

# SEAFOOD<sup>TOMORROW</sup>



**Nutritious, safe and sustainable seafood for consumers of tomorrow**

Grant agreement no: 773400

## **Deliverable D3.1**

**Protocol for analytical platform, samples shipping and quality assurance strategy**

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## 1. Summary

### **Objective(s):**

WP3, led by ANFACO with contributions from 17 partners, intends to validate through chemical/biological analyses, laboratory assays and predictive modelling from independent partners the claims and impacts on human health, nutrition and safety of the sustainable production and processing eco-innovative solutions developed in WPs 1 and 2. The impact will be evaluated both in terms of nutritional quality as well as food safety by investigating biological and/or chemical contaminants.

WP3 makes avail of a technological platform which investigates:

- a) Validated methods to detect relevant agents (e.g. nutrients, toxicological and biological hazards).
- b) Proof of concept-based methods to detect biological activities (e.g. *in vitro* bio accessibility).
- c) Database integrating WP3 results, as well as other parameters relevant for labelling from WP4 and 5.
- d) Models and scenario analysis to assess and quantify the exposure, benefits and risks (e.g. shelf-life) for human health that are foreseen for each eco-innovative solution.

Each validation study will be targeted to the specific health-relevant claim of each eco-innovative solution. Each seafood product, strategy or process optimized in WP1 and 2 will be assessed in comparison with the "business-as-usual" scenario, i.e., the current seafood products and/or production methods. Special attention will be paid to population subgroups particularly liable to benefits or vulnerable to risks, e.g. youths, seniors and pregnant women.

The present Deliverable is the result of an effort led by ISS that involved all WP1, WP2 and WP3 participants with the objective to set up the WP3 analytical protocol, i.e., to establish the work plan of each *ad hoc* validation study targeted to each eco-innovative solution optimized in WPs 1 and 2. To this end, a technological platform was created detailing a consistent and harmonized strategy plan for quality assurance and shipping samples from WPs 1 and 2 partners to the relevant WP3 partners. The plan includes protocols defining the sample type, sampling procedure, sample preparation, amount of sample, labelling, storage, transport, analyses/assays to be performed, reporting and data archiving. The plan is to be adopted in T3.2, dealing with the laboratory assays, and will support the creation of the database dealt with in T3.3.

### **Rationale:**

The Deliverable presents the set-up of the WP3 analytical protocol, i.e., the work plan of each *ad hoc* validation study targeted to each eco-innovative solution optimized in WPs 1 and 2. A technological platform was created detailing a consistent and harmonized strategy plan for quality assurance and shipping samples from WPs 1 and 2 partners to the relevant WP3 partners. The plan includes protocols defining the sample type, sampling procedure, sample preparation, amount of sample, labelling, storage, transport, analyses/assays to be performed, reporting and data archiving. The plan is to be adopted in T3.2, dealing with the laboratory assays, and will support the creation of the database dealt with in T3.3.

## 2. Background

SEAFOOD<sup>TOMORROW</sup> aims to validate and optimize commercial solutions for improving the socioeconomic and environmental sustainability of the seafood production and processing industry, while contributing to product quality and safety. Activities focus on the sustainable production and processing of nutritious and safe seafood products through the demonstration and first application in the market of eco-innovative, sustainable solutions of marine and aquaculture-derived food products. The project takes into account impacts across different regions and population segments, as well as the specificities of different types of seafood. The consortium is built on interdisciplinary research teams of 19 RTDs integrated by 4 IAGs and 13 SMEs with diverse and complementary interests in the solutions to be validated and optimized.

The present Deliverable builds upon the vast experience of the partners in the fields of (i) seafood production and processing industry, (ii) surveying of seafood products for assessing chemical composition, nutrient profile and levels of chemical and biological contaminants, and (iii) food safety and risk/benefit-to-risk assessment. It takes into account previous collaborative efforts in the field of designing fit-for-purpose sampling plans aimed at providing reliable and robust data on seafood quality and safety, e.g. within the FP7 ECsafeSEAFOOD project.

## 3. Partners' surveys

### 3.1. Survey on assays

A survey was carried out to check the analytical skills of WP3 partners and the capability to carry out the specific assays required to validate the claims and impacts on human health, nutrition and safety of the eco-innovative solutions developed in WPs 1 and 2 by comparisons with the corresponding 'business-as-usual' products. The survey was aimed to refine and improve the general scheme set up at the stage of project proposal in collaboration with the T3.2 Task Leader and deliver a fit-for-purpose and cost-effective plan for analyses.

For each eco-innovative solution, whenever relevant, the list of assays was established keeping in mind the parameters for which maximum levels or guidance values are set by EU Regulations and Recommendation for fish feed and seafood products. Contaminants/nutrients for which legal limits/recommendations do not exist were targeted as appropriate for the *ad hoc* assessment of the impact of the eco-innovative solutions.

The range of assays covered is extensive. Chemical analyses entails, among others, characterization of the composition (e.g. proximates, fatty acids pattern), nutrient profile (e.g. minerals and vitamins), levels of environmental and processing contaminants (e.g. toxic trace elements, PAHs, BFRs). Other assays targets specific biologically-relevant properties (e.g. water activity, antioxidant activity, lipid oxidation, *in vitro* bio-accessibility). Microbiological analyses are featured for the characterization of specific biological hazards and the determination of the growth rate of spoilage and pathogenic bacteria (e.g. *Listeria monocytogenes*) throughout the products shelf life. Finally, sensory attributes of the products are targeted, either based on the evaluation of sensory panels (e.g. visual appearance, texture, mouth feel, smell, taste) or instrumental measurements (e.g. colour and texture).

The survey was carried out as an inclusive and iterative process, where each WP3 partner had the possibility to repeatedly refine the type of contribution on the basis of the proposals made by other partners, by liaising with specific partners and by continuous consultation within and among the three WPs involved (1, 2 and 3). The final draft was validated by WP1 and WP2 task leaders. In the definition of the list of partners carrying out the specific assays for each eco-innovative solution, both scientific issues and practical constraints (e.g. the laboratory throughput, the shipping costs that are charged to the partner carrying out the analyses) could be adequately taken into account.

In some cases where multiple partners proposed to perform the same activity and the activity entailed a substantial analytical burden, the latter was shared so as to provide a timely and effective processing of samples. Partners responsible for analyses will deliver their results to the partners developing the respective eco-innovative solutions to enable their improvement/optimization.

### 3.2. Survey on samples

A complementary survey has been carried out among WP1 and WP2 Task Leaders to define all aspects related to sampling and delivery of samples to the analytical partners in WP3. A detailed questionnaire was administered asking to specify the type of samples to be analysed in WP3, their storage and shipping conditions, the number of specimens and their amount in weight. The Excel questionnaire was composed by 2 sheets, one for the 'business-as-usual' scenario and one for the improved products.

Careful consideration was given to aspects such as the representativeness of samples, the method of sampling, the seasonal issues and the potential need for repeated sampling. The complete route of the samples, from the collection site/production plant to the analytical laboratory was defined, including the conditions for sample storage post-shipment and associated shelf-life, and the subsequent handling (i.e. removal of inedible portion, if any). All associated details (e.g. sample coding) were clearly defined. The foreseen date for availability of the analytical results was established as well. Also in this case the survey was carried out as an inclusive and iterative process, which entailed an extensive consultation within and among the partners of three WPs involved (1, 2 and 3).

## 4. Results and Discussion

Table 1 shows the biological species that will be dealt with for the development of the eco-innovative solutions. The list has to be considered provisional since in some cases (T1.4, T2.2, and T2.4) the exact species have not been identified yet.

**Table 1 – List of the biological species used in the eco-innovative solutions.**

| Common name             | Scientific name                  | FAO 3-Alpha Code |
|-------------------------|----------------------------------|------------------|
| <b>Seaweeds</b>         |                                  |                  |
| Laminaria (oarweed)     | <i>Laminaria digitata</i>        | Not available    |
| Saccharina (sugar kelp) | <i>Saccharina latissima</i>      | Not available    |
| <b>Bivalve molluscs</b> |                                  |                  |
| Mussel                  | <i>Mytilus galloprovincialis</i> | MSM              |
| Pacific oyster          | <i>Crassostrea gigas</i>         | OYG              |

**Fish**

|                   |                            |     |
|-------------------|----------------------------|-----|
| Atlantic salmon   | <i>Salmo salar</i>         | SAL |
| Common carp       | <i>Cyprinus carpio</i>     | FCP |
| Gilthead seabream | <i>Sparus aurata</i>       | SBG |
| Rainbow trout     | <i>Oncorhynchus mykiss</i> | TRR |

**4.1. Sampling plan and analytical efforts for each eco-innovative solution**

**4.1.1. Utilization of novel sustainable feed materials in aquafeeds towards the fortification of farmed fish (T1.1)**

The eco-innovative solutions of T1.1 are shown in Table 2. The sampling plan and the analytical plan for T1.1 products are show in **HUVY 5%** and **5&** of the Annexes, respectively.

