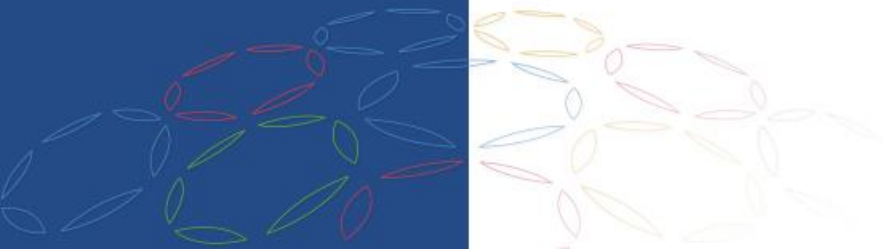

Guðbjörg Ólafsdóttir, Matis

Rapid methods to detect undesirable microbes in fish

Authenticate: Workshop on food integrity and available methods for detecting food fraud

Faroe Islands, Tórshavn
November 14th 2017





Introduction

Microbial communities in food and food production establishments have highly versatile structure based on various extrinsic factors.

Food type, temperature, salinity, pH, water content, packaging conditions, storage conditions etc.

Molecular methods have been developing rapidly in recent years both for specific detection of single species and screening methods that allow species composition of a given sample.

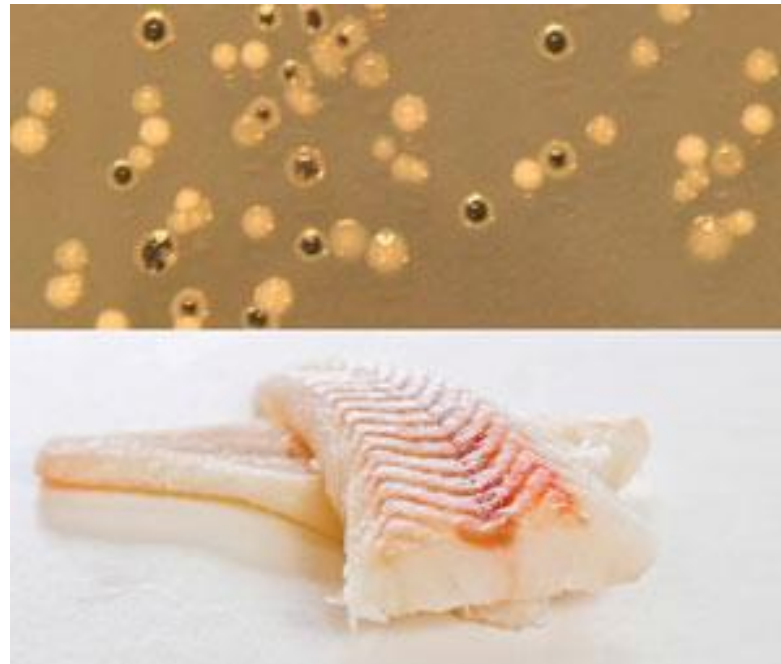


Introduction

The spoilage process

The spoilage process of fish is composed of complex interactions between bacteria, the raw material itself and environmental factors

Research activities have revealed the main bacterial spoilers which have been referred to as the specific spoilage organisms (SSO)



Monitoring of spoilage bacteria in the supply chain

What are the advantages?

Whatever storage time or conditions have been applied, the number of spoilage bacteria does not lie

Gives an independent observation on product quality and estimate of freshness

Could be a valuable addition to quality management at production sites and an unbiased quality control for buyers of fresh food

