Traceability and Quality Monitoring throughout the Fish Value Chain

D2.4 Pilot Design and Piloting plan (v2.0)

DELIVERABLE NUMBER	D2.4
DELIVERABLE TITLE	Pilot design and piloting plan (v0.2)
RESPONSIBLE AUTHOR	George-John Nychas (AUA)

TraceMyFish is part of the ERA-NET Cofund BlueBio with funding provided by national sources [i.e., General Secretariat for Research and Innovation in Greece, Research Council of Norway, Innovation Fund Denmark and Icelandic Centre for Research in Iceland] and co-funding by the European Union's Horizon 2020 research and innovation program, Grant Agreement number 817992.

PROJECT ACRONYM	TraceMyFish
PROJECT FULL NAME	Traceability and Quality Monitoring throughout the Fish Value Chain
STARTING DATE (DUR.)	01/11/2021 (24 months)
ENDING DATE	31/10/2023
COORDINATOR	Panagiotis Zervas
COORDINATOR EMAIL	panagiotis@scio.systems
WORKPACKAGE N. | TITLE	WP2 | Tracking hazards & potential measures
WORKPACKAGE LEADER	NTNU
RESPONSIBLE AUTHOR	George-John Nychas (AUA)
RESPONSIBLE AUTHOR EMAIL	gjn@aua.gr
DATE OF DELIVERY (CONTRACTUAL)	28/02/2023
DATE OF DELIVERY (SUBMITTED)	31/03/2023
VERSION | STATUS	1.0 | FINAL
NATURE	REPORT
DISSEMINATION LEVEL	PUBLIC
AUTHORS (PARTNER)	Jørgen Lerfall (NTNU), Hildur Inga Sveinsdottir (MATIS), Anastasia Lytou (AUA)
CONTRIBUTORS	Sine Marie Moen Kobbenes (NTNU), Anita Nordeng Jakobsen (NTNU), Lemonia Fengou (AUA), Panagiotis Tsakanikas (AUA), Nette Schultz (VIDEOM), Alessia del Genio (VIDEOM), María Guðjónsdóttir (UoI)
REVIEWER	Panagiotis Zervas (SCiO)

VERSION	MODIFICATION(S)	DATE	AUTHOR(S)
0.1	ToC and draft	17/02/2023	Anastasia Lytou
0.2	Updates on section 2.1 and 3.1	03/03/2023	Jørgen Lerfall
0.3	Updated on section 2.2, 3.2	20/03/2023	Hildur Inga Sveinsdóttir
0.4	Updates on section 2.1	27/03/2023	Nette Schultz
0.5	Consolidation and final version	28/03/2023	Anastasia Lytou, Panagiotis Tsakanikas, George-John Nychas
1.0	Review and submission	31/03/2023	Panagiotis Zervas (SCiO)

PARTICIPANTS		CONTACT PERSON
SCiO P.C. (SCiO, Greece) **Coordinator**		Panagiotis Zervas E-mail: panagiotis@scio.systems
Department of Food Science and Human Nutrition, Agricultural University of Athens (AUA, Greece)		George-John Nychas E-mail: gjn@aua.gr
Department of Biotechnology and Food, Norwegian University of Science and Technology Science (NTNU, Norway)		Jørgen Lerfall E-mail: jorgen.lerfall@ntnu.no
Videometer A/S (VIDEOM, Denmark)		Nette Schultz E-mail: NS@videometer.com
Faculty of Food Science and Nutrition, University of Iceland (UoI, Iceland)		Maria Guðjónsdóttir E-mail: mariagu@hi.is
Matis (MATIS, Iceland)		Hildur Inga Sveinsdóttir E-mail: hilduringa@matis.is

ACRONYMS LIST

CA	Consortium Agreement
EB	Executive Board
MS	Monitoring Secretariat
PB	Project Board
PCO	Project Coordinator
WPL	Work Package Leader
TMF	TraceMyFish

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

EXECUTIVE SUMMARY

The objective of WP2 entitled as "Tracking hazards and potential measures" is to define and outline the corresponding settings of all three pilots of TMF project, so as to enable tracing the potential hazards across the Bluebio value chain affecting food quality and safety along three crucial value chains in Europe, and more precisely: 1) the Atlantic salmon value chain, 2) the Atlantic whitefish value chain, and 3) the Mediterranean seabream/seabass value chain. In addition to tracking potential hazards, WP2 is targeting to map the critical parameters affecting the quality and also the safety, by employing non-invasive sensors (VideometerLab and VideometerLite), laboratory respectively portable multispectral imaging instruments; meaning the use of non-destructive measures to be included in the iFish Management System (iFMS) for hazard control, prevention and alerting mechanism to be developed within the project. In this report, we present the modifications and adaptations of the previous Delivarable D2.3 concerning the identified variables, as they stem out from the mapping of the needs of the related stakeholders in tandem with the potential/envisioned hazards inherent along the three value chains considered in the project; a work performed in Task 2.1 (please refer to D2.1 for more details). Thus, moving forward from D2.3 and the outputs from Task2.1, we herein report the modification on the pilots' design and plans in terms of defining more precisely the appropriate parameters, measures, and sensor strategies to be implemented into the second iFMS pilot. The need for adaptation, extension and/or exclusion of some of the aforementioned measure and parameters has become apparent during the first piloting phase (which was based on D2.3). Since the selected three fish value chains have their specific challenges, the three responsible research partners; namely NTNU, AUA, and UoI have focused their efforts on the value chain of Atlantic salmon, Gilthead seabream/European seabass, and Atlantic whitefish, respectively. This deliverable consists the second and final version of the pilots' planning and design that will be performed, mainly focusing to the feasibility of the proposed scheme of tracking and quality assessment along the food chain with the employed, and updated according to the emerging needs after the first piloting phase, non-invasive instrument under real or close to real conditions. Additionally, as with the previous phase of the pilots, the goal is to populate a reference lake of appropriate datasets for model development. Models that will be able to predict, identify and report quality and tracking information along the food, fish "journey" from the initial production to the consumers' hands.

