Supporting a future with safe, nutritious, and sustainable seafood

SEAFOOD^{TOMORROW} Final Event, 15.04.2021

Rapid screening tools for seafood authentication

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www.seafoodtomorrow.eu

This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 773400 (SEAFOOD^{TOMORROW}). This output reflects the views only of the author(s), and the European Union cannot be held responsible for any use which may be made of the information contained therein.

Setting the scene…

Revealed: seafood fraud happening on a vast global scale

The Guardian

16 March 2021

▲ A chemist working to identify a fish at a laboratory in Marseille, France. The Guardian analysed 44 studies on seafood fraud, many of which used DNA analysis techniques. Photograph: Anne-Christine Poujoulat/AFP/Getty

A Guardian Seascape analysis of 44 recent studies of more than 9,000 seafood samples from restaurants, fishmongers and supermarkets in more than 30 countries found that 36% were mislabelled, exposing seafood fraud on a vast global scale.

Why is seafood substituted?

- Economic gain/loss
- Limited availability
- Visually similar species
- Conceal IUU fisheries
- Quota limited

Consequences of seafood fraud?

- Financial gain/loss
- Conservation efforts are threatened
- Health risk for consumers (allergies, toxins)
- Consumer loss of confidence

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Seafood authentication

Unprocessed

Processed

Problem with processed food: visual identification no longer possible

DNA-based identification

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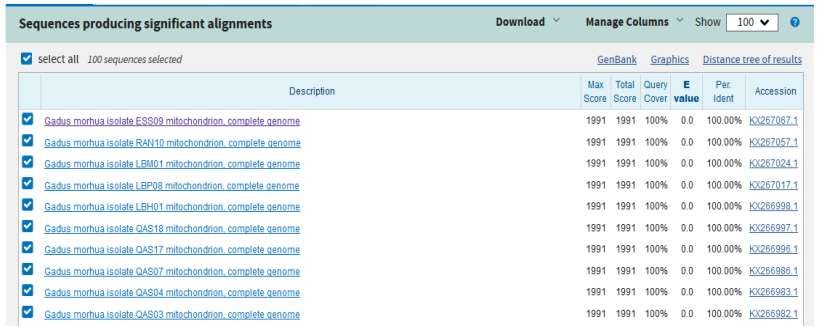
DNA-based identification

Premise of DNA barcoding: each species has a unique DNA profile

Unidentified sample

Reference database

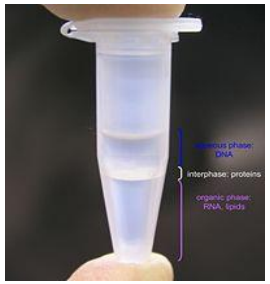


Sequences producing significant alignments — Download — Manage Columns — Show 100

select all *100 sequences selected* — GenBank — Graphics — Distance tree of results

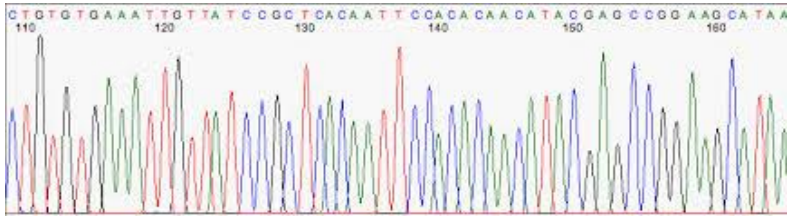
Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Gadus morhua isolate ESS09 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX267067.1
Gadus morhua isolate RAN10 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX267057.1
Gadus morhua isolate LBM01 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX267024.1
Gadus morhua isolate LBP08 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX267017.1
Gadus morhua isolate LBH01 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX266998.1
Gadus morhua isolate QAS18 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX266997.1
Gadus morhua isolate QAS17 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX266996.1
Gadus morhua isolate QAS07 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX266986.1
Gadus morhua isolate QAS04 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX266983.1
Gadus morhua isolate QAS03 mitochondrion, complete genome	1991	1991	100%	0.0	100.00%	KX266982.1

DNA extraction



PCR

Sequence (Sanger)