**Table 2 – Eco-innovative solutions of T1.1.**

| <b>Matrix</b>  | <b>Expected nutritional and sensorial benefit</b>   | <b>Expected impact on food safety</b> |
|--|---|---------------------------------------|
| Macroalgae products  | Se and I content                                    | Limited contaminants levels           |
| Feed (trout)<br>Feed (seabream)<br>Feed (carp)   | Enhanced Se, I and n-3 PUFAs (EPA and DHA) content* | No increase in contaminants levels    |
| Fortified trout (pilot-scale)<br>Fortified salmon (farm-scale)<br>Fortified seabream (pilot-scale)<br>Fortified seabream (farm-scale)<br>Fortified carp (pilot-scale)<br>Fortified carp (farm-scale) | Enhanced Se, I and n-3 PUFAs (EPA and DHA) content  | No increase in contaminants levels    |
| Fortified salmon (farm-scale)<br>Fortified seabream (farm-scale)<br>Fortified carp (farm-scale)  | Maintenance or improvement of sensorial traits      | Not applicable                        |

\* Fe levels will be monitored in the blends but no specific fortification is foreseen, as Fe is a pro-oxidant compound thus not being appropriate to target in the trials

For the 'business-as-usual products' commercial feed used in farm-scale trials will be collected in M18, fortified fish in M21 (fillets initial fish) and M24 (fillets final fish), whereas the whole final fish will be collected in M24. Once received in the analytical laboratories the latter will be subjected to standard filleting in order to remove the inedible portion. Tentative dates for the availability of the analytical results are M20 for feeds and M26 for the other matrixes (fillets and whole fish).

For the eco-innovative solutions, dry macroalgae will be collected as raw materials to be used in experimental fish feeds along with the feeds used in pilot-trials and farm-scale trials and the faeces of rainbow trout (sampled at the end of trial). For the edible products, fillets from initial and final fish will

be collected at different months as shown in Table A1 whereas the whole final fish will be collected at M24. The latter will be subjected to standard filleting in order to remove the inedible portion once in the analytical laboratories. Tentative dates for the availability of the analytical results are M8 (seaweeds), M24 (feeds pilot scale), M12 (fish samples pilot-scale), M20 (feeds farm-scale) and M26 (fish samples shipped on M24).

#### 4.1.2. Integrated multitrophic aquaculture (IMTA) for sustainable production (T1.2)

The eco-innovative solutions of T1.2 are shown in Table 3. The sampling plan and the analytical plan for T1.2 products are show in **HUVY'5'** and **5(** of the Annexes, respectively.

**Table 3 – Eco-innovative solutions of T1.2.**

| Matrix          | Expected nutritional and sensorial benefit   | Expected impact on food safety     |
|-----------------|--|------------------------------------|
| IMTA salmon     | Maintenance of sensorial properties and nutrient levels (fatty acid profile, vitamins, minerals) | No increase in contaminants levels |
| IMTA macroalgae |  |                                    |

For the 'business-as-usual products' salmon and seaweeds from monoculture fish farm (non IMTA) will be collected. A tentative date for the availability of the analytical results is M11.

For the eco-innovative solutions, at M7 seaweeds and filets from fish grown at three IMTA farms, i.e. Sulefisk (Solund, Norway), Engesund (Masfjorden, Norway), Osland (Bjordal, Norway), will be collected. A tentative date for the availability of the analytical results is M10.

#### 4.1.3. Sustainable management of shellfish production areas (SPAs) through delineation of buffer zones (T1.3)

The eco-innovative solutions of T1.3 are shown in Table 4. The sampling plan and the analytical plan for T1.3 products are show in **HUVY'5)** and **5\*** of the Annexes, respectively.

**Table 4 – Eco-innovative solutions of T1.3.**

| Goal  | Expected nutritional and sensorial benefit | Expected impact on food safety   |
|---|--|--|
| Predictive model for norovirus and <i>E. coli</i> | None specific                              | Control of microbiological ( <i>E. coli</i> , NoV) and HAB contamination |
| Predictive model for Harmful algal blooms         |  |  |

For the 'business-as-usual products' commercially sized oysters (12-18 individuals) will be collected from 4 sites in the Fal Estuary and 3 sites in Alfacs Bay and tested for human norovirus and *E. coli*. The shellfish will be tested according to ISO 15216-1:2013 and ISO 16649-3:2015, respectively. A tentative date for the availability of the analytical results is M13.

For the eco-innovative solutions, commercially sized oysters will be collected from the same production areas as above and tested for *E. coli* and Norovirus. Tests will be made on samples of 12-18 oysters taken from sampling stations. A tentative date for the availability of the analytical results is M13.

The activity pertaining the HAB model improvement will use historical monitoring data and no analytical testing will be carried out.

#### 4.1.4. Integration of fast screening methods in the management of seafood production systems (T1.4)

The eco-innovative solutions of T1.4 are shown in Table 5. The sampling and analytical plans for T1.4 products are show in **HUZY 5+** and **5**, of the Annexes, respectively.

**Table 5 – Eco-innovative solutions of T1.4.**

| Goal                              | Expected nutritional and sensorial benefit | Expected impact on food safety  |
|-----------------------------------|--|---|
| Tetrodotoxins sensor              | None specific                              | Control of tetrodotoxins contamination level  |
| Xenobiotic sensor                 |  | Control of xenobiotics (PAHs, BFRs and PFCs) contamination level                        |
| Multiplex regulated toxins sensor |  | Control of regulated marine toxins (lipophilic, ASP and PSP toxins) contamination level |

As far as the 'business-as-usual products' are concerned, samples of oysters / mussels will be collected in different areas for the activity pertaining the tetrodotoxins (TTX) and the multiplex regulated toxins sensors. TTX presence in shellfish has been proposed to be related with the presence of bacteria of the genus *Vibrio*, which is more abundant in warm months. For the marine toxins, sampling months have been chosen considering that sample collection would be fine all year long for ASP, whereas for STX, DOM and OA preferably warm months (May to September) should be selected. The number of specimens has been chosen according to values used in monitoring programs, whereas the number of analytical samples has been chosen according to previous experience in validation studies. Shell removal will be performed once the samples reach the analytical laboratory (assays are performed mainly in-house).

For the xenobiotic sensor activity, low and high fat content fish species will be selected. Number of specimens has been chosen considering previous experience in optimisation and validation studies.



For the eco-innovative solutions, the same considerations as above apply. Availability of the analytical results for the xenobiotic sensor activity is expected within 6 months after samples reception. The assays related to the two other activities are mainly performed in-house.

#### 4.1.5. Sodium reduction in seafood products (T2.1)

The eco-innovative solutions of T2.1 are shown in Table 6. The sampling plan and the analytical plan for T2.1 products are show in **HUVY 5-** and **5%\$** of the Annexes, respectively.

**Table 6 – Eco-innovative solutions of T2.1.**

| Matrix      | Expected nutritional and sensorial benefit                              | Expected impact on food safety   |
|-------------|---|--|
| Fish paté   | Sodium reduction; Maintenance of sensory properties and nutrient levels | Maintenance of microbiological status (spoilage and pathogenic microorganisms) |
| Smoked fish |   |  |

As far as the first sample type is concerned, for both the 'business-as-usual product' and the eco-innovative solution a paté based on Norwegian salmon will be produced according to a standardized recipe.

As far as the second sample type is concerned, the number of samples will be more finely tuned according to specific parameters in order to characterize the raw material. At least, two samples per treatment will be evaluated in the former trials. For the final trial and treatment, at least three samples will be produced and subsamples will be sent for analyses.

For all samples a tentative date for the availability of the analytical results is M24.

#### 4.1.6. Digestible, attractive, functional, sustainable and nutritionally adapted food to specific population groups (T2.2)

The eco-innovative solutions of T2.2 are shown in Table 7. The sampling plan and the analytical plan for T2.2 products are show in **HUVY 5%&** and **5%&** of the Annexes, respectively.

**Table 7 – Eco-innovative solutions of T2.2.**

| Matrix           | Expected nutritional and sensorial benefit   | Expected impact on food safety  |
|------------------|--|---|
| Youth product 1  | High levels of bioaccessible nutrients; Reduced oxidation levels; Sensorial acceptance | No increase of spoilage and pathogenic bacteria contamination, biogenic amines, and chemical contaminants |
| Youth product 2  |  |   |
| Senior product 1 |  |   |
| Senior product 2 |  |   |

Woman product 1

Woman product 2

Within this task, 6 ready to eat products targeted at specific population groups will be produced at semi-industrial scale. The production will be distributed between IDmer, RISE and possibly ILVO, according to the selected products and the technical resources of those institutions. The exact biological species on which the products will be based have not been defined yet, but preference will be given - as far as possible - to available, sustainable and non-endangered species from inland and marine fisheries and aquaculture with low or medium commercial value available in different European countries, including fatty and lean species, and covering the need for diversification of consumers choices. Most of the products are expected to be based on fish; should molluscs/crustaceans be used this will be taken into account in terms of the panel of contaminants analysed (see Table A12).

