Malta. During this event, topics such as the results from stakeholders' survey in the Mediterranean or the new sensors developed in the EnviGuard project were tackled. The workshop was attended by 10 people and was also announced as live stream which allowed some of the registered participants followed through live stream.

- Project dissemination material: banner, two flyers, poster, invitations to events, bags, pens, notebooks, 2 videos, were developed in the EnviGuard project frame.

In EnviGuard, also activities devoted to cooperating and find synergies with other OCEAN projects have been performed:

- TTZ attended the BRAAVOO kick-off meeting in Lausanne (December 2013), the workshop “European Marine Policy and its implementation through Projects for monitoring of marine environment for Blue Growth” in Barcelona (November 2014).
- BAZ and AWI participated in the 2nd Interproject Workshop Meeting of the OCEAN2013 projects at the same time of the 2015 ASLO Aquatic Sciences Meeting in Granada, Spain (26th of February 2015).
- CTN participated in the first public BRAAVOO Workshop and Creative Design Course particularly targeting PhD and Master students and postdoctoral researchers at the interface between biology and micro-engineering in Lausanne (31st January to 2nd of February 2016).
- TTZ attended the 2nd SEA-on-a-CHIP Progress Workshop: Monitoring for a sustainable management of marine resources” in Ferrara, Italy (13th of April 2016).
- TTZ attended a 3-day scientific and technical workshop, in Telde, Gran Canari, Spain (from 2nd to 4th November 2016) organised by AtlantOS project.
- TTZ participated in Oceanology International 2016 in London and shared stand with other OCEAN projects.
- TTZ and NBS attended the MTS/IEEE OCEANS’ 1Aberdeen, Scotland, (21st to 22nd June 2017). EnviGuard participated at the Oceans of Tomorrow workshop held as part of the conference.

- MT attended the BRAAVOO workshop in Switzerland (24th to 25th November 2016). Both projects – EnviGuard and BRAAVOO were about detection of pollutions in seawater using different technologies.

Besides taking part in common events, a common newsletter was prepared by the sister projects. BAZ collected the necessary information from the EnviGuard partners to be included in the newsletters.

With regards to project exploitation strategy, 24 KERs were identified by the partners at the beginning of the projects for which different exploitation means were foreseen such as commercial exploitation, scientific publications, further research, technology transfer and licenses. By the time the ESS took place, and according to the expert advice, the list of KERs was reduced to 11. Based on the work performed during the seminar (dealing with ownership, potential exploitation routes, characterisation of the market, IPR protection measures) and the information reported by the partners, ESS expert prepared a final report and included the issues detected and associated recommendations (confidential information).

It can be concluded at the end of the project, that the partners have revised during the project duration the most relevant project results, some have been neglected and some others have been added. For the following KERs, detailed information has been provided by the partners involved regarding potential exploitation. The final KER list includes 13 results of the EnviGuard project of different nature and with different stages of development. An Exploitation session was scheduled during the final meeting to further discuss the pending issues relevant for the results ownerships, protection and exploitation.

In general, partners have chosen for the Intellectual Protection Rights (IPR) protection to keep the knowhow internally, as trade secrets. No patents are foreseen at this stage on project results. Partners have also prepared publications on the developments to claim priority over future applied patents from others. The owners of almost all reported KERs are research centres and universities. Several results are ready for its commercialisation and/or to be used for further activities/research. Different exploitation routes have been considered, such as licences to SME partners as BIOD, direct commercialisation to clients (Business to clients, B2C) and to manufacturer (Business to business, B2B).

Potential impact WP8: As WP8 combined the general project coordination and included task such as scientific management. It had not socio-economic impact by itself or results to exploit. It kept track of the amount of trainings - more than 50, predominantly young researchers were trained at partnering entities of the project ranging from weeks up to three month – and scientific degrees associated to the work within the project - one BSc, two MSc and five PhD.