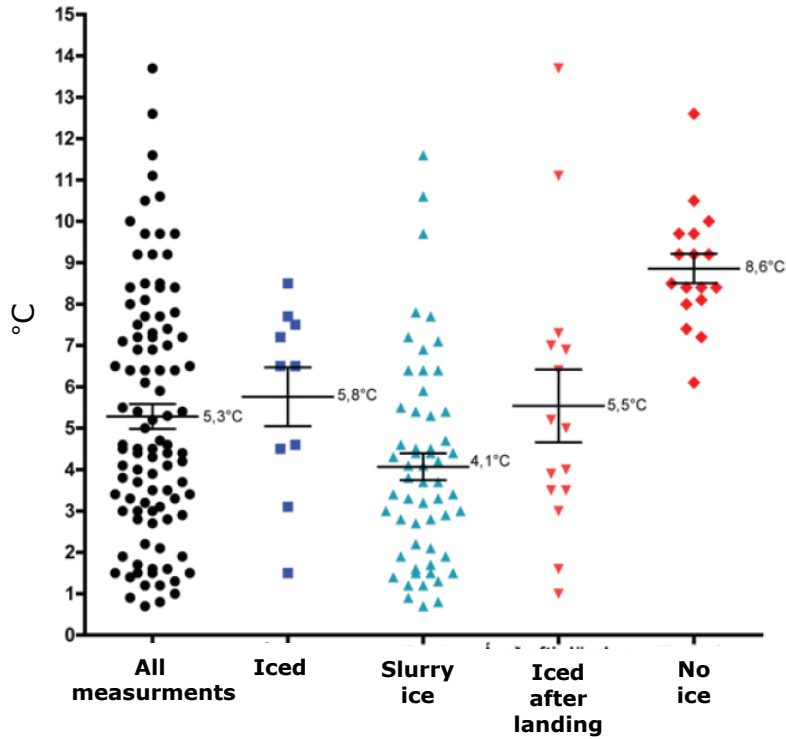


First steps in the supply chain

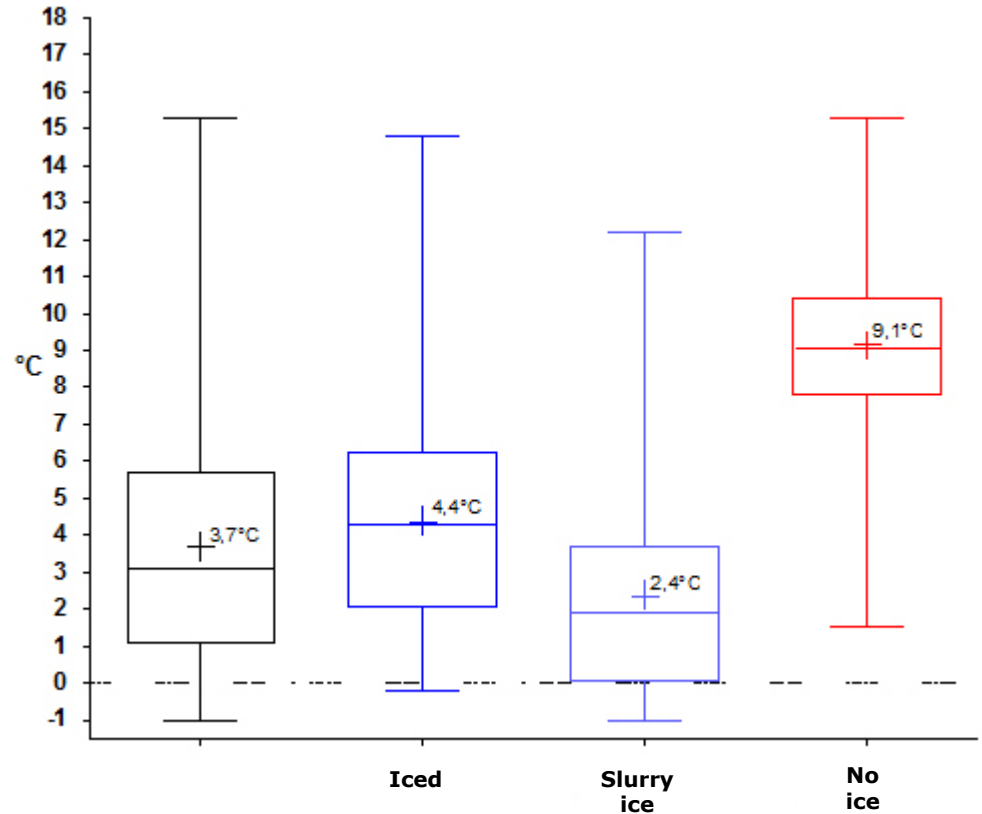
- Large corporations
 - With their own supply chains
 - Large vessels – processing (onboard/in land) – transportation
 - secondary processing abroad – distribution
 - Catch can be few days old when landed
 - Streamlined production and quality of raw material
- Small corporations
 - Small vessels
 - Catch is sold through fish markets
 - Catch landed same day
 - Diverse handling of fish and therefore on quality of raw material
- How is the quality monitored?



Temperature of landed catch



July 2010



July 2011



What controls microbial quality?

What controls microbial quality?

