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⁴ The initials of the revising individual in capital letters

Deliverable D4.4

Report on second development phase for IoT-enabled sensors

23/08/2022

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Executive summary

In WP4 of the AquaVitae project, “Sensors, data integration and Internet of Things”, three different sensors will be developed by DTU, Biolan and Norce. The physical sensors are already created, but in this WP the purpose is to develop them further in order to meet the practical needs and requirements of the aquaculture sector, in particular the low trophic aquaculture producers. To accomplish this task, stakeholders have been interviewed to ascertain their needs regarding the use of the sensors developed in the project. Based on this feedback, the scope and a plan of development for each sensor was created. The first sensor prototypes were delivered by month 12, and were tested and evaluated by the stakeholders and AquaVitae’s partners.

This deliverable describes the result of the second development phase, incorporating the feedback from stakeholder evaluation of the first prototype.

During the lifetime of the project, WP4 delivers four reports documenting the work on the sensor development:

- D4.1: Scope, plans and initial stakeholder feedback, M5
- D4.3: Report on first development phase, M12
- **D4.4: Report on second development phase, M36**
- D4.5: Report on final development phase, M46

The current deliverable follows on from Deliverable 4.1 “Scope, plans and initial stakeholder feedback”, that gave a detailed overview of the feedback obtained. It also contains the development plan for the first and second iteration of each of the different sensor prototype development processes. In summary, the stakeholder’s main requirements regarding use of the sensors are (from D4.1) better process control, better yield, and lower costs. The main requirements for the sensors themselves are accuracy, affordability, and robustness.

This deliverable reports the status of the three sensors at month 36, at the end of the second development phase.

Datasets used to produce the deliverable

- WP4: Sulphite monitoring device technical documents (Biolan)
- WP4: Sulphite analyses (Biolan)
- CS9: Mussel hatchery production (Ireland & Denmark) (DTU)

Both datasets from Biolan are confidential due to commercial reasons and will not be made public. The one from DTU is still under work and is therefore not uploaded yet to a public repository but is planned to be uploaded to Zenodo under the AquaVitae community. The description of those 3 datasets can be found in the Data Management Plan deliverable M36 (D4.7).

1. Introduction

1.1 Scope of AquaVitae

AquaVitae is a research and innovation project funded by the EU's Horizon 2020 programme, BG-08-2018. The project consortium consists of 35 partners, from 16 different countries, spread across four continents. In addition to Europe, partners are situated in countries bordering the Atlantic Ocean, including Brazil, South Africa, Namibia, as well as in North America. Its broad objective is to introduce new low trophic species, products and processes in marine aquaculture value chains (VCs) across the Atlantic.

1.2 Scope of WP4 Sensors, data integration and Internet of Things

The objective of WP4 is to develop or improve and test new or existing sensors for use in the aquaculture industry using an Internet of Things (IoT) approach; this includes biochemical sensors, biomass sensors, and the integration and visualisation of data from environmental sensors (SO5). Specific objectives are:

- To develop new methods for biomass monitoring in offshore aquaculture sites by combining underwater laser cameras with computer vision and machine learning algorithms
- To design and develop a smart sulphite biosensing device for use in aquaculture production
- To develop an IoT platform for integration and analysis of sensor data related to aquaculture production
- To develop a Data Management Plan (DMP) for the data generated in AquaVitae, and to update the DMP at the end of every reporting period

1.3 T4.1 Scoping, planning, and eliciting stakeholder feedback for sensor development.

In WP9 the AquaVitae multi-actor platform will be created and put into operation, and this involves numerous stakeholder meetings on CS level. AV will employ a multi-actor approach to ensure that project outcomes are co-created with extensive involvement of users and other stakeholders, to ensure relevance and acceptability. In this task, the scope and a detailed multi-actor plan for the sensor development work that is linked to WP4 will be defined, based on the overall AV / WP project description, the priorities of the industry participants and the scientists, and the feedback from the stakeholders. The plan for the sensor development will contain descriptions of who will do what when and where, and will be synchronised with the plans made for the CSs in WP1-3.

2. Sensor development tasks

2.1 DTU – 3D camera for mussel biomass monitoring

Develop new methods for biomass monitoring in offshore aquaculture sites (DTU, Norce)

The initial aim of this task was to use and to adapt next generation range-gated camera technology based on state-of-the art “time of flight” image sensors and innovative pulsed laser illumination (LiDAR). This is referred to as an Underwater Time Of Flight Image Acquisition (UTOFIA) camera, and it was an output from the H2020 UTOFIA project which ended in 2018. DTU has performed initial trials with UTOFIA to optimize range gating methods applied to mussel production, and to develop specific software for volumetric reconstruction of the mussel lines including the use of machine learning functionality.

However significant deviations occurred to this task, which are explained in the section 4.1.2.

In AquaVitae, we develop and test the system in CS8 (and CS9), but it is also applicable in other CSs where biomass monitoring is relevant, e.g IMTA (Integrated multi-trophic aquaculture) production and finfish production. The monitoring device could be easily integrated with the IoT platform to enable data storage, visualisation, and data analysis (machine learning). The encoding and exchange format for all data to be transferred to the IoT platform will be defined. Application program interface (APIs) will be defined to enable the collection of data from the biosensor.

2.2 Biolan – sulphite sensor for shrimps

Design and develop a smart sulphite biosensing device for use in aquaculture production (Biolan, Norce)

This task delivers a prototype of a high-performance, battery-operated, portable and connected biosensor for sulphite monitoring, aimed to be used in shrimp production. The electronics are designed to achieve technical specifications by first running simulations, and then testing different circuit architectures. The layout of the printed circuit boards are defined and created for mounting the prototypes. For software development, the tool-chain is selected and configured, and signal processing and calibration processes coding are defined. The mechanical structure have been designed and prototyped by 3D printing. The final cast has been designed and produced. The biosensing device is starting to be integrated with the IoT platform to enable data storage, visualization, and analysis (machine learning). The encoding and exchange format for all data to be transferred to the IoT platform are under analysis. APIs is developed to enable the collection of data from the biosensor.

2.3 Norce – integrated sensor data platform

IoT platform for integration and analysis of sensor data (Norce, Nofima)

Data gathering is based on local infrastructure for power efficient and resilient communication between low power IoT devices without fixed infrastructure and to enable integration with existing IoT devices and platforms at the test sites. Machine learning functionality are developing for sensor data fusion, sensor calibration, sensor monitoring and prognosis. A flexible and simple visual tool (dashboard) to create AI training sets with automated training, validation and deployment of AI based monitors / virtual sensors is developing. Edge computing support for sensors and AI trained monitors/virtual sensors will be provided for sites with no or unstable Internet access. The IoT platform will be tested in IMTA, shellfish, and finfish production cases.

3. Method

The method applied is the spiral method (see D4.1 for more detail). At month 36, we are at the end of the second iteration loop. According to the development process, this is the end of the second development phase when a prototype has incorporated stakeholders' feedback in their development.

During the first iteration loop, the three partners have interviewed many stakeholders to gather their needs and priorities regarding the sensors. The stakeholders' expectations are listed in the deliverable D4.1: "Scope, plans and initial stakeholder feedback WP4" M5.

The prototypes were developed in accordance with the requirement specifications and tested by the stakeholders at M12. The results of the first development phase are described in the deliverable D4.3: "Report on first development phase for IoT-enabled sensors" M12.

With that first check point in place the second iteration loop started. The feedback from the stakeholders at M12 on user acceptance were gathered and the prototypes were developed further in accordance with the expectations. This deliverable reports the status of the three sensors at month 36, at the end of the second development phase.

4. Results

The progression of each partner is presented separately: DTU, then Biolan and finally Norce.

4.1 DTU

Objectives Planned to be achieved M36	M36 achievements
Test UTOFIA system on mussels line	The effects of both COVID-19 and the global chip shortage have resulted in significant repair delays of over 12 months for the UTOFIA. To maintain our deliverable schedule, we shifted focus to alternative 3D reconstruction devices

4.1.1 Analysis and tests performed the last 18 months

Solution Description

The target application is to obtain an estimate of the volume occupied by mussels growing on a longline. Regularly timed volume estimates will then provide a volumetric growth-rate metric as an indicator of the mussels' health and productivity. Obtaining a volume estimate comes in two major steps: first the longline must be scanned with a sensor platform to obtain an adequate spatial reconstruction in 3D, next the reconstruction must be segmented and refined to remove noise, and a meshing algorithm must be applied to generate a volume from the refined reconstruction. These steps are outlined below.

State of the Art

Three-dimensional spatial reconstruction of underwater scenes has been achieved with acoustic sonar, laser time-of-flight cameras, and multiple view visual spectrum camera systems such as stereo or tri-ocular cameras. When compared to the cost of acoustic and time-of-flight solutions, stereo cameras present a much cheaper solution with greater potential to scale as a commercial product. Stereo cameras have been used for 3D reconstruction tasks in a wide variety of in situ underwater environments such as shallow water and reefs (Marouchous et al. 2015), benthic habitats (Shortis et al. 2008), and shipwrecks (Nornes et al. 2015). The data output of these cameras allows for modelling of structure and texture of underwater scenes, which has been recognized and developed by the aquaculture research community for specific applications. In particular, stereo cameras have been used to obtain biometrics of free-swimming fish in farms (Torisawa et al. 2010; Vale et al. 2020) which is used to monitor the growth rate and size distribution of the farm population. The same biometric monitoring technology can be applied to oysters and mussels. Indeed, a New Zealand national research project on precision farming technologies for aquaculture (Cornelisen et al. 2022), with similar goals to AquaVitae, has investigated the use of stereo cameras mounted on a remotely operated vehicle for the purposes of mussel longline volumetric estimations. The project has yet to present peer-reviewed results, but we have improved upon the idea by reducing the size of the stereo camera payload so that it can be easily integrated into a variety of platforms. The main improvement we have made to the

existing works is a cheaper and more flexible solution that has greater potential to scale across the aquaculture market (but not exclusively).