This task incorporates the formulation and selection of recipes based on intermediary products at national and European level in order to meet consumer and market acceptance. The products will be processed on September/October 2019 after the selection of the 6 winning recipes. For each product, the final formulation will be compared to reference products for the “business-as-usual” scenario.

A tentative date for the availability of the analytical results is M27 for all of the six products.

#### 4.1.7. Strategies to reduce contaminants from seafood products (T2.3)

The eco-innovative solutions of T2.3 are shown in Table 8. The sampling plan and the analytical plan for T2.3 products are show in **HUVY 5%** and **5%** of the Annexes, respectively.

**Table 8 – Eco-innovative solutions of T2.3.**

| Matrix                      | Expected nutritional and sensorial benefit | Expected impact on food safety |
|-----------------------------|--|--------------------------------|
| Pacific oyster              | None specific                              | Reduction of <i>Norovirus</i>  |
| Atlantic Salmon             |  | Reduction of <i>Listeria</i>   |
| Processed bivalves (canned) |  | Reduction of PSP               |

For the activity related to *Norovirus* reduction, a batch of 1600 commercially sized oysters will be collected from production areas and placed in depuration tanks for purification. Tests will be made for selection of process efficiency parameters. Sub-samples of 15 oysters will be taken from the tanks and tested for human norovirus, FRNA bacteriophage and *E. coli*. Sampling will be carried out during the norovirus season (October-March) and will potentially target multiple sites, depending on norovirus prevalence and results of bioaccumulation tests. A tentative date for the availability of the analytical results is M13.

For the activity related to *Listeria* reduction, for each type of non-treated/control fish products (2) of the business-as-usual scenario, i.e. fresh salmon fillet or cold smoked salmon fillet, 4 samplings over the shelf life will be performed with 3 samples per sampling (for a total of 24 control samples). Likewise, for the eco-innovative solution, for each type of treated fish product (2 salmon fillets), 4 samplings over the shelf life will be performed with 3 samples per sampling (for a total of 24 treated samples). A tentative date for the availability of the analytical results is M21-M23.

For the activity related to PSPs reduction, mussels or other bivalves (e.g. clams, oysters, pectinids) will be used as samples depending on the natural contamination. Reference products for the "business-as-usual" scenario will be processed bivalves (canned) from the market, which will be submitted to sensorial analyses only in order to compare with detoxified products (samples analysed by the same partner producing the latter, i.e. ANFACO). A tentative time frame for the availability of the analytical results is 3 months after sample collection.

#### **4.1.8. Reduction of energy and water in seafood processing (T2.4)**

The sampling plan and the analytical plan for T2.4 products are shown in **HUVY 5%** and **5%** of the Annexes, respectively.

Within this task, samples of fish soup and dried fish will be subjected to analytical activities for the edible matrixes. Waste water from the treatment plant will be additionally collected and analysed. For both the 'business-as-usual' products and the eco-innovative solutions, 3 samples (triplicates) for each experiment will be collected.

Tentative dates for the availability of the analytical results are M12 (dried fish) and M18 (fish soup and water effluents).

#### **4.2. Generalised sampling guideline**

Sampling has to be documented and properly recorded. Sampling date, location, number (weight) of samples and all the accompanying/ancillary information have to be recorded immediately and appropriately stored.

In the case of field sampling, recording of the sampling sites, geographic coordinates and other relevant information for the specific task to be performed (e.g. hour, date, atmospheric conditions, characteristics of site, sampling devices, sample containers) has to be taken care of. It is required that the collection team has knowledge and experience in the collection and identification of seafood.

Individuals of the selected target species should be rinsed in tap water to remove any foreign material from the external surface, handled using clean nitrile gloves, and placed in clean holding containers (plastic bags) to prevent contamination, labelled in the outer bag and immediately stored in the appropriate conditions (e.g. ice) for the transport to the laboratory. The latter should be as timely as possible.

For the determination of specific substances special provisions may apply (e.g. some plastic polymers may be a source of brominated flame retardants). In such cases, the analytical laboratory dealing with the determination of the implicated substances has to give advice and agree in advance with the sampling team what type of materials are safe to be used during the sampling procedures.

In case of sampling from the market or production lines it is important to record all the data (e.g. state of the sample, package, quantity, lot number) that might be needed to properly interpret the analytical

results. Photographic documentation should be also taken when necessary. Other information can be recorded in the form of notes.

### 4.3. Sample shipment and storage

Samples have to be stored and shipped according to the conditions detailed in the Tables A1, A3, A5, A7, A9, A11, A13, A15 of the Annexes. Deviations have to be properly justified and, after agreement of the sender and the receiving laboratory, appropriately recorded.

For shipment of frozen samples, insulating boxes and all other appropriate technical means have to be used. Reputable couriers/shipping companies have to be selected and must guarantee compliance with the relevant shipping conditions (e.g. refrigerated samples, frozen samples, delivery within a specific time frame). An exact duplicate of the shipped sample should be kept by the sender until the end of the project, in case a replicate or counter-analyses should be needed.

Laboratories have to provide the exact shipping address and the details of the reference person taking care of sample reception, one month prior to the sample shipment. As a general rule, the institution receiving the samples should pay for the shipment.

Before shipment, samples have to be coded using the sample codes detailed in the above mentioned Tables of the Annexes. Deviations have to be properly justified and, after agreement of the sender and the receiving laboratory, recorded. The sender may opt to keep the coding blind (i.e., the laboratory does not know what each sample correspond to).

### 4.4. Analytical quality assurance

Laboratories have to comply with the general rules of analytical quality assurance. Fit-for-purpose analytical methods have to be used and experienced personnel should oversee the analytical procedures. The laboratory has to specify if the analytical method used is an official method, a reference/standard method, an accredited method, or an internally validated method. Internal quality control, and especially use of appropriate (certified) reference materials, should be in place.

Sample preparation of fish or other seafood whole specimens will require - if not performed earlier - recording of individual weight, total length (sex when appropriate), and each specimen should have a specific identification (e.g. scientific name, date, number). Sample preparation should allow to separate the flesh from inedible parts avoiding contamination. Sample preparation has to be performed in a clean laboratory environment (including use of, e.g., clean knives and boards) and using tools and containers that do not introduce sample contamination. Whenever possible, sample treatment should be performed in laboratories with filtered air (ideally in clean room conditions).

Subsampling has to take place to maintain representativeness of the analytical aliquots. An adequate number of sample and reading replicates have to be performed, so that reliable and accurate analytical data are produced.

## 4.5. Reporting of analytical results

It is essential that the analytical data generated in T3.2 are reported in a coherent and harmonised way either to be transmitted to WP1 and WP2 partners developing the eco-innovative solutions or to be archived in the database dealt with in T3.3.

In order to harmonise reporting and facilitate efficient data archiving, an Excel form for reporting results has been created and the relevant sheets are shown in **HUVYg'5%+!5%**. This file will be available at Basecamp.

## 5. Conclusions

A comprehensive protocol for the SEAFOOD<sup>TOMORROW</sup> analytical platform, samples shipping and quality assurance strategy has been set up. The main elements of the protocol are summarized in the 19 Tables in the Annexes representing the practical guidelines for ensuring the obtainment of coherent and reliable data within the project and their proper transmission and archiving.