As mentioned in under the description of WP7, TTZ was invited and participated in a variety of meetings in front of different stakeholders from SME/industry to governmental and science.

Public Website: www.EnviGuard.net
Coordinator contact: Björn Suckow
ttz Bremerhaven
Am Lunedeich 12
27572 Bremerhaven
bsuckow@ttz-bremerhaven.de

- ## ANNEX I FIGURES

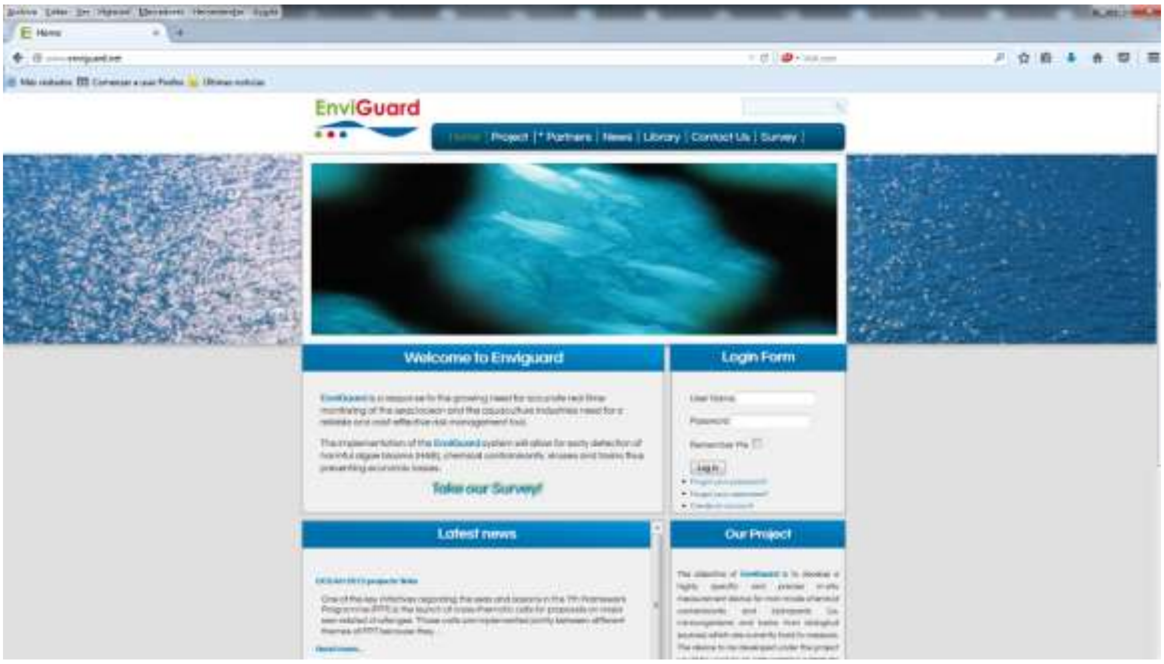