Spatial Reconstruction

Three-dimensional spatial reconstruction of underwater scenes has been achieved with acoustic sonar, laser time-of-flight cameras, and multiple view visual spectrum camera systems such as stereo or tri-ocular cameras. In our case, we have selected a compact stereo camera, OAK D Lite (<https://docs.luxonis.com/projects/hardware/en/latest/pages/DM9095.html>) that is able to perform the reconstruction in real-time. We have integrated the camera with an underwater robotic platform, the BlueROV2 (<https://bluerobotics.com/>) to allow an operator to remotely pilot the stereo camera underwater (see Figure 1).



Figure 1: Left: Prototype BlueROV2/MyntEye D1200 Nose Camera Integration with Bubbledome. Right: Final Oak D Lite Nose Camera Integration with Flat Glass Plate.

The camera comes factory calibrated for a sensor-lens-air environment and had to be recalibrated to account for distortions associated with a sensor-lens-air-glass-water environment. The 3D reconstruction output of the stereo camera driver is a grayscale image taken from the left camera lens, coupled with a depth image measured in mm registered to the left camera lens.

These two images are then used to obtain a cloud of points with 4 dimensions, three spatial dimensions and a fourth intensity dimension obtain from the grayscale image. Each new image taken by the camera will provide a unique perspective of the scene and will produce a separate 3D point cloud. Each point cloud must be registered with the others to produce a unified point cloud representation of the scene. To perform this registration, we depend upon the Real-Time Appearance-Based Mapping project (RTAB-Map), an open-source BSD licensed platform designed for mapping of 3D environments with camera and laser scanner devices. RTAB-Map is capable of performing unified point cloud registration in real-time and was chosen because of its maturity.

Together with the stereo camera and RTAB-Map, we are able to aggregate point cloud data in real-time (Figure 2). RTAB-Map calculates and tracks features from the grayscale data to obtain an estimate of the camera's trajectory through the scene, with the assumption that the camera is moving, and that the scene is static with only minor changes. This can be challenging in the marine environment where scenery is usually quite dynamic (e.g. fluctuations of the mussels line, seaweed changes shape, marine life moves through the scene, sand and mud are "blown", sunlight refracted through surface waves

creates patterns). It is up to the operator to pick and choose the right scene, lighting and weather conditions to minimize dynamic effects while they perform the scan.

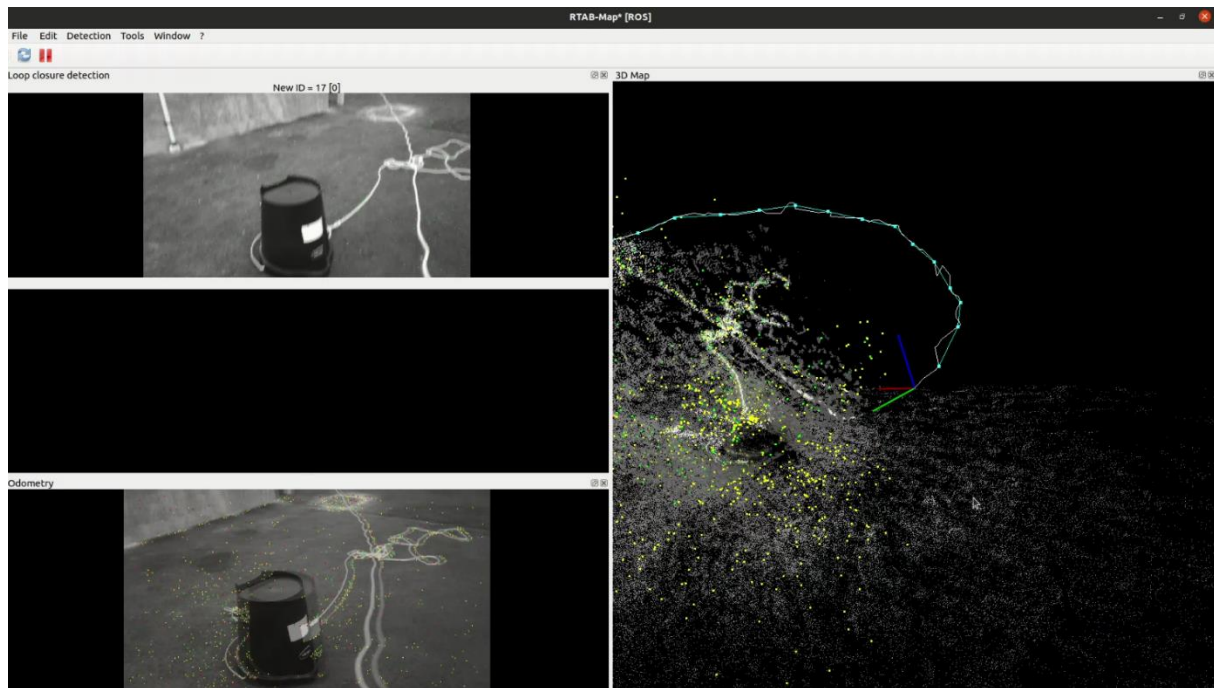


Figure 2: RTAB-Map aggregating point cloud data (right panel) of an inverted bucket at the autonomous systems testing area, DTU. The cyan line indicates the estimated trajectory of the camera provided by the feature-based motion model. Since enough features are present, the trajectory estimation is good

Point Cloud Refinement and Meshing

RTAB-Map exports the unified point cloud in a standardized native format (“.ply” format file), which can be read by many spatial modelling programs. We import the point cloud into MeshLab (<https://www.meshlab.net/>), which is GPLv3 licensed and contains many points cloud manipulation, denoising and meshing algorithms to refine the model into a single closed surface. Care must be taken to eliminate outliers such that the spatial accuracy of the model is not affected. Distinguishing between noisy outliers and inlier points is non-trivial. Simple methods, such as resampling and voxel decimation, can appear to reduce noise but are also liable to remove inliers and reduce the accuracy of the model.

During this period, we have found no satisfactory automated denoising method that works consistently for complicated geometries. We expect that, for the target mussel application, that the modeler must have some expert knowledge and familiarity with mussel longlines to perform constructive denoising with machine learning support methods.

The cleaned point cloud is then converted to a mesh through Delauney triangulation. The estimated volume occupied by the mesh surface can then be estimated through numerical integration. It is likely that the mesh surface will contain holes and other imperfections, these can be closed manually or with convex hull calculations. The refined model and volume estimate are saved for future reference.

Results

Stereo Camera Viability

Over Fall 2021, a student project was created to investigate the viability and implementation of stereo cameras for scanning of underwater objects. ZED and MyntEye-D1200 stereo cameras were provided as a starting point. During the internship, the student was able to demonstrate the viability of both stereo cameras for spatial scanning by obtaining 3D reconstructions and meshes of objects in a

controlled air environment (Figure 3) but was unable to integrate either camera into an underwater platform. The outcomes of the report identified that the OAK D stereo camera series performs best on lightweight computer systems (such as those present on underwater robots), as it is capable of performing heavy stereo reconstruction and encoding tasks onboard the camera's Vision Processing Unit.

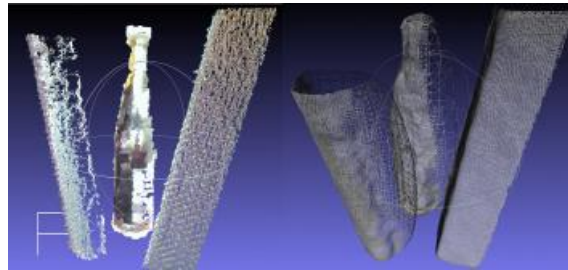


Figure 3: Left: Point Cloud Reconstruction of Simple Objects (cylinders, bottles and rectangular bars). Right: Meshed Surface Models of the Objects

Calibration and Reconstruction in Laboratory Conditions

Major changes to the stereo camera's optical properties were introduced when it was integrated into a watertight enclosure. Additional distortions, particularly around the edges and corners of the camera's image view, affect the accuracy and precision of the output reconstruction point cloud. These distortion effects can be modelled and compensated for by recalibrating the integrated camera with a standard checkerboard calibration placard while underwater. Calibration (Figure 4) was performed in controlled conditions at the testing pool within the Autonomous Systems Testing Arena at DTU. The calibration information is used by the stereo 3D reconstruction algorithm to account for the additional distortions present in underwater imagery.



Figure 4: A sample used in the underwater stereo calibration process. Corresponding corners of the checkerboard pattern are matched between the left and right cameras. The calculated transformation is used to estimate intrinsic, extrinsic and distortion information about each camera

Further, 3D printed geometric shapes of known dimensions were positioned within the testing pool for the purposes of evaluating the stereo camera's performance at underwater 3D reconstruction, and to evaluate the volume estimation pipeline (Figure 5). We found that the white, relatively featureless walls and floor of the pool made the point cloud aggregation component of RTAB-Map less accurate

as there are far less features available in the image for robust camera motion estimation. The resulting trajectory is jittery, and RTAB-Map improperly aggregates the point clouds making the unified point cloud very unlike the actual object. We surmise that in an underwater environment with more features, such as what we expect in the mussel longline farm where the object of interest contains many textures and the background will contain both moorings, sea bottom and other feature-rich objects, that RTAB-Map will have better motion estimation.