# Annexes

## Key to the tables A1-A16

RTE = Ready To Eat

RT = Room temperature

R = Refrigerated

F = Frozen

RUI = Refrigerated and used immediately

**Table A1. Sampling plan for T1.1 - Utilization of novel sustainable feed materials in aquafeeds towards the fortification of farmed fish**

| Matrix                            | Type of product | Biological species: scientific name | Partner providing the sample | Sample origin       | Method of sampling  | Month(s) of sample delivery to labs | State of the sample | Number of specimens | Number of analytical samples    | Total weight of the sample | Shipping conditions | Sample coding              | Sample storage post-shipment (shelf-life) |
|-----------------------------------|-----------------|-------------------------------------|------------------------------|---------------------|---------------------|-------------------------------------|---------------------|---------------------|---------------------------------|----------------------------|---------------------|----------------------------|---|
| <b>Business-as-usual products</b> |                 |                                     |                              |                     |                     |                                     |                     |                     |                                 |                            |                     |                            |   |
| Feed (trout)                      | Feed pellets    | Not applicable                      | SPAROS                       | Feed mill, Portugal | Random (final feed) | M18                                 | Feed pellets        | 1                   | 1                               | 200 g                      | RT                  | FEEDAS1                    | RT (24 months)                            |
| Feed (seabream)                   | Feed pellets    | Not applicable                      | SPAROS                       | Feed mill, Portugal | Random (final feed) | M18                                 | Feed pellets        | 1                   | 1                               | 200 g                      | RT                  | FEEDSB1                    | RT (24 months)                            |
| Feed (carp)                       | Feed pellets    | Not applicable                      | SPAROS                       | Feed mill, Portugal | Random (final feed) | M18                                 | Feed pellets        | 1                   | 1                               | 200 g                      | RT                  | FEEDCC1                    | RT (24 months)                            |
| Fortified salmon (farm-scale)     | Fish fillets    | <i>Salmo salar</i>                  | TL/MF                        | Fish farm (Norway)  | Random              | M21, M24                            | Fresh fish fillet   | 2                   | 2                               | 250 x 2 = 500 g            | F (-18°C)           | FILLETAS1 to FILLETAS2     | F at -18°C (3 months)                     |
| Fortified seabream (farm-scale)   | Fish fillets    | <i>Sparus aurata</i>                | SKALOMA                      | Fish farm (Greece)  | Random              | M21, M24                            | Fresh fish fillet   | 2                   | 2                               | 250 x 2 = 500 g            | F (-18°C)           | FILLETASB27 to FILLETASB28 | F at -18°C (3 months)                     |
| Fortified carp (farm-scale)       | Fish fillets    | <i>Cyprinus carpio</i>              | ICR/ZUT                      | Fish farm (Poland)  | Random              | M21, M24                            | Fresh fish fillet   | 2                   | 2                               | 250 x 2 = 500 g            | F (-18°C)           | FILLETCC27 to FILLETCC28   | F at -18°C (3 months)                     |
| Fortified salmon (farm-scale)     | Whole fish      | <i>Salmo salar</i>                  | TL/MF                        | Fish farm (Norway)  | Random              | M24                                 | Whole fresh fish    | 25                  | Samples for the sensorial panel | 25 market size fishes      | R (4°C)             | WFAS1 to WFAS25            | RUI                                       |
| Fortified                         | Whole fish      | <i>Sparus</i>                       | SKALOMA                      | Fish farm           | Random              | M24                                 | Whole               | 25                  |                                 | 25 market                  | R (4°C)             | WFSB1 to                   | RUI                                       |



|                                  |                |  |               |                          |                     |                                  |                     |    |                 |                       |           |                           |                       |
|----------------------------------|----------------|--|---------------|--------------------------|---------------------|----------------------------------|---------------------|----|-----------------|-----------------------|-----------|---------------------------|-----------------------|
| seabream (farm-scale)            |                | <i>aurata</i>  |               | (Greece)                 |                     |                                  | fresh fish          |    | might be needed | size fishes           |           | WFSB25                    |                       |
| Fortified carp (farm-scale)      | Whole fish     | <i>Cyprinus carpio</i>                                   | ICR/ZUT       | Fish farm (Poland)       | Random              | M24                              | Whole fresh fish    | 25 |                 | 25 market size fishes | R (4°C)   | WFCC1 to WFCC25           | RUI                   |
| <b>Eco-innovative solutions</b>  |                |  |               |                          |                     |                                  |                     |    |                 |                       |           |                           |                       |
| Macroalgae products              | Dry macroalgae | <i>Laminaria digitata</i><br><i>Saccharina latissima</i> | SPAROS and MF | Market (Portugal)        | Final product       | M6                               | Seaweed powder      | 2  | 2               | 200 x 2 = 400 g       | RT        | MA1 & MA2                 | RT (24 months)        |
| Feed (trout)                     | Feed pellets   | Not applicable   | SPAROS        | Feed mill, Portugal      | Random (final feed) | M7 pilot trials, M18 farm trials | Feed pellets        | 5  | 5               | 200 x 5 = 1000 g      | RT        | FEEDAS2 to FEEDAS6        | RT (24 months)        |
| Feed (seabream)                  | Feed pellets   | Not applicable   | SPAROS        | Feed mill, Portugal      | Random (final feed) | M7 pilot trials, M18 farm trials | Feed pellets        | 5  | 5               | 200 x 5 = 1000 g      | RT        | FEEDSB2 to FEEDSB         | RT (24 months)        |
| Feed (carp)                      | Feed pellets   | Not applicable   | SPAROS        | Feed mill, Portugal      | Random (final feed) | M7 pilot trials, M18 farm trials | Feed pellets        | 5  | 5               | 200 x 5 = 1000 g      | RT        | FEEDCC2 to FEEDCC6        | RT (24 months)        |
| Fortified trout (pilot-scale)    | Faeces         | <i>Oncorhynchus mykiss</i>                               | DTU           | Research site (Denmark)  | Stripping           | M10                              | Freeze dried faeces | NA | 12              | 5-10 g                | RT        | FECESRBT1 to FECESRBT12   | RT (desiccator)       |
| Fortified trout (pilot-scale)    | Fish fillets   | <i>Oncorhynchus mykiss</i>                               | DTU           | Research site (Denmark)  | Random              | M10                              | Fresh fish fillet   | 90 | 30              | 100 x 30 = 3000 g     | F (-18°C) | FILLETRBT1 to FILLETRBT30 | F at -18°C (3 months) |
| Fortified salmon (farm-scale)    | Fish fillets   | <i>Salmo salar</i>                                       | TL/MF         | Fish farm (Norway)       | Random              | M24                              | Fresh fish fillet   | 3  | 3               | 250 x 3 = 750 g       | F (-18°C) | FILLETAS3 to FILLETAS5    | F at -18°C (3 months) |
| Fortified seabream (pilot-scale) | Fish fillets   | <i>Sparus aurata</i>                                     | IPMA          | Research site (Portugal) | Random              | M10                              | Fresh fish fillet   | 26 | 26              | 100 x 26 = 2600 g     | F (-18°C) | FILLETSB1 to FILLETSB26   | F at -18°C (3 months) |
| Fortified                        | Fish fillets   | <i>Sparus</i>  | SKALOMA       | Fish farm                | Random              | M24                              | Fresh fish          | 3  | 3               | 250 x 3 =             | F (-18°C) | FILLETSB29                | F at -18°C            |





|                                 |              |                        |         |                        |        |     |                   |    |   |                     |           |                          |                       |
|---------------------------------|--------------|------------------------|---------|------------------------|--------|-----|-------------------|----|---|---------------------|-----------|--------------------------|-----------------------|
| seabream (farm-scale)           |              | <i>aurata</i>          |         | (Greece)               |        |     | fillet            |    |   | 750 g               |           | to FILLETSB31            | (3 months)            |
| Fortified carp (pilot-scale)    | Fish fillets | <i>Cyprinus carpio</i> | ICR/ZUT | Research site (Poland) | Random | M10 | Fresh fish fillet | 26 | 26  | 100 x 26 = 2600 g   | F (-18°C) | FILLETCC1 to FILLETRBT26 | F at -18°C (3 months) |
| Fortified carp (farm-scale)     | Fish fillets | <i>Cyprinus carpio</i> | ICR/ZUT | Fish farm (Poland)     | Random | M24 | Fresh fish fillet | 3  | 3   | 250 x 3 = 750 g     | F (-18°C) | FILLETCC29 to FILLETCC31 | F at -18°C (3 months) |
| Fortified salmon (farm-scale)   | Whole fish   | <i>Salmo salar</i>     | TL/MF   | Fish farm (Norway)     | Random | M24 | Whole fresh fish  | 25 | Samples for the sensorial panel might be needed | 25 market size fish | R (4°C)   | WFAS26 to WFAS50         | RUI                   |
| Fortified seabream (farm-scale) | Whole fish   | <i>Sparus aurata</i>   | SKALOMA | Fish farm (Greece)     | Random | M24 | Whole fresh fish  | 25 |   | 25 market size fish | R (4°C)   | WFSB26 to WFSB50         | RUI                   |
| Fortified carp (farm-scale)     | Whole fish   | <i>Cyprinus carpio</i> | ICR/ZUT | Fish farm (Poland)     | Random | M24 | Whole fresh fish  | 25 |   | 25 market size fish | R (4°C)   | WFCC26 to WFCC50         | RUI                   |

