Spoilage microbiota of fresh fish: identification and characterisation of the spoilage potential

Bederfflora van verse vis: identificatie en karakterisatie naar bederfpotentieel

Flores d'altération du poisson frais : de l'identification et la caractérisation au potentiel d'altération

Katrien Broekaert, Bert Noseda & Frank Devlieghere

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Spoilage of fish

- Post mortem changes in fish:
  - Rigor mortis
  - Autolytic & bio-chemical changes
  - **Bacteriological growth and activity → SPOILAGE OF FISH (95%)**
  - Chemical changes (rancidity)
  - Physical changes

- Initial quality loss: autolytic → makes nutrients available for bacterial growth.

Microbiological parameters

Parameter	Goal (cfu/g)	Tolerance (cfu/g)	End of shelf life (cfu/g)
Total aerobic psychrotrophic count	10^5	10^6	10^7
Psychrotrophic lactic acid bacteria	10^2	10^3	10^7 (a)
Yeasts	10^2	10^3	10^5
Moulds	10^2	10^3	No visual growth
E. coli	10^2	10^3	10^3
Coagulase positive staphylococci	10^2	10^3	10^3
Sulfite reducing bacteria	10^2	10^3	10^5
Salmonella spp.	Absent in 25g	Absent in 25g	Absent in 25g
Listeria monocytogenes	Absent in 25g	Absent in x g	10^2

Total Viable Count analysis

TVC is defined as the number of bacteria (cfu/g) in a food product which develop into clearly visible colonies when the test is carried out under standard conditions

TVC is only a measurement of the fraction of the microbiota able to produce colonies in the medium used under the conditions of incubation

LIMITED INFORMATION

TVC:
- no measure of “total” bacterial population
- no differentiation between types of bacteria
- doubtful in the examination of frozen fish

SPECIFIC SPOILAGE ORGANISMS

the total number of microorganisms on fish
≠ microorganisms responsible for fish spoilage

microorganisms responsible for fish spoilage
= rather only a small fraction of the microbiota
the “specific spoilage organisms” or SSOs (Dalgaard, 1995)

SSOs must be enumerated (and identified) for quality control or determination of the remaining shelf life of fish.

SSOs are specific to each fish species,
and are still unknown for many fish species.

TVC growth media

Growth media used for TVC of fish and fishery products

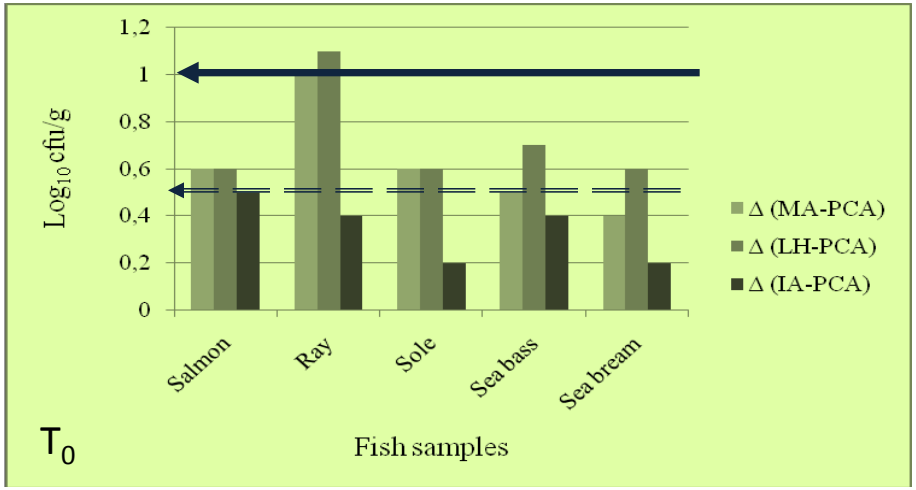
✓ Plate Count Agar (PCA) ISO – ICMSF	3d 21 °C
✓ Iron Agar (IA) Total viable counts (TVC) and counts of H_2S-producers	5d 15°C
✓ Marine Agar (MA)	3d 21 °C
✓ Long and Hammer Agar (LH) - NMKL 184 modification by Van Spreekens (1974) with an additional 1% w/v NaCl.	5d 15°C