1 INTRODUCTION

Herein, we present the second version of the deliverables for Task 2.2., dedicated to the design of the overall pilot schemes that will be applied during the second piloting phase of the project, in terms of the three use cases considered in TMF. In this second and final version we present the adjustments occurred during the first pilots extending the first version, i.e., D2.3. To this end, the research partners, namely UoI, NTNU and AUA along with the suggestions of the industrial partners identified new or exclude some old parameters/conditions from the workplans, for a meaningful design of a continuous non-invasive monitoring of the products and/or processed products considered within the project (please refer to D2.1 and D2.3). The three use cases are harmonized under a common pilot design and in the previous deliverable since apart from the raw first material (type of fish), the rest of the value chain remains more or less the same, exhibiting small differences as the will be depicted next in detail. Briefly, the pilot variables that need to be defined correspond to the time and place of performing the data acquisition, where time and place refer to the different/several sites along the value chain; i.e., the production, transportation, food processing and storage in between the former ones. The definition of those variables is very critical in order to enable a sufficient and efficient, in terms of data completeness and coverage of the whole value chain.

Additionally, specific technological, sensor related issues and variables are identified during the evaluation and relative experimentation during phase 1 of the pilots, in a way that the applicability of the sensor along the value chain is ensured and judged as trustworthy in terms of results. This procedure is enabling the downstream effective design of the cloud services that will be provided to the end user of the TraceMyFish platform/solution. As before, regarding the aforementioned variables (in the monitoring phase), the definition of the technical variables for the pilots are mandatory for the design of a system suitable for efficient product monitoring and tracing, including the requirements in terms of accessibility, usability and acquaintance to the use technologies from the specific persons acquiring and inferring the data. Thus, in this second and final version of the pilots' design and variable identification we present the final, modified in some cases designs of the pilot studies and particularly their design and specifications (concerning mainly at data acquisition calibration and prediction targets), while the second will serve as an adaptation and finalizations of the pilots towards the acquisition of the evaluation and demonstration data for the TraceMyFish platform.

2 VARIABLES IDENTIFICATION ALONG THE FOOD CHAIN

Herein, we present our initial mapping of the critical parameters that affect, have profound impact on the quality and safety of the products along the fish value chain, as it will be followed by the corresponding three (3) pilots; i.e., 1) the Atlantic salmon value chain, 2) the Atlantic whitefish value chain, and 3) the Mediterranean seabream/seabass value chain. To this end, we outline the identified non-destructive measures to be included in the iFish Management System (iFMS) for hazard control, detection, and prevention, as planned to be developed and applied downstream in the project. We should mention that the below mentioned variables has been recognized during Task 2.1 and Task 2.2 and the accompanying deliverable D2.1 and D2.2, where the corresponding hazards and stakeholders' needs are mapped, for each pilot case independently. The identified variables will be employed and used towards the continuous non-invasive monitoring of the products as they assumed in the project.

The pilot variables that need to be defined correspond to the time and place of data acquisition, where time and place are referring to the different sites of the value chain (i.e., production, transportation, food processing and storage). In addition, any emerging hazards and unwanted incidents, e.g., changes in temperature while transportation or at storage, capable of causing adverse effects in the final or intermediate products across the food chain are identified and taken under consideration. Finally, hazard characterization, where the qualitative and/or quantitative evaluation of the nature of the adverse effects of contaminants (e.g., bacteria, parasites, etc.) present in the above-mentioned products (raw, processed, in/at storage facilities) along the value chain, are undertaken.
In this updated version some of the variables that will be examined throughout the project have been revised in order to be in accordance with Aquaculture concerns. Additionally, following partners discussion, the investigation of some parameters in common was decided, so as any comparisons among different species and value chains to be feasible.

2.1 SENSOR EMPLOYED THROUGHOUT THE PILOTS - UPDATED VERSION

Throughout the Pilots, Videometer's spectral imaging technology was employed for the collection, processing and analysis of data.
Spectral imaging is a scientific methodology which combines imaging with spectroscopy, allowing for the measurement of different spectra within the same image (Carstensen, 2018).

Spectral imaging, in the Videometer case, is achieved by placing a camera on the top of an integrating sphere, with LEDs of different wavelengths placed around its equator. The integrating sphere comprises of a hollow sphere with a highly reflective coating, which allows for uniformity in the scattering and diffusing effects of the LEDs' light rays (Figure 1). The LEDs strobe sequentially to capture a stack of images recorded at different wavelengths. Consequently, each pixel in the captured image represents a reflectance spectrum. The range of the Videometer technology includes ultraviolet, visual, and near-infrared wavelengths. Additionally, fluorescence can be captured by placing different filters in front of the camera. (Carstensen & Folm-Hansen, 2006).

Videometer's flagship instrument, the VideometerLab (Figure 1), captures images with up to 20 different wavelengths ranging from 365 nm to 970 nm.
In the TraceMyFish project, Videometer will provide a modified version of the VideometerLite system (VideometerLite - Videometer), shown in Figure 1, that will be used to collect data as input for the project iFMS. VideometerLite is a portable and wireless spectral imaging device designed for easy, straightforward, and accurate image analysis.
The analytical power of the technology offers a unique potential for fast characterisation of food integrity in terms of color, surface chemistry, texture, shape, and size without touching the sample and with little or no sample preparation.

Figure 1. VideometerLite, portable handheld spectral imaging device.