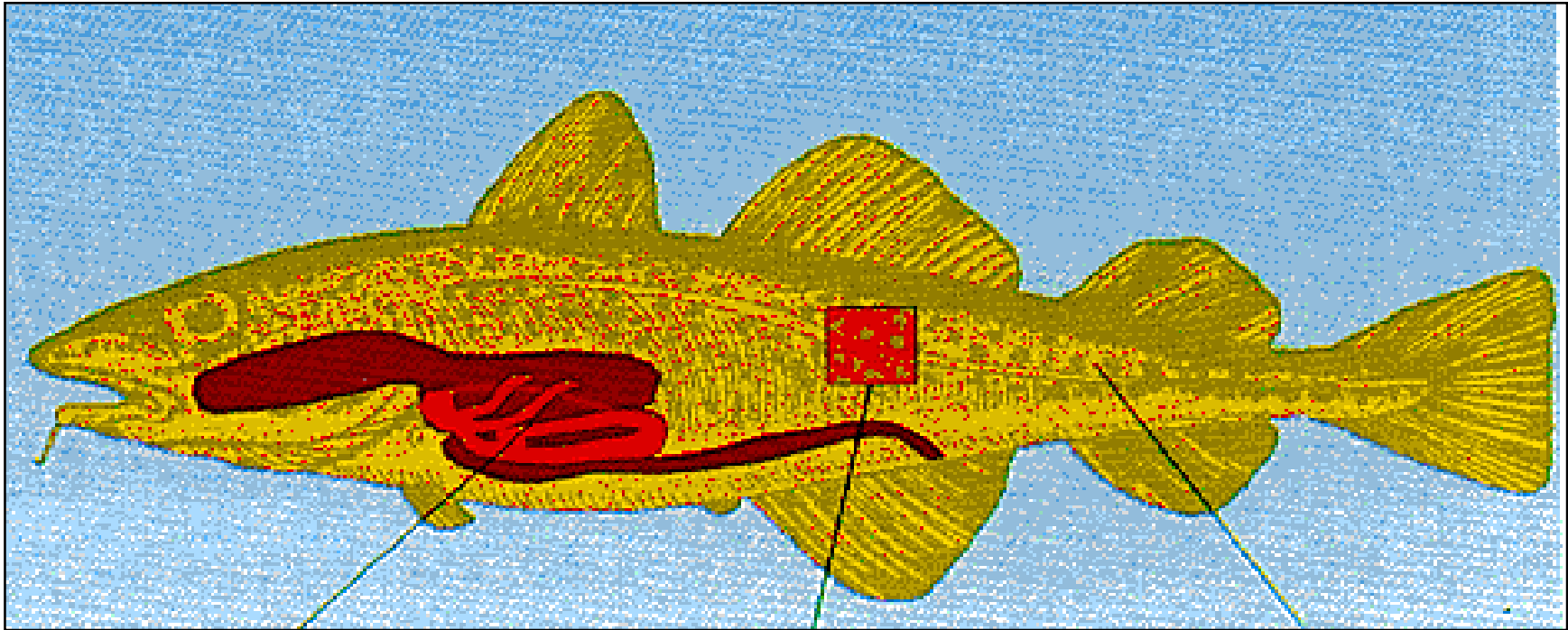
- Temperature in the whole process
- Time of bleeding
- Time of gutting
- Handling
- Time of storage
- Processing methods
- Processing conditions
- Storage conditions

Is it possible to use a single microbial parameter as a reference for quality control?

No



Bacteria on fresh cod



Intestine:
10-100.000.000/g

Skin:
100-
100.000/cm²

Flesh:
No bacteria



Microbial indicators: Specific Spoilage Organisms (SSO)