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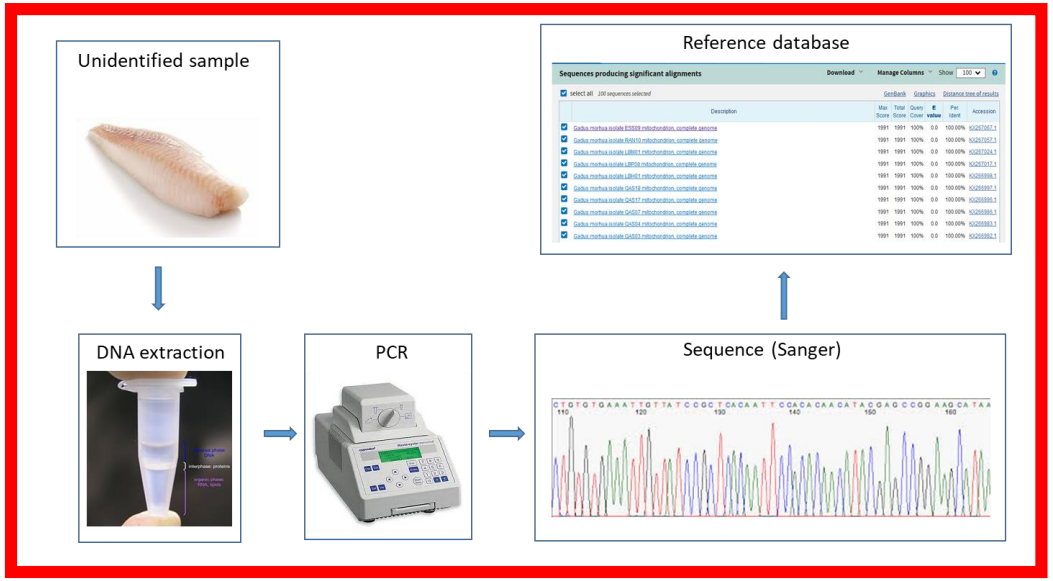
DNA-based identification



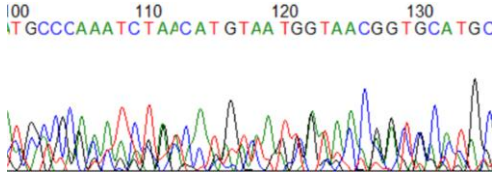
PROBLEMS with DNA barcoding:

=> errors in public databases

=> 5 days until results

=> does not allow quantification

=> does not work for food products with multiple species



Clear need for

- high quality reference database
- fast screening tools, also for mixed food products
- tool to quantify % of species in mixed seafood products

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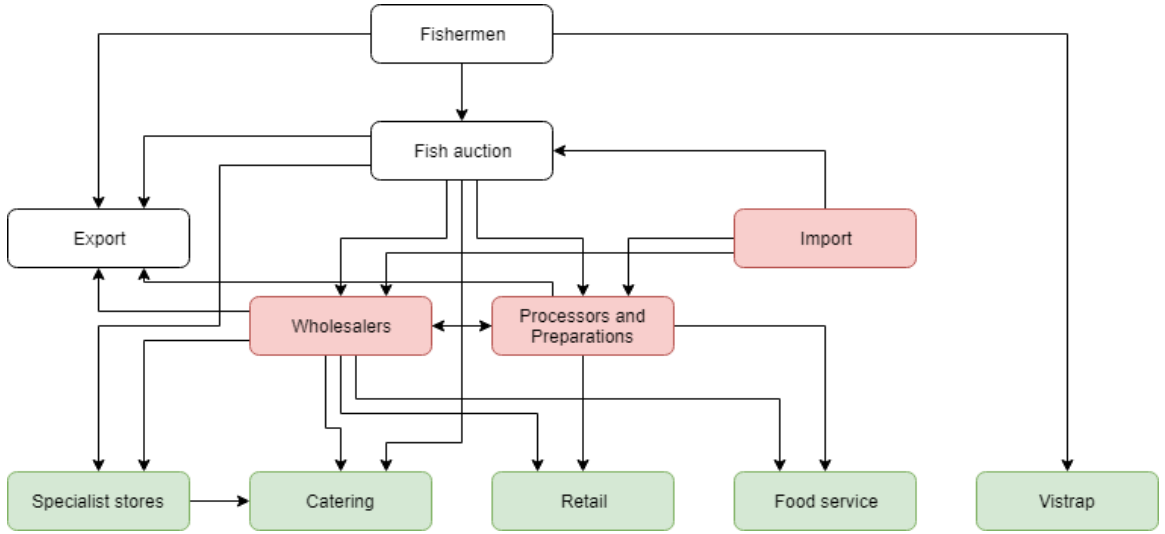
1/ A reliable reference database