Using strobed LED systems, the VideometerLite efficiently combines the measurements of up to 11 wavelengths into a single spectral image, where each pixel corresponds to a different reflectance spectrum, wavelength range [365-850 nm]. Furthermore, it includes the presence of fluorescence filters, allowing fort the capturing, processing and analysis of fluorescence in food products. Thus, just as the VideometerLab, it includes UV, visual and NIR wavelengths for a precise, accurate, and thorough quality inspection foods.

2.2 THE ATLANTIC SALMON VALUE CHAIN

The Atlantic value chain, defined in D2.2, includes all steps from farming to convenient, consumer-friendly value-added retail salmon products. Unwanted incidents and hazards along the value chain were identified in D2.2 and are the basis for the pilot design presented in section 3.1.

2.2.1 Site of sampling/data acquisition – Use case 1

Data acquisition will be conducted from laboratory-designed experiments simulating different scenarios of unwanted incidents in the salmon value chain. Unwanted incidence includes factors such as 1) stress prior to slaughtering, and rough handling resulting in deviation of quality parameters, e.g., texture and colorimetric properties; 2) poor bleeding resulting in residual blood in muscle challenging the visual perception of portionated raw- and cold-smoked salmon products; 3) melanin spots, and; 4) loss of freshness and faster spoilage and a challenged food safety due to contamination or a broken cold-chain. To understand the potential to use the VideometerLite technology to measure changes in freshness and spoilage, data will be acquired through several storage experiments following both whole head-on-gutted (HOG) fish and fillets throughout their shelf-life.

2.2.2 Technological and sensor related issues and variables

Non-destructive analysis applying VideometerLight and the VideometerLab instrument will be conducted in tandem with traditional chemical, physicochemical, and microbiological laboratory methodologies (2.1.3) to assess the VideometerLight device's performance to measure different quality parameters of salmon along the value chain. Temperature and pH will be monitored with in-house portable loggers.

Different instruments will be applied to verify the results obtained by VideometerLight. Examples are DigiEye System to measure the fillet's appearance (e.g., melanine- and blood spots) and colour, and the samples texture properties will be measured by Texture Analyser TA-XT

2.2.3 Qualitative and/or quantitative variables

Traditional chemical, physicochemical, and microbiological laboratory methodologies that fit the specific experimental setup will be conducted. Relevant quality parameters include colour, texture, melanin- and blood spots, and fillet gaping. Microbiological quality will include analysis of aerobic plate counts (APC) and *Pseudomonas* counts, while *Listeria monocytogenes* will be used as a food safety indicator. The collected data from traditional wetlab-analysis will play an essential role in assessing the VideometerLight device.

2.3 THE ATLANTIC WHITEFISH VALUE CHAIN

The Atlantic whitefish value chain, defined in D2.2, includes all steps following catching to consumer retail products. Unwanted incidents and hazards along the value chain were identified in D2.2 and are the basis for the pilot design presented in section 3.2.

2.3.1 Site of sampling/data acquisition – Use case 2

Data acquisition will be and is being performed through laboratory and controlled and monitored industrial experiments simulating real circumstances. The experiments focus is to evaluate ways to implement multispectral imaging solutions, VideometerLite mainly, into the value chain as a monitoring and/or diagnostic tool. Within the value chain there are various quality parameters and hazards monitored and some could be evaluated using this technology, e.g.:

- Freshness of whole fish through changes in the visual appearance of fish, e.g., eyes, gills, using the QIM scale and chemical analysis as reference.
- Detection of fillet flaws, such as blood spots and skin remnants, and parasites, nematodes, in/on fillets
- Evaluation of processing level and quality of salted cod products, e.g., identification of yellowing due to oxidation on the surface.
- Evaluation of side raw material quality and composition for processing management

2.3.2 Technological and sensor related issues and variables

Non-destructive analysis applying VideometerLight and the VideometerLab instrument will be conducted alongside chemical, physicochemical, sensorial and microbiological testing to determine the VideometerLight device's ability to measure, detect and/or predict relevant parameters along the value chain.

2.3.3 Qualitative and/or quantitative variables

The reference analysis performed will vary based on the subject at each time. The most relevant chemical and microbiological parameters evaluated will be composition, total volatile nitrogen (TVB-N), products of lipid oxidation and hydrolysis, evaluation of total viable count and other specific microorganisms. Further analysis of physical properties of samples will be performed, e.g., texture analysis and pH, and finally sensorial methods will be used to evaluate changes in flavour, odour, texture and appearance when relevant. During sensory analysis a trained sensory panel will be used.

2.4 THE MEDITERRANEAN SEABREAM/SEABASS VALUE CHAIN

The Mediterranean seabream value chain, defined in D2.2, includes all steps from fish farming to seabream products on retail available for consumers. Unwanted incidents and hazards along the value chain were identified in D2.2 and are the basis for the pilot design presented in section 3.3.

2.4.1 Site of sampling/data acquisition – Use case 3

For data acquisition, industrial experiments simulating real circumstances will be performed in laboratory. The experiments will focus on the investigation and evaluation of ways to implement multispectral imaging solutions, VideometerLite mainly, into the value chain as a monitoring and/or diagnostic tool. Within the seabream value chain there are various quality parameters and hazards monitored and some could be evaluated using this technology, e.g.

- Microbiological quality and freshness
- Freshness based on sensory evaluation of the products
- Estimation of freshness based on the changes in fish eye appearance
- Changes in textural parameters etc.

2.4.2 Technological and sensor related issues and variables

Non-destructive analysis applying VideometerLab, VideometerLite 1 and VideometerLite 2 instruments will be conducted alongside physicochemical, sensorial and microbiological testing to determine the VideometerLite device's ability to measure, detect and/or predict relevant parameters along the value chain.
Texture analysis will be performed using a TA HD plus, Texture Analyzer (Stable micro systems).