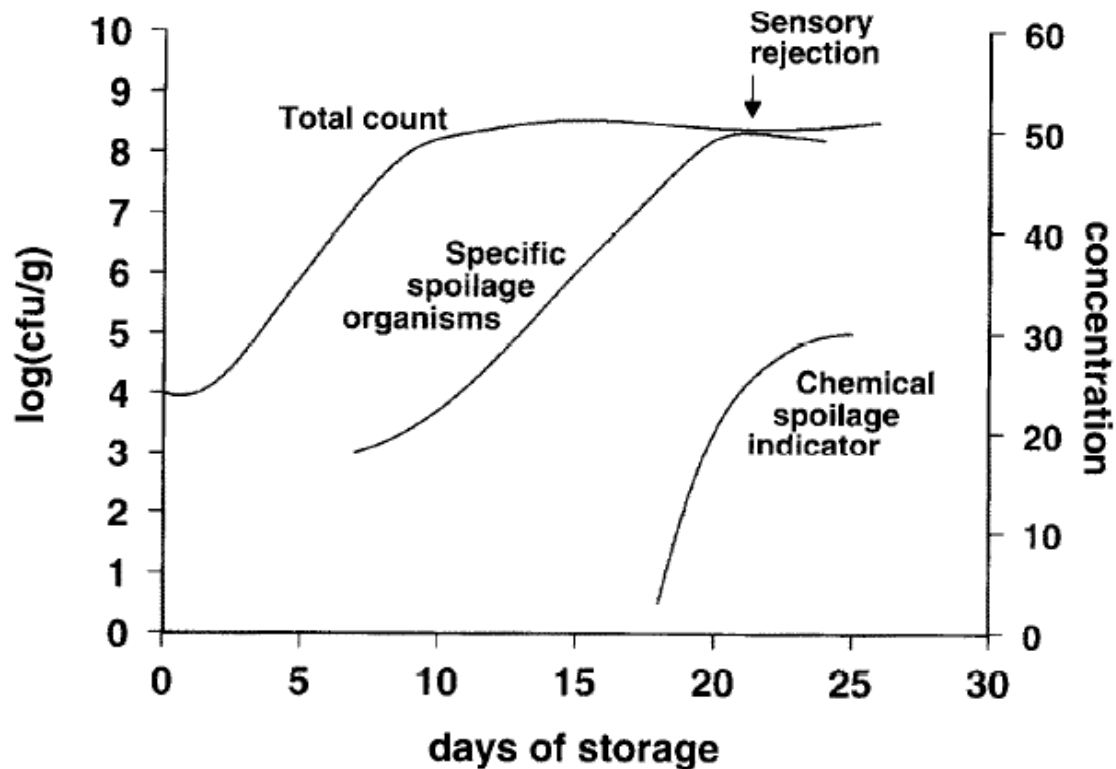
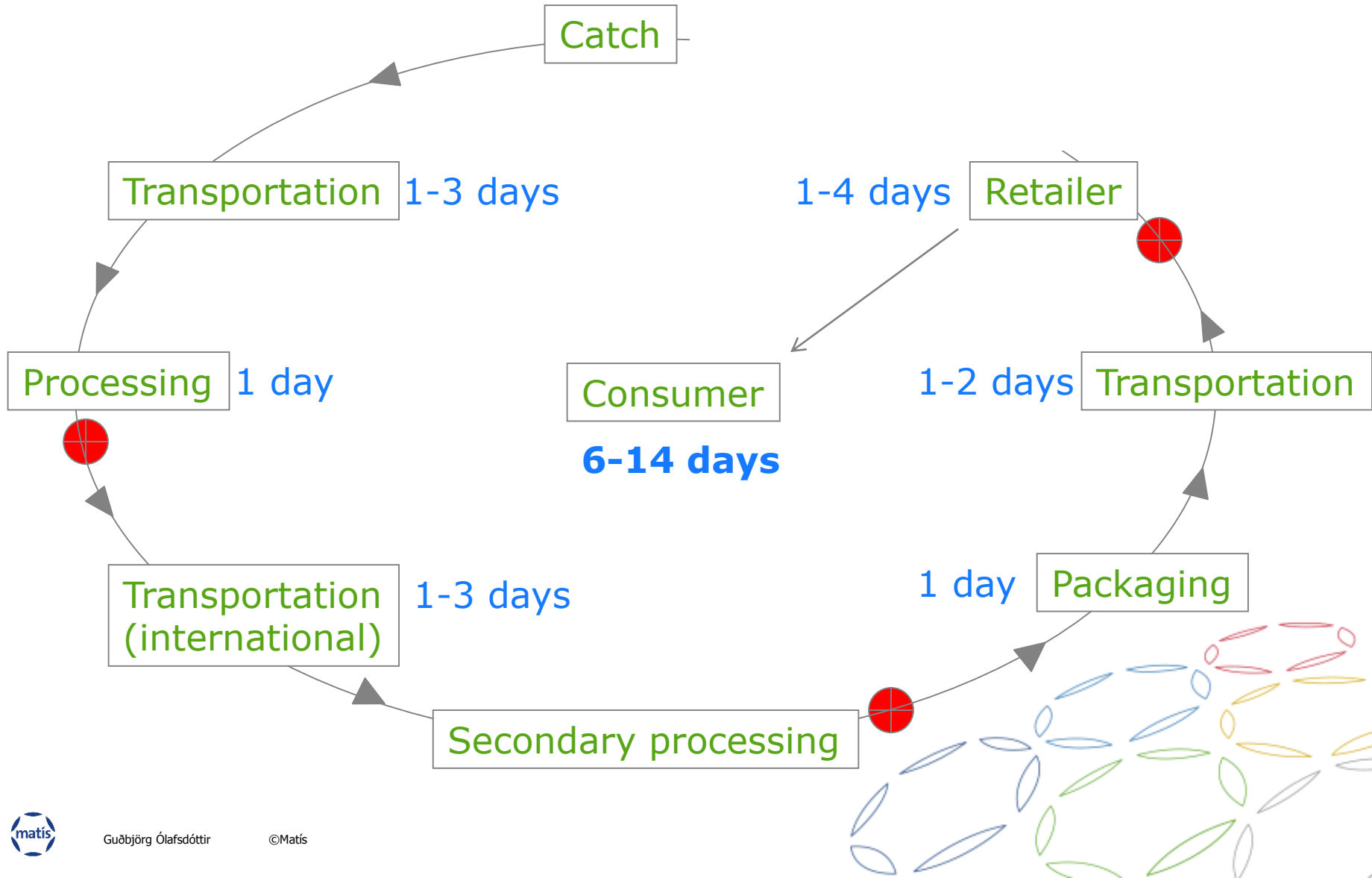


Fig. 1. Model of changes in total count (TVC), specific spoilage organisms (SSO) and chemical spoilage indices during chill storage of a fish product (modified from Huss et al., 1996).



