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Smart4Sea

D1. Sampling and Quality Assurance Protocol (WP3, Activity No 2)

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1. SAMPLES FROM FISHING GROUNDS RELEVANT TO THE EUROPEAN CONSUMER

1.1. Objectives

The purpose of sampling fishing grounds is to target relevant commercial fish species consumed in European countries. From each fish, the dorsal muscle will be collected to quantify:

1. Microplastics (MPs) and stable isotope signatures;
2. Co-occurring endocrine-disrupting chemicals (EDCs) – *e.g.*, bisphenols, phthalates, polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), UV filters and musks;
3. Nutrients (protein, fatty acid, amino acid, and mineral contents).

The levels of MPs and EDCs, and their potential relationships and impacts on seafood's nutritional value, will be used to inform potential exposure and toxicological risks to consumers.

2. GUIDELINES FOR SAMPLE COLLECTION

Duration: Mar-Apr 2026

Responsible partner: ARVI-INNOVAPESCA

The criteria used for selecting target fish species were the following:

a) Only species in the "Least Concern" category of the International Union for Conservation of Nature (IUCN Red List);
b) Relevance to the European capture fisheries and to the European consumer;
c) Wide geographic distribution, covering benthic and pelagic environments;
d) Easy to identify taxonomically;
e) Large enough to provide adequate tissue for the analyses;
f) Susceptible of accumulating contaminants.

Tips for fish/squid samples collection from capture fishing vessels:

g) Specimens should be captured in permitted fishing grounds for the target species being sought (Table 1.1);
h) The collected specimens should satisfy any legal requirements of harvestable size or weight, or at least be of consumable/commercial size if no legal harvest requirements are in effect;
i) Fish/squid with visible signs of disease or lesion should not be included in the composite sample and, if included by any chance, these will not be considered for the analyses to preserve the integrity and quality of the other samples;
j) Specimens should be of similar size so that the smallest specimen in a composite is no less than 75% of the total length of the largest specimen (if possible);
k) All fish of one species should be collected during a fishing gear deployment (i.e., if this is not possible, samples may be combined from other deployment events conducted as close in time as possible, but no more than one week apart);
l) For conducting the analysis, a minimum number of specimens is required is 25 specimens for hake and monkfish; 50 specimens for sardine, mackerel, and squid (Table 1.1);
m) If feasible, the collection team participating in sampling needs to ensure a limited time lag between fish entrapment and sample preservation;

n) Sampling information should accompany the specimens to the finest detail possible with the following information in a Field Record Form (Table 1.2):
- collection location/GPS data,
- physicochemical parameters (water temperature),
- day and hour of collection,
- duration between capture and processing,
- type and composition of fishing gear,
- time and number of deployments,
- approximate sampled area in square meters,
- characteristics of site,
- total number of specimens;

o) Hake and monkfish & hake fisheries: Before being frozen, viscera must be removed carefully, avoiding the rupture of any organs, such as digestive tract and gallbladder, and their contact with the remaining carcass. These are organs where MPs and EDCs are retained and their rupture can interfere with further analyses;

p) Mackerel, sardine & squid: Fish can be frozen whole, requiring minimal preparation during their collection in the fishing vessels;

q) Contamination of the sample during collection and transport must be avoided: possible sources of contamination are smoking, exhaust engines, dust;

r) Frozen blocks with fish will be wrapped in aluminium to reduce the contact between fish and the box (i.e., potential presence of plastic lining);

s) Every composite sample should be properly identified and accompanied by a Field Record Form before sending it to the University of Porto (correspondence details in Page 2).

Table 1. List of the species selected.

Name	Sci name	FAO code	Min size (cm)*	Classification	Min # indv
Monkfish	*Lophius* spp.	MON		Demersal	25
Hake	*Merluccius merluccius*	HKE	≥27.0	Demersal	25
Mackerel	*Scomber scombrus*	MAC	≥20-30.0	Benthopelagic	50
Sardine	*Sardinia pilchardus*	PIL	≥11.0	Benthopelagic	50
Argentine shortfin squid	*Illex argentinus*	SQA		Benthopelagic	50

*Minimum commercial size

Table 2. Example of Field Record Form (modify to meet project needs).