2.4.3 Qualitative and/or quantitative variables

Conventional physicochemical, microbiological and sensory laboratory methodologies relevant with the specific experimental design will be conducted. Relevant quality parameters include texture, sensory and microbiological quality. Microbiological quality will include analysis of aerobic plate counts (APC), *Pseudomonas* counts, sulphur producing bacteria, bacteria of Enterobacteriaceae family, *B. thermosphacta* etc., while *Listeria monocytogenes* will be used as a food safety indicator. Data that will be acquired from conventional analysis will be used for the investigation of the VideometerLite device potential to assess seabream quality.

3 PILOT DEFINITION & DESIGN

This section is about the overall design of the pilots' scheme that will be applied across the project, in terms of the three use cases considered in the project. Thus, by incorporating also the information extracted and accumulated in Tasks 2.1 and 2.2 (as reflected and expressed in D2.1 and D2.2). The three use cases will be harmonized under a common pilot design since apart from the raw first material (type of fish), the rest of the value chain remains more or less the same. As state in the proposal, Task2.2 and consequently the two related deliverables are divided in terms of time, into two periods. Herein, i.e., the very early period, will be dedicated to the design of the pilot studies and particularly their design and specifications (for the calibration data acquisition), while the second one (D2.4) will serve as an adaptation and finalization of the pilots towards the acquisition of the evaluation and demonstration data towards the evaluation of the TMF platform.

3.1 THE ATLANTIC SALMON VALUE CHAIN

In this Section we provide the designed workplan, which also will serve as a data acquisition process pipeline, data that will be used for the development and validation of the prediction/estimation models in the case of the Atlantic salmon value chain. The sampling protocols include small and medium scale experiments simulating unwanted incidents and hazards along the value chain (the sampling plan is tentative and will be updated according to collaboration with other ongoing projects/activities).

3.1.1 Issues related to pre-slaughtering stress and fish handling

Stress and rough handling of fish affects textural and colorimetric properties. To obtain fish with a texture gradient related to stress and rough handling, fish from an ongoing project funded by the research council of Norway (project 321586) will be used. After conducting a feeding trial with three experimental fish feed, a stress experiment was performed over an intensive period of 14 days, having unstressed fish as controls (Figure 2). The design was then used to evaluate the potential of VideomterLite and VideometerLAB2 to evaluate textural- and colorimetric parameters. The results were validated by comparing multispectral data obtained from the Videometer devices with data obtained from traditional methodologies measuring textural properties (penetration test) and colorimetric properties (DigiEye imaging, SalmoFan, and muscle pigment concentrations). All measurements were performed on the Norwegian quality cut of fresh fillets at day one postmortem, as well as after six days refrigerated storage:

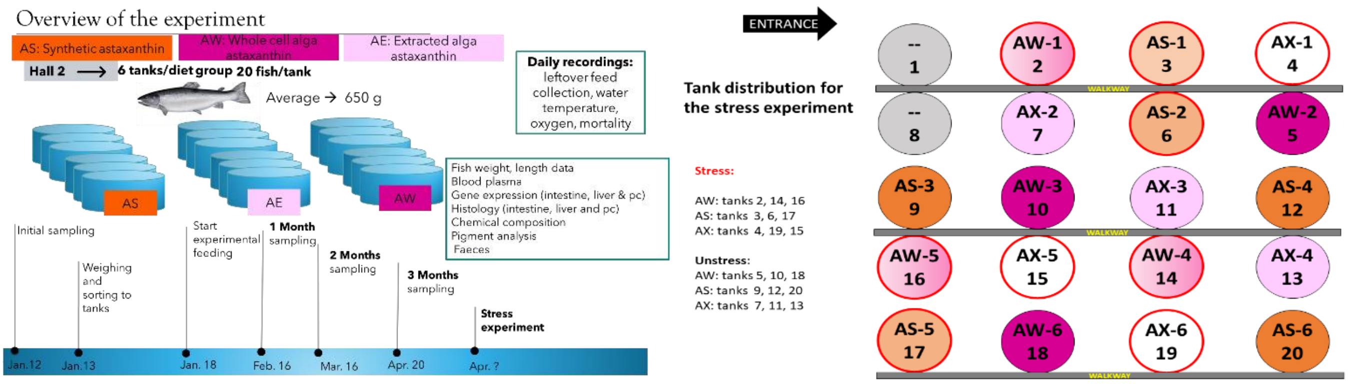

Figure 2. The experimental design of which the fish was used to study the potential of Videometer devices to measure the textural and colorimetric properties of Atlantic salmon.

3.1.2 Issues related poor bleeding and residual blood

Poor bleeding happens, and residual blood in the salmon fillets is a concern for the salmon processing industry. An experiment is designed with different bleeding protocols to obtain fish with a muscle blood gradient (Figure 3). The worst-case scenario will be unbleed gutted fish, filleted, with no further handling to remove the blood. The other groups will consist of gill-cutted gutted fish bled in a make-shift Chilled Seawater System (CSW) for either 15, 30, 45, 60, or 90 min before filleting. The sample size will be a minimum of five individuals, giving 10 fillets per group, of which each experimental group has different residual blood levels. Additionally, fillets will be vacuum packaged and stored for 21 days to realise muscle blood to the fillet surface for improved calculations. The experiment will be conducted in April/May 2023 at the NTNUs salmon research farm in Romsdalsfjorden, Norway. We will use the VideomterLite to measure residual fillet blood on-site and repeat the measurements after arrival NTNU's laboratory on day one post-bleeding. When measurements are repeated, the multispectral blood data will be compared to the visual blood score and DigiEye measurements.