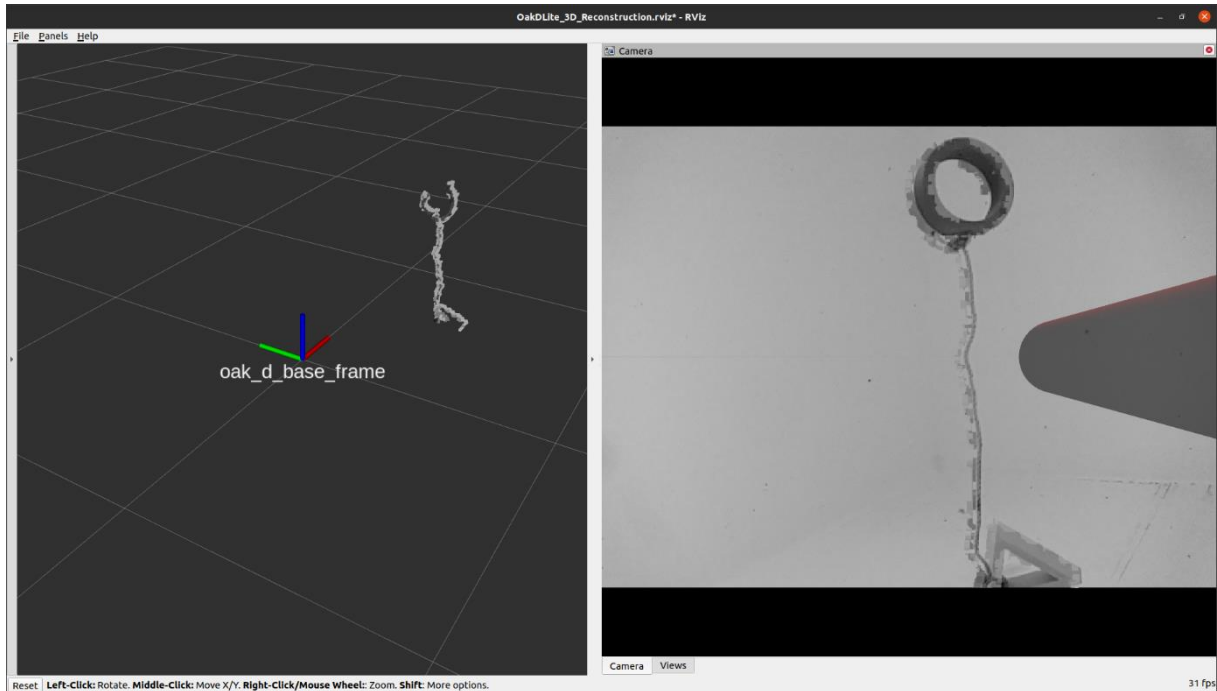


Figure 5: Left: Point cloud reconstruction of the 3D printed hoop and triangle shape with rope. Right: corresponding left camera view of the objects with point cloud and reference axis present as a virtual overlay

Summary

Our results have shown that the integrated stereo camera robotic platform is capable of performing 3D reconstruction and unified point cloud aggregation in real-time. Laboratory testing conditions show that the resulting point cloud of known geometric shapes is noisy and needs significant segmentation, denoising and refinement to obtain a mesh surface of sufficient quality for accurate volume estimation.

4.1.2 Deviation, adaptation of the sensor in relation to the initial plans

Significant deviations occurred to this task. At the outset of Aquavita, there was one UTOFIA system in existence which was highly demanded amongst the original project partners. The effects of both COVID-19 and the global chip shortage have resulted in significant repair delays of the unit to over 12 months to access the device (which as today cannot yet be accessed).

To maintain our deliverable schedule, we then shifted focus to alternative 3D reconstruction devices. During an explorative phase conducted by an intern at DTU Aqua, we assessed different stereo cameras to perform 3D reconstructions of scenes in real-time. In particular, stereo cameras with small baselines (less than 10 cm), are able to fit in small watertight enclosures and are power efficient hence serving the need of users' applications. Small baseline stereo cameras, such as the ZED-m, OAK D, MyntEye-D, are much cheaper than the UTOFIA system (around 300x cheaper), are very compact (less than 150 mm in maximum length) and can operate at an acceptable range of 0.5 - 5 m. We selected the OAK-D-Lite stereo camera for integration with an underwater robotic platform.

This new system can provide additional opportunities for innovation in AQUAVITAE since the solutions, once tested in real environment, can be transferred to several markets including aquaculture, fishery, and ocean observation technology sectors.

4.1.3 Changes to Impact

By adopting the change from the UTOFIA system to a stereo camera system, we are presented with two additional points of impact that were previously considered small. Unlike UTOFIA, the stereo camera system is well within the price range of the target mussel longline industry. This means the final product could scale across the entire market, ensuring that the majority of mussel farms are obtaining volumetric growth rate estimates for their stock, greatly affecting the sustainability of production. Additionally, the camera system is self-contained and does not require to be integrated with a robotic platform, nor does it require to be underwater to obtain 3D reconstructions. This opens the potential for the system to be used in various aquaculture and fisheries related industries, and potentially transfer into agriculture and other terrestrial markets.

4.1.4 Collaboration done and planned

Collaboration planned with Blue Atlas Robotics. The company has developed a specialized underwater robotic platform capable of capturing 6 - 8 simultaneous images for the purposes of robust 3D reconstruction (i.e. better outlier detection and correction capabilities than a stereo camera system). They have developed their system for harbor and ship hull inspections, but we believe the system could be adapted to aquaculture application such as mussel longlines. We are in close contact with Blue Atlas Robotics and have arranged for them to demonstrate their system at the Danish Shellfish Centre longline facilities.

4.1.5 TRL level at the moment and expected at the end of the project

At this stage, the integrated system is estimated at TRL 3-4 as it has been validated up to 3D reconstruction within a laboratory environment. A field campaign was arranged with the Danish Shellfish Centre at their mussel longline installations in May / June 2022 to elevate the solution into TRL 5-6. This campaign has been delayed to late August due to lack of overlap in acceptable weather conditions, vessel, and technician availability.

Table 1: TRL evolution during the project

Timeline	M0 start of the project	M18	M36	M54 end of the project
TRL	2	2	3-4	5-6
Explanation/ justification	Camera technology present, but underwater mapping components to be developed.	Underwater enclosure and integration on ROV conceived, designed and developed with bench tests performed.	Stereo camera system operated in underwater conditions in laboratory environment.	Data collection performed in sea trials, data processing produces to match needs of the users.

The new planned field campaign in late August is expected to bring the stereo camera system up to a TRL of 5-6. The main activity of the campaign is to visit the mussel long lines in the Limfjord owned and maintained by the Danish Shellfish Centre (DTU) and survey the mussel lines with the robotic stereo

camera system. Sample sections of the mussel lines will be transported to a new site in the Øresund in the following week, where an additional survey will be conducted in different water conditions. The collected video data will be processed to generate 3D models of the mussel long lines, which will show the system's robustness to variance in conditions (lighting, turbidity, weather). Because the system is being directly tested on the target aquaculture sector (mussels) and is tested across two separate conditions (robustness), we believe the system will achieve a TRL of 5 or 6 depending on whether the produced data can be successfully integrated with the IoT technology produced by Norce.

4.1.6 Collection of user's feedbacks

Following the field campaign, we will then take the gathered results to present to potential end-users and obtain valuable feedback from them. Targeted end users are:

1. Danish aquaculture industry with e.g. focus on mussel longline production (e.g., Musholm A/S)
2. Robotics companies that could license the developed technology for their own services (e.g., EIVA, Blue Robotics)
3. Mussel longline research performing organization
4. Mussel longline production businesses and researchers based in other countries (e.g. New Zealand)
5. Pelagic and demersal fisheries businesses to improve species classification (e.g. Danihs Pelagic Fishery Organization)

The following sample questions will be used to obtain quality, targeted feedback:

1. Are 3D maps and volume estimates of underwater objects (i.e., species, infrastructures) useful for your business?
2. Is the resolution and image quality achieved by our system sufficient to support your activities?
3. Are the operational conditions (shape and size of the device, robustness, lighting, battery consumption, etc.) sufficient to use the device in your work?
4. What metrics are the best indicators for monitoring conditions of e.g. mussel growth, fish behaviour, infrastructure maintenance ?
5. Please describe an example of operations with this device.
6. Do you need a robotic system, or could it be achieved with a simpler method (e.g. stand alone camera deployment)?
7. Could this system be adapted for other target applications relevant to your activities?

A scoring system applied to questions 1-3 will serve the needs to finalise the technical development around the hardware components, while questions 4-6 will give us indication about suitable user guides and user interface, while question 7 provide indications about future developments.

4.1.7 Plan for the next 18 months

The next developing phase will be to complete validation of the integrated stereo camera within the target environment, showing that it is possible to obtain 3D reconstructions of mussel lines and obtain biovolume estimates with the integrated system.

We plan also to compare the results with other commercially available systems like for example the multicamera system developed by Blue Atlas Robotics (<https://blueatlasrobotics.com/>).