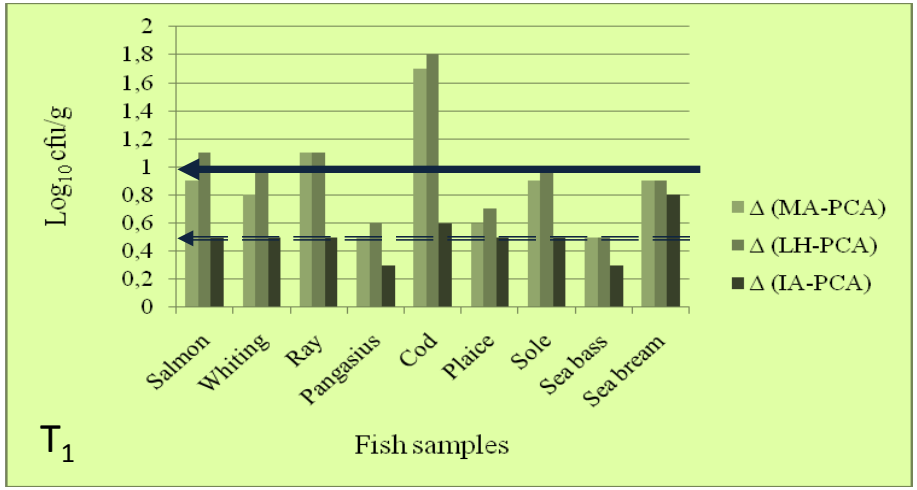
EXAMPLE: DETECTION OF SPOILAGE MICROBIOTA

TVC > 7 log cfu/g ➔ spoilage organoleptically detectable
➔ no longer acceptable for consumption

LARGE DIFFERENCES BETWEEN GENERAL MEDIA!

- PCA lowest TVC, after storage on ice often > 1 $\log_{10}$ difference
- Results on MA and LH mostly alike (difference < 0.5 $\log_{10}$)





EXAMPLE: DETECTION OF SPOILAGE MICROBIOTA

Not growing on PCA	Only growing on PCA	Not growing on MA	Not growing on LH	Not growing on IA
Photobacterium spp.				
Shewanella spp.				
Vibrio spp.				
Pseudoalteromonas spp.				
Psychrobacter spp.	*Psb. cibarius*	*Psb. cibarius*	*Psychrobacter* spp.	*Psb. cibarius*
Pseudomonas fluorescens	*Ps. fragi*	*Ps. fragi*	*Ps. fragi*	*Ps. fragi*
	Acinetobacter spp.	*Acinetobacter* spp.	*Acinetobacter* spp.	*Acinetobacter* spp.
		Flavobacterium spp.		
				Brochotrix thermosphacta

MICROBIOTA = FISH SPECIFIC

The shelf life of aerobically stored seafood depends on several factors (Jay, 1986):

1) the storage conditions,
2) the intrinsic factors of the seafood, and
3) the qualitative and quantitative composition of the initial microbiota, which is related to the environment, the water temperature, area of catch and the early handling and processing procedures.

Freshly caught seafood is naturally contaminated with a diversity of microbiota: e.g. *Aeromonas*, *Pseudomonas*, *Moraxella/Acinetobacter*, *Shewanella*, *Photobacterium* and *Flavobacterium* species (Liston, 1980)

These microbial populations may shift during storage (Shewan and Georgala, 1957).

MICROBIOTA = FISH SPECIFIC

Type of product	Spoilage bacteria
Fresh chilled aerobic storage - high TMAO, pH>6	*Shewanella* spp.
- low TMAO, or low pH	*Pseudomonas* spp.
Fresh chilled and modified atmosphere packaged Marine products with TMAO	*Photobacterium phosphoreum*
Warm water low TMAO	Lactic acid bacteria; *Brochotrix thermosphacta*
Tropical freshwater fish	*Aeromonas* spp.
Fresh and lightly preserved seafood at ambient temp	*Aeromonas* spp., *Vibrio* spp., *Photobacterium* spp., *Enterobacteriaceae*
Lightly preserved and chilled products	Lactic acid bacteria; *B. thermosphacta, P. phosphoreum*, *Vibrio* spp., Enterobacteriaceae

EXAMPLE: BROWN SHRIMP (*Crangon crangon*)