Fish in the supplied chain



Relevant microbial parameters to estimate spoilage

Until now, no single method is available for the rapid detection or quantification of these bacteria.

Cultivating the most important bacteria can give a good estimate on product quality during processing, transportation or storage

The time frame however is too large to be able to use it for processing management purposes.

Species	Method	# days
Pseudomonas	Cultivation on CFC agar at 22°C	3
Photobacterium	Malthus conductance method	2
Shewanella	Cultivation iron agar at 17°C	5



New Methods to study fish microbiota

rt-PCR methods: rapid tests developed for

Estimation of P. phosphoreum and Pseudomonads

Cell count by flow cytometry: FACS (Aria II)

Community analysis (molecular level):

16S rRNA gene sequence analysis

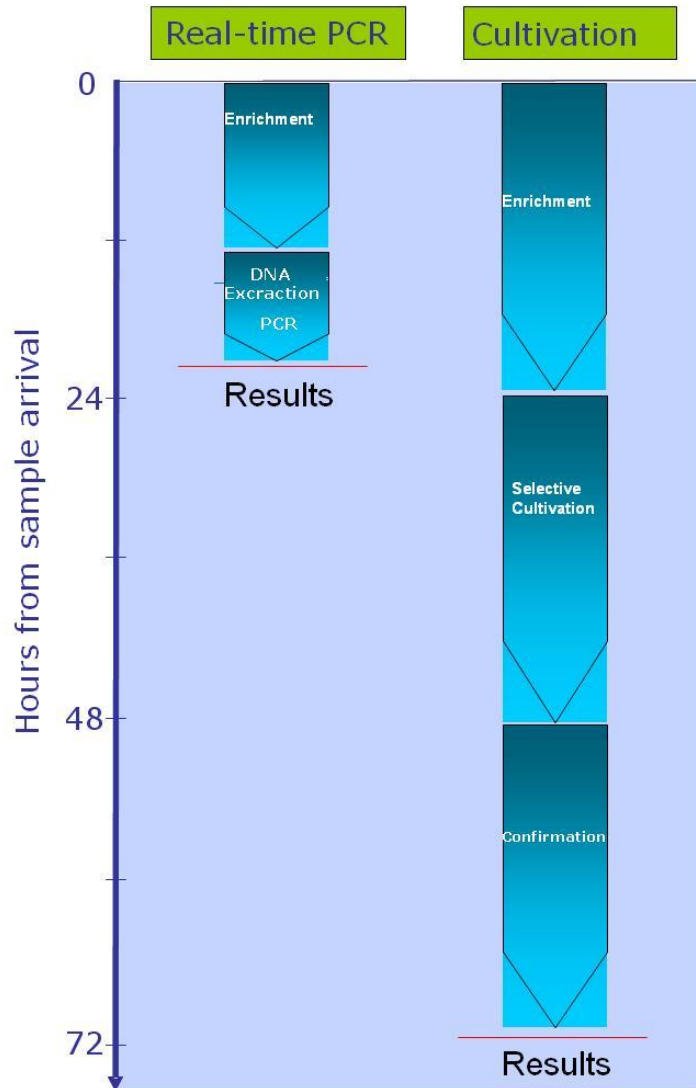
Next Generation Sequencing (NGS)

Sensory evaluation

Smell, taste, texture, appearance, colour



Analysis procedures



Analysis procedure and time (no preenrichment)

- 25g food sample diluted in 225mL buffer 15 min
- DNA extraction 90 min
- PCR analysis 120 min
- Results analysis 15 min

Total

4 hours



Real-time PCR technology

Required instrumentation:

Stomacher



Real-time PCR cycler



DNA extraction robot

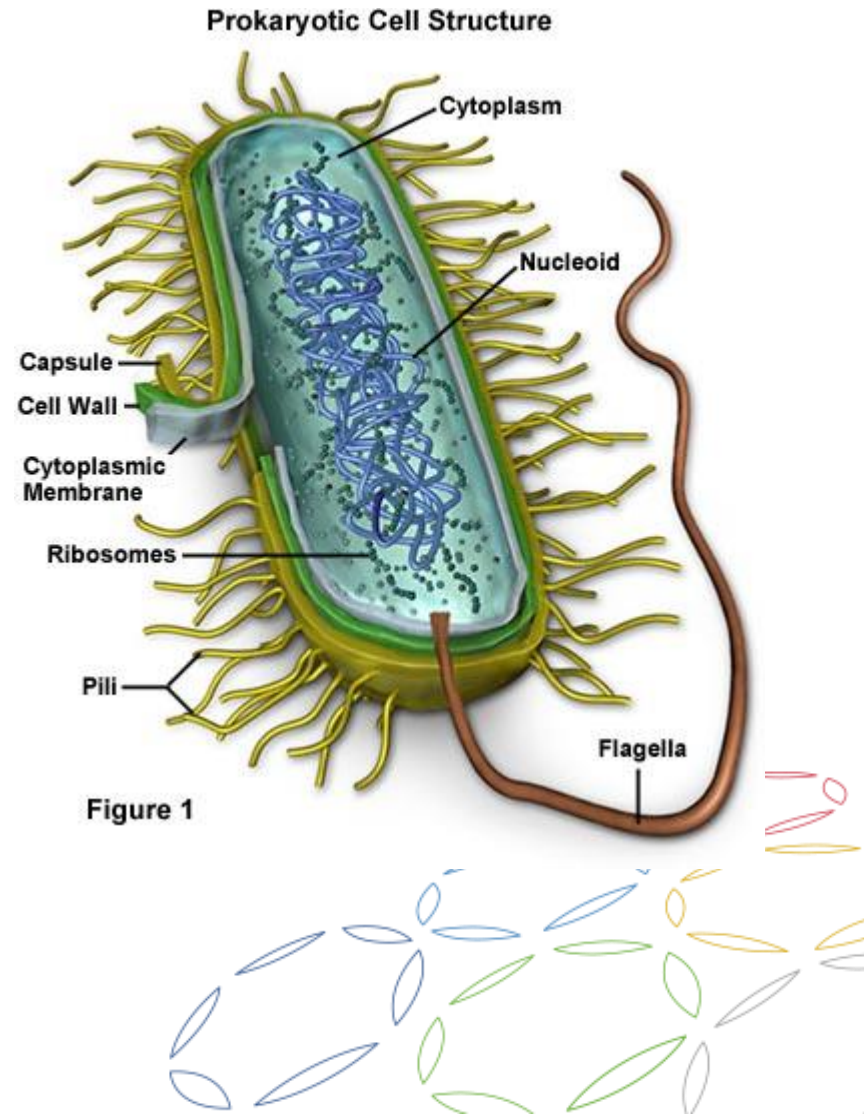
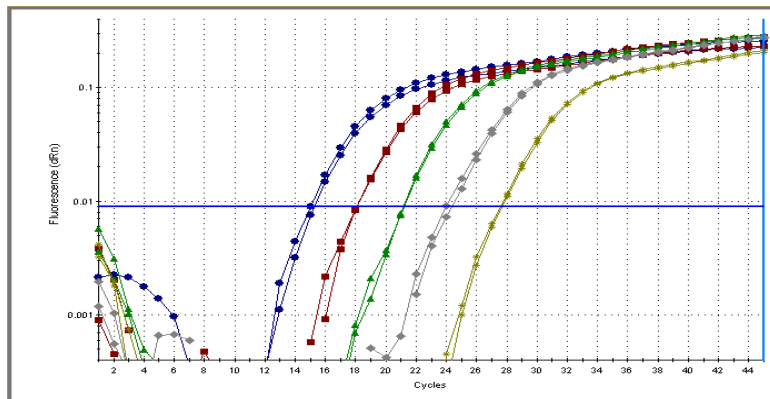


Real-time PCR technology

Small part of the DNA molecule from the bacteria is amplified using DNA polymerase

