Anisakids Detection Methods
I. UV-Press method

This project has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration under Grant Agreement no. 312068.

CETMAR
CENTRO TECNOLÓGICO DEL MAR

Parasite PArasite Risk ASsessment with Integrated Tools in EU fish value chain

- February 2013-January 2016
- 21 partners (15 RTDs; 6 SMEs)
  - ✓ 13 countries (10 Europe+ 3 Asia)
  - ✓ interdisciplinary

Objective: provide new scientific evidence and technological developments to detect, monitor and mitigate the impact of zoonotic parasites, mainly anisakid nematodes, in European and imported fishery products

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What is a **zoonotic parasite?**

It is a parasite that can be naturally transmitted from animals to humans and vice versa (WHO, World Health Organization).

Fishery products can be affected by more tan 50 zoonotic parasite species (cestodes of the genus *Diphyllobothrium*, digenetic trematodes of the families Heterophyidae, Opisthorchiidae and Echisnostomatidae, etc)

In Europe, anisakids nematodes are the most relevant group regarding the safety of fishery products, mainly those species included in the genera *Anisakis* and *Pseudoterranova*.

Anisakiasis is the accidental infection of humans after the ingestion of *Anisakis* larvae.

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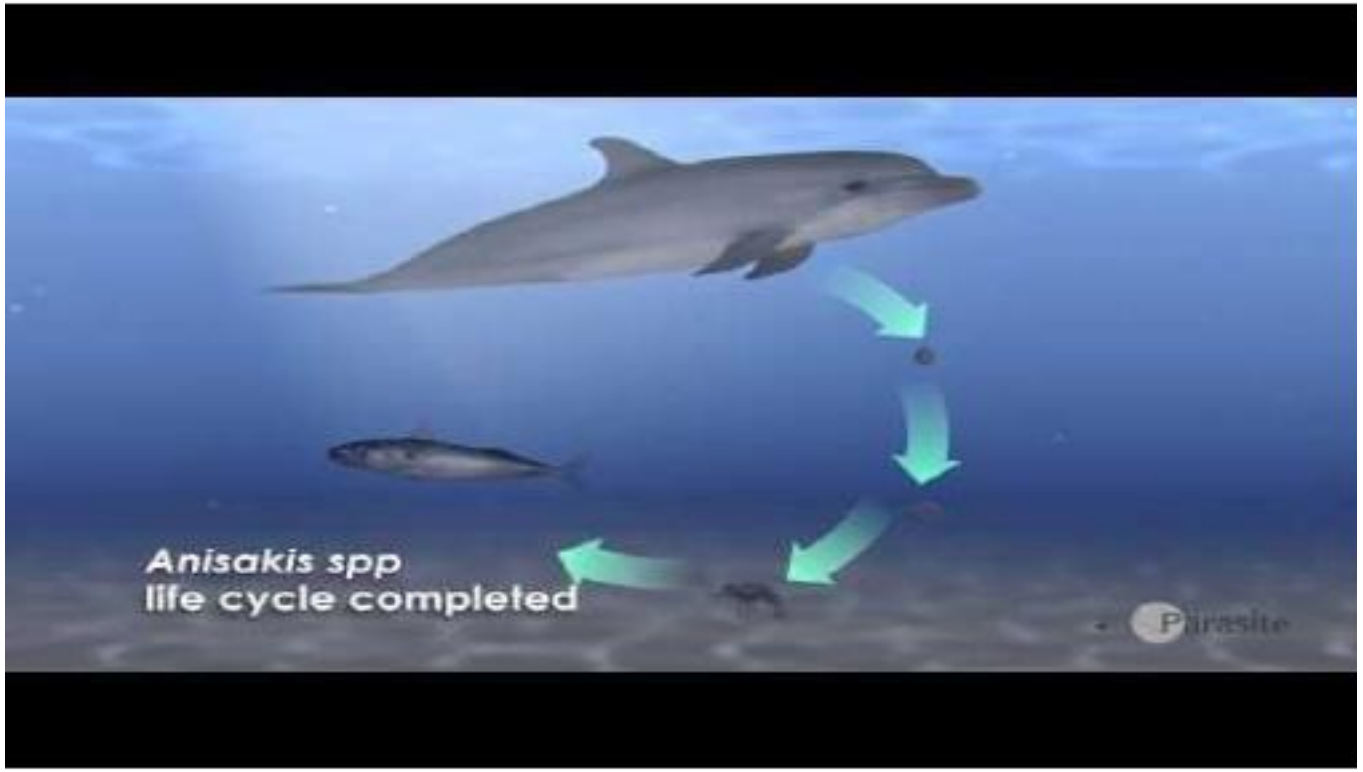
But…How do fishes and humans become infected by *Anisakis*?