Dominant microbiota of shrimp during storage under several conditions/processing:

***Pseudoalteromonas* spp.**
***Psychrobacter* spp.**

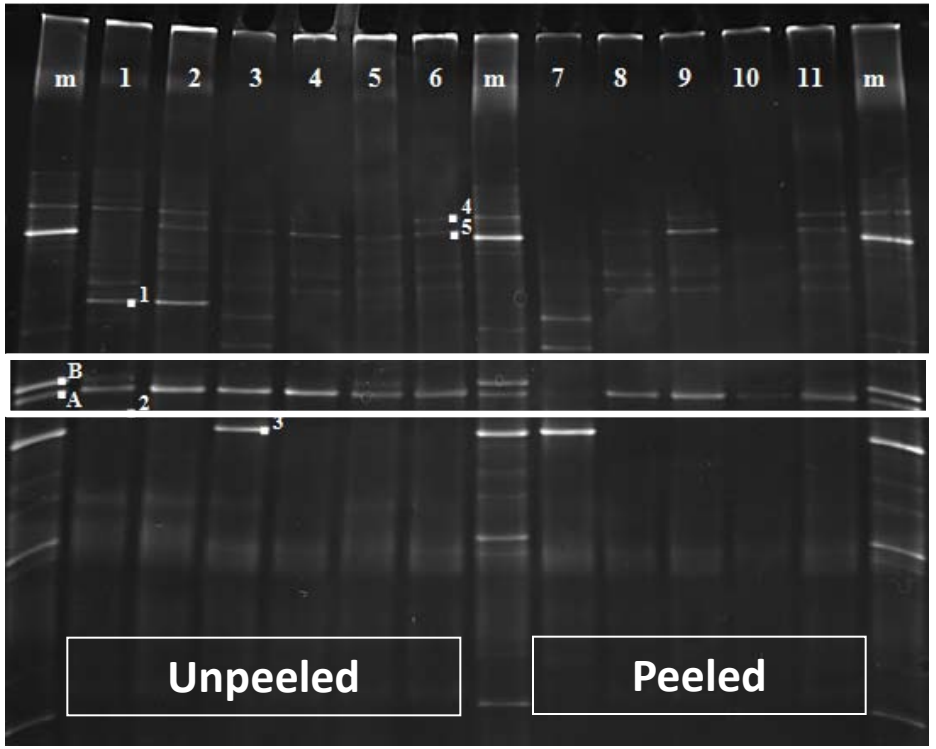


Fig: DGGE pattern from the microbiota of cooked shrimp swabbed from modified Long and Hammer medium (LH) under different storage and processing conditions.
m= marker, A= *Pseudoalteromonas* spp., B= *Psychrobacter* spp..

EXAMPLE: RAY (*Raya sp.*)

Dominant microbiota of ray during storage on ice:

Day 1: ***Pseudomonas* spp.**
***Psychrobacter* spp.**
***Pseudoalteromonas* spp.**
***Flavobacterium* spp.**
Day 3: + *Shewanella* spp.
Day 9: + *Arthrobacter* spp.