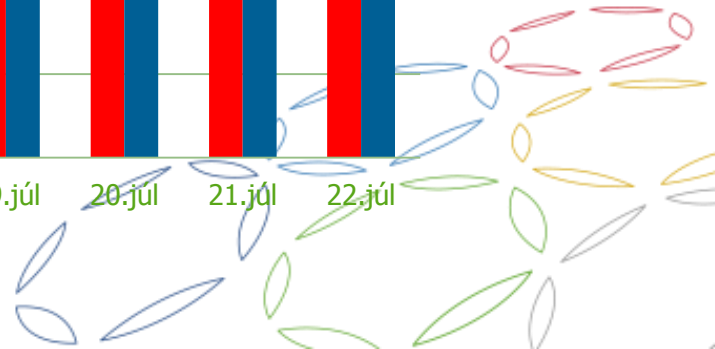
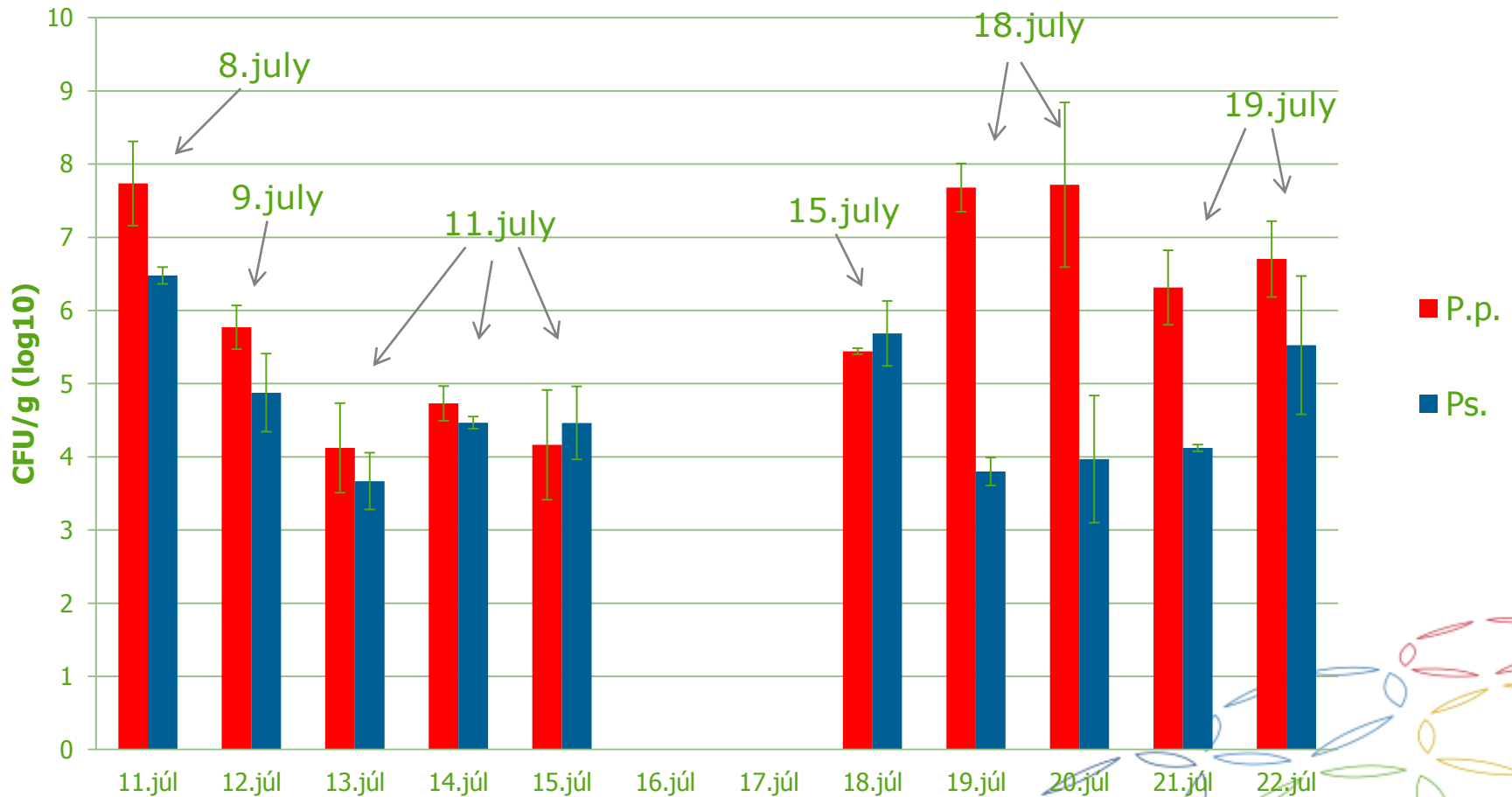
During amplification fluorogenic substances in the reaction emit light and is detected by the instrument