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…And which are the consequences for humans?

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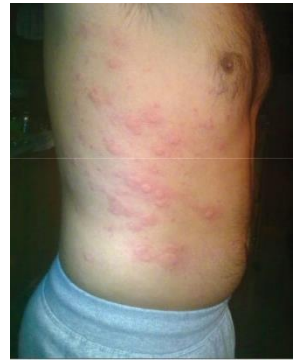
Social and Health impact: *GASTRO-ALLERGIC ANISAKIASIS and ALLERGY*



- Increasing health problem:

- ✓ **Spain**: recognized as "health problem" in the Real Decreto 1420/2006, 1st December. The prevalence of *Anisakis* hypersensitivity detected ranged from 12,4% in the population of Madrid to 0,43% in Galicia.

- ✓ **Italy**: Doctors working in 34 allergy centers across Italy agreed to participate in a multicenter, prospective epidemiologic survey. Results revealed that sensitization rate showed marked geographic differences (range: 0.4–12.7%), being highest along the Adriatic and Tyrrhenian coasts (AAITO-IFIACI *Anisakis* Consortium, 2011).

- ✓ **Croatia**: Mladineo et al (2014) showed that IgE sensitization to *Anisakis* reached 3.5% in the population of higher fish consumers (islands). The study underlines the necessity of incorporating *Anisakis* spp. allergens in routine hypersensitivity testing of coastal population.

- ✓ Lin et al. (2014) observed a low seroprevalence of anti-Anisakis IgE antibodies in a **Norwegian** population.

- ✓ Kim Jung et al. (2011) showed for the first time the seroprevalence of anisakiasis in **Korea**, being the seropositive rate of 6.6%.

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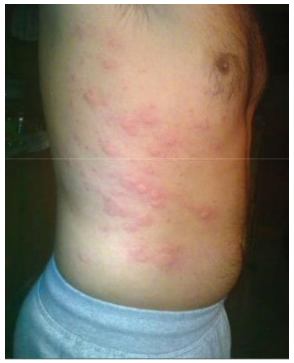
Social and Health impact: *GASTRO-ALLERGIC ANISAKIASIS and ALLERGY*



Anisakiasis, both gastric and allergic, is caused by the ingestion of live *Anisakis* larvae.

However, the subsequent allergic responses may be triggered by dead larvae and their antigens.

- **Incidence in the population:**

  ► After the first case of anaphylaxis described in the 90ies, the improvement of the diagnostic methods have allowed to have more reliable data about the incidence in the population.

  ► According to Spanish Society of Allergology and Clinical Immunology-SEAIC (2011) in **some regions of Spain, *Anisakis* is considered to be the main factor associated with urticaria/angioedema in adults following fish and shellfish consumption**. Besides, more than 50% of the patients required emergency treatment and almost 100% hospital admission.