Results of sea trials will be collated to engage with potential users and obtain feedback for the fine tuning of the hardware and software.

Expected time line is: field campaign (August 2022), data processing (September 2022), collection of users' feedbacks (October- November 2022), product tailoring (November – December 2022).

4.1.8 Challenges, delays

The data transfer between the underwater robot host computer and the user's topside computer is challenging to optimize. For the moment, we record the raw data onboard the robot host computer to minimize frame dropping and latency. However, the logged data grows fast (approximately 1 GB every 30 seconds). Larger storage space is required for this approach if the scanning activity lasts more than a few minutes.

There is a trade-off between using the host computer's processing power to compress the image data to save on transmission bandwidth. If no compression is performed, then image and depth frames may be dropped due to data transport latency. If too much compression is performed, then the transmission rate (i.e. the number of image and depth frames sent per second) drops. If too many frames are dropped or if the transmission rate is too low, then RTAB-Map is unable to perform point cloud aggregation. Further testing and optimization of the integrated system (for example, replacing the host computer with a more powerful GPU accelerated machine capable of fast image encoding) for different expected use cases (such as recording, or online scanning) is required.

4.1.9 IP issue

Concerning the collaboration between DTU and Blue Atlas Robotics, the intellectual property of both parties is preserved as no proprietary information (e.g. designs, software, or algorithms) is shared, the aim is to evaluate the effectiveness of existing high TRL multi-view camera solutions on the mussel longline volume estimation application.

4.2 Biolan

Objectives Planned to be achieved M36	M36 achievements
Development of an improved biosensing device based on System on Chip (SoC) electronics.	Development of a IoT and miniaturized analytical device
Integration of the Bluetooth (BT) communication system	BT enabled for data acquisition and storage in cloud platform, App development
Development of an App for data capture	First connection to NORCE platform
	First trials on field by stakeholders

4.2.1 Analysis and tests performed the last 18 months

Miniaturization of sulphite biosensing device achieved based on System on Chip

A prototype of a miniaturised potentiostat device has been designed and developed in which the electronic components have been drastically reduced. The reduction has been based mainly on the use of a single chip (System-on-chip, SoC), which has made it possible to avoid the application of tantalum capacitors and to reduce the number of components from hundreds of references to a few dozens. In addition, the migration from electronic ink-based displays to TFT (Thin Film Transistor)-based displays has been carried out. As a result, a prototype with the following characteristics has been obtained:

- PCB (printed circuit board) including the SoC (Figure 6)
The SoC module controls and measures the biosensors and electrochemical sensors. It is a very low power consumption controller based on a microprocessor. The module has current, voltage and impedance measurement capabilities. The different elements are linked to the other modules via BLE (Bluetooth Low Energy) interfaces.
- TFT screen designed specifically for the device according to the mechanical structure according to specifications, LVGL (Light and Versatile Graphics Library) graphic library, landscape scree, defined resolution, black screen background (Figure 7)
- Housing according to the specification of the battery, the PCB and the display (Figure 7)

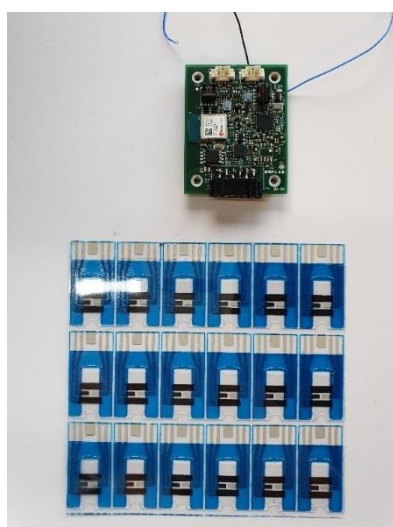


Figure 6: PCB including the SoC



Figure 7: Potentiostat device with the TFT screen

Method for sulphite monitoring in water based on new device developed and analytical verification

The developed device is applied for the quantification of sulphite in shrimp processing water by means of an enzyme-based electrochemical biosensor platform. The enzymatic biosensor is a system that incorporates a biochemical sensing element (based on a specific oxidative enzyme) in intimate contact with or in close proximity of a transducer system that relates the concentration of an analyte to a measurable signal. The addition of the analyte causes sequential redox reactions that involve a release of electrons proportional to the concentration of the analyte. In the case of the new developed device, the enzyme specific for sulphite detection is immobilized on screen printed electrodes, that are for one single use.

The screen-printed electrodes have been designed according to the new device. The new electrodes incorporate a plastic substrate cover that allows the sample to capillary enter and flow to the electrodes where the electrochemical measurement will be taken (Figure 8).

After the design of the new platforms, the screens were designed for the printing of the electrodes and the printing and assembly by plotter cutting and lamination of a small batch that was verified by electrochemical characterisation. Satisfactory results were obtained in terms of sample input and electrochemical measurements.

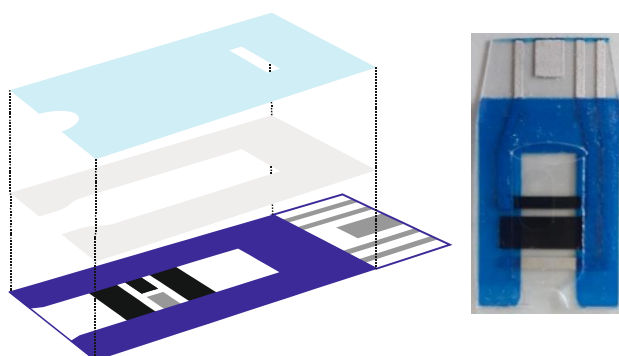


Figure 8: Design of screen printed electrodes to be applies to the new developed potentiostat

Based on this new design, the method for sulphite quantification in shrimp processing water was adapted and optimized. Two ranges have been developed:

Application	Ranges	LOQ	Recommended Matrix
Sulphite in water	5-30 g/L	5 g/L	Water
Sulphite in water	20-75 g/L	20 g/L	Water

Linearity of the method has been verified for both defined ranges by performing calibration curves (Figure 9)

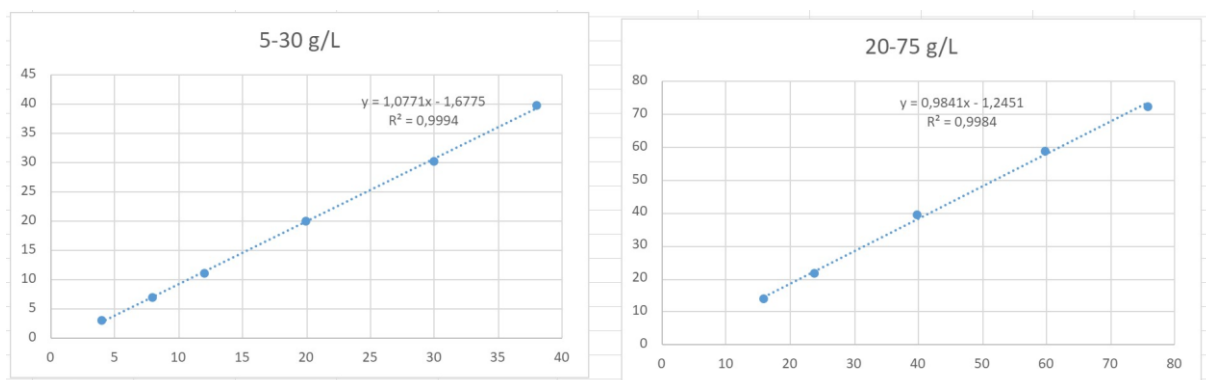


Figure 9: Calibration curves for the 5-30 g/L and 20-75 g/L ranges.

The performance of the new potentiostat device developed applied to the measurement of sulphite in shrimp processing water was verified by means of a reproducibility test, performing a calibration with 3 different potentiostat devices (Figure 10), and by carrying out a comparison test between the developed device and another potentiostat that is commercially available for research activities (Figure 11). Reproducibility and proper performance were demonstrated.

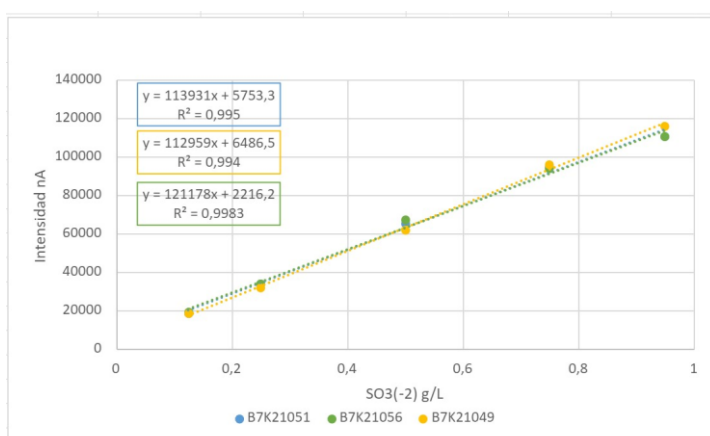


Figure 10: Calibration curves with 3 different devices

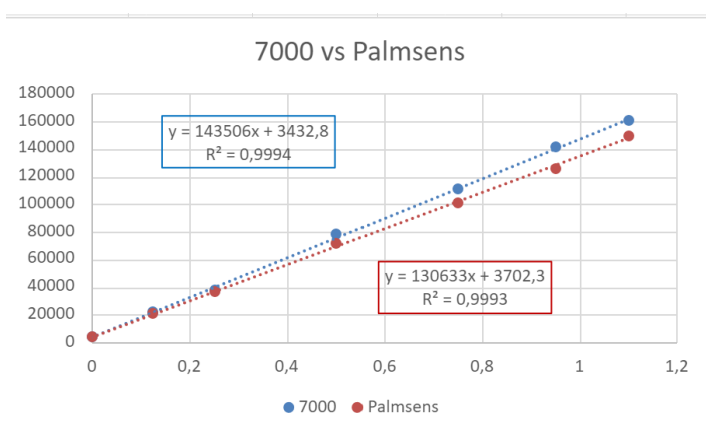


Figure 11: Comparison between developed potentiostat and a research device commercially available (Palmsens)