- 42 commercially traded European fishes; 300 sequences
- Specimens, tissues, DNA, sequences are all linked
- Open access: www.seafoodtomorrowdata.eu/authentication/
- Video tutorial: https://drive.google.com/open?id=1B8rnQTgGPMDt6iJYe-GB1jV379zRiYIv

Market application: substitution of cod and sole along the Belgian supply chain



Deconinck et al. 2020, Food and Chemical Toxicology 141

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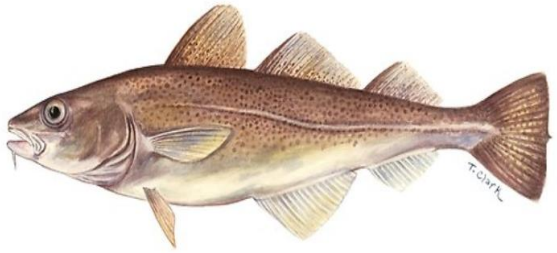
1/ A reliable reference database

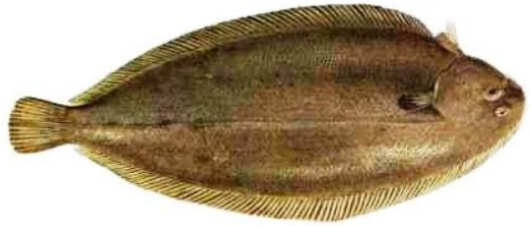


3/132 (2%)

Gadus chalcogrammus

Melanogrammus aeglefinus

Pollachius virens



7/41 (17%)

Cynoglossus sp.