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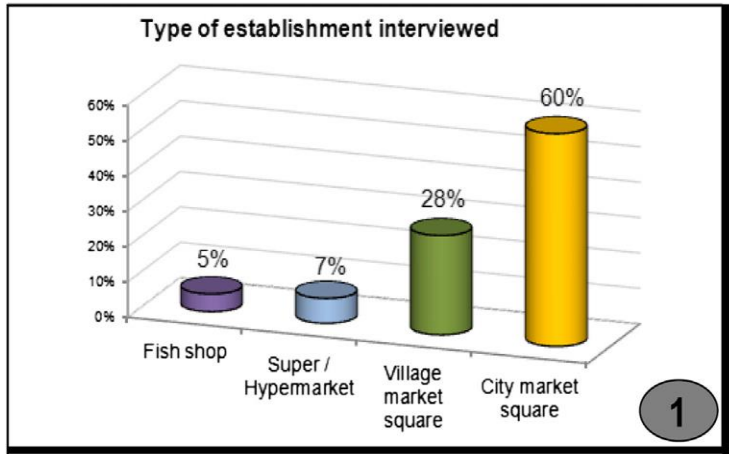
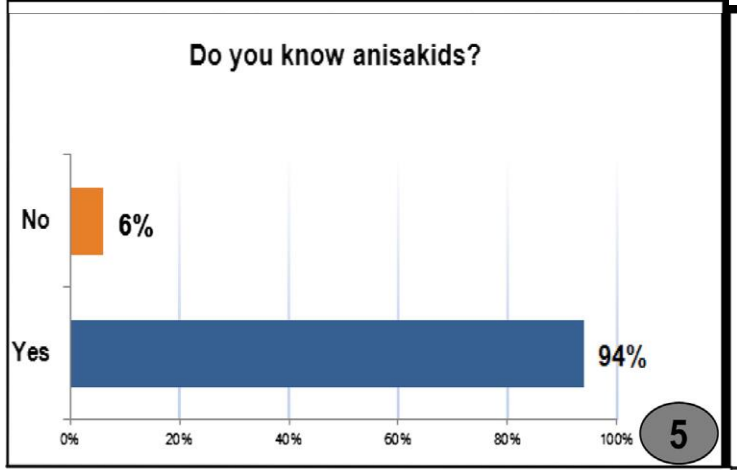
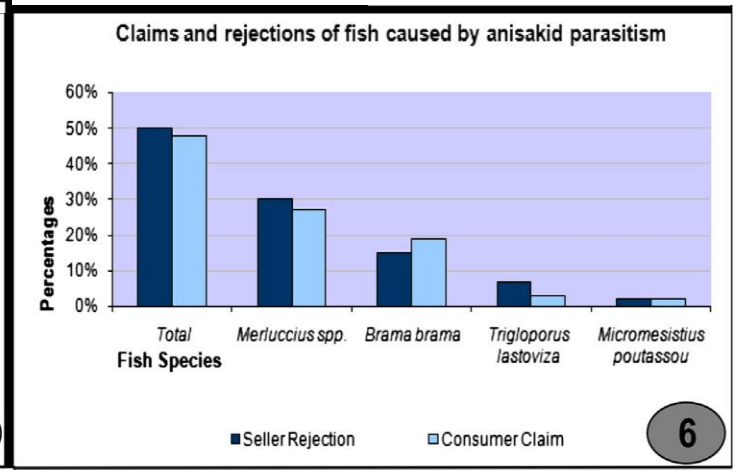
Social and Economic impact: *REJECTION OF CONTAMINATED SEAFOOD PRODUCTS*

Survey made to 108 fishmongers in Galicia (Spain) to get information about (1) their awareness of risks posed by *Anisakis*, (2) handling practices, (3) self-control and preventive measures.







Llarena-Reino et al. 2015. Horizon scanning for management of emerging parasitic infections in fishery products. Food Control 49: 49-58.

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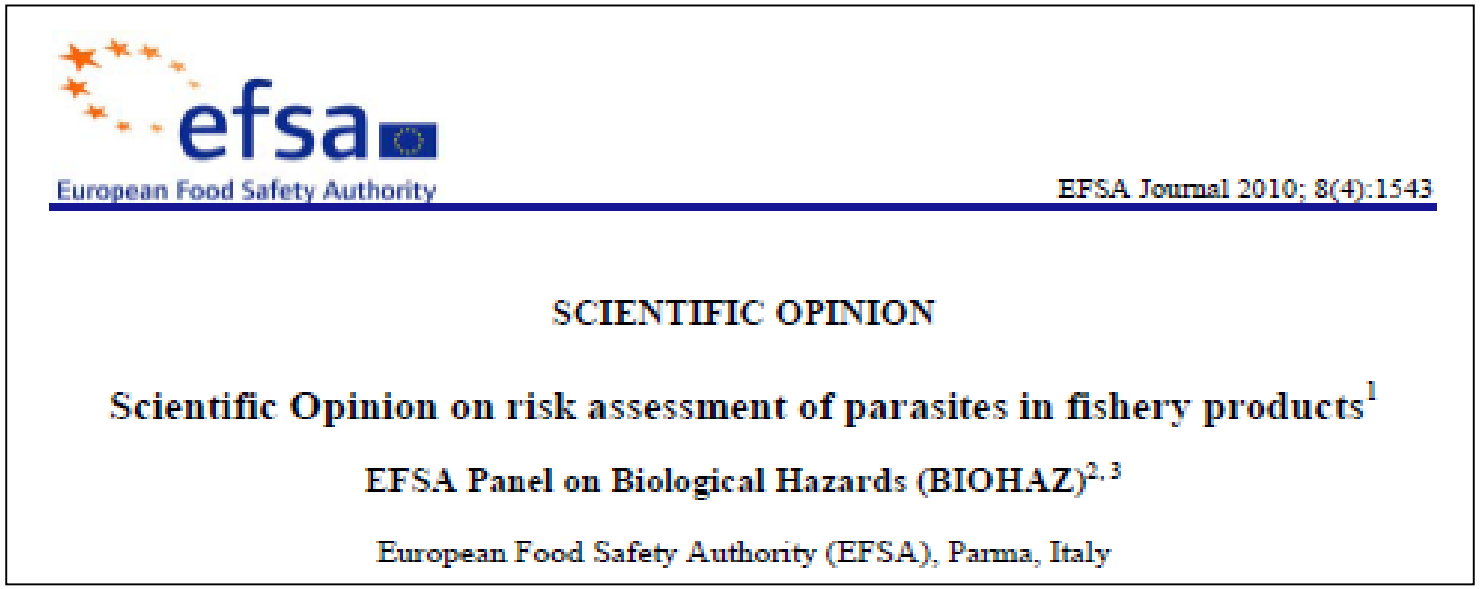
The presence of parasites in fishery products is an increasing worldwide problem(EFSA, European Food Safety Agency).