The stability of the electrodes modified with the enzyme for the specific detection and quantification of sulphite was assayed in an accelerated stability assay, demonstrating stability for 1 year at 4 °C (Figure 12)

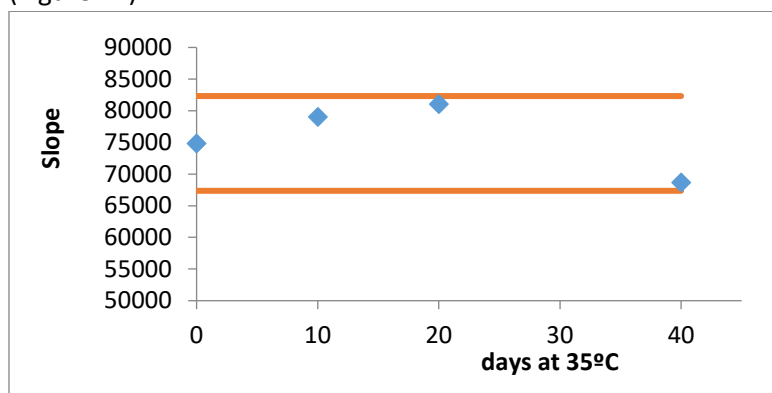


Figure 12: Calibration slope performed with electrodes from the same batches stored at 35 °C

Design of a calibration tool for device control

A calibrator, dummy cell, that allows customers to verify its correct operation has been developed (Figure 13). The PCB and its external design were designed. It is an electronic circuit that must be inserted into the equipment. Under the application of specific potential, the device has to obtained specific intensity values.



Figure 13: Dummy cell calibrator

Connectivity and data collection

BT was included for connectivity and enabled. A first version of an App (Android) has been developed to send the data from BIO7000 to the cloud (Figure 14).

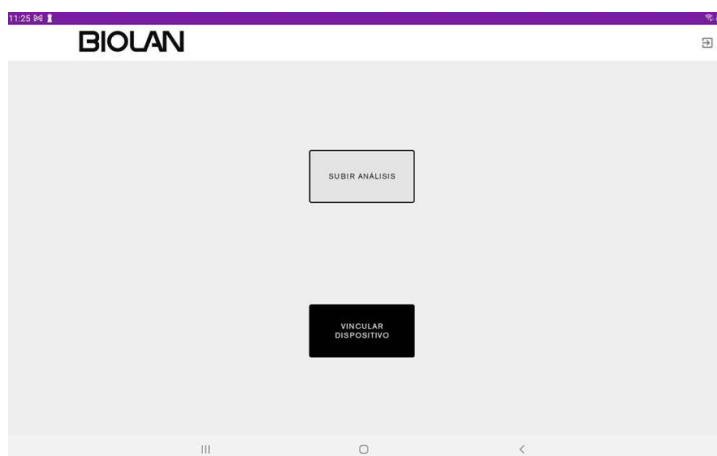


Figure 14: Screenshot of the BIO7000 App

BIOLAN has developed BIOLANGLOBAL, a cloud platform to collect all the data retrieved by the biosensors. The platform is already applied for collection of the data retrieved by the benchtop biosensor series (BIO3000) (Figure 15).

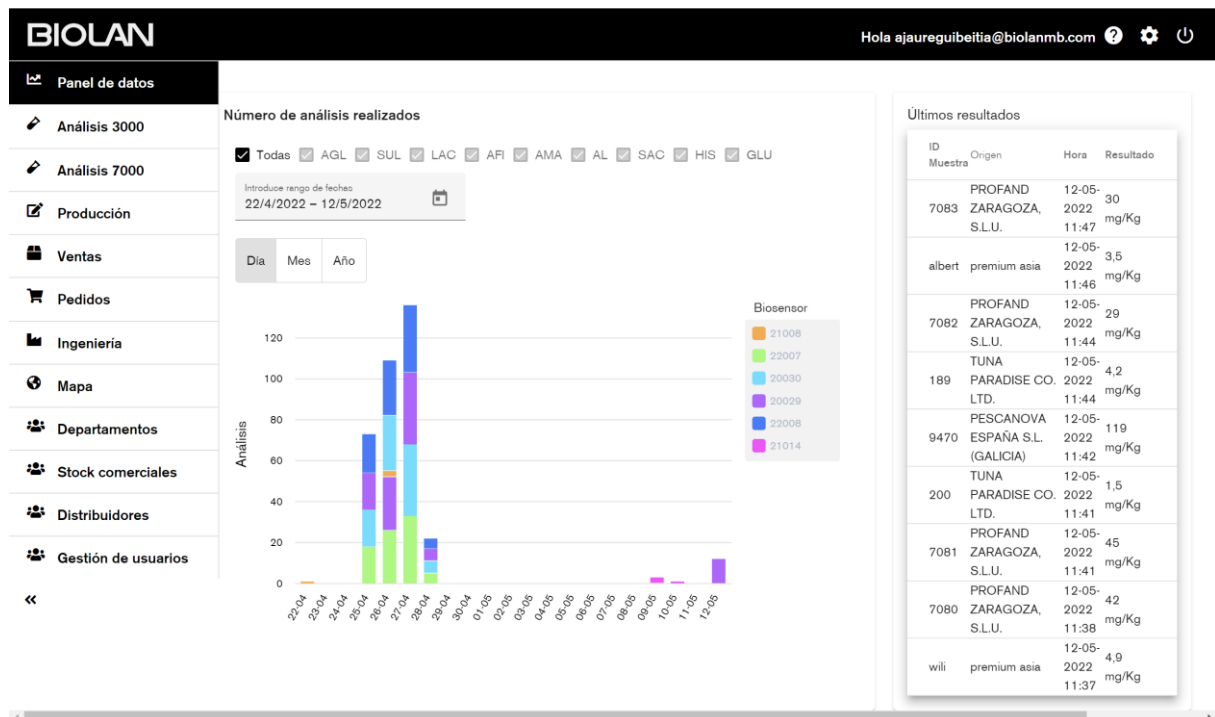


Figure 15: Screenshot of BIOLANGLOBAL cloud platform

During this period the use case of BIO7000 was developed in the platform in order to collect the data from the new devices through the developed App (Figure 16).

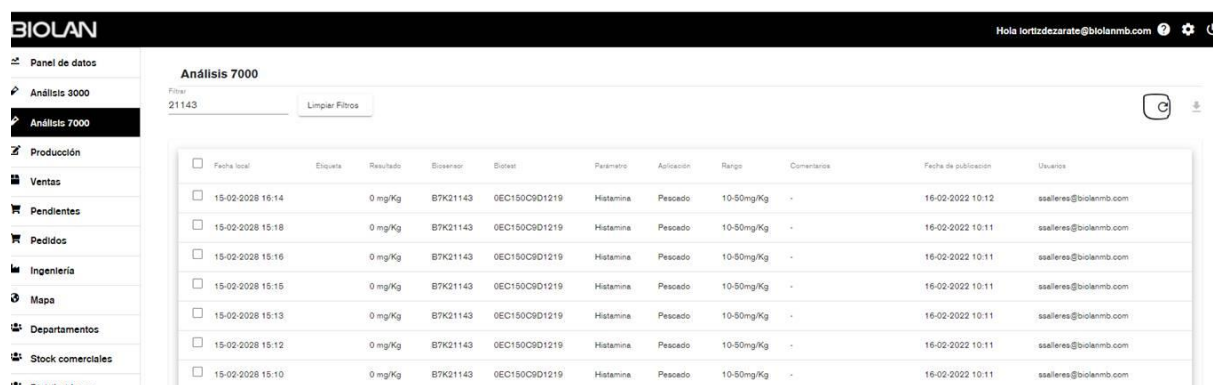


Figure 16: Screenshot of the BIO7000 App

Validation on field

First trials have been performed on field (Figure 17 and 18). The devices and associated reagents have been applied on field to measure the content of sulphite in the processing water where shrimps are introduced after harvesting.



Figure 17: Shrimp processing water measurement on field



Figure 18: BIO7000 showing the result of sulphite measurement on field

The applied methodology was as follows:

- Previous training in the lab in order to show field workers how to use the new device with trials applying solutions with specific concentration of sulphite following the instructions manual.
- After this initial training, analyses were performed close to a shrimp pool, detecting sulphite concentrations in the water where the shrimps are collected.
- Analyses were performed in the water in which shrimps were treated before adding the metabisulphite, in the first addition, after removing the first shrimps, in the second addition, and after removing this first addition.
- Samples were diluted in given tubes: 50 µl of water is added to 5ml of dilution solution
- Previous to add the sample to the strip: the code of the strip and the sample identifier have to be entered with the keyboard displayed by the device.
- The strip is inserted in the device with the electrical contacts facing the instrument and the sample channel facing upwards.
- 50 µl of this mixture is added to the strip with the help of a micropipette
- Once the device detects that the channel of the strip is completely full, the measurement process will start automatically and the result is displayed on the screen after 35 seconds, expressed as grams of sulphite per litre.