**Table A2. Analytical plan for T1.1 - Utilization of novel sustainable feed materials in aquafeeds towards the fortification of farmed fish**

| Assay class  | Specific analyte(s)/ assay(s)  | Partner            | Assay class             | Specific analyte(s)/ assay(s)           | Partner    | Assay class             | Specific analyte(s)/ assay(s) | Partner | Assay class  | Specific analyte(s)/ assay(s)  | Partner      | Assay class                     | Specific analyte(s)/ assay(s) | Partner               |
|--|--|--------------------|-------------------------|---|------------|-------------------------|-------------------------------|---------|--|--|--------------|---------------------------------|-------------------------------|-----------------------|
| Proximate composition and fatty acids (all matrices) | Protein, carbohydrates, total fat, moisture, ash, fiber, SAT, MUFA, PUFA, cholesterol, Trans | ICETA / IPMA / DTU | Minerals (all matrices) | I, Se, Na, Mg, P, Zn, Cl, Ca, Cu, Fe, K | DTU / IPMA | Vitamins (all matrices) | Vitamins (E, D)               | ICETA   | Environmental / processing contaminants (all matrices) | PBDEs (incl. congeners 28, 47, 99, 100, 153, 154, 183 and 209)<br><br>As, Inorganic As, Cd, Pb, MeHg, Hg | CSIC / ICETA | Sensory analyses (all matrices) | Sensory attributes            | IPMA / ANFACO / ICETA |



**Table A3. Sampling plan for T1.2 - Integrated multitrophic aquaculture (IMTA) for sustainable production**

| Matrix                            | Type of product | Biological species: scientific name | Partner providing the sample | Sample origin            | Method of sampling | Month(s) of sample delivery to labs | State of the sample | Number of specimens | Number of analytical samples | Total weight of the sample | Shipping conditions | Sample coding        | Sample storage post-shipment (shelf-life) |
|-----------------------------------|-----------------|-------------------------------------|------------------------------|--------------------------|--------------------|-------------------------------------|---------------------|---------------------|------------------------------|----------------------------|---------------------|----------------------|---|
| <b>Business-as-usual products</b> |                 |                                     |                              |                          |                    |                                     |                     |                     |                              |                            |                     |                      |   |
| Non-IMTA salmon                   | Fish fillets    | <i>Salmo salar</i>                  | Can be provided by TL/MF     | TL                       | Random             | M10                                 | Fresh fish file     | 3                   | 3                            | 3 Kg                       | F (-18°C)           | T1.2-XXX             | F at -18° (3 months)                      |
| Non-IMTA macroalgae               | Macro-algae     | <i>Saccharina latissima</i>         | Can be provided by TL/MF     | TL                       | Random             | M10                                 | Dried macro-algae   | 3                   | 3                            | 600 g                      | RT                  | T1.2-XXX             | RT (1 year)                               |
| <b>Eco-innovative solutions</b>   |                 |                                     |                              |                          |                    |                                     |                     |                     |                              |                            |                     |                      |   |
| IMTA salmon                       | Fish fillets    | <i>Salmo salar</i>                  | TL/MF                        | Fish farm (Norway)       | Random             | M7                                  | Fresh fish fillet   | 15                  | 15                           | 1 kg x 15 = 15 kg          | F (-18°C)           | T1.2-001<br>T1.2-015 | F at -18° (3 months)                      |
| IMTA macroalgae                   | Macro-algae     | <i>Saccharina latissima</i>         | TL/MF                        | Macroalgae form (Norway) | Random             | M7                                  | Dry macro-algae     | 9                   | 9                            | 200 g x 9 = 1.8 kg         | RT                  | T1.2-016<br>T1.2-024 | RT (1 year)                               |

**Table A4. Analytical plan for T1.2 - Integrated multitrophic aquaculture (IMTA) for sustainable production**

| Assay class  | Specific analyte(s)/ assay(s)  | Partner | Assay class             | Specific analyte(s)/ assay(s)           | Partner    | Assay class             | Specific analyte(s)/ assay(s) | Partner | Assay class  | Specific analyte(s)/ assay(s)  | Partner         | Assay class                     | Specific analyte(s)/ assay(s) | Partner               |
|--|--|---------|-------------------------|---|------------|-------------------------|-------------------------------|---------|--|--|-----------------|---------------------------------|-------------------------------|-----------------------|
| Proximate composition and fatty acids (all matrices) | Protein, carbohydrates, total fat, moisture, ash, fiber, SAT, MUFA, PUFA, cholesterol, Trans | ICETA   | Minerals (all matrices) | I, Se, Na, Mg, P, Zn, Cl, Ca, Cu, Fe, K | IPMA / DTU | Vitamins (all matrices) | Vitamins (E, D)               | ICETA   | Environmental / processing contaminants (all matrices) | PBDEs (incl. congeners 28, 47, 99, 100, 153, 154, 183 and 209)<br><br>As, Inorganic As, Cd, Pb, MeHg, Hg | CSIC<br><br>DTU | Sensory analyses (all matrices) | Sensory attributes            | ANFACO (IPMA / ICETA) |



**Table A5. Sampling plan for T1.3 - Sustainable management of shellfish production areas (SPAs) through delineation of buffer zones**

| Goal                              | Type of product           | Biological species: scientific name  | Partner providing the sample                | Sample origin   | Method of sampling                         | Month(s) of sample delivery to labs | State of the sample | Number of specimens  | Number of analytical samples           | Total weight of the sample | Shipping conditions    | Sample coding | Sample storage post-shipment (shelf-life)     |
|-----------------------------------|---------------------------|--|---|---|--|-------------------------------------|---------------------|----------------------|--|----------------------------|------------------------|---------------|---|
| <b>Business-as-usual products</b> |                           |  |   |   |  |                                     |                     |                      |  |                            |                        |               |   |
| Norovirus/E. coli model           | Raw mollusc (with valves) | <i>Crassostrea gigas</i>   | Industry partner (WCM, ) or other supplier) | Production areas Fal Estuary (UK) or Alfacs Bay (Spain) | Random by hand from cultivation rafts/bags | M12                                 | Whole shellfish     | Approx. 1600 oysters | 12 samples from each site (84 samples) | To be defined              | T must not exceed 10°C | T1.3-XXX      | Samples should not be frozen prior to testing |
| HAB model improvement             |                           | This element of the task will use historical monitoring data. No analytical testing will be carried out. |   |   |  |                                     |                     |                      |  |                            |                        |               |   |
| <b>Eco-innovative solutions</b>   |                           |  |   |   |  |                                     |                     |                      |  |                            |                        |               |   |
| Norovirus/E. coli model           | Raw mollusc (with valves) | <i>Crassostrea gigas</i>   | Industry partner (WCM, or other supplier)   | Production areas Fal Estuary (UK) or Alfacs Bay (Spain) | Random by hand from cultivation rafts/bags | M12                                 | Whole shellfish     | Approx. 1600 oysters | 12 samples from each site (84 samples) | To be defined              | T must not exceed 10°C | T1.3-XXX      | Samples should not be frozen prior to testing |
| HAB model improvement             |                           | This element of the task will use historical monitoring data. No analytical testing will be carried out. |   |   |  |                                     |                     |                      |  |                            |                        |               |   |

**Table A6. Analytical plan for T1.3 - Sustainable management of shellfish production areas (SPAs) through delineation of buffer zones**

| Assay class             | Specific analyte(s)/ assay(s)  | Partner |
|-------------------------|--|---------|
| Biological contaminants | Human norovirus (genogroups I and II); F-specific RNA bacteriophage; <i>Escherichia coli</i> | Cefas   |



**Table A7. Sampling plan for T1.4 - Integration of fast screening methods in the management of seafood production systems**

| Goal                              | Type of product                              | Biological species: scientific name                  | Partner providing the sample | Sample origin   | Method of sampling          | Month(s) of sample delivery to labs       | State of the sample                      | Number of specimens                           | Number of analytical samples                 | Total weight of the sample | Shipping conditions | Sample coding | Sample storage post-shipment (shelf-life) |
|-----------------------------------|--|--|------------------------------|---|-----------------------------|---|--|---|--|----------------------------|---------------------|---------------|---|
| <b>Business-as-usual products</b> |  |  |                              |   |                             |   |  |   |  |                            |                     |               |   |
| Tetrodotoxins sensor              | Raw bivalve molluscs                         | <i>Crassostrea gigas / Mytilus galloprovincialis</i> | IRTA                         | Alfacs and Fangar Bays (Catalonia, Spain) and other areas | Random sampling along rafts | Preferably warm months (May to September) | Whole or flesh only                      | 10 oysters per sample / 25 mussels per sample | 50 (25 oyster samples and 25 mussel samples) | 1 kg                       | F (-18°C)           | T1.4-IRTA-XXX | F (12 months)                             |
| Xenobiotic sensor                 | Fish fillets or edible parts of the products | Not defined yet                                      | AZTI                         | To be defined   | Random sampling             | M6, M12, M18                              | Fish fillet, homogenized and frozen      | 6 (M6), 25 (M12), 25 (M18)                    | Equal to number of specimens                 | 30 g/sample                | F (-18°C)           | T1.4-AZTI-XXX | F (12 months)                             |
| Multiplex regulated toxins sensor | Raw bivalve molluscs                         | <i>Crassostrea gigas / Mytilus galloprovincialis</i> | QUB, IRTA                    | Alfacs and Fangar Bays (Catalonia, Spain) and other areas | Random sampling along rafts | M6, M12, M18                              | Whole or flesh only, homogenized samples | 10 oysters per sample / 25 mussels per sample | 50 (25 oyster samples and 25 mussel samples) | 1 kg                       | F (-18°C)           | T1.4-QUB-XXX  | F (12 months)                             |
| <b>Eco-innovative solutions</b>   |  |  |                              |   |                             |   |  |   |  |                            |                     |               |   |
| Tetrodotoxins sensor              | Raw bivalve molluscs                         | <i>Crassostrea gigas / Mytilus galloprovincialis</i> | IRTA                         | Alfacs and Fangar Bays (Catalonia, Spain) and other areas | Random sampling along rafts | Preferably warm months (May to September) | Whole or flesh only                      | 10 oysters per sample / 25 mussels per sample | 50 (25 oyster samples and 25 mussel samples) | 1 kg                       | F (-18°C)           | T1.4-TTX-XXX  | F (12 months)                             |
| Xenobiotic sensor                 | Fish fillets or edible                       | Not defined yet                                      | AZTI                         | To be defined   | Random sampling             | M6, M12, M18                              | Fish fillet, homogenized and             | 6 (M6), 25 (M12), 25                          | Equal to number of                           | 100 g/sample               | F (-18°C)           | T1.4-AZTI-XXX | F (12 months)                             |