EnviGuard

EnviGuard project logo

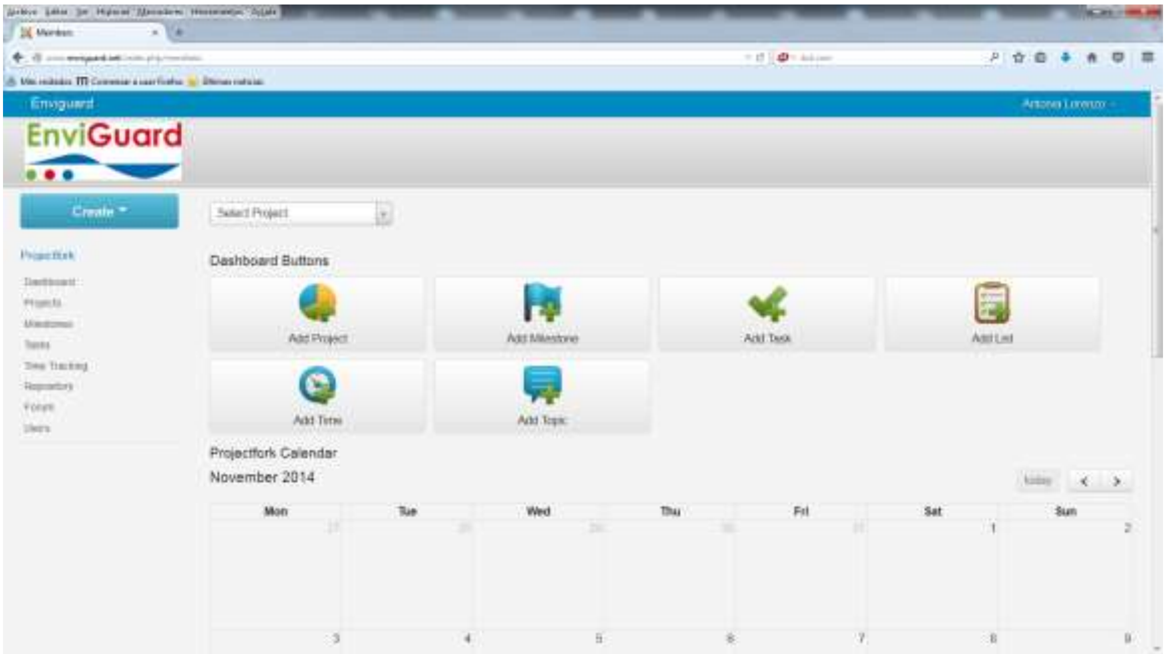
EnviGuard

EnviGuard EnviGuard EnviGuard

EnviGuard port and units logos



EnviGuard website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EnviGuard
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Select Project
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November 2014