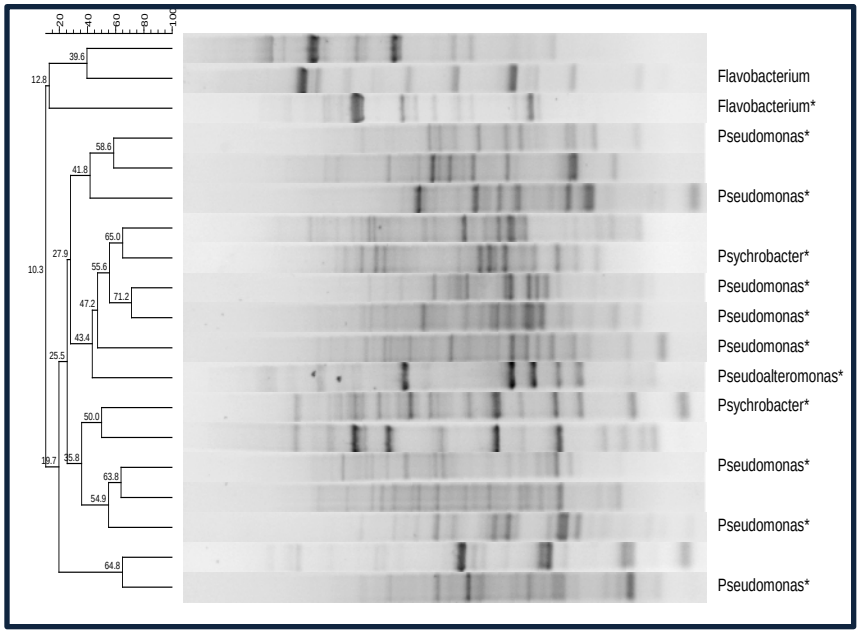


Fig: $(GTG)_5$ rep- fingerprint from the microbiota of fresh ray stored on ice at day 1.

Bacterial spoilage of fish

Freshwater fish

Scientific grouping	Biological characteristics	Technological characteristics	Examples
Teleostei or bony fish		fatty fish (store lipids in body tissue)	trout, eel
		lean (white) fish (store lipids in liver only)	Tilapia, perch, pike

Marine fish

Scientific grouping	Biological characteristics	Technological characteristics	Examples
Cyclostomes	jawless fish		lampreys, slime-eels
Chondrichthyes	cartilaginous fish	high urea content in muscle	sharks, skate, rays
Teleostei or bony fish	pelagic fish	fatty fish (store lipids in body tissue)	herring, mackerel, sardine tuna, sprat
	demersal fish	lean (white) fish (store lipids in liver only)	cod, haddock, hake grouper, seabass

Bacterial spoilage of fish

- Initially (aerobic conditions): aerobic organisms use carbohydrates and lactate and produce CO_2 and H_2O $\rightarrow$ decrease in Eh (surface)

- In marine fish species, a decreased Eh favoures facultative anaerobic bacteria (e.g. *Shewanella putrefaciens* and *Enterobacteriaceae*), reducing TMAO to TMA (unpleasant fishy odour):

$$CH_3\,CHOHCOOH + (CH_3)_3\,NO \xrightarrow{\text{TMAO-reductase}} CH_3\,COCOOH + (CH_3)_3\,N + H_2O$$