efsa
European Food Safety Authority

EFSA Journal 2010; 8(4):1543

SCIENTIFIC OPINION

Scientific Opinion on risk assessment of parasites in fishery products[1]

EFSA Panel on Biological Hazards (BIOHAZ)[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

EFSA Panel on Biological Hazards (BIOHAZ) concluded in 2010 that ***Anisakis*** *simplex* is, so far, the only anisakid that has been clearly implicated with **allergic reactions**.

It was recommended also to **improve research** in several aspects regarding this topic.

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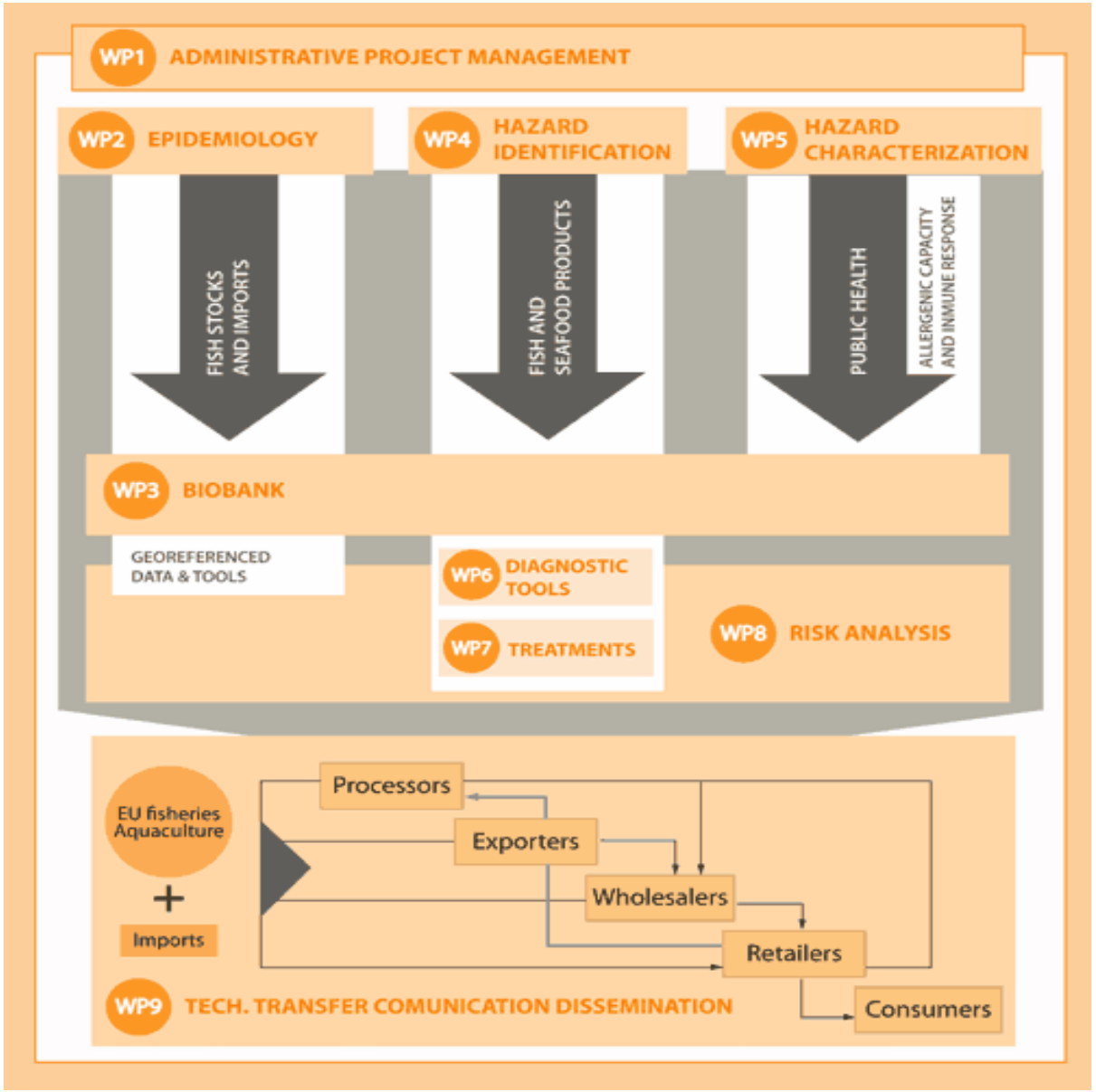
Parasite address the problem all along the value chain:



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Parasite addresses the problem all along the value chain:

1) Epidemiological study: monitoring of species and fishing grounds

2) Improvement of diagnostic methods to protect sensitized patients

3) **Improvement of detection methods for the industry**

4) Interventions along the production chain to remove parasites (on board: TEDEPAD/ industry: new freezing systems, among others)

5) Integration of all this information to carry out a comprehensive risk analysis

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3) Improvement of detection methods for the industry

Currently, according to European regulation (EC nº 853/2004): "*Food business operators must ensure that fishery products have been subjected to a* ***visual examination*** *for the purpose of detecting visible parasites before being placed on the market. They must not place fishery products that are obviously contaminated with parasites on the market for human consumption*". And "*Where* ***candling*** *of fillets is necessary from a technical viewpoint, it must be included in the sampling plan*" (EC nº 2074/2005).

But these methods are no effective (Levsen et al. 2005).

✓ **RT-PCR:** This is a molecular method that is very sensitive and allows detection of anisakids traces (DNA detection) in processed fishery products (surimi, baby products, etc…

✓ **Protein detection by immunoassays:** This is a molecular technique that is very sensitive and allows the detection of anisakids traces and antigens in processed fishery products (surimi, baby food, etc.…). Specially relevant for sensitized patients and for labelling of *Anisakis*-free products, for example.

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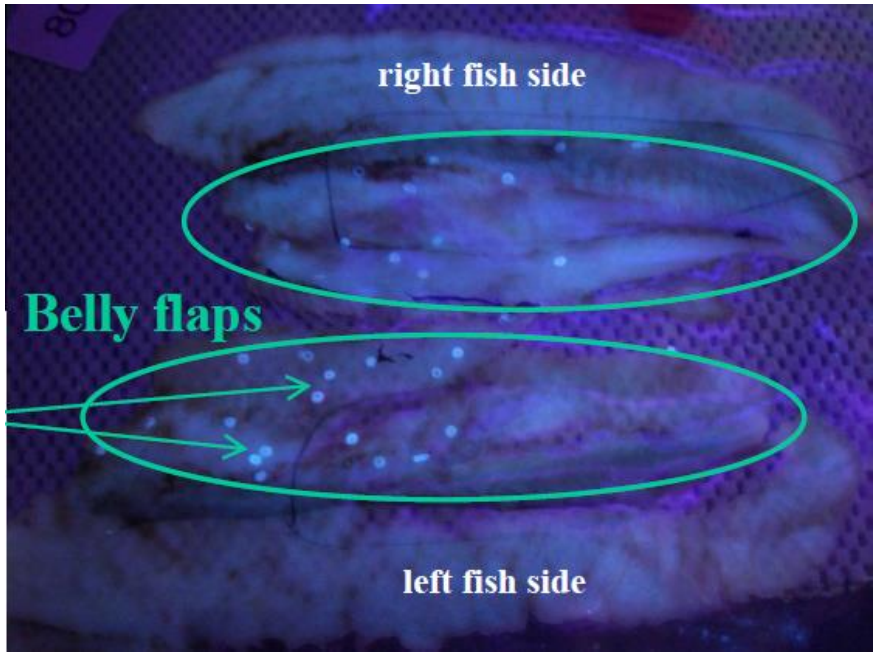
3) Improvement of detection methods for the industry

✓ **UV-Press**: this method is based on anisakids capacity to show fluorescence under UV light.