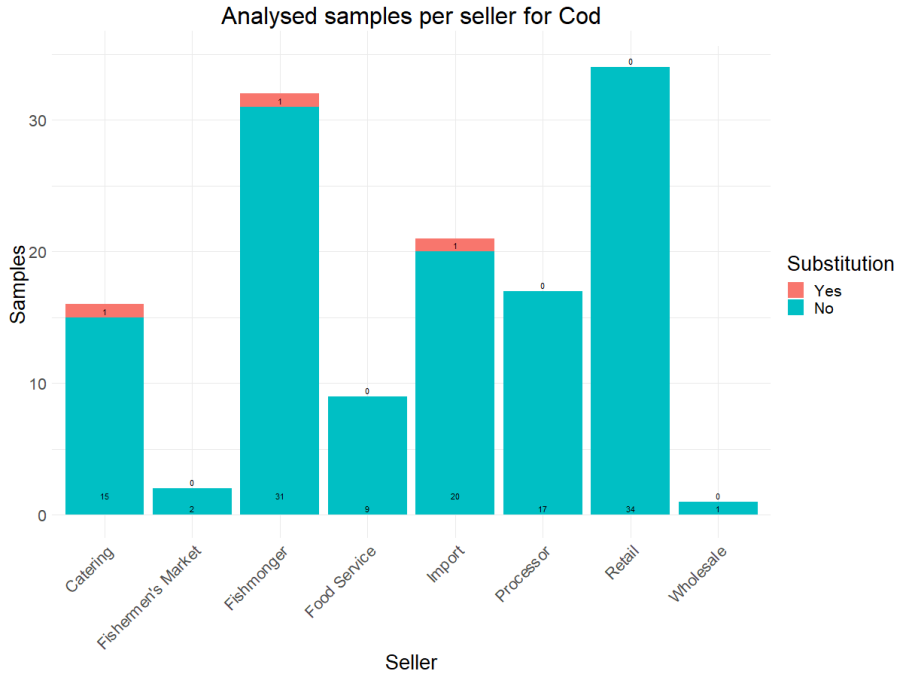
Lepidopsetta polyxstra

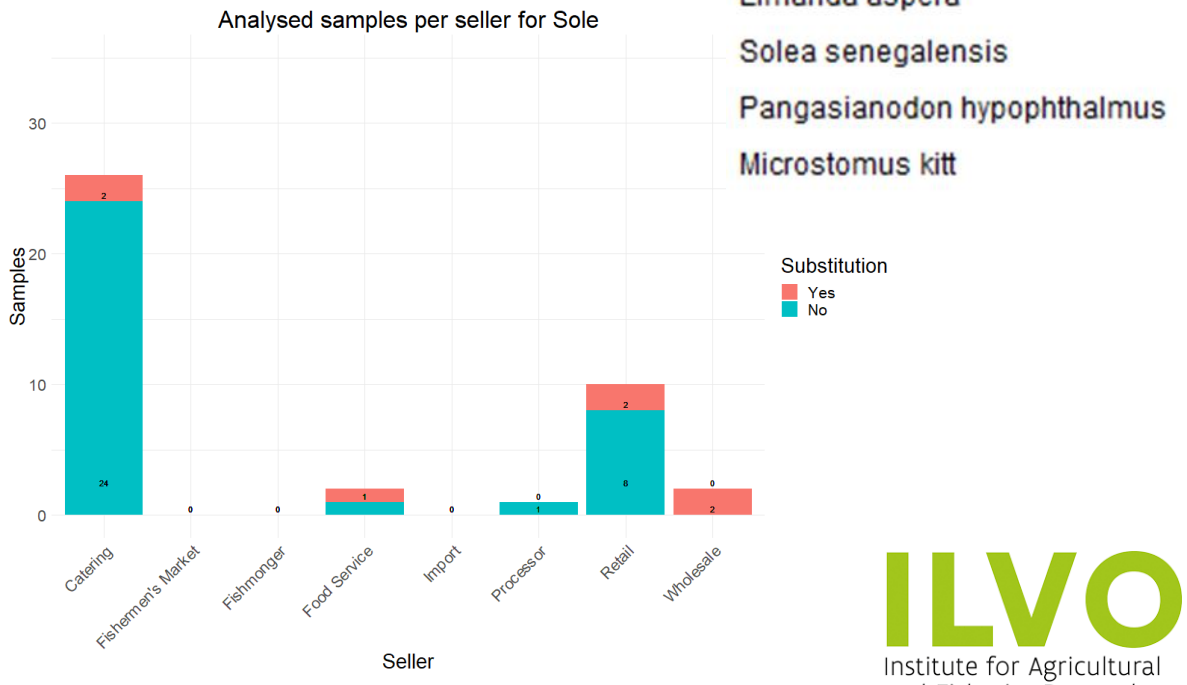
Limanda aspera

Solea senegalensis

Pangasianodon hypophthalmus

Microstomus kitt





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Institute for Agricultural and Fisheries Research

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2/ Fast screening tool for salmon

ATLANTIC SALMONS
3 % substitution rate

Salmo salar

90% of the farmed salmon

50% of the global salmon market

Salmo trutta (brown trout)

farmed

Oncorhynchus mykiss (rainbow trout)

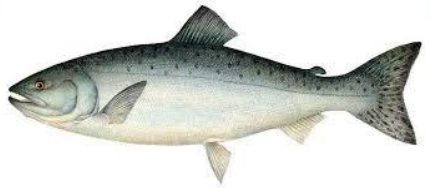
PACIFIC SALMONS
17 % substitution rate

mostly wild caught

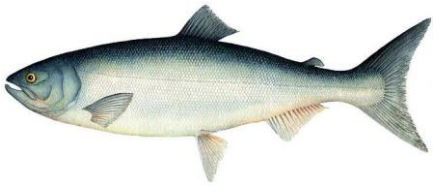
O. tshawytscha (chinook)

O. keta (chum)

O. kisutch (coho)



O. gorbuscha (pink)



O. nerka (sockeye)

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2/ Fast screening tool for salmon

SEAFOOD TOMORROW

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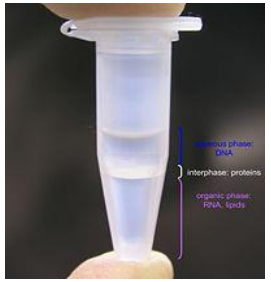
Instituto de Ciências, Tecnologias e Agroambiente da Universidade do Porto