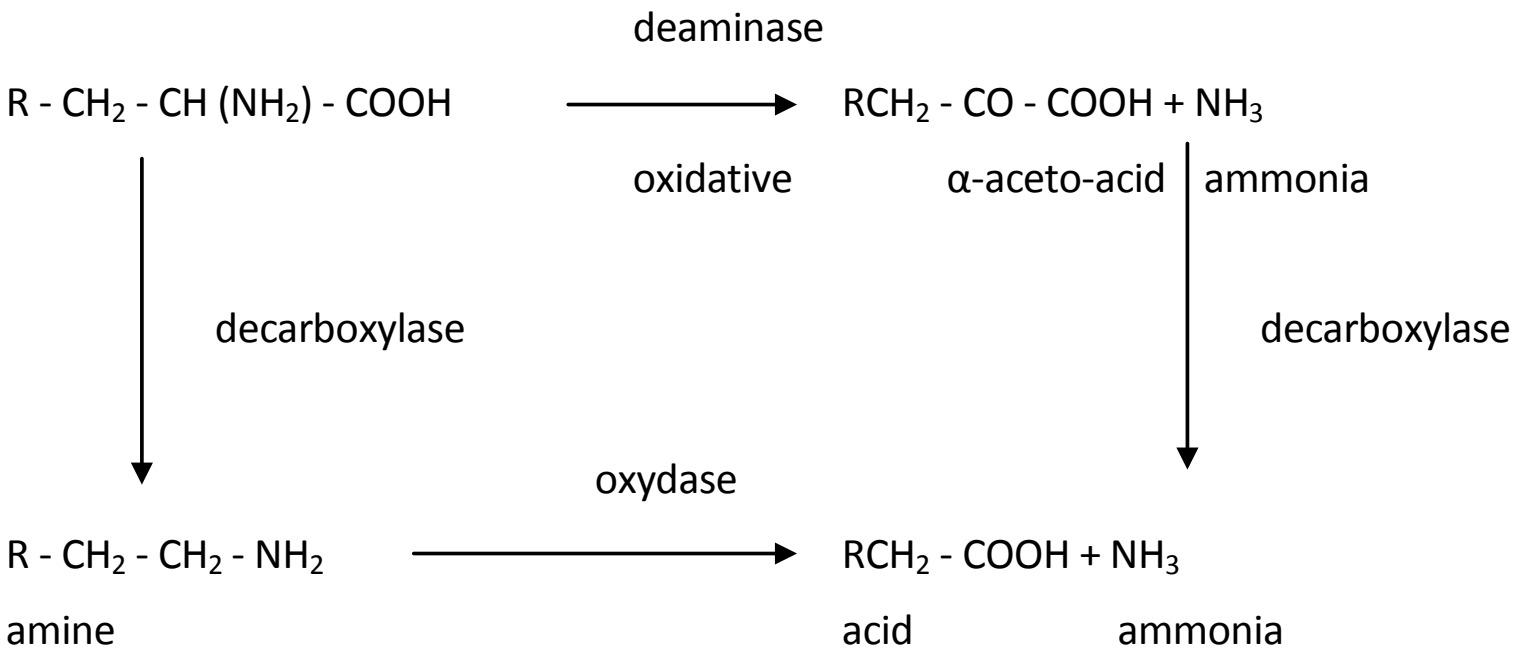
lactic acid TMAO pyruvate TMA

$$CH_3\,COCOOH + (CH)_3\,NO + H_2O \longrightarrow CH_3\,COOH + (CH_3)_3\,N + CO_2 + H_2O$$

pyruvate TMAO acetic acid TMA

Bacterial spoilage of fish

- Breakdown of amino acids:



Bacterial spoilage of fish

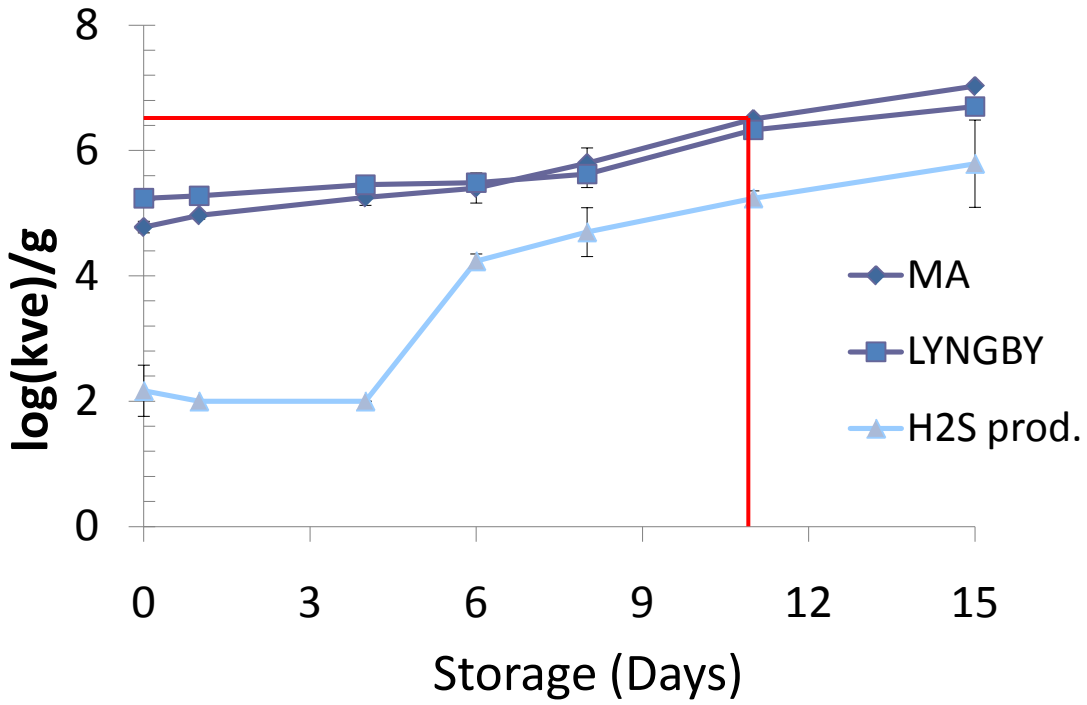
- Breakdown of S-containing amino acids cysteine and methionine to H_2S, CH_3SH (methanethiol) and $(CH_3)_2S_2$ (e.g. *Shewanella putrefaciens)*
  - detectable even at low ppb levels
  - low quantities have a considerable effect on the sensorial quality