Composite Sample ID:_____ Date and hour of collection:_____

Collection method:_____ Nr of deployments:_____ Duration (*in min*):_____

Type/composition of fishing gear:_____

Location data

ICES Fishing Area Code (*area / subarea / division*): _____

* Note subarea and division if equal to all specimens

Latitude: _____ Longitude:_____

Collection depth range:_____

Site characteristics (proximity to coast, relevant meteorological conditions, others):_____

_____

Physicochemical parameters (water)

Temperature:_____

Sample description

Fish species:_____ Total nr of individuals:_____

Specimen #	Length (mm)	Weight (g)	Date/time	Notes
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____
_____	_____	_____	_____	_____

Time between capture and processing:_____

Additional comments:_____

_____

Collection responsible:_____ Contact:_____

Affiliation:_____

Address to send:_____

_____

3. GUIDELINES FOR SEAFOOD MUSCLE COLLECTION

Duration: Mar-Oct 2026

Responsible partner: FFUP

The focus on collecting muscle samples from fish and squid is to characterise MP and EDC levels not only in the tissue that represents the largest proportion of the organism's biomass, but also in the tissue most relevant to human consumption. Furthermore, the characterisation of MP and EDC levels in seafood muscle in the mentioned species provides an overview of the European consumer intake from seafood species relevant to the capture fisheries. The analyses of stable isotope signatures and nutritional components allow to further explore the influence of ecological traits, and how MPs and EDCs may affect nutritional value.

Tips for collection muscle samples from seafood:

3.1. Safety and contamination control

a) The working stations where muscle samples will be collected must be thoroughly cleaned with ethanol and free of MP sources to mitigate procedural contamination (e.g., no ventilation, area with limited access, no textiles). The conduction of sample handling inside a fume hood in a less busy area of the laboratory is preferred;
b) Reduce MP sources by wearing 100% cotton lab coats and powder-free nitrile gloves;
c) Replace plastic equipment by metal/glass alternatives whenever possible, such as cutting boards, dissection tools, among others;
d) Procedural blanks (Petri dishes with clean fibreglass micromembrane filters) must accompany sample handling to account for potential sample contamination by airborne MPs;

3.2. Sample preparation and morphometric data

e) Defrost frozen blocks of fish/squid before sample collection;
f) If the frozen block was not wrapped in aluminium foil and has >50 specimens, those in contact with the box should not be considered for the analyses. If those specimens in contact with the box are needed to ensure the number of specimens required, register that information;
g) Rinse the skin of the fish/squid with distilled water to remove adhered impurities;
h) Record the total body weight and standard length (fish) and dorsal mantle length (squid);
i) For non-eviscerated species (only performed after muscle sample extraction!) – eviscerate and record the weight of the liver and visceral fat (fish) and digestive gland (squid) for the calculation of organosomatic indexes;
j) Since hake and monkfish are eviscerated onboard of the fishery vessel, total body weight will derive from the recorded weight (fish without viscera) and the estimated average visceral weight per specimen (data provided by ARVI-INNOVAPESCA);

3.3. Muscle sampling – Fish

k) Place the specimen with the left flank upwards and, using a scalpel, cut into the muscle in the entire dorsal region (avoid the abdominal region to prevent contamination from viscera where MPs are easily retained);

l) Collect dorsal muscle tissue for A – characterising MP load (10-20 g) and B – stable isotope signatures (-2 g). Remove the skin of both A and B samples;
m) Weigh each sample, wrap in aluminium foil properly labelled, and store at –20 °C;
n) Place the specimen with the right flank upwards and, using a scalpel, cut into the muscle in the entire dorsal region (also avoiding the abdominal region);
o) Collect dorsal muscle tissue for C – characterising EDC load (~8 g), D – characterising nutritional value, and E – quantify total lipid & protein contents (~5 g). Use muscle trimmings for E samples if needed. Remove the skin of both D and E samples;
p) Weigh each sample and wrap in aluminium foil properly labelled. The samples for C and D must be lyophilised before storage (weigh the samples after lyophilisation again). Preserve the samples at –80 °C.

3.4. Muscle sampling - Squid

q) Using a scalpel, cut longitudinally in the ventral part of the mantle and carefully remove the viscera (be sure not to rupture the ink sac) and fins. Divide the mantle in two, isolating the right side for A and B, and the left side for C, E, and D;
r) Collect and process mantle samples following steps l) and m) for A and B samples, and steps o) and p) for C, D, and E samples;

Note: The amount of sample required for characterising EDC load (C) will be used on demand, aiming to ensure that sufficient material remains preserved for subsequent analysis (D).

Table 3. Detailed sample list collected from fish and squid.

Analytical Purpose	Approx. mass	Without skin	Storage
A – Quantify microplastic load	10-20 g	X	–20 °C
B – Stable isotope signatures	2 g	X	–20 °C
C – Quantify EDC load	8 g		–80 °C (lyophilised)
D – Characterise nutritional value	10 g	X	–80 °C (lyophilised)
E – Total lipid & protein content	5 g	X	–80 °C

Table 1 - Caption table 1

Labelling details

Each sample will be labelled during the sample preparation at the University of Porto. The labels, entitled "MICROPLASTICS", "STABLE ISOTOPES", "EDCs", "NUTRITIONAL VALUE", "TOTAL LIPIDS", "TOTAL PROTEIN" will be colour-coded per species and will show the sample title.