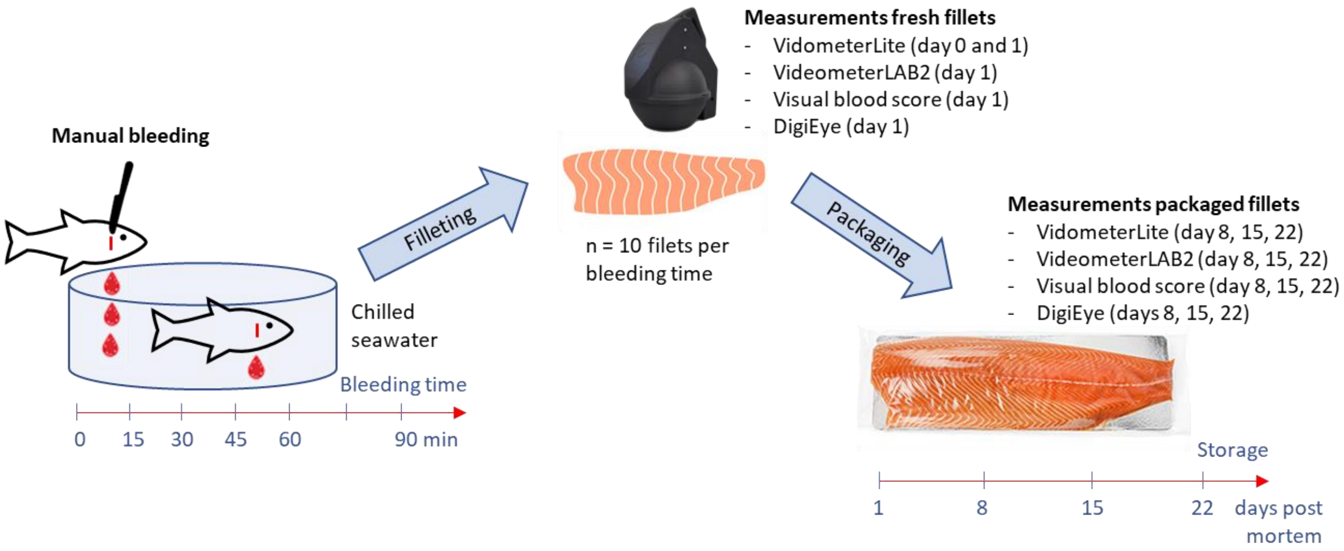

Figure 3. The experimental design of which the fish was used to study the potential of Videometer devices to measure residual blood in Atlantic salmon filets

3.1.3 Issues related melanin spots

Downgraded fillets (fillets containing melanin) were obtained from a nearby salmon processing plant to obtain multispectral data of fillets showing melanin spots. The experiment was conducted twice, of which the first was evaluated using VideometerLAB2, whereas VideometerLite, VideometerLAB2, and DigiEye were used to obtain data in the second one. In both experiments, images of the melanin spot itself were captured, and a nearby area was used as a reference (Figure 4). Fillets used in these experiments were selected to give a visual gradient melanin score from light grey to dark black.

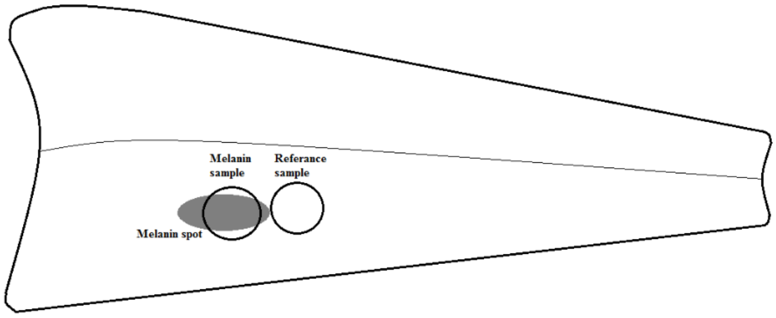

Figure 4. Schematic illustration of sampling locations on fillets containing melanin spots

3.1.4 Issues related to loss of freshness and microbial spoilage

Laboratory experiments will be conducted to simulate loss of freshness and microbial spoilage for salmon products packed and distributed under different conditions (whole fish on ice, vacuum -and modified atmosphere packed). Atlantic salmon will be slaughtered at a nearby slaughterhouse and transported to NTNU for further freshness and microbial spoilage evaluation. To ensure a similar chemical composition of the experimental samples, the back- and mid-loin running from the gills to the Norwegian quality cut (NQC) will be used to prepare uniform experimental samples that will be randomly distributed within the planned storage designs.

The first experiment (Figure 5) was designed to test the potential of the VideometerLite and VideometerLab2 to evaluate quality changes (freshness and microbial spoilage) as a function of storage time for the whole salmon stored on ice. The sample size will be a minimum of ten individuals. The traditional Quality Index Method (QIM) scheme for raw, farmed HOG salmon will be compared to VideometerLite and LAB2 images of eyes, gills and skin. Traditional QIM measurements includes a sensorial evaluation of colour/appearance of skin folds, mucus, and smell of skin; colour and form of eyes; cut surface and blood in the abdomen, membrane on the inside of abdomen and odour of abdomen; colour/appearance of mucus and odour of gills; and texture (elasticity) of flesh. In addition to QIM scores, the microbial growth on skin and gills will be correlated against imaging data obtained from the Videometer devices to better understand the potential of the VideometerLite prototype for measuring loss of freshness and spoilage of HOG salmon as an alternative to the QIM evaluation currently used along the Atlantic salmon value chain.