|                                   |                       |  |           |   |                             |              |  |   |  |      |           |              |               |
|-----------------------------------|-----------------------|--|-----------|---|-----------------------------|--------------|--|---|--|------|-----------|--------------|---------------|
|                                   | parts of the products |  |           |   |                             | frozen       | (M18)                                    | specimens                                     |  |      |           |              |               |
| Multiplex regulated toxins sensor | Raw bivalve molluscs  | <i>Crassostrea gigas / Mytilus galloprovincialis</i> | QUB, IRTA | Alfacs and Fangar Bays (Catalonia, Spain) and other areas | Random sampling along rafts | M6, M12, M18 | Whole or flesh only, homogenized samples | 10 oysters per sample / 25 mussels per sample | 50 (25 oyster samples and 25 mussel samples) | 1 kg | F (-18°C) | T1.4-QUB-XXX | F (12 months) |

**Table A8. Analytical plan for T1.4 - Integration of fast screening methods in the management of seafood production systems**

| Type of product                              | Assay class | Specific analyte(s)/ assay(s)  | Partner             | Assay class                           | Specific analyte(s)/ assay(s) | Partner                    |
|--|-------------|--|---------------------|---------------------------------------|-------------------------------|----------------------------|
| Raw bivalve molluscs                         | Biotoxins   | Tetrodotoxins  | IRTA                | Environment / processing contaminants |                               |                            |
| Fish fillets or edible parts of the products |             |  |                     |                                       | PAHs<br>PFCs<br>PBDEs         | ICETA<br>DTU<br>CSIC, AZTI |
| Raw bivalve molluscs                         |             | Lipophilic toxins (okadaic acid, dinophysistoxins, yessotoxins, azaspiracids), ASP toxins (domoic acid) and PSP toxins (saxitoxin and analogues) | ANFACO / QUB / IRTA |                                       |                               |                            |



**Table A9. Sampling plan for T2.1 - Sodium reduction in seafood products**

| Matrix                            | Type of product             | Biological species: scientific name | Partner providing the sample | Sample origin                                    | Method of sampling | Month(s) of sample delivery to labs | State of the sample | Number of specimens | Number of analytical samples | Total weight of the sample | Shipping conditions | Sample coding | Sample storage post-shipment (shelf-life) |
|-----------------------------------|-----------------------------|-------------------------------------|------------------------------|--|--------------------|-------------------------------------|---------------------|---------------------|------------------------------|----------------------------|---------------------|---------------|---|
| <b>Business-as-usual products</b> |                             |                                     |                              |  |                    |                                     |                     |                     |                              |                            |                     |               |   |
| Fish paté                         | Fish paté (based on salmon) | <i>Salmo salar</i>                  | RISE                         | Norwegian salmon delivered fresh to the producer | Random sampling    | According to milestones             | Vacuum packaged     | One                 | Whatever is required         | 1 kg                       | R (4°C)             | T2.1-XXX      | R (4°C), 6 weeks from production          |
| Smoked fish                       | Smoked salmon               | <i>Salmo salar</i>                  | IRTA                         | Market   | Random sampling    | According to milestones             | Vacuum packaged     | ≥3                  | Whatever is required         | 1 kg                       | R (4°C)             | T2.1-XXX      | R (4°C), 2 weeks from production          |
| <b>Eco-innovative solutions</b>   |                             |                                     |                              |  |                    |                                     |                     |                     |                              |                            |                     |               |   |
| Fish paté                         | Fish paté (based on salmon) | <i>Salmo salar</i>                  | RISE                         | Norwegian salmon delivered fresh to the producer | Random sampling    | According to milestones             | Vacuum packaged     | One                 | Whatever is required         | 1 kg                       | R (4°C)             | T2.1-XXX      | R (4°C), 6 weeks from production          |
| Smoked fish                       | Smoked salmon               | <i>Salmo salar</i>                  | IRTA                         | Market   | Random sampling    | According to milestones             | Vacuum packaged     | ≥3                  | Whatever is required         | 1 kg                       | R (4°C)             | T2.1-XXX      | R (4°C), 2 weeks from production          |

**Table A10. Analytical plan for T2.1 - Sodium reduction in seafood products**

| Type of product             | Assay class              | Specific analyte(s)/ assay(s)       | Partner | Assay class                             | Specific analyte(s)/ assay(s)     | Partner       | Assay class      | Specific analyte(s)/ assay(s) | Partner |
|-----------------------------|--------------------------|-------------------------------------|---------|---|-----------------------------------|---------------|------------------|-------------------------------|---------|
| Fish paté (based on salmon) | Fatty acids              | SAT, MUFA, PUFA, cholesterol, Trans | ICETA   | Water activity, pH and organic acids    | Water activity, pH<br>Lactic acid | RISE<br>ICETA | Minerals         | Na                            | IPMA    |
| Smoked salmon               |                          | SAT, MUFA, PUFA, cholesterol, Trans | ICETA   |   | Water activity, pH<br>Lactic acid | IRTA<br>ICETA |                  | Na                            | IRTA    |
| Type of product             | Assay class              | Specific analyte(s)/ assay(s)       | Partner | Assay class                             | Specific analyte(s)/ assay(s)     | Partner       | Assay class      | Specific analyte(s)/ assay(s) | Partner |
| Fish paté (based on salmon) | Microbiological analyses | Biological contaminants             | RISE    | Environmental / processing contaminants |                                   |               | Sensory analyses | Sensory attributes            | RISE    |
| Smoked salmon               |                          | Biological contaminants             | IRTA    |   | PAHs                              | CSIC          |                  | Sensory attributes            | IRTA    |





**Table A11. Sampling plan for T2.2 - Digestible, attractive, functional, sustainable and nutritionally adapted food to specific population groups**

| Matrix                            | Type of product                        | Biological species: scientific name | Partner providing the sample | Sample origin   | Method of sampling | Month(s) of sample delivery to labs | State of the sample  | Number of specimens | Number of analytical samples | Total weight of the sample | Shipping conditions | Sample coding | Sample storage post-shipment (shelf-life) |
|-----------------------------------|--|-------------------------------------|------------------------------|-----------------|--------------------|-------------------------------------|----------------------|---------------------|------------------------------|----------------------------|---------------------|---------------|---|
| <b>Business-as-usual products</b> |  |                                     |                              |                 |                    |                                     |                      |                     |                              |                            |                     |               |   |
| Youth product 1                   | RTE fish product, processed and packed | Not defined yet                     | Not defined yet              | Not defined yet | Random sampling    | M24 / 25                            | Final cooked product | 3                   | 3                            | 200 g approx.              | Not defined yet     | T2.2-XXX      | Not defined yet                           |
| Youth product 2                   | RTE fish product, processed and packed | Not defined yet                     | Not defined yet              | Not defined yet | Random sampling    | M24 / 25                            | Final cooked product | 3                   | 3                            | 200 g approx.              | Not defined yet     | T2.2-XXX      | Not defined yet                           |
| Senior product 1                  | RTE fish product, processed and packed | Not defined yet                     | Not defined yet              | Not defined yet | Random sampling    | M24 / 25                            | Final cooked product | 3                   | 3                            | 200 g approx.              | Not defined yet     | T2.2-XXX      | Not defined yet                           |
| Senior product 2                  | RTE fish product, processed and packed | Not defined yet                     | Not defined yet              | Not defined yet | Random sampling    | M24 / 25                            | Final cooked product | 3                   | 3                            | 200 g approx.              | Not defined yet     | T2.2-XXX      | Not defined yet                           |
| Woman product 1                   | RTE fish product, processed and packed | Not defined yet                     | Not defined yet              | Not defined yet | Random sampling    | M24 / 25                            | Final cooked product | 3                   | 3                            | 200 g approx.              | Not defined yet     | T2.2-XXX      | Not defined yet                           |
| Woman product 2                   | RTE fish product, processed and packed | Not defined yet                     | Not defined yet              | Not defined yet | Random sampling    | M24 / 25                            | Final cooked product | 3                   | 3                            | 200 g approx.              | Not defined yet     | T2.2-XXX      | Not defined yet                           |
| <b>Eco-innovative solutions</b>   |  |                                     |                              |                 |                    |                                     |                      |                     |                              |                            |                     |               |   |
| Youth                             | RTE fish                               | Not defined                         | IDmer                        | production      | Random             | M24 / 25                            | final                | 3                   | 3                            | 200 g                      | Not                 | T2.2-XXX      | Not                                       |