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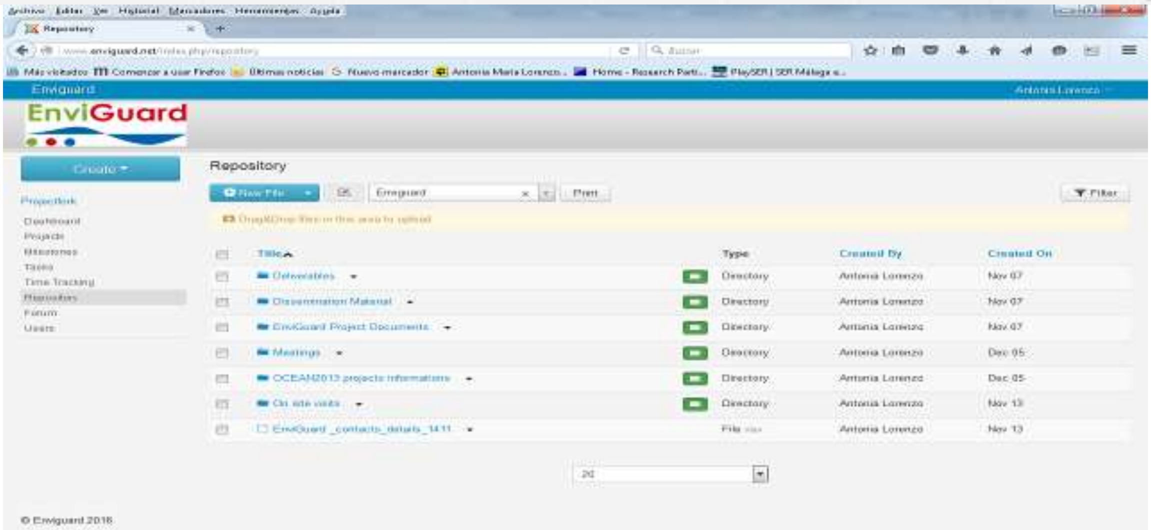
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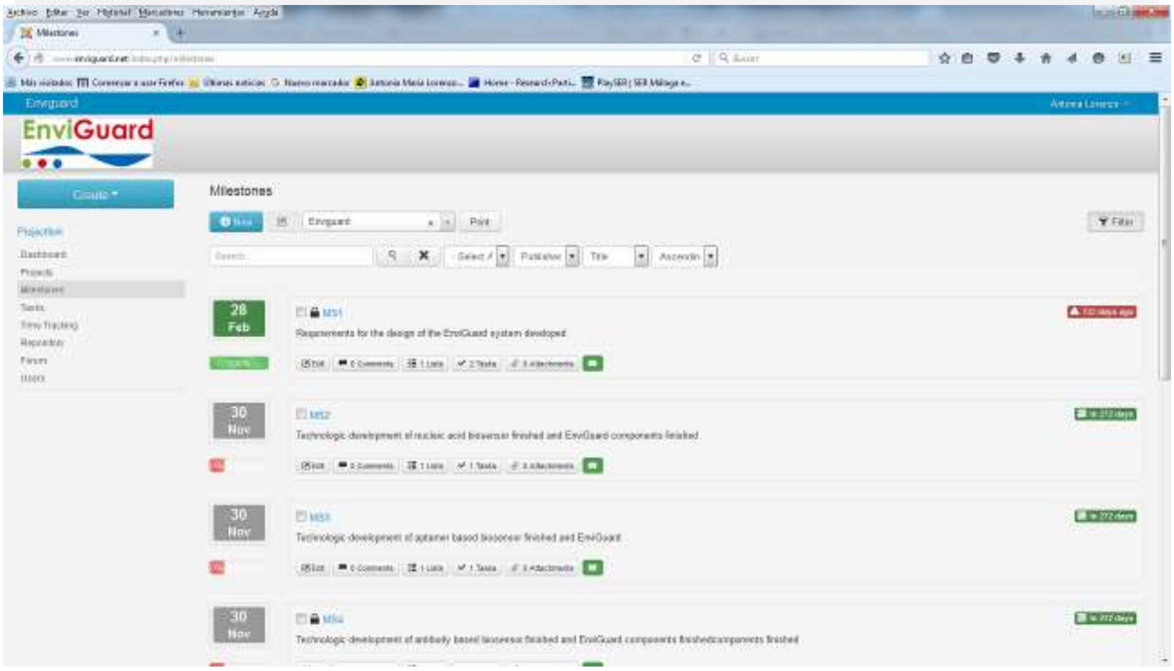
Directory
Antonia Lorenzo
Nov 07
Dissemination Material
EnviGuard Project Documents
Meetings
Dec 05
On site visits
Nov 13
© Enviguard 2016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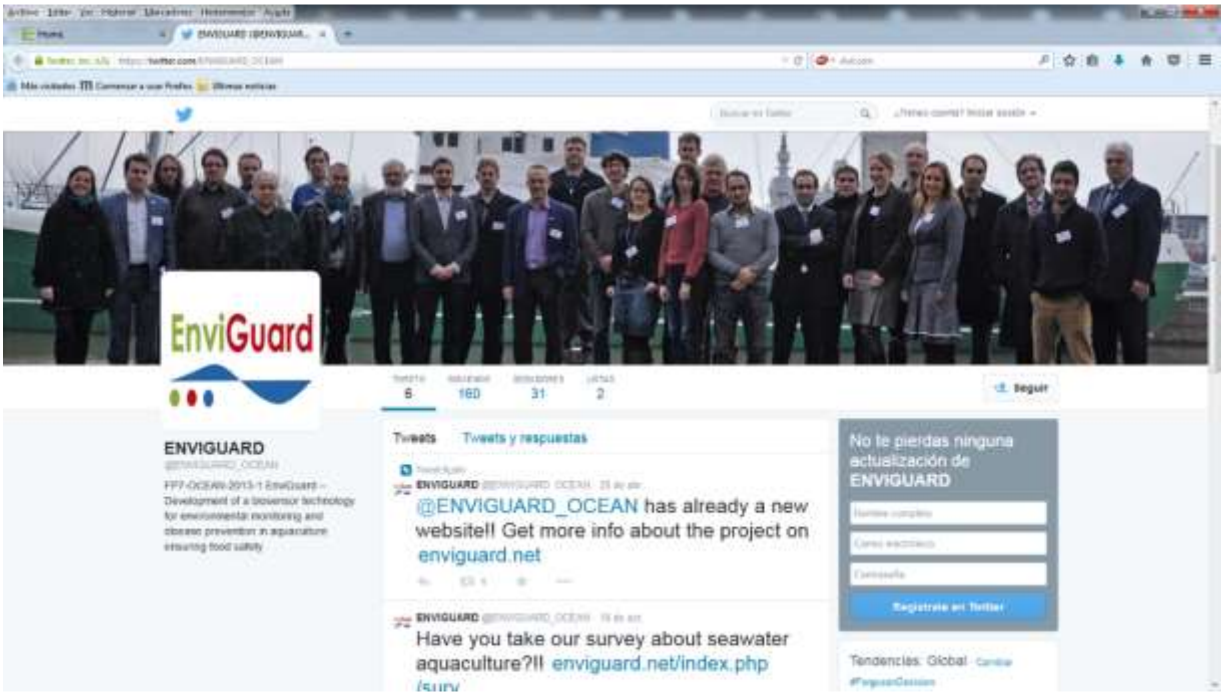
28
Feb
MS1
Requirements for the design of the EnviGuard system developed
30
Nov
MS2
Technologic development of nucleic acid biosensor finished and EnviGuard components finished
MS3
Technologic development of aptamer based biosensor finished and EnviGuard
MS4
Technologic development of antibody based biosensor finished and EnviGuard components finishedcomponents finished