The more bacteria present in a sample – the sooner the light is detected



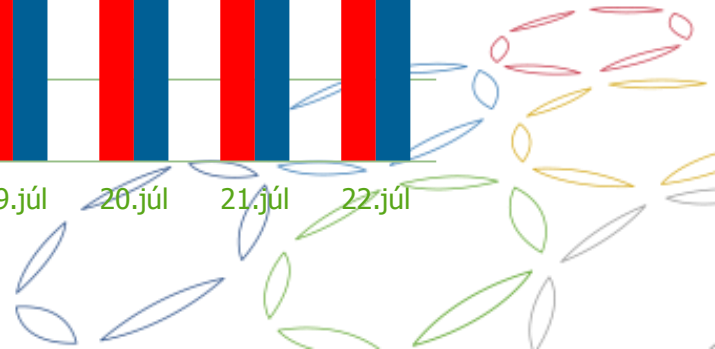
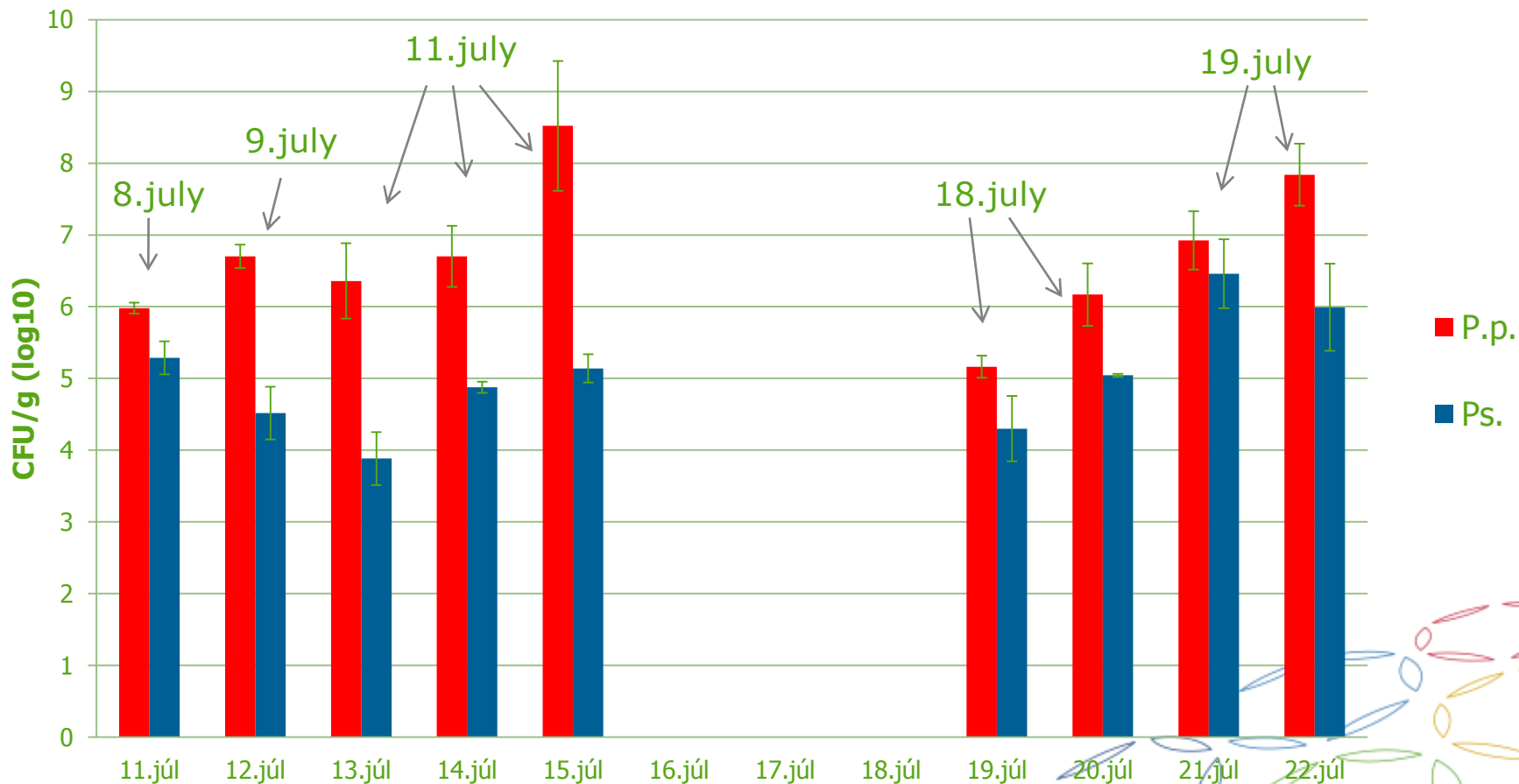
The situation in retail – week days in same store

Haddock at retailer



The situation in retail – week days in same store

Cod at retailer



Conclusions

- Large variation in number of spoilage bacteria can be expected to be present in fresh fish
- Direct monitoring of spoilage potential is rare. Data on number of spoilage bacteria in flesh upon landing and processing is not available.
- Methods have been developed for rapid quantification
- Can provide pressure to the industry to handle the material in the best way
- Higher overall quality, increased shelf life and higher value.
- Can be of use in shelf life prediction where bacterial load is needed.



Ræktunargreiningar

- Örverugreiningar eru framkvæmdar á viðurkenndum (faggildum) rannsóknarstofum
- Hefðbundnar ræktunargreiningar taka 3-7 daga

Bakteríur	Aðferð	# Daga
<i>Salmonella</i>	NMKL 71, jákvætt/neikvætt	3
<i>Campylobacter</i>	NMKL 119, jákvætt/neikvætt	4
<i>Listeria</i>	NMKL 136, jákvætt/neikvætt	5



- **Notkun hraðvirkra aðferða til örverugreininga hefur færost mikið í vöxt undanfarin ár**
- **Með hraðvirkum greiningaraðferðum má stytta greiningartíma úr nokkrum dögum niður í 4-24klst eftir aðferðum**
- **Real-time PCR aðferðafræði er notuð í ýmsar rannsóknir og greiningar í sameindalíffræði**