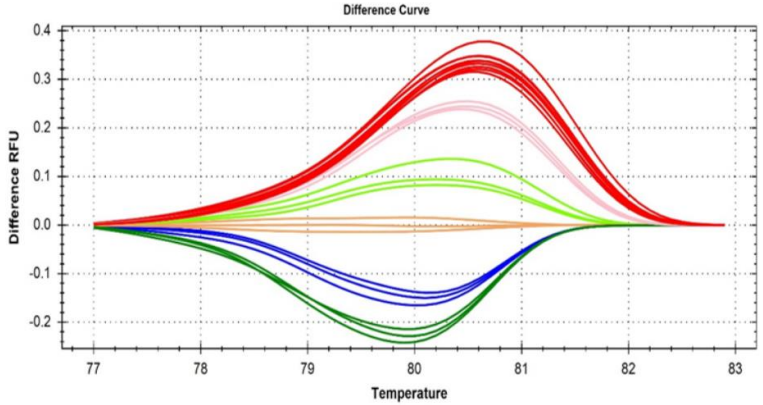
High Resolution Melting Analysis (HRMA) => TRL 7

Unidentified product

DNA extraction



qPCR



Advantages:

- Results within 4 hours
- 24 to 48 samples can be analysed simultaneously
- Food processing does not affect results, except for canning
- All 8 salmonids can be identified with the same kit (in contrast to other commercial kits)

Biorex food diagnostics

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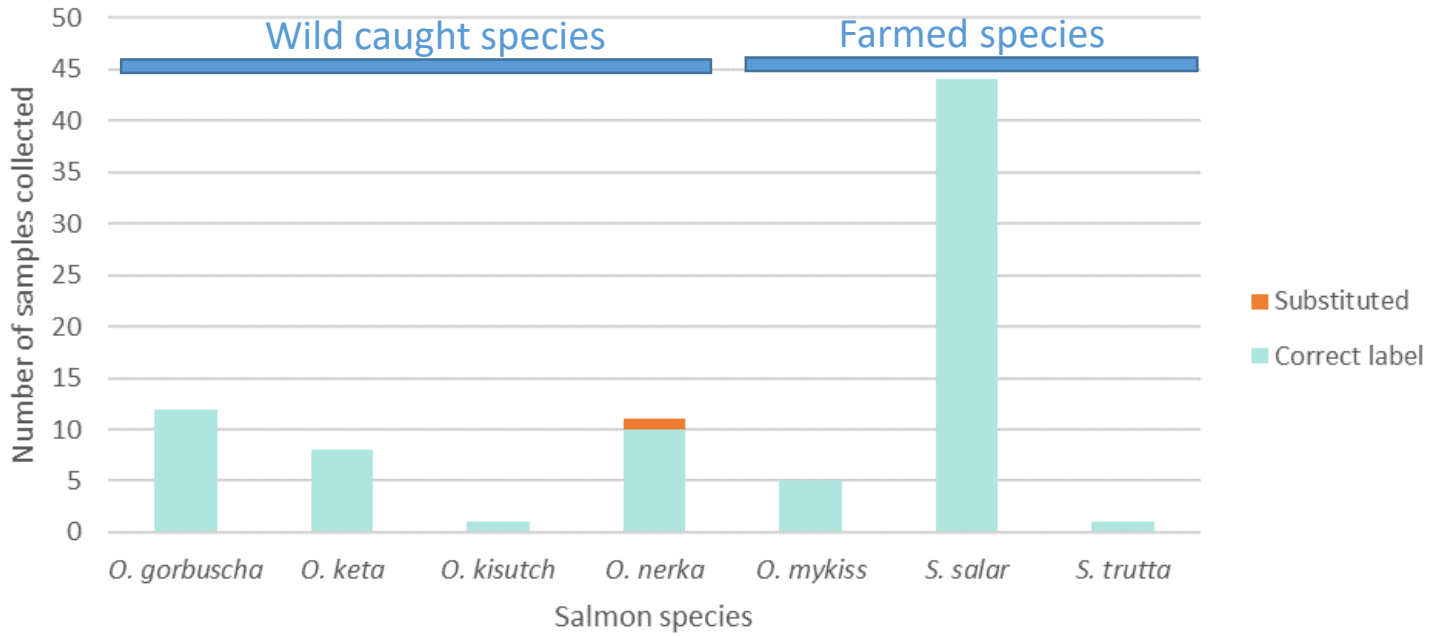
2/ Fast screening tool for salmon