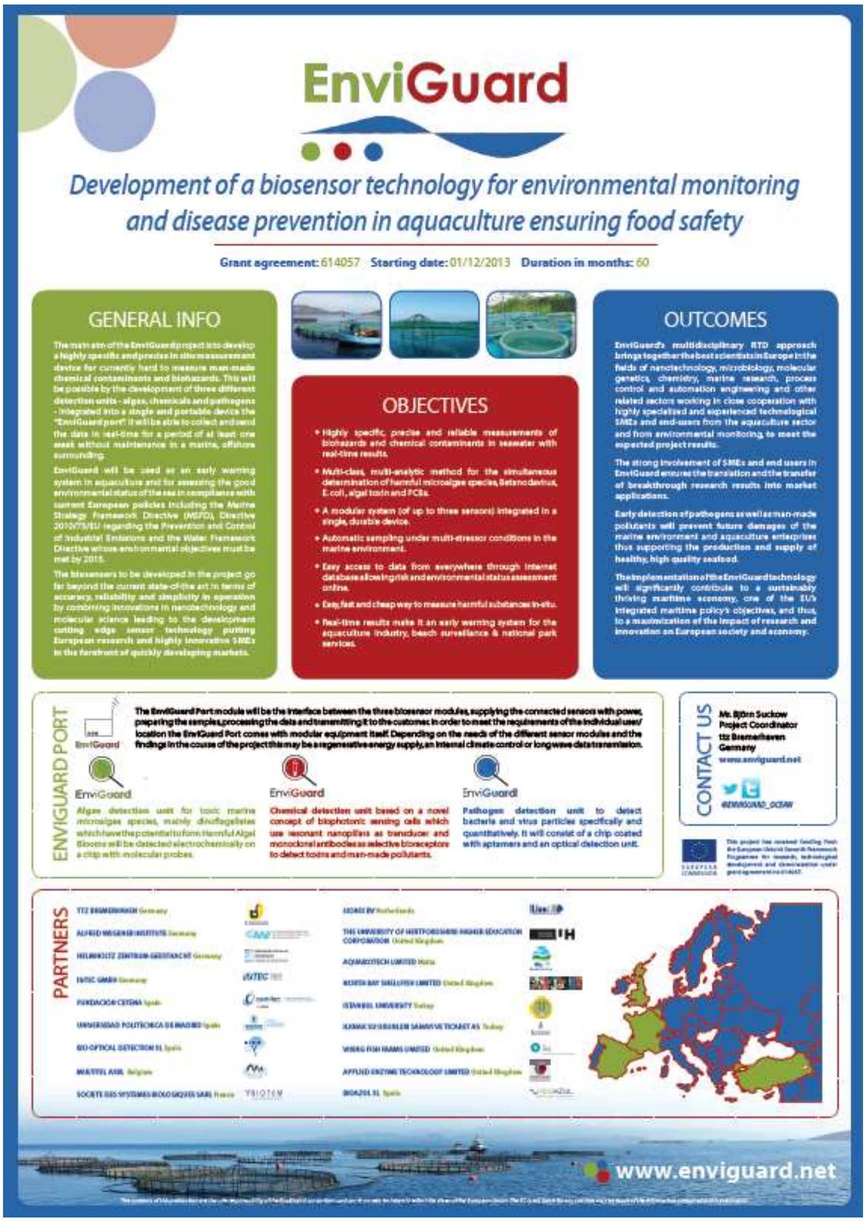
EnviGuard website member area



EnviGuard twitter

1st EnviGuard project brochure

2nd EnviGuard project brochure

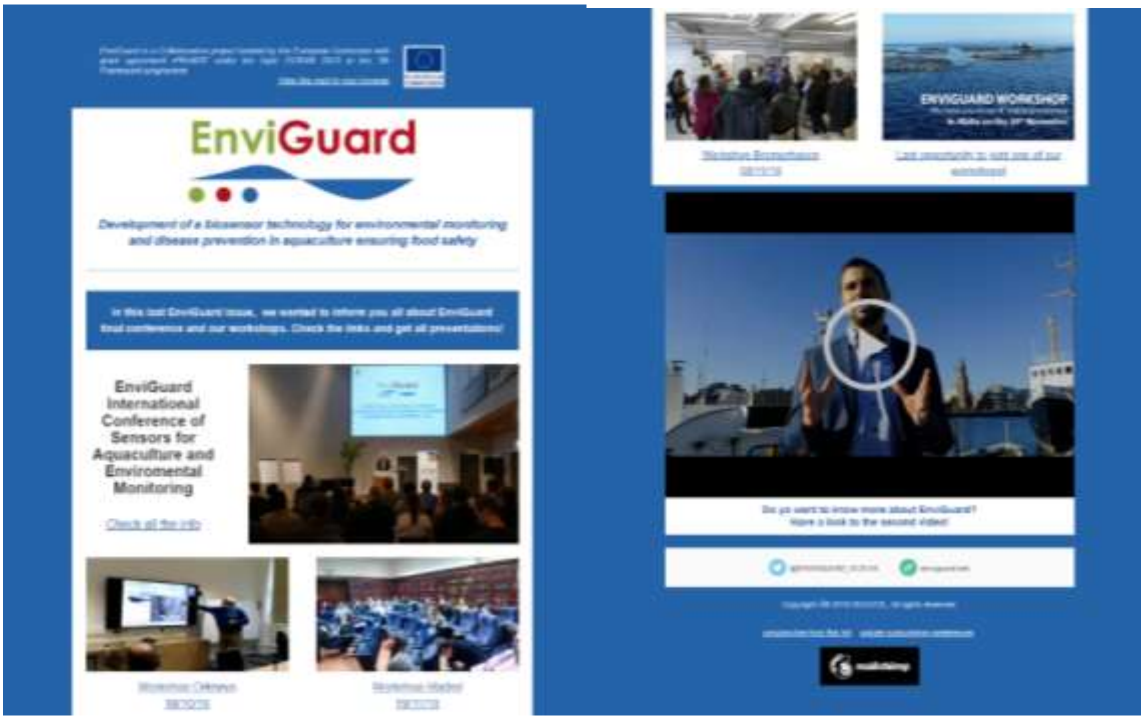


EnviGuard project poster


EnviGuard
Development of a biosensor technology for environmental monitoring and disease prevention in aquaculture ensuring food safety
1st Results on EnviGuard Survey
Read more
EnviGuard
SCHeMA
COMMON SENSE
Mario
NEXOS
EnviGuard
The Ocean of Tomorrow Projects (2010-2013)


EnviGuard
Development of a biosensor technology for environmental monitoring and disease prevention in aquaculture ensuring food safety
EnviGuard's trials are ongoing and we want to share the results with all of you
We invite you to join us to learn more about it during the EnviGuard training workshops:
VENUE Orkney Islands, UK
DATE July 25, 2018 10:00 am
VENUE Madrid, Spain
DATE October, 2018 10:00 am
VENUE Bremerhaven, Germany
DATE November 7, 2018 10:00 am
VENUE Malta
DATE November, 2018 10:00 am
More details soon on the website!!


EnviGuard video will be available soon! Stay tuned to our website!!
Save the date!
EnviGuard Final Conference



EnviGuard project newsletters

EnviGuard International Conference and workshops dissemination materials


EnviGuard Workshop
Registration through this e-mail:
dennis@researchrelay.com
Venue:
The Commodore Restaurant & Bar