|                  |  |                 |       |                                |                                      |          |                      |   |   |               |                 |                             |
|------------------|--|-----------------|-------|--------------------------------|--------------------------------------|----------|----------------------|---|---|---------------|-----------------|-----------------------------|
| product 1        | product, processed and packed          | yet             |       | plant IDmer, France            | sampling of the final product        |          | cooked product       |   |   | approx.       | defined yet     | defined yet                 |
| Youth product 2  | RTE fish product, processed and packed | Not defined yet | IDmer | production plant IDmer, France | Random sampling of the final product | M24 / 25 | final cooked product | 3 | 3 | 200 g approx. | Not defined yet | T2.2-XXX<br>Not defined yet |
| Senior product 1 | RTE fish product, processed and packed | Not defined yet | IDmer | production plant IDmer, France | Random sampling of the final product | M24 / 25 | final cooked product | 3 | 3 | 200 g approx. | Not defined yet | T2.2-XXX<br>Not defined yet |
| Senior product 2 | RTE fish product, processed and packed | Not defined yet | RISE  | production plant IDmer, France | Random sampling of the final product | M24 / 25 | final cooked product | 3 | 3 | 200 g approx. | Not defined yet | T2.2-XXX<br>Not defined yet |
| Woman product 1  | RTE fish product, processed and packed | Not defined yet | IDmer | production plant IDmer, France | Random sampling of the final product | M24 / 25 | final cooked product | 3 | 3 | 200 g approx. | Not defined yet | T2.2-XXX<br>Not defined yet |
| Woman product 2  | RTE fish product, processed and packed | Not defined yet | RISE  | production plant IDmer, France | Random sampling of the final product | M24 / 25 | final cooked product | 3 | 3 | 200 g approx. | Not defined yet | T2.2-XXX<br>Not defined yet |



**Table A12. Analytical plan for T2.2 - Digestible, attractive, functional, sustainable and nutritionally adapted food to specific population groups**

| Assay class  | Specific analyte(s)/ assay(s)   | Partner       | Assay class                                     | Specific analyte(s)/ assay(s)   | Partner    | Assay class             | Specific analyte(s)/ assay(s)                   | Partner       |
|--|---|---------------|---|---|------------|-------------------------|---|---------------|
| Proximate composition and fatty acids (all matrices)                   | Protein, carbohydrates, total fat, moisture, ash, fibre, aminoacids (total and free), TVB-n and TMA, water activity | IPMA/ANFACO   | Biologically-relevant properties (all matrices) | Water activity, antioxidant activity, lipid oxidation   | IPMA/ICETA | Minerals (all matrices) | Se and Se species, Ca, Cu, Fe, Zn               | ISS           |
|  | SAT, MUFA, PUFA, cholesterol, Trans   | ICETA         |   | <i>in vitro</i> bio-accessibility (trace elements, e.g., those in which the products are fortified) | ISS        |                         | Na, Mg, P, Cl, K                                | IPMA          |
| Assay class  | Specific analyte(s)/ assay(s)   | Partner       | Assay class                                     | Specific analyte(s)/ assay(s)   | Partner    | Assay class             | Specific analyte(s)/ assay(s)                   | Partner       |
| Vitamins and molecules in which the products are going to be fortified | Vitamins (E, D) (all matrices)  | ICETA         | Chemical and biological contaminants            | As, inorganic As, Cd, Pb, Hg, MeHg, Ni* (all matrices)  | ISS        | Sensory analyses        | Sensory attributes (products developed by RISE) | RISE          |
|  | Vitamin A (in products fortified with Vit. A)   | To be defined |   | PAHs, PBDEs (all matrices)  | CSIC       |                         | Sensory attributes (all other products)         | IPMA / ANFACO |
|  |   |               |   | Biological contaminants (products developed by RISE)  | RISE       |                         |   |               |
|  |   |               | Biological contaminants (all other products)    | ANFACO  |            |                         |   |               |

\* For toxic elements, the following panel is valid for RTE products based on Teleosts: Cd, Pb, Hg, and MeHg. If a RTE product is based or contains Crustaceans/Molluscs the following analytes are of special importance: As and inorganic As, Cd, Pb, Hg, and Ni.



**Table A13. Sampling plan for T2.3 - Strategies to reduce contaminants from seafood products**

| Matrix                            | Type of product                             | Biological species: scientific name                   | Partner providing the sample             | Sample origin   | Method of sampling                           | Month(s) of sample delivery to labs        | State of the sample                             | Number of specimens                         | Number of analytical samples | Total weight of the sample | Shipping conditions               | Sample coding | Sample storage post-shipment (shelf-life)     |
|-----------------------------------|---|---|--|---|--|--|---|---|------------------------------|----------------------------|-----------------------------------|---------------|---|
| <b>Business-as-usual products</b> |   |   |  |   |  |  |   |   |                              |                            |                                   |               |   |
| Norovirus reduction               | Raw mollusc with valves                     | <i>Crassostrea gigas</i>                              | Industry partner (WCM or other supplier) | Production area in the UK (specific origin not known at this stage) | Collected randomly by hand from growing bags | M12  | Whole shellfish                                 | 300   | 56                           | To be defined              | T must not exceed 10°C            | T2.3-NoVXXX   | Samples should not be frozen prior to testing |
| Listeria reduction                | fresh fish fillet / cold smoked fish fillet | <i>Salmo salar</i>                                    | AZTI or other industrial supplier        | Industrial supplier or Market - Basque Country (Spain)              | Random sampling                              | From M20 to M22. AZTI will do the analysis | Fresh salmon fillet / cold smoked salmon fillet | 24  | 24                           | Approx. 100 g              | Not applicable (analysis at AZTI) | T2.3-ListXXX  | R (4°C)                                       |
| PSPs reduction*                   | Processed bivalves (canned)                 | <i>Mytilus galloprovincialis</i> / others (depending) | ANFACO                                   | Market or industrial processor                                      | Random sampling                              | M23-24                                     | Canned molluscs / preserved                     | 3 (different brands) x 2 (different sauces) | 3 x 2                        | 100 g each sample          | RT                                | T2.3-PSP-XX   | RT  |
| <b>Eco-innovative solutions</b>   |   |   |  |   |  |  |   |   |                              |                            |                                   |               |   |
| Norovirus reduction               | Raw mollusc with valves                     | <i>Crassostrea gigas</i>                              | Industry partner (WCM or other supplier) | Production area in the UK (specific origin not known at this stage) | Collected Randomly by hand from growing bags | M12  | Whole shellfish                                 | 1640  | 320                          | To be defined              | T must not exceed 10°C            | T2.3-NoVXXX   | Samples should not be frozen prior to testing |
| Listeria reduction                | fish fillet                                 | <i>Salmo salar</i>                                    | AZTI or other industrial supplier        | Industrial supplier or Market - Basque                              | Random sampling                              | From M20 to M22. AZTI will do the          | Fresh salmon fillet / cold                      | 24  | 24                           | Approx. 100 g              | Not applicable (analysis at AZTI) | T2.3-ListXXX  | Under refrigeration                           |



|                   |   |   |                                 | Country<br>(Spain)                        |   | analysis   | smoked<br>salmon<br>fillet        |                          |    |                         |                     |                 |                  |
|-------------------|---|---|---------------------------------|---|---|--|-----------------------------------|--------------------------|----|-------------------------|---------------------|-----------------|------------------|
| PSPs<br>reduction | Raw<br>bivalves<br>molluscs<br>(with<br>valves) | <i>Mytilus<br/>galloprovincialis</i><br>/ others<br>(depending) | IRTA, Cefas,<br>IPMA,<br>ANFACO | Depends on<br>the PSP<br>toxic<br>episode | collection<br>from<br>rafts/harve<br>sting area | Depends<br>on the<br>occurrenc<br>e of toxic<br>episodes | Fresh or<br>frozen<br>shellfish   | 25 mussels<br>per sample | 80 | I kg<br>each<br>sample  | R(4°C)/<br>F(-18°C) | T2.3-<br>PSP-XX | F (12<br>months) |
| PSPs<br>reduction | Processed<br>bivalves<br>(canned)               | <i>Mytilus<br/>galloprovincialis</i><br>/ others<br>(depending) | ANFACO                          | Depends on<br>the PSP<br>toxic<br>episode | collection<br>from<br>rafts/harve<br>sting area | Depends<br>on the<br>occurrenc<br>e of toxic<br>episodes | Canned<br>molluscs /<br>preserved | 30                       | 30 | 100 g<br>each<br>sample | RT                  | T2.3-<br>PSP-XX | RT               |

\* Only sensorial analyses, comparison with detoxified products.