Results were only obtained in the device and not sent to connected devices. Collected data are shown in Table 1 with anonymized results since they are owned by the company where validation is performed.

Table 2: sulphite data collected from two companies

Company	Result	Device	Biostest	Parameter	Matrix	Range
	1 67g/L	B7K21160	1ABE001FBE02F	Sulfite	Water	20-75g/L SO2
	1 72g/L	B7K21160	1ABE001FBE02F	Sulfite	Water	20-75g/L SO2
	1 57g/L	B7K22001	21FBD0BCFF149	Sulfite	Water	20-75g/L SO2
	1 77g/L	B7K21091	1ABE001FBE02F	Sulfite	Water	20-75g/L SO2
	1 58g/L	B7K21154	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 38g/L	B7K21060	1ABE001FBE02F	Sulfite	Water	20-75g/L SO2
	2 24g/L	B7K21060	1ABE001FBE02F	Sulfite	Water	20-75g/L SO2
	2 33g/L	B7K21060	1ABE001FBE02F	Sulfite	Water	20-75g/L SO2
	2 54g/L	B7K22008	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 67g/L	B7K22008	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 50g/L	B7K22008	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 63g/L	B7K22008	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 64g/L	B7K22008	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 82g/L	B7K22008	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 86g/L	B7K22008	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 87g/L	B7K22008	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 41g/L	B7K21060	1B007020EA05E	Sulfite	Water	20-75g/L SO2
	2 62g/L	B7K21060	1B007020EA05E	Sulfite	Water	20-75g/L SO2

4.2.2 Collection of user's feedbacks

In addition, since the new device has been analytically validated, the most interesting results of this field validation were to collect the user experience. A survey was conducted among the companies that used the device for field validation (Fig 19).

SURVEY

**APPLICATION OF NEW PROTOTYPE OF BIOSENSOR
FOR SULFITE MONITORING IN SHRIMP
AQUACULTURE**

IDENTIFICATION OF USER

Company		
Country		
Activity	Harvesting	Processing
Annual production		

MEASUREMENT OF SULFITE

Do you perform sulfite measurements?	
Current method	
Concentration range	
Cost of analysis	
N° of samples analyzed/year	

USER FEEDBACK EXPERIENCE-NEW BIO7000

Where you using the previous portable device of BIOLAN to measure sulfite, BIO700?	Y/N
Do you find the new device easier to use than the previous one? Please, specify.	Yes, the sample application is easier Yes, the menu is easier Yes, the handling of the device is easier




Figure 19: survey on user's experience

Two companies were asked to fill this questionnaire. Both companies used the previous BIOLAN equipment to measure sulfite in the field. Their responses were:

- 2/2 found the method simpler
- 2/2 found the device menu simpler
- 2/2 found the device operation simpler

Both companies were willing to give this data, but anonymously.

Both companies are interested in the new evolution of the device and its connection to the cloud for data submission and integration.

Thus, the user experience with the new device and strips was very satisfactory. The users concluded that the method was much simpler than with the previous one.

4.2.3 Deviation, adaptation of the sensor in relation to the initial plans

The prototype as result of loop2 is not fully developed for some problems with BT pairing. Connection with Android devices is achieved, but still working for pairing with other devices. Work on this issue will be performed in the following months till a fully working version is achieved. The prototype is ready for field demonstration with PESCANOVA applying Android devices. Thus, the delay is not very critical.

4.2.4 Collaboration done and planned

- NORCE, we are collaborating in the connection of BIO7000 device with NORCE's platform.
- PESCANOVA, the new technology has been presented to PESCANOVA and we are planning the validation of the whole solution with them.

4.2.5 TRL level at the moment and expected at the end of the project

We have reached a TRL 5-6 and a TRL 8 is planned as a result of the project

4.2.6 Plan for the next 18 months

- Optimization of BT connection
- App optimization
- final design of communication with NORCE platform
- demonstration with stakeholders (PESCANOVA)

4.2.7 Challenges, delays

- Challenges with BT pairing.
- Adaptation to PESCANOVA's communication strategy or limitations.

4.2.8 IP issues

BIOLAN has the opportunity to implement the technology developed in the frame of AV in the shrimp aquaculture industry, as a first step to expand to new future applications. Due to the innovation and applicability of the device, other further applications will be sought by the company. BIOLAN already

has a registered patent that protects the electrochemical method for measuring sulphite in different samples based on enzymatic detection. The rights for further exploitation of results obtained by BIOLAN in WP4 concerning the sulphite measuring device will be owned by BIOLAN.

4.3 Norce

Objectives Planned to be achieved M36	M36 achievements
Stable version of the IoT platform, with support for automatic transfer and storage of data and simple visualization V2.	Achieved, now more stable, improved performance and with new functionality.
Test and evaluate the IoT platform in selected production cases.	This has been hampered due to covid and limited availability of sensors and sensor data from the AV CSs to integrate in the sensor platform. NORCE has designed a macroalgae PoC with own external sensors (GPS, accelerator sensors on buoys) and use these to provide data. Stakeholders are interested and involved.
Integration with UTOFI-camera and BIOFISH biosensor.	-UTOFI-camera replaced by 3D-cam, and not yet integrated. -BIOFISH 700 and BIOFISH 7000 integrated.

4.3.1 Analysis and tests performed the last 18 months

Integration of Biolan BIOFISH devices onto the sensor platform.

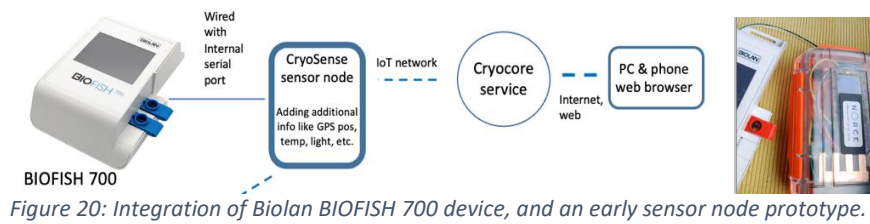
Integrating the Biolan BIOFISH devices with the sensor platform have opened several opportunities to the handling and enhancing of the data from the BIOFISH. The CryoSense sensor node have the potential to retrieve the data automatically and continuously from the BIOFISH and store it online in CryoCore, and with its onboard GPS it can tag each test sample result with accurate GPS position and time stamp. These test results will then be available to be viewed and managed on any computing device (phone, tablet, and computer) both during and after the BIOFISH sampling process. Using a dedicated CryoSense sensor node to retrieve data from the BIOFISH also alleviate the need for having a company or private smartphone available.

The GPS tagging and timestamping can be especially useful as it groups co-located sample results together and has the potential to provide simple overview of the location where the values are from. For farms with multiple pools or areas for sensor measurements, this will ensure a higher data quality and improve on ease of use compared to manual tagging.

Results with the BIOFISH 700

The BIOFISH 700 is the old device and is to be replaced by the 7000, and therefor this integration was just an early exercise for how to work with these devices. The 700 was integrated into the sensor platform using the Pycom sensor nodes but had to be demonstrated using a wired serial connection as it turned out to be difficult to get the Bluetooth working between the BIOFISH 700 and Pycom node. However, it was demonstrated that data can be automatically retrieved from the BIOFISH and transmitted onto the sensor platform, decoded, and shown in web browser on a PC or smart phone

(Figure 19). The effort of getting Bluetooth to work properly was not pursued further as the 700-device was to be replaced by a newer BIOFISH 7000 device.



Results with the BIOFISH 7000

The work on integrating the new BIOFISH 7000 device with the sensor platform is also ongoing. This time it has been demonstrated over Bluetooth, where the data is automatically retrieved from the BIOFISH 7000, transmitted onto the sensor platform, and available for access from PC or Smart Phones (Figure 20). However, currently this has been demonstrated using a Linux based sensor node and not the Pycom based sensor node. Limitations of the current Pycom Bluetooth implementation combined with the BIOFISH implementation makes the devices unable to connect to each other. NORCE is working together with Biolan towards solving this issue, and looking into workarounds.