Main Street, Holm, Orkney KW17
United Kingdom
09:30 Registration and coffe
09:40 Welcome and introduction of the EnviGuard team
10:00 Project presentation:
General overview
What is EnviGuard Port
10:30 Transfer to prototype site
10:40 Introduction to site and goal of the trials
Trial run of prototype
Practical examination
Explanation of the functionality of the units
12:00 Return to seminar venue and lunch breack
13:00 Investigate areas for the prototype use
Participants questionnaries
14:30 End of the event
Find all the information on www.enviguard.net


EnviGuard Workshop
Registration through this e-mail:
dennis@researchrelay.com
Venue:
The Commodore Restaurant & Bar
Main Street, Holm, Orkney KW17
United Kingdom
Workshop agenda (9:30 - 14:30)
– Short introduction of EnviGuard project.
– General overview of the EnviGuard Port.
– Visit to the prototype site: trials goals, practical examination, explanation of the functionality of the algae, chemical and pathogen units.
– Discussion and feedback from attendees on areas for prototype use.
Coffee will be served at 9:30 and lunch at 12:15.
Find all the information on www.enviguard.net


EnviGuard Workshop
JOIN US IN BREMERHAVEN ON THE 8TH NOVEMBER
ADDRESS:
ttz Bremerhaven
Am Lunedeich 12,
27572 Bremerhaven
(Germany)
TIME:
9:30H - 14:00H
Organizers:
Click here to register
BIOAZUL
EnviGuard