Specie's FAO code + "_" + specimen number

Figure 1. Scheme for the sample labels sorted by analytical purpose.

Monkfish

SMART4SEA
MICROPLASTICS
MON_05 *(e.g. sample code)*

Hake

SMART4SEA
MICROPLASTICS
HKE_05 *(e.g. sample code)*

Sardine

SMART4SEA
MICROPLASTICS
PIL_05 *(e.g. sample code)*

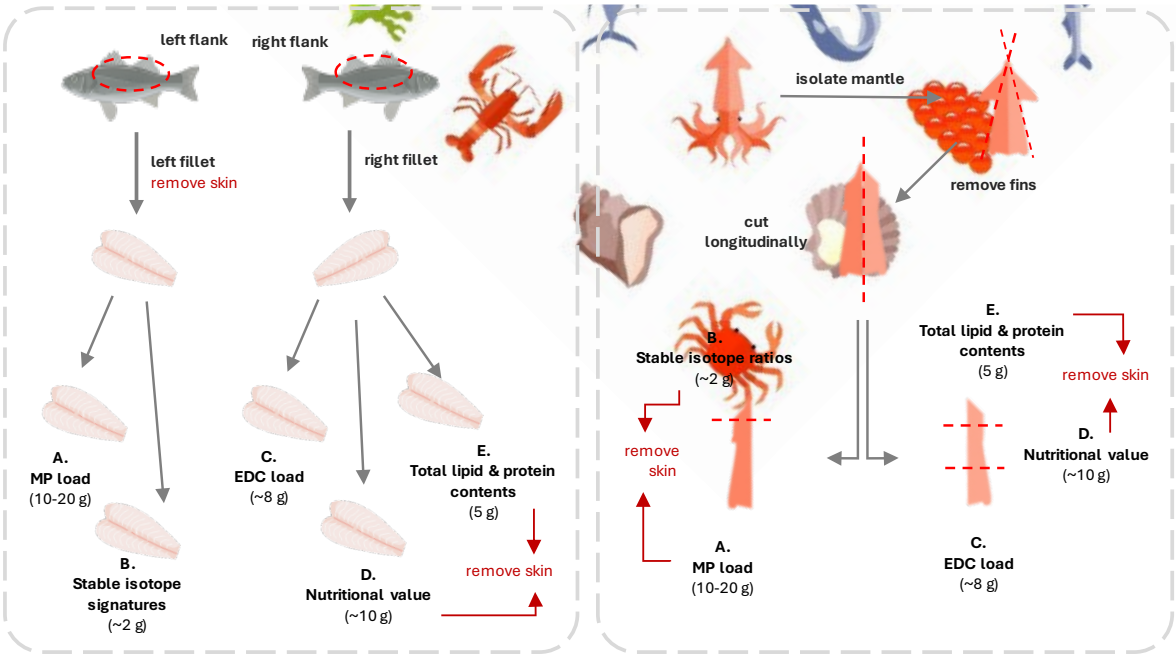
Mackerel

SMART4SEA
MICROPLASTICS
MAC_05 *(e.g. sample code)*

Squid

SMART4SEA
MICROPLASTICS
SQA_05 *(e.g. sample code)*

Figure 2. Scheme for the muscle sample collection



4. GUIDELINES FOR NUTRITIONAL VALUE ANALYSIS OF SEAFOOD MUSCLE

Duration: Sep-Dec 2026

Responsible partner: FFUP and IPMA

For each species, two groups will be established based on the mean MP levels found in the muscle of the 50 specimens (25 specimens in the case of monkfish and hake). The two groups establish are defined by the least and most contaminated specimens (MPs/g) and comprised by a minimum of 10 specimens. EDC levels will be used as criterion for group attribution if MP levels reveal inefficient. Therefore, the following activities will be performed after obtaining MP and EDC results.

Tips for the sample preparation to nutritional value analysis:

a) The lyophilised samples for D – characterise nutritional value will be sent in dry ice to IPMA (correspondence details in Page 2). The samples for E – quantify total lipid & protein contents will be analysed at FFUP, thus not requiring transportation;
b) Each pool will be prepared with the homogenisation of the D samples from the selected specimens (10 indv. per pool) together using a stainless-steel kitchen grinder;
c) Three replicates per pool will be collected and stored in falcon tubes, with the respective labelling, and stored at -80 °C.

Labelling details

Each sample will be labelled during the sample preparation at the University of Porto. The labels, entitled "NUTRITIONAL VALUE", will be colour-coded per species and will show the sample title. "LOW" or "HIGH" will be used to distinguish the least and most contaminated groups, respectively.