To show fluorescence, it is essential that nematode cells break (after parasite death) and release lipofuscine.

Freezing and thaw is an easy and efficient way to break nematode cells.



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UV-PRESS DETECTION METHOD: *The Ring-trial exercise*

This is not an innovative method (Karl & Lienemann, 1993) but it is **very promising**, both from an industrial and control point of view

↓

To test its efficiency

↓

Ring-trial: inter-laboratory exercise where 5 partners were involved (IIM-CSIC, ANSES, NIFES, MRI and UT-URS)

Results: Accurate, sensitive and specific method.

	UV-Press	Artificial Digestion
Accuracy	100%	97%
Sensitivity	100%	96%
Specificity	100%	100%

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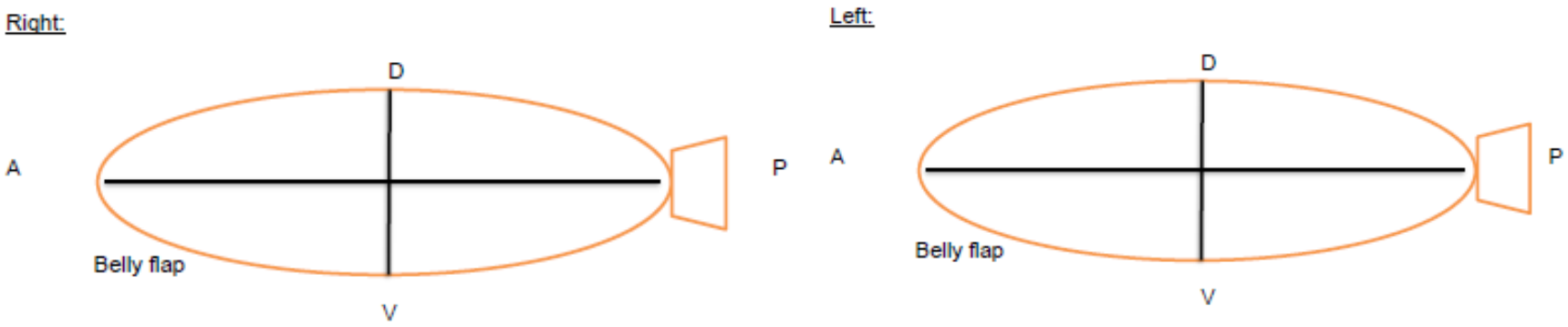
UV-PRESS DETECTION METHOD: *Application of the method*

1) Big species (e. g. Atlantic hake)

Cut 4 fillets by side and introduce them in a labelled plastic bag each (*)



2) Small fishes (e.g. anchovy)

After removing the head and spine bone, introduce the whole fish in a labelled plastic bag (*)

(*) offals have to be removed in both cases, since this method is not efficient for their analysis

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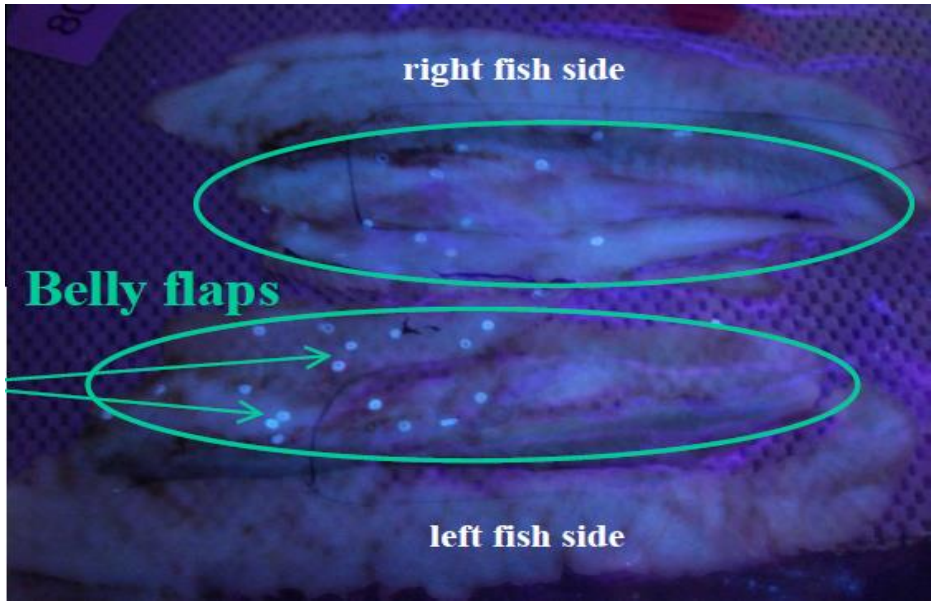
UV-PRESS DETECTION METHOD: *Application of the method*