EnviGuard workshops invitations

ENVIGUARD FINAL CONFERENCE
Wednesday, 7th of November 2018, Bremerhaven

9:00 AM	REGISTRATION
9:30 AM	CONFERENCE OPENING AND KEY NOTES **Mr. Björn Suckow**, ttz Bremerhaven **Mr. Guido Kebschau**, BIS Bremerhaven (Bremerhaven Economic Development Company) **Dr. Björn Kempken**, BEG logistics GmbH
10:00 AM	INTRODUCTION TO THE ENVIGUARD PROJECT **Mr. Björn Suckow**, ttz Bremerhaven
10:15 AM	1st SESSION: ENVIGUARD PROJECT OUTCOMES Chairman: **Mr. Björn Suckow**, ttz Bremerhaven • PDU: "Aptamers as new detection molecules in pathogen analysis". **Dr. Ingo Klarholz** (ttz Bremerhaven, Germany). • ADU: "Automated approaches in regular observation of marine microbe". **Dr. Katja Metfies** (AWI, Germany). • CDU: "Development of a multiplexed biosensor for the simultaneous detection of toxins and PCBs in marine waters". **Dr. Paula Ciaurriz** (NAITEC, Spain). • EnviGuard Port as a modular measurement System, including automatic sampling and filtration (AutoFiM). **Mr. Thomas Hanken** (ISITEC GmbH, Germany).
11:30 AM	COFFEE BREAK
12:00 PM	2nd SESSION: MICROFLUIDIC FOR SENSING PURPOSES Chairman: **Dr. Iñaki Cornago**, NAITEC • Arrays of Resonant Nanopillars as Multiplexed Optical Transducers. **Dr. Ana López Hernández** (UPM, Spain). • On the design and performance of a custom fluidic rotary switching valve. **Dr. Theo Veenstra** (Lionix International, Netherlands). • A multichannel spectrometer for the interrogation of resonant optical sensors. **Dr. Fabian Dortu** (Multitel, Belgium). • On-chip monitoring of oxygen consumption of cells. **Ms. Camilla Konermann** (IMSAS Bremen, Germany).
13:15 PM	LUNCH BREAK
14:15 PM	3rd SESSION: SENSORS FOR MONITORING MARINE ENVIRONMENT Chairman: **Björn Suckow**, ttz Bremerhaven • The SMS project: on-line monitoring of the quality of marine waters. **Dr. Sergio Bodini**, (SYSTEA, Italy). • Monitoring the seafloor with new eyes: underwater hyperspectral imaging. **Dr. Chennu** (Max Planck Institute for Marine Microbiology (MPIMM), Bremen, Germany). • Absorptiometry, fluorometry & radiometry – State of the art technology in environmental optical sensing. **Dr. Yvonne A. Lipsewers & Annemarie Köppen** (TriOS Mess und Datentechnik GmbH, Germany). • FerryBox: From physical to biogeochemical and biological measurements. **Dr. Wilhelm Petersen** (HZG – Helmholtz Zentrum Geesthacht Centre for Materials and Coastal Research GmbH, Germany).
15:30 PM	COFFEE BREAK
16:00 PM	4th SESSION: AQUACULTURE PERSPECTIVE AND MARKET POTENTIAL Chairman: **Ms. Inês Pimparel**, AquaBioTech Group (Malta) • Use of EnviGuard prototype as the end-user and future prospects. **Dr. Dennis Gowland**, (North Bay Shellfish, United Kingdom). • Intelligent processing of sensory data for an automated control system for denitrification filters. **Mr. Mirko Bögner** (AWI, Germany) • Intelligent Fish-Welfare Monitoring. **Mr. Dominik Ewald** (MonitorFish, Germany). • TAPAS Case Study: IMTA feasibility in Malta. **Dr. Alexia Massa-Gallucci**, (AquaBioTech Group, Malta). • Continental Aquaculture in Latin America. A potential market for new technologies. **Dr Alonso González Mejía**, (Colombia). • Remote Sense in Aquaculture - Opportunities in LATAM, **Dr. Luiz Storino Filho**, (Agertek, Brazil).
17:45 PM	CLOSING REMARKS **Mr. Björn Suckow**, ttz Bremerhaven
18:00 PM	NETWORKING SESSION- OPPORTUNITIES FOR FUTURE COLLABORATION
19:30 PM	GALA DINNER

EnviGuard final conference programme

Björn Suckow presenting at the *EnviGuard International Conference*

EnviGuard 1st workshop

EnviGuard 2nd workshop

EnviGuard 3[rd] workshop

EnviGuard 4th workshop

Mr. Sergio Bodini presenting the results of OCEAN 2013 project SMS at EnviGuard 's international conference