LOW/HIGH + "_" + Specie's FAO code + replicate #

Figure 3. Scheme for the sample labels destined for nutritional analysis sorted by contamination levels.

Monkfish (pool with least contaminated fish)

SMART4SEA
NUTRITIONAL VALUE

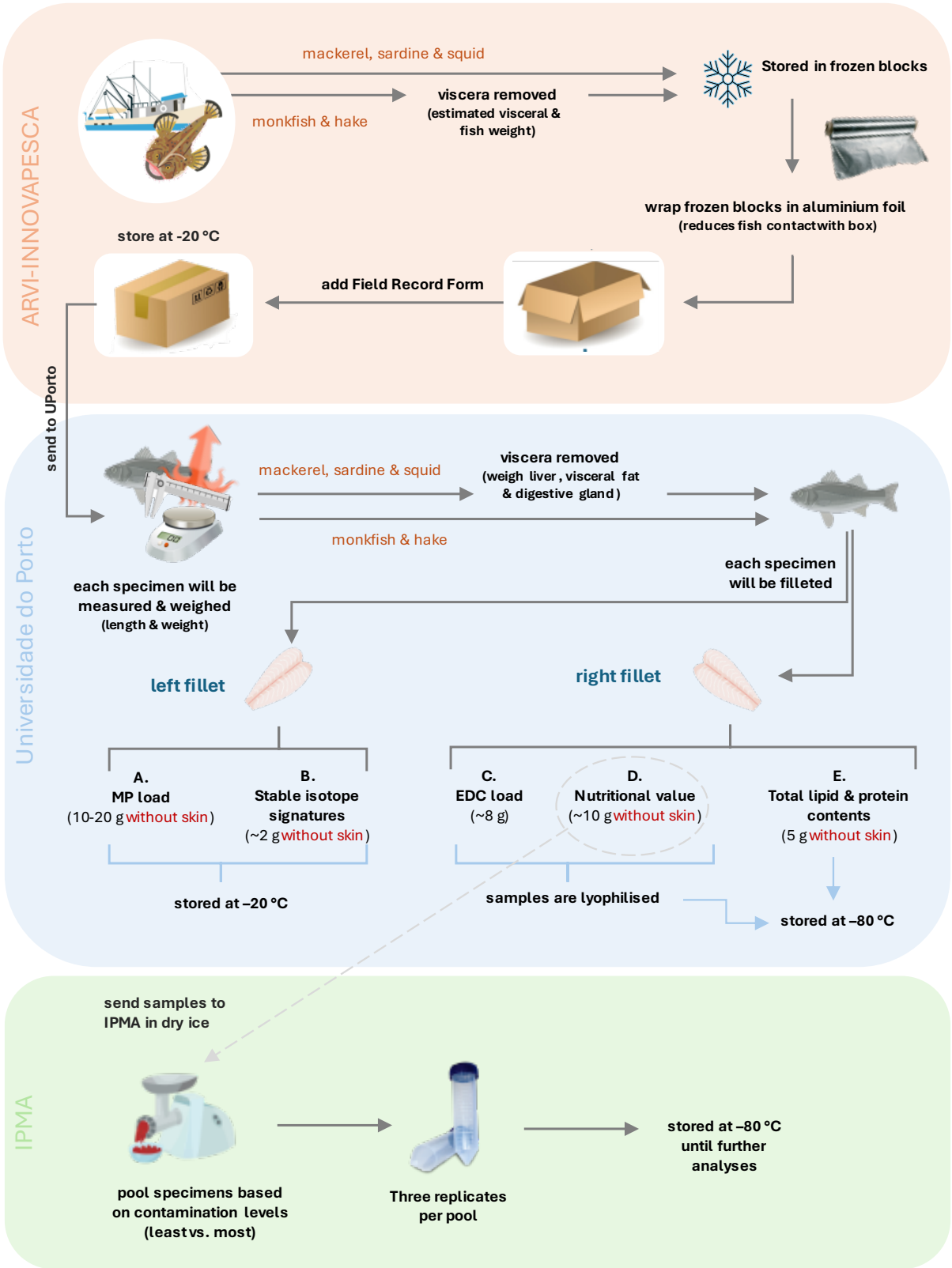
LOW_MON 1 *(e.g. sample code)*

Monkfish (pool with most contaminated fish)

SMART4SEA
NUTRITIONAL VALUE

HIGH_MON 1 *(e.g. sample code)*

Figure 4. Scheme for the overall sample collection workflow



5. DETAILS OF THE PARTNERS INVOLVED IN THE ACTIVITY

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