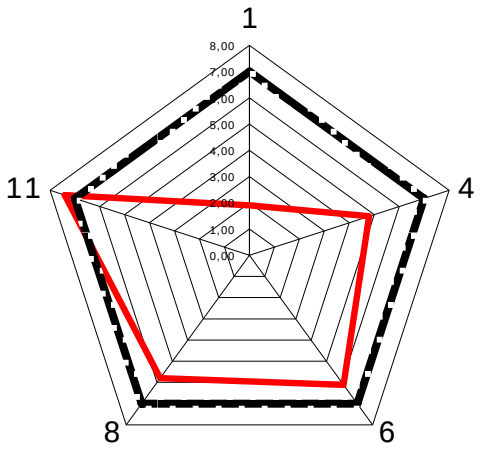
- Decomposition of urea in *elasmobranchii* during storage:

$$(NH_2)_2CO + H_2O \xrightarrow{\text{urease}} CO_2 + 2NH_3$$

Bacterial spoilage of grey shrimp

- Example:
  - Storage of MAP (50% CO_2-50% N_2) Grey shrimp (*Crangon crangon)* at 4°C:





Bacterial spoilage of grey shrimp

- Example:
  - Main metabolites originating from bacterial spoilage of MAP (50% CO_2-50% N_2) *Crangon crangon* stored at 4°C (determined with Thermal Desorption-GC-MS):
    - Trimethylamine
    - Acetaldehyde
    - Ethanol
    - Aceton
    - Isopropanol
    - Dimethylsulfide
    - Dimethyldisulfide
    - Carbondisulfide

Bacterial spoilage of grey shrimp

- Example: Why did spoilage occur so early?

- At a high product pH, amines tend to volatilize more =>

At pH ± 8.0, a minimal amine production might already cause sensorial deviations

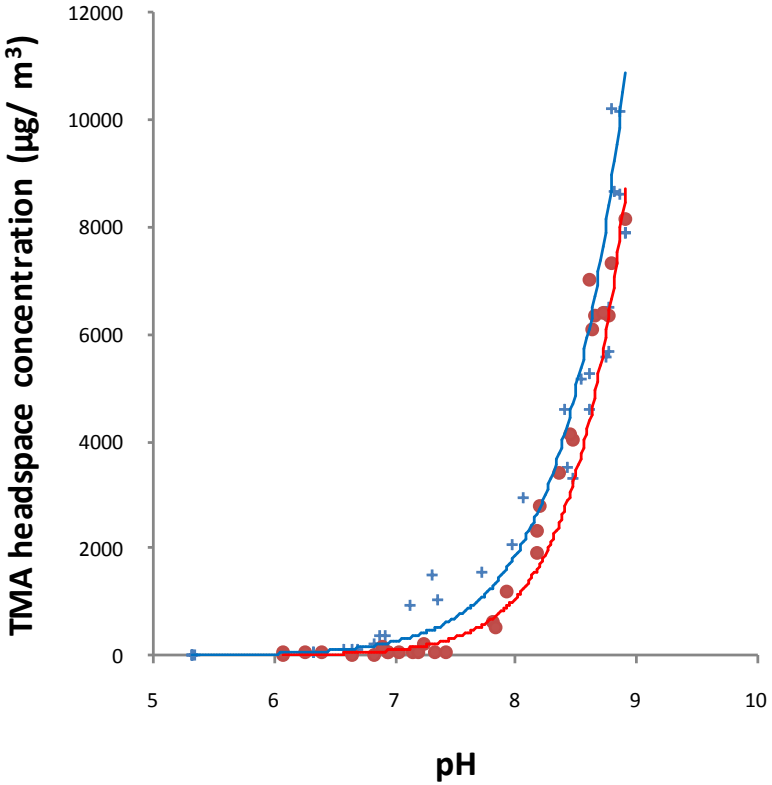


Figure. TMA headspace concentrations (µg/m³) of 5.0 mg spiked N in 50.0 g water (+) and in 50g mixed shrimp (●), in function of the pH. (determined with Selective Ion Flow Tube Mass Spectrometry SIFT-MS)