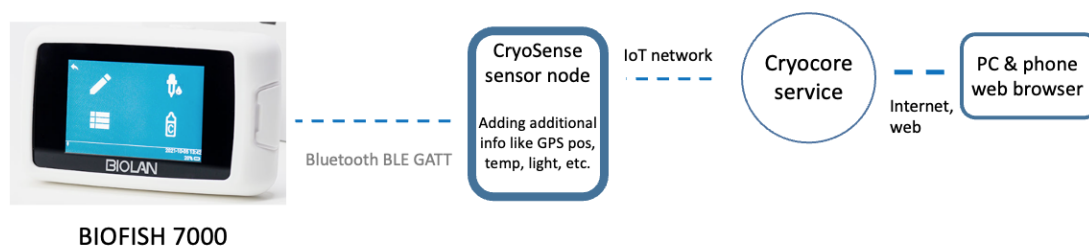
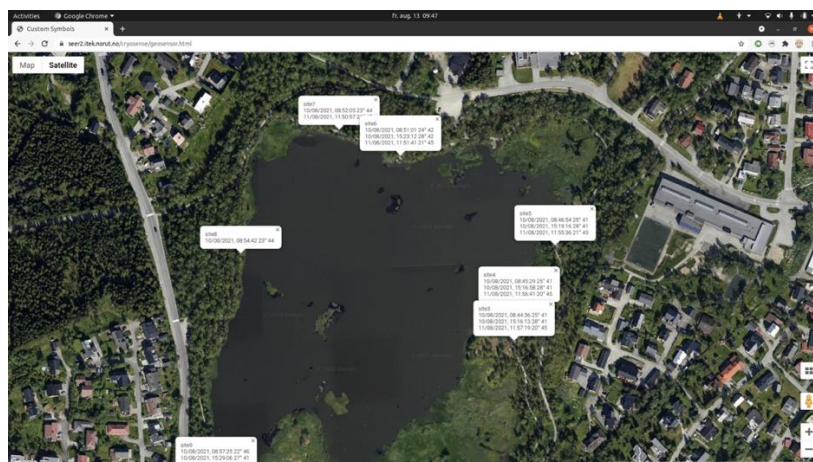
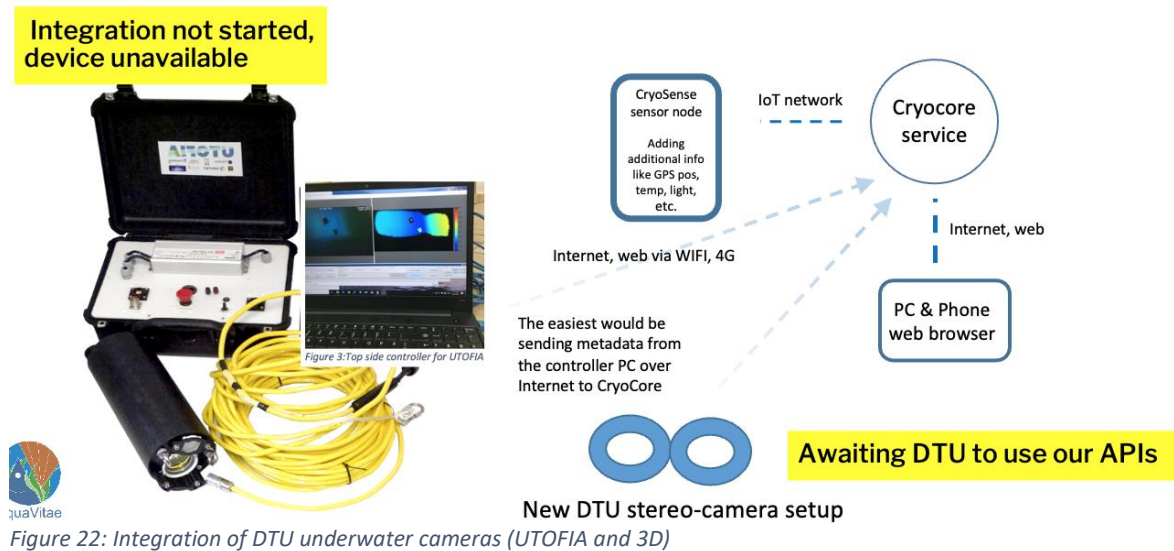


Figure 21, show how the GPS tagged data from the CryoSense sensor node is mapped onto a satellite image of a small lake.



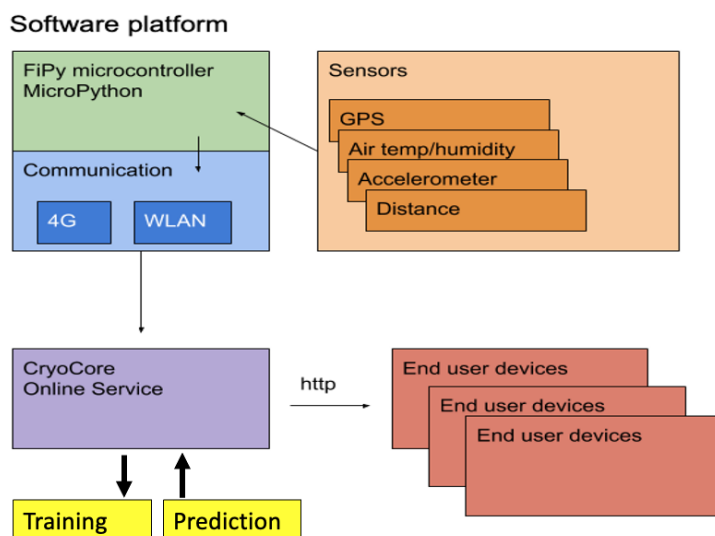
Integration of the DTU underwater camera system

This work on integrating underwater cameras to the sensor platform (Figure 22) has been delayed. Mainly due to the breakdown of the DTU UTOFIA-camera, and that the replacement 3D-camera is still under development. However, one approach for integration will be that the computer controlling the cameras will submit meta-data into the sensor platform over either Internet using CryoCore APIs, or via a sensor node and over an IoT-wireless network like LTE-M. DTU and NORCE are to discuss the details of this when the use case is ready.



Further development of the sensor platform

The IoT platforms (Figure 23) has been developed and tested further during the last period, and there has been performance and functionality improvements to both CryoCore and CryoSense. CryoSense now also support enhanced sensor nodes with embedded GPS and accelerometer (Pycom Pytrack 2.0 X). In addition, support for export of data to training has been implemented and will later be the base for prediction. The platform has been prepared for data export for AI training, and our proof-of-concept (described below) will seek to explore these possibilities when a dataset has been collected.



The sensor platform has now also been expanded to supporting the MQTT (Message Queuing Telemetry Transport) universal protocol, which is a standard widely supported in industry IoT cloud platforms. This has been demonstrated by NORCE with the sensor nodes sending data using MQTT to Pycom's cloud-based device management platform Pybytes 2.0, which again exported the data on to CryoCore. However, this gives less control of the working of the sensor node, particularly during variable communication coverage, so NORCE will continue to develop and use CryoSense on the sensor nodes. Both transfer mechanisms can be used inter-changeably.

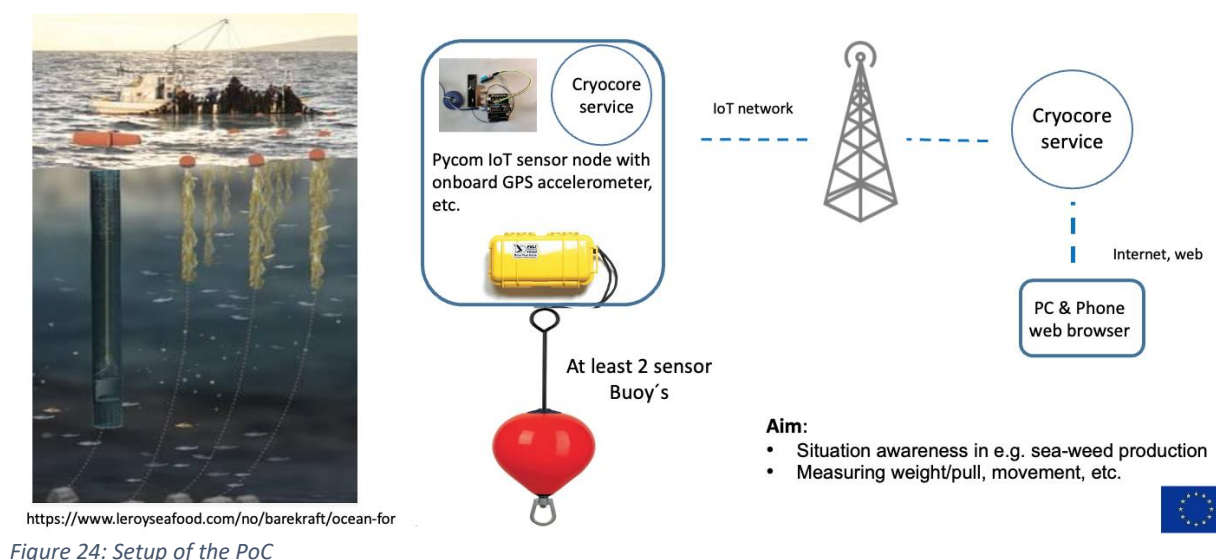
Design and implementation of proof-of-concept use case

NORCE is currently developing a PoC use-case to demonstrate and evaluate the applicability of the sensor platform on situation awareness in macroalgae production. A use-case that several of the partners in AquaVitae working with macroalgae has expressed their interest in.

The concept is to use sensor nodes to do long term measure of both biomass growth and monitor for unexpected movements due to weather/wear on macroalgae farm. The goal is to have a viable system that can run for 7 months without maintenance on a single battery. The Figure 24 shows the setup of the PoC.

The plan is to have at least two buoys, each with its CryoSense sensor node mounted on top. One of the buoys will be used for macroalgae farming, while the other buoy will act as reference and have no additional weight pulling on it. By using both GPS and accelerometer data from the buoys, we aim to train AIs to estimate biomass growth as well as monitor for unexpected movements – possible indicators of damage or high wear due to environmental factors.

The development of the POC will take place in several stages, where some are still ongoing during the summer of 2022. When this is satisfactory NORCE will set up test with two buoys in the sea, hopefully autumn of 2022. One possible location is the NOFIMA center for marine aquaculture at Kraknes outside Troms, where NOFIMA has offered space for doing the field tests.