High Resolution Melting Analysis (HRMA) => TRL 7

Market application: authentication of salmon in retail samples from Portugal, Poland and Belgium

- 81 samples tested
- 1/11 *O. nerka* substituted by *S. salar* (9 %)



Monteiro et al, under review, Food and Chemical Toxicology

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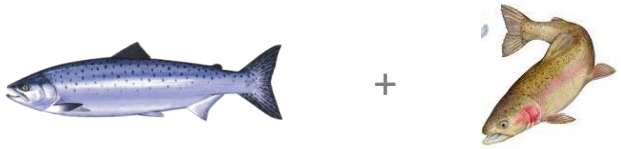
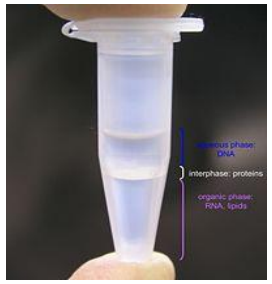
3/ Identification and quantification of *Salmo salar* in mixed seafood

ILVO
Institute for Agricultural and Fisheries Research

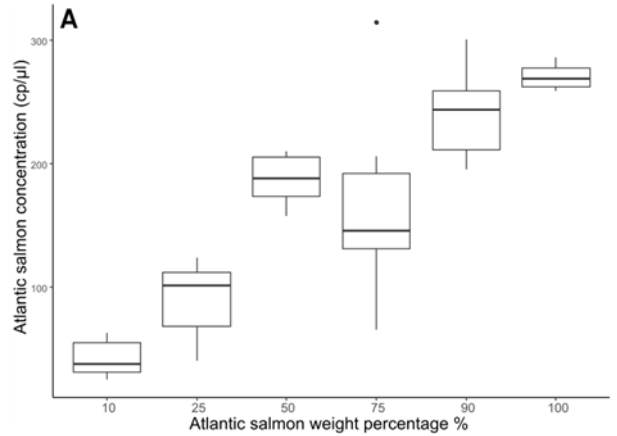


Mixed and processed food product

DNA extraction



ddPCR assay



Advantages:

- Allows to semi-quantify the amount of *S. salar* in a mixed food product
- Can also be used to identify *S. salar* in mixed food products (including other salmonids)
- 42 samples can be analysed simultaneously, within 5 hours

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3/ Identification and quantification of *Salmo salar* in mixed seafood

Market application: authentication of salmon in retail samples from Belgium and Poland

- 46 samples tested
- 3 samples with no results (all were canned)
- Many retail samples were poorly labelled (no scientific name, wrong customery name)
- No evidence of substitution (0 %):
  - All *S. salar* food products were correctly identified
  - No *S. salar* detected in food products labelled as pacific salmon
- Quantification in fully homogenised samples semi-quantitative

Deconinck et al., under revision, Food and Chemical Toxicology

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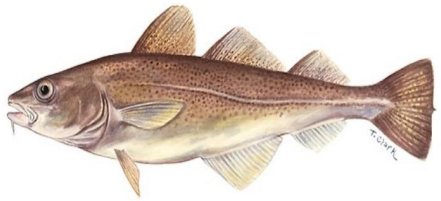
Conclusions

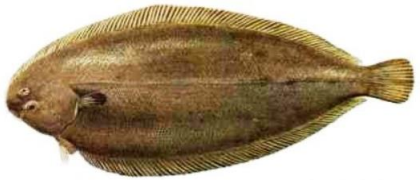
- Seafoodtomorrow database: allows reliable identification of single species food products
- HRMA tool: quick identification of 8 salmon species in single species food products within 4 hours
- Proof of concept for Atlantic salmon in mixed food products:

  => identification of *Salmo salar* within 5 hours

  => quantification: semi-quantification possible
- Market studies show that substitution occurs
  - in the European market
  - in different steps of the supply chain
  - with large differences between species

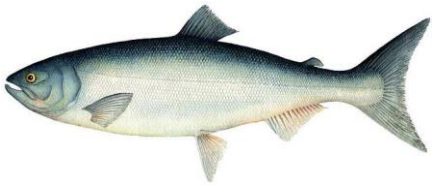


2%



17%

0%



9%

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Thank You

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