✓Introduce plastic bags (with samples inside) into the press until getting a thin layer of about 2 mm.

✓ Freeze the pressed samples at -20ºC for at least 24h. **NOTE**: *this step is not necessary if the fish has been frozen previously*.

✓ After freezing, the samples can be observed under UV light.



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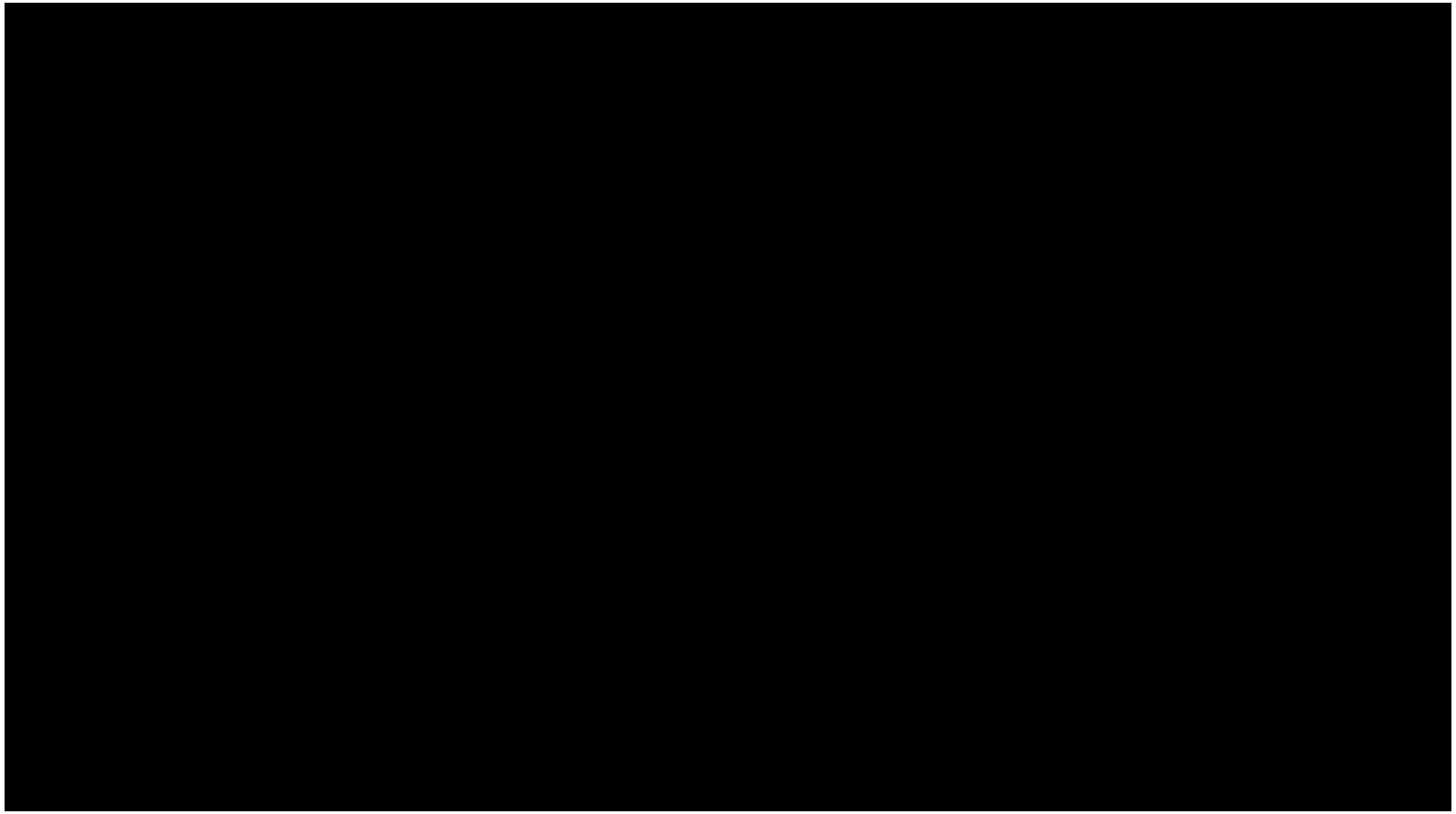
UV-PRESS DETECTION METHOD: *Application of the method*