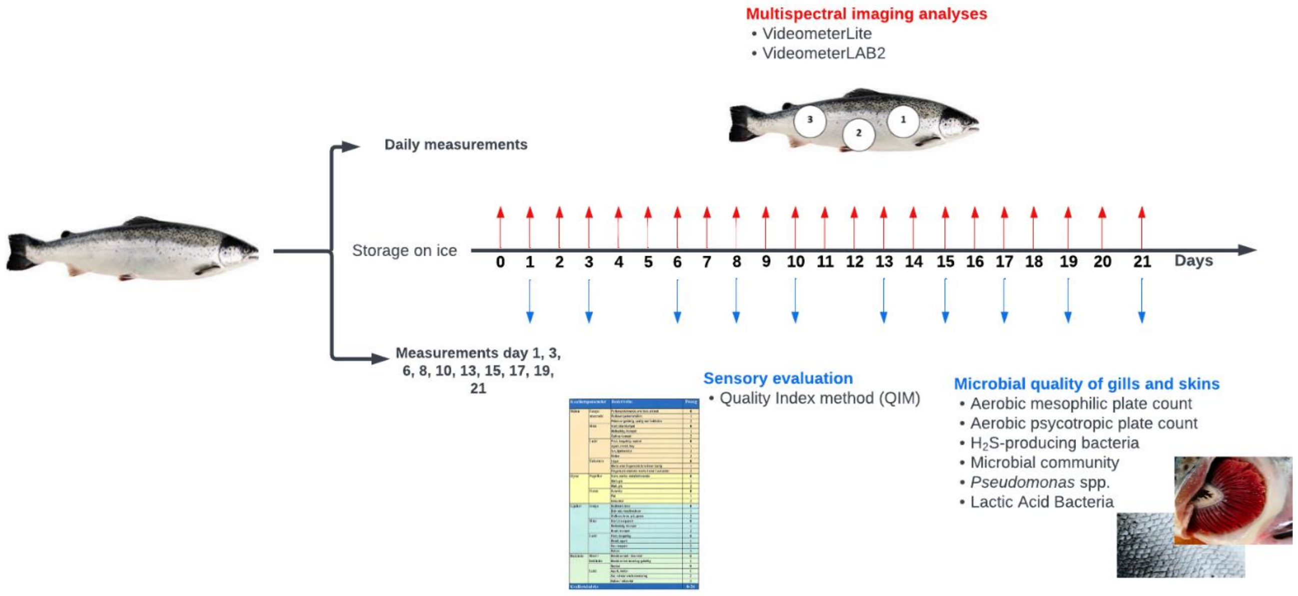

Figure 5. The experimental design of the fish was used to study the potential of Videometer devices to evaluate quality changes (freshness and microbial spoilage) of HOG salmon stored on ice as a function of storage time.

The second experiment (Figure 6) will be designed to test the potential of the VideometerLite and VideometerLab2 to evaluate quality changes (freshness and microbial spoilage) as a function of storage time for Atlantic salmon fillets distributed under different conditions (on ice or packaged in either vacuum or a modified atmosphere). The storage condition for packaged portions will be 4 and 8 °C to simulate different storage scenarios. Based on the results from the second experiment we have an option to initiate a third experiment as a challenge study, where salmon fillets are inoculated with known spoilage bacteria (e.g., *Pseudomonas*, *Photobacterium*), and quality parameters will be assessed during storage at different storage conditions (vacuum -and modified atmosphere packed). Un-inoculated salmon will then be applied as a control group for inoculated samples.

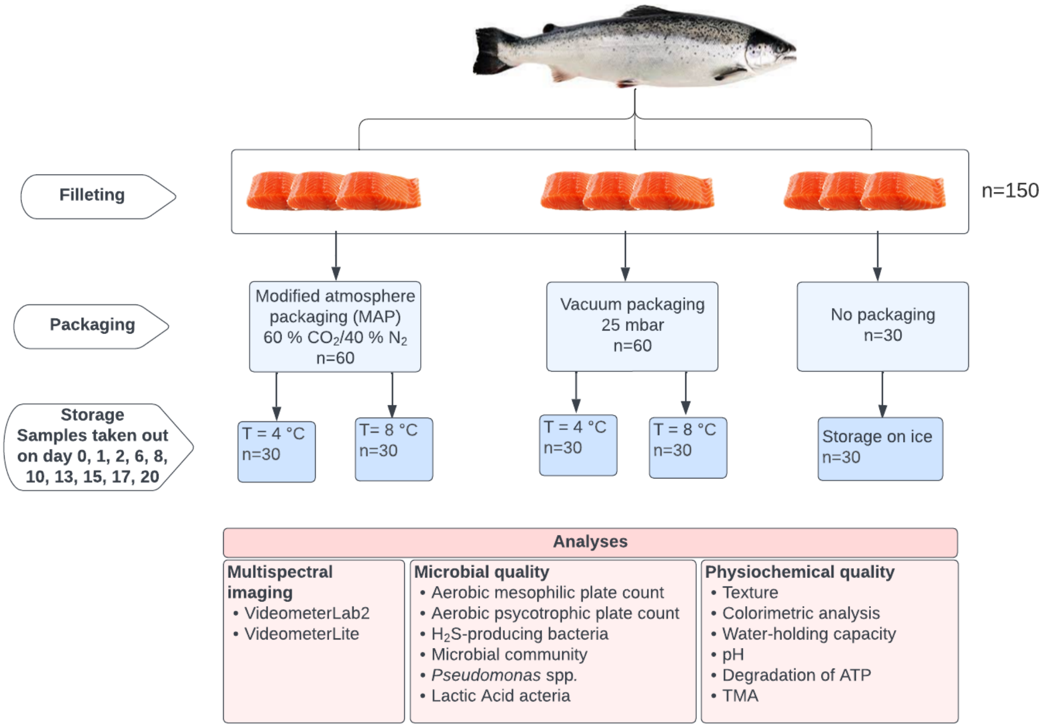

Figure 6. The experimental design of which the fish was used to study the potential of Videometer devices to evaluate quality changes (freshness and microbial spoilage) of salmon fillets as a function of storage time.

Microbiological- and physiochemical quality parameters will be evaluated using VideometerLab2, VideometerLite, and traditional wet-chemistry and microbiological methodologies to monitor quality changes during storage time (6-10 times) in all experiments.

Microbiological analysis: The microbiological profile of the samples will be analysed using a variety of agars (Table 1). Molecular analysis of microbial communities will be applied if required.