**Table A14. Analytical plan for T2.3 - Strategies to reduce contaminants from seafood products**

| Type of product                                   | Assay class                 | Specific analyte(s)/<br>assay(s)                                       | Partner       | Assay class      | Specific analyte(s)/<br>assay(s) | Partner |
|---|-----------------------------|--|---------------|------------------|----------------------------------|---------|
| Raw mollusc with valves<br>(Norovirus reduction)  | Microbiological<br>analyses | Human norovirus (genogroups I and<br>II); F-specific RNA bacteriophage | Cefas         | Sensory analyses |                                  |         |
| Fish fillet<br>(Listeria reduction)               |                             | <i>Listeria</i>  | AZTI          |                  | instrumental colour              | AZTI    |
| Processed bivalves,<br>canned<br>(PSPs reduction) |                             | PSP toxins   | ANFACO / IRTA |                  | Sensory attributes               | ANFACO  |

**Table A15. Sampling plan for T2.4 - Reduction of energy and water in seafood processing**

| Matrix                            | Biological species: scientific name | Partner providing the sample | Sample origin                   | Method of sampling   | Month(s) of sample delivery to labs | State of the sample | Number of specimens | Number of analytical samples | Total weight of the sample | Shipping conditions                               | Sample coding | Sample storage post-shipment (shelf-life)         |
|-----------------------------------|-------------------------------------|------------------------------|---------------------------------|--|-------------------------------------|---------------------|---------------------|------------------------------|----------------------------|---|---------------|---|
| <b>Business-as-usual products</b> |                                     |                              |                                 |  |                                     |                     |                     |                              |                            |   |               |   |
| Fish soup                         | Not defined yet                     | IRTA                         | CENTA, Monells, Girona, (Spain) | Random sampling at the end of the treatment plant          | M12-M18                             | Liquid soup         | 3 x 2               | 3 x 2                        | 6 kg                       | R (4°C) for pasteurized product/RT for sterilized | T2.4-XXX      | R (4°C) for pasteurized product/RT for sterilized |
| Water effluents                   | Not applicable                      | IRTA                         | CENTA, Monells, Girona, (Spain) | Random sampling of the waste waters of the treatment plant | M12-M18                             | Liquid (water)      | 3 x 2               | 3 x 2                        | 6 kg                       | RT  | T2.4-XXX      | RT  |
| Dried fish                        | Not defined yet                     | ILVO                         | ILVO production unit, Melle     | Random sampling of dried product                           | M12-M18                             | Dried fish          | 3 x 3               | 3 x 3                        | 1 - 2 kg                   | RT  | T2.4-XXX      | RT  |
| <b>Eco-innovative solutions</b>   |                                     |                              |                                 |  |                                     |                     |                     |                              |                            |   |               |   |
| Fish soup                         | Not defined yet                     | IRTA and ANFACO              | CENTA, Monells, Girona (Spain)  | Random sampling at the end of the treatment plant          | M12-M18                             | Liquid soup         | 3 x 4               | 3 x 4                        | 12 kg                      | R (4°C) for pasteurized product/RT for sterilized | T2.4-XXX      | R (4°C) for pasteurized product/RT for sterilized |
| Water effluents                   | Not applicable                      | IRTA                         | CENTA, Monells, Girona (Spain)  | Random sampling of the waste waters of the treatment plant | M12-M18                             | Liquid (water)      | 3 x 2               | 3 x 2                        | 6 kg                       | RT  | T2.4-XXX      | RT  |
| Dried fish                        | Not defined yet                     | ILVO                         | ILVO production unit, Melle     | Random sampling of dried product                           | M12-M18                             | Dried fish          | 3 x 3               | 3 x 3                        | 1 - 2 kg                   | RT  | T2.4-XXX      | RT  |



**Table A16. Analytical plan for T2.4 - Reduction of energy and water in seafood processing**

| Type of product | Assay class                                | Specific analyte(s)/ assay(s)                          | Partner       | Assay class              | Specific analyte(s)/ assay(s) | Partner      | Assay class      | Specific analyte(s)/ assay(s) | Partner     |
|-----------------|--|--|---------------|--------------------------|-------------------------------|--------------|------------------|-------------------------------|-------------|
| Fish soup       | Proximate composition and other properties | Lipid oxidation  | IPMA/ICETA    | Microbiological analyses | Biological contaminants       | IRTA /ANFACO | Sensory analyses | Sensory attributes            | IRTA/ANFACO |
| Water effluents |  | Organic load, pH, CQC and CBO                          | IPMA/ICETA    |                          | Biological contaminants       | IRTA         |                  |                               |             |
| Dried fish      |  | Protein, total fat, ash                                | ILVO          |                          |                               |              |                  | Sensory attributes            | ILVO        |
|                 |  | MUFA, PUFA, Omega-3 PUFA, lipid oxidation, amino acids | To be defined |                          |                               |              |                  |                               |             |

**Table A17. Excel form for reporting results: page 1.**

|                    |  |  |
|--------------------|--|--|
|                    |  | SEAFOOD <sup>TOMORROW</sup><br>Grant agreement no: 773400<br>WP3<br>Task 3.1 |
| <b>LABORATORY</b>  |  |  |
| Partner short name |  |  |
| Institution        |  |  |
| Lab. Name          |  |  |
| Address            |  |  |
| Contact Person     |  |  |
| Tel.               |  |  |
| e-mail             |  |  |

Lab info | Samples | Results |



**Table A18. Excel form for reporting results: page 2.**

| SAMPLES TO BE ANALYZED  |                                 |                                   |                    |
|-------------------------|---------------------------------|-----------------------------------|--------------------|
| Matrix/Sample           | Sample code                     | Date of arrival at the Laboratory | Storage conditions |
| e.g. Salmon fish fillet | aaa<br>bbb<br>ccc<br>ddd<br>eee | xx yy 20zz                        | (description)      |

  

| PARAMETER/PROPERTY TO BE MEASURED |                              | METHOD <i>(please specify if official method, reference/standard method, accredited method, internally validated method)</i> |                   |
|-----------------------------------|------------------------------|--|-------------------|
| Assay class                       | Specific analyte(s)/assay(s) | Analytical Technique   | Analytical Method |
|                                   |                              |  |                   |
|                                   |                              |  |                   |
|                                   |                              |  |                   |
|                                   |                              |  |                   |
|                                   |                              |  |                   |
|                                   |                              |  |                   |
|                                   |                              |  |                   |
|                                   |                              |  |                   |

  

Lab info
Samples
Results



**Table A19. Excel form for reporting results: page 3.**

| REPORTING FORM  |                        | sample code       |         |         |         |         |         |                         |                 |                  |
|---|------------------------|-------------------|---------|---------|---------|---------|---------|-------------------------|-----------------|------------------|
| <b>Moisture content (%)<sup>1</sup></b>   |                        |                   |         |         |         |         |         |                         |                 |                  |
| <i>1: If determined<br/>2: Approximative weight in grams of the test portion<br/>3: Parameter/property measured<br/>4: Concentration and LOQ to be reported in the same unit<br/>5: Automatically calculated<br/>6: Limit of Quantification</i> |                        |                   |         |         |         |         |         |                         |                 |                  |
| Test portion (g) <sup>2</sup>   | Measurand <sup>3</sup> | Unit <sup>4</sup> | Value 1 | Value 2 | Value 3 | Value 4 | Value 5 | Mean value <sup>5</sup> | SD <sup>5</sup> | LOQ <sup>6</sup> |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
|   |                        |                   |         |         |         |         |         | #DIV/0!                 | #DIV/0!         |                  |
| REFERENCE MATERIALS or CERTIFIED REFERENCE MATERIALS USED   |                        |                   |         |         |         |         |         |                         |                 |                  |
| Please describe the name od the material, the producer, the matrix, the reference/certified values and the found values   |                        |                   |         |         |         |         |         |                         |                 |                  |
| ▶ ◀   Lab info   Samples   Results   🔍  |                        |                   |         |         |         |         |         |                         |                 |                  |