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UV-PRESS DETECTION METHOD: *Advantages*

ADVANTAGES:

- ✓ **accurate, sensitive and specific** method, as revealed in the ring trial exercise carried out in the project.
- ✓ **simple and quick** method that allows certain automatization.
- ✓ it can be applied in **non-processed fishery products**.
- ✓ **industrially applicable**, both in processing and fishing sectors. E. g.: Norwegian fleet(HERMES).

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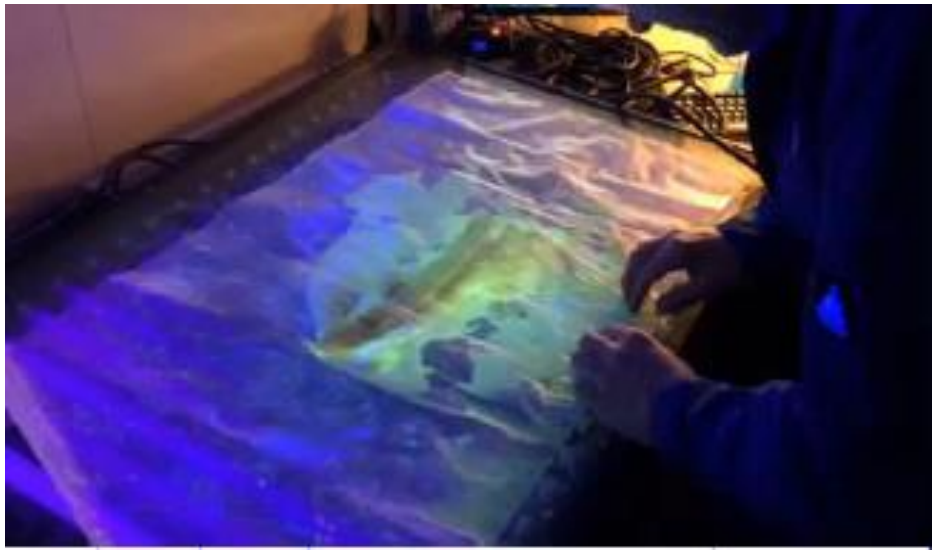
UV-PRESS DETECTION METHOD: *Advantages*



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UV-PRESS DETECTION METHOD: *β-testing exercise*

Before its application, the method has to be proved as useful at industrial level and correctly applied by industry operators

β-testing exercise

- β tests are conducted by **end users** in their **own working places**
- tests have to be carried out in real conditions, used by the industries in their working routine
- testers should **register all the problems/inconveniences** faced during the exercise in order to improve the methodology or their training

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UV-PRESS DETECTION METHOD: *β-testing exercise*

Participants: ARVI, HERMES and NEDERLOF's

Suggested dates: last week of May (25-29 May 2015)

- ✓ Each participant will receive:
  - ➢ **set of samples** to be tested (15 fish samples)
  - ➢ **detailed instructions** to carry out the β-test.

- ✓ Samples will have to be analyzed by the participants **within three days** after the arrival of the samples.
- ✓ The results obtained will have to be **sent to CETMAR** for being analyzed.
- ✓ Industrial partners will evaluate the **applicability and usefulness** of this method, assessing its **potential incorporation** into working routine.

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Parasite

Thank you very much for your attention!

Questions?

CETMAR
CENTRO TECNOLÓGICO DEL MAR

Contact:
rfernandez@cetmar.org
cmartinez@cetmar.org

More information: ***http://parasite-project.eu***
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