Table 1. **Culture media, target microorganisms, and incubation conditions for the microbial profile**

Culture media	Target microorganisms	Incubation conditions
Iron agar	Aerobic mesophilic bacteria and H_2S-producing bacteria	22°C for 3 days
Long and Hammer agar	Psychrotrophic aerobic bacteria	15°C for 6 days
Man, Rogosa and Sharp agar	Lactic Acid Bacteria	25°C for 5 days
Pseudomonas Agar + CFC (centrimide,fucidin,cephaloridine)	*Pseudomonas* spp.	25°C for 2 days

Physiochemical analyses: The salmon fillet's textural, colorimetric, and water-holding properties will be evaluated as affected by storage time. These data will be obtained using a texture analyser, imaging, and in-house methodology for water holding capacity (based on centrifugation), as well as VideometerLite and VideometerLAB.

Sensory evaluation: In some experiments, simple sensory evaluation of salmon samples will be conducted by a semi-trained panel of 6-8 members. The freshness of the samples will be evaluated using a 5-point scale (1 - like very much, 2 - like moderately, 3 - neither like nor dislike, 4 - dislike moderately, 5 - dislike very much).

The experiments will be conducted in Q1-Q2 in 2023 in NTNU's laboratory.

3.2 THE ATLANTIC WHITEFISH VALUE CHAIN

In this Section we provide the designed workplan, which also will serve as a data acquisition process pipeline, data that will be used for the development and validation of the prediction/estimation models in the case of the Atlantic cod value chain. The samplings include small and medium scale experiments simulating quality changes along the value chain. Following discussions within the consortium and Icelandic pilot partners a slight change in focus was made to ensure more harmony between pilots, allowing more comparisons to be made between the different species being evaluated within the project. Therefore, analysis of fully salted cod and side streams was set aside and a more detailed plan focusing on the fresh fish value chain, both whole fish and fillets, set in place.

The main focus in the TraceMyFish project will be laid on the analysis and monitoring of the fresh fillet production at BRIM in Reykjavík and the freshness/quality of whole fish during storage. Samples will be taken throughout the processes and analysed with the VideometerLite and VideometerLab instruments, along with a range of traditional microbiological, physicochemical and sensorial reference analyses to obtain a detailed overview of the quality changes occurring and identifying potential hazard points during processing. The obtained data from the Videometer technologies will be compared to the traditional quality assessment method results to investigate the strength and accuracy of potential quality prediction models built from the acquired spectroscopic and image analysis data. The sensitivitiy of the TraceMyFish technologies to identify parasites (such as nematodes) will also be assessed in collaboration with the Icelandic research project "Greining á hringormum í flökum / Assessment of nematodes in fillets", which is funded by the Icelandic Food Research Fund/Matvælasjóður. The planned and ongoing activities focus mainly on the following topics:

- **Freshness of whole fish:** Whole fish freshness is generally evaluated through sensory evaluation, visual inspection of eyes, gills and skin and evaluation of odour of gills (QIM scale). This type of evaluation is labour intensive and employees need to be trained to ensure replicability and consistency between assessors. Therefore, trials have been planned in alongside trials within the Norwegian salmon pilot to compare evaluation of sensory analysis as well as relevant physicochemical and microbiological analysis to spectral data from the VideometerLab and VideometerLite. The trials will take into consideration factors such as fish size, storage conditions, chilling methods and are planned for spring and summer of 2023.

- **Parasite detection:** Trials have been performed evaluation the possibility of using VideometerLab and/or VideometerLite for parasite detection (nematodes) in fresh fillets. The trials were performed first in laboratory conditions evaluation the possibility for detection (based on e.g. appearance of the nematode and the depth at which it is embedded in the fillet) and to determine if artificial intelligence could be used for the data analysis and detection. Further, trials were performed in industrial conditions where the technology was compared to traditional methods, nematode removal (candling) by a trained employee. Data analysis is ongoing.

- **Blood spots:** Possibilities for detection of blood spots in and on fillets will be evaluated. A pre-trial has been performed and data analysis is underway, the results of the pre-trial will be used to finalise a sampling plan for a larger trial. Further, participants in the Icelandic white fish pilot and Norwegian salmon pilot have been collaborating on sampling protocols and methodologies used to ensure the data can be compared between the species.

Fillet freshness: A trial has been planned to test the potential of the VideometerLite and VideometerLab2 to evaluate quality changes (freshness and microbial spoilage) in cod fillets throughout its shelf life in cold storage. The fillets will be kept under different conditions and the storage conditions set to emulate known possible temperature ranges in the value chain. The trial set up takes sampling protocols of the Norwegian salmon pilot into consideration to ensure the data is as comparable as possible.

3.3 THE MEDITERRANEAN SEABREAM/SEABASS VALUE CHAIN

The initial workplan designed for the seabream/seabass value chain was presented in D2.2. The main focus of the first plan was the estimation of the microbiological quality of seabream samples by using multispectral imaging analysis (VideometerLab2, VideometerLite). In this Deliverable, the final workplan and overall experimental procedures relevant with this workplan are provided. Following discussions with TMF partners and aquaculture industry, some modification in the first workplan were decided in order to be harmonized with the other two pilots and also investigate quality parameters that are of Aquaculture's interest. Data that will be acquired from these experimental procedures will be used for the development and validation of the prediction/estimation models in the case of the seabream and seabass value chain.

3.3.1. Microbiological quality of seabream

Fish samples will be monitored for microbiological contamination using the VideometerLab and VideometerLite instruments, while they will also be analyzed with conventional microbiological techniques, that will be used as the reference metrics, forming the "ground truth" of microbiological contamination levels.