Results developing proof-of-concept use case

Sensor nodes design

NORCE has assembled two sensor pack where each CryoSense box is equipped with a FiPY with a PyTrack sensor board hosting a L76GNSS GPS and a LIS2HH12 accelerometer as well as a 12000mAh battery pack. The sensors are placed in a small watertight Peli-case (Figure 25).



Figure 25: Sensor pack

Connectivity tests

The aim of this test was to get a very first impressions on challenges and opportunities when deploying and running the CryoSense boxes in the ocean. We would like to investigate robustness, connectivity, sensing, fault tolerance and consistency.

A kayak trip was done with both sensor boxes placed together to validate that they provide consistent measurements. GPS data was collected as well as accelerometer data.

Figure 26, shows part of the route. The GPS accuracy is < 2.5m CEP. The blue Box1 failed at the end due to a software bug, likely in the communication reconnection code.



Figure26: part of the route

The devices report positions that are fairly accurate. Only internal antennas were used, and they were mounted inside the kayak. The red device seems slightly more accurate.

Also, accelerometer data was collected from both devices, and as the figure 27 shows they are measuring a high level of similarity. The whole dataset is about 25 minutes long. The large changes are due to the waves coming from the southeast, hence there were no waves on the left side (northwest) of the island but substantially higher waves on the right (southeast).

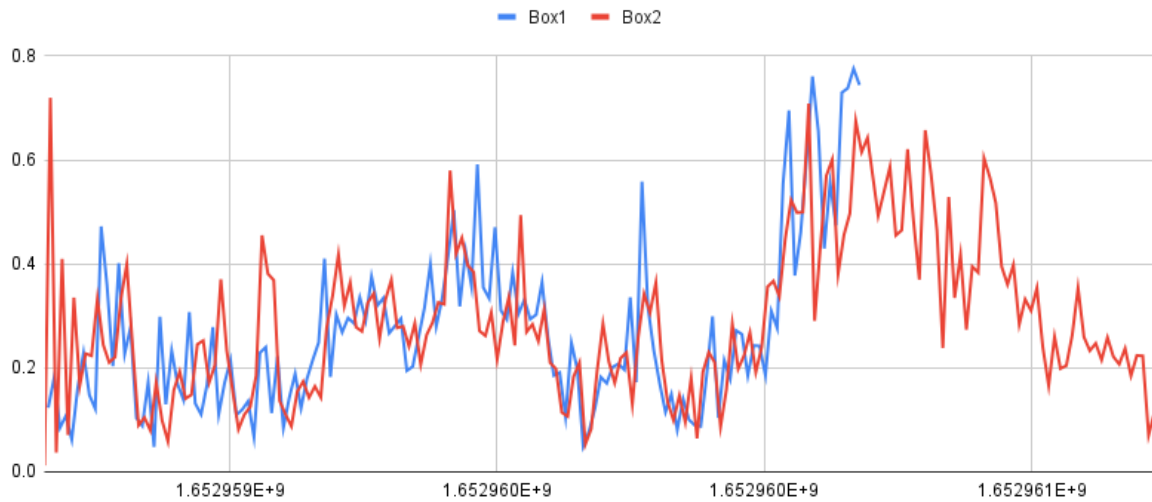


Figure 27 Accelerometer data (combined all axes) shows a high level of similarity

Test of sensors and buoys



Figure 28 Buoy test site

A test was performed using two 33cm diameter buoys placed close to shore in calm waters (Figure 28). Each buoy was mounted using rope to the sea floor. The tidal variance in the area is about 2.25 metres. They were also weighed down with a stretch of chain each to ensure that they were floating relatively straight up.

One of the buoys had an additional 25x25x25cm block of Leca attached close to the surface (Figure 29). This block has almost natural buoyancy but is severely affected by movement in the water such as waves. It was mounted using rope.



Figure 29 Leca block

On top of each buoy, a CryoSense box was fastened by a simple hook. This means that they are not mounted 90 degrees on the buoy, and as such, the x,y,z forces are not correctly depicted in any of the graphs. The buoys were left to measure for about 19.5 hours, experiencing some larger events as passing boats and ships created various waves.

The buoys were then deployed close to the shoreline (the image is taken at low tide). They were mounted a few metres apart, and the accuracy of the GPS means that the positioning data is somewhat overlapping. Also, the large tidal difference means that they were moving a fair bit during the period.

Figure 30 shows that Box2 (red) is slightly lower (to the south) than the Box1 (blue).



Figure 30 GPS reported positions of the sensor boxes (box1 blues, box2 red)

Figure 31 is taken from land, so the sensor boxes can be easily seen, with Box1 on the left and the Box2 to the right.



Figure 31 Sensor boxes and boyu's seen from land

The area is quite well protected from weather, so only very small background waves were present during the trial. Only a part of the collected data is visualised below, as the whole deployment is too long for detailed viewing. Several boat-passings can however be clearly seen, as they created events with larger and very well-defined waves. In the chart below (Figure 32), Box1 clearly has a higher level of movement than Box2. Box1 had the additional Leca block and was visually more active in the waves.

Box1 and Box2

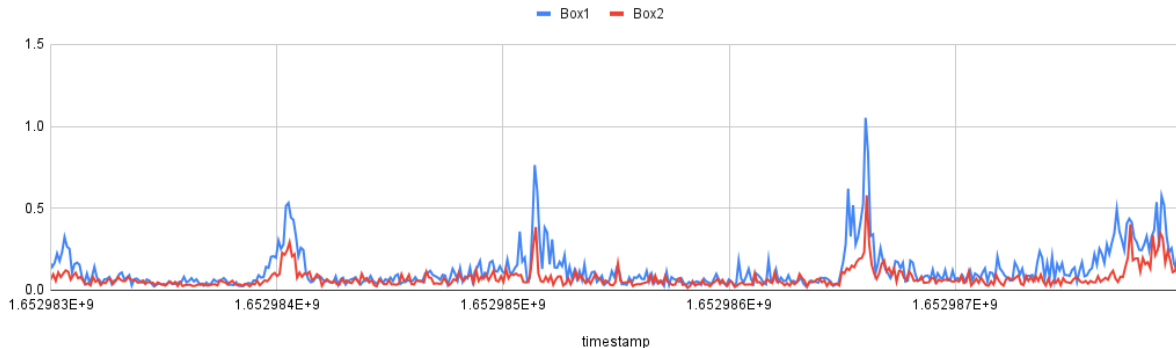


Figure 32 Sensor accelerometer readings (box1 blues, box2 red)

4.3.2 Deviation, adaptation of the sensor in relation to the initial plan

Access to test sites and identification of use-cease has been a challenge, as not many partners have ready-made sensors that could easily add value if integrated with the sensor platform. Therefore, NORCE decided to develop and evaluate an aquaculture proof-of-concept that contains embedded sensors that can provide a low-cost alternative to otherwise expensive sensors or costly manual observations. This setup has been deemed as relevant by several partners in the project as well as external companies, and we thus regard it as a useful and suitable change to the initial plan.

4.3.3 Collaboration done and planned

- AquaVitae partner NOFIMA has expressed interest in testing the buoy sensors related to macroalgae, both for site planning and production, and can provide NORCE access to initial testing site at their Kraknes marine aquaculture site outside Tromsø.
- A local macroalgae startup in Tromsø has shown interest for the buoy sensor system and have invited NORCE to evaluate it at their test locality when ready.
- AquaVitae partner Ocean Rain Forrest has also expressed interest in testing and evaluating the buoy sensor concept at some of their farms on the Faroe Islands.

4.3.4 TRL level at the moment and expected at the end of the project

- High TRL 5-6 on sensor nodes, network, protocols, and sensor and cloud platform as these has been evaluated and stable over long time, expect TRL 6 at the end.
- Lower TRL 4-5 on measurement method, accuracy, and analytics as these has not yet been evaluated in the macroalgae proof-of-concept, expect TRL 6 at the end.

4.3.5 Plan for the next 18 months

- Finalizing BIOFISH 7000 integration over Bluetooth
- Designing and building proof-of-concept multi buoy use-case
- Initial evaluation of proof-of-concept in sea
- Long(er) term evaluation of buoy-setup with stakeholders (Kraknes/NOFIMA, Ocean Rain Forrest Faroe Islands)
- Improve Data collection, analytics support, and visualization
- Integrate DTU-cameras with sensor platform

4.3.6 Challenges, delays

Access to test sites could still be a challenge, robustness of sensing method has uncertainties, and durability of sensor nodes and communication at sea could be a challenge.

4.3.7 IP issues

There are no particular issues with IP protection for Norce. They own the rights to the code written for the programming of the sensor integration platform.

5. Conclusion

The 3 partners have elevated their TRL level during the second development phase. DTU is making good progress with their mitigating solution to opt for a stereo camera as the initial UTOFIA camera was not available. The stereo camera being more affordable and smaller might offer more market possibilities turning this deviation into an advantage. Biolan have made progress with the connectivity of the device, developed their cloud platform, and miniaturized their device. They work in close connection with their main stakeholder PESCANOVA and will test their device on field soon. NORCE, due to a challenge regarding access to site and partners having ready-made sensors to test their sensor integrating platform on, has developed a proof-of-concept containing embedded sensors which provide a low-cost alternative for aquaculture producers compared to expensive sensors or manual observations. They have raised a lot of interest among several partners in AquaVitae and external ones, so this deviation was turned into an advantage, as was the case for DTU. To conclude, the development of the 3 sensors is on track and expected to have high TRL level at the end of the project and a high market value.

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