In an attempt to collect data by representative samples from the whole seabream value chain, samples from both the Aquaculture site directly (~ 200 samples from two different harvest periods) and from different selling points (~150 samples) both aerobically and vacuum packaged, stored at different temperature conditions (2 and 4 °C) will be analyzed. The total aerobic population will be estimated at certain time points of storage, while at specific time points the population of specific microbial groups related to seabream spoilage will be also assessed. In the first concept (samples from Aquaculture directly) we had the opportunity to analyze fresh samples throughout storage, up to the time point that they are considered as spoiled. The second concept, apart from being representative of another stage of the value chain- it can also serve as a survey for this type of products, as samples with different shelf-life from various selling points will be analyzed.
In parallel with these analyses, 8 specific fish samples (two replications (4x2)) stored at 4 °C will be also subjected to microbiological and MSI analysis using VideometerLite 1 and VideometerLite2. The difference from the previous scenarios was that in this case, the same sample (of the same fish) was analyzed every day in order to monitor changes occur in the same organism throughout storage. In this way, we exclude from the analysis any variation originates from the fish organism (texture, shape, color etc.).
Covering the above-mentioned scenarios, we managed to analyze samples from different stages of the value chain, including the origin spot (analysis of whole fish), the processing/ manufacturing and packaging stage as

well as the finished product placed on the markets. The acquired datasets will be provided to Scio to develop models and investigate any correlation between MSI and microbiological data. More detailed information about the analytical techniques and experimental procedures are provided in Deliverables 6.1 and 6.2.

3.3.2. Sensory quality

In order to meet complimentary stakeholders' needs (please refer to D2.1), subjective quality metrics such as odor and overall appearance of the products, sensory based quality evaluation/reporting will be performed simultaneously to the analytical microbiological assessment, following the same experimental design. Sensory based quality evaluation, stands for questionnaire filling by a semi-trained panel of 5 members concerning the quality of the products. This acquired information, although subjective and in many cases biased, will be used as reference towards the apparent overseen quality, in tandem with the quantitative assessment of microbial populations via traditional microbiological in lab analysis.
The freshness of the samples will be evaluated using a 5-point scale (1 - like very much, 2 - like moderately, 3 - neither like nor dislike, 4 - dislike moderately, 5 - dislike very much).

3.3.3. Other quality parameters (texture, eye color)

Apart from the aforementioned analysis, some further quality parameters will be also investigated, including textural parameters and fish eye color as well. Both of them are important for the estimation of freshness in several fish species. Texture analysis will be performed using a TA HD plus, Texture Analyzer (Stable micro systems). The same samples were also subjected to MSI analysis using VideometerLite1 and VideometerLite2. For the analysis of fish using the VIDEOMETER instruments, the fish samples will be purchased whole, ungutted and stored at 2 or 4°C for up to 10 days. At regular intervals two images will be acquired for each fish head (i.e., back and front). In parallel, microbiological analysis will be conducted for the enumeration of total viable counts (TVC - Plate Count Agar, incubation time: 30oC for 2-3 days) as reference indicator of fish quality. More information about the analytical techniques and experimental procedures will be provided in Deliverables 6.2.

In general, traditional analytical strategies will be followed to gain quality and safety data of the chosen products throughout their value chain. The conventional, current state-of-the-art wet-lab protocols for chemical, physiochemical, and microbiological measurements will be used as input to calibrate measures obtained by the sensing technology that the project aims to implement. To this end, experimental investigation of samples with gold-standard equipment (NTNU, AUA, UoI) will work closely together for the purpose of data collection across the blue value chain, in terms mainly to the possible hazards on microbial contamination and spoilage, quality assessment, and/or any other contaminants and pesticides introduced throughout the chain (as depicted in Section 2). Furthermore, prevention of food waste due to doubts in the cooling chain and pesticides of the process waste streams throughout the food chain.
The information provided by the laboratory measurements and with the coupled portable spectral imaging instrument by Videometer, will and should be used in an ad-hoc mode, depending on the fish species and the type of quality and safety hazard considered (e.g., spoilage, contamination, origin identification etc.). Depending on the checking/measurement of samples site (along and in the considered food chain), the quality/safety across the food chain, different properties of deterioration extracted by the non-invasive sensor can be considered depending on the efficiency offered at each specific case, e.g., detection of fraud after

product processing and prior distribution of the processed product or detection of safety hazards either after product processing or storage of the product. All the predicted properties, along with the use of available, custom and tailor-made data analysis approaches, for each type of fishes in tandem with the considered sampling point across the food chain, will be employed and serve as an efficient and robust tool for practical decision-making (helping the “intelligent” logistic of the products) and potentially even as an early warning system. It is obvious that this constitutes of a rapid, non-invasive, portable, analytical monitoring and tracking system that can be used at ‘real time’, when coupled with validated machine learning models, to monitor and predict safety at each stage of food production and even further, along the food chain, and to facilitate the prediction of the safety (concerning various hazards) of the products. It is thus obvious that herein, we focus on providing a framework along with a “closed”/standardized multi-sensor instrument for a novel food safety and quality monitoring decision-making system. This will be done by linking the current state of a product tested via the non-invasive Videometer MSI sensors (data collection), along to the history of the products (storage time and temperature across different time points).

4 CONCLUSIONS

This Deliverable (D2.4) is an update of the variables and activities described in D2.2, with regard to the parameters relevant with the needs and requirements of the fish value chain actors. Following discussions among pilot partners and Aquaculture industry as well, slight modifications in the experimental designs were decided so as all the three pilots to be as much as aligned as possible, covering at the same time Aquaculture's substantial needs. Towards this direction, the identified variables along the three considered fish value chains, namely 1) the Atlantic salmon value chain, 2) the Atlantic whitefish value chain, and 3) the Mediterranean seabream/seabass value chain are described. Additionally, the information about the sensors that will be employed in the TMF project is updated, as a new version of VideometerLite will be also employed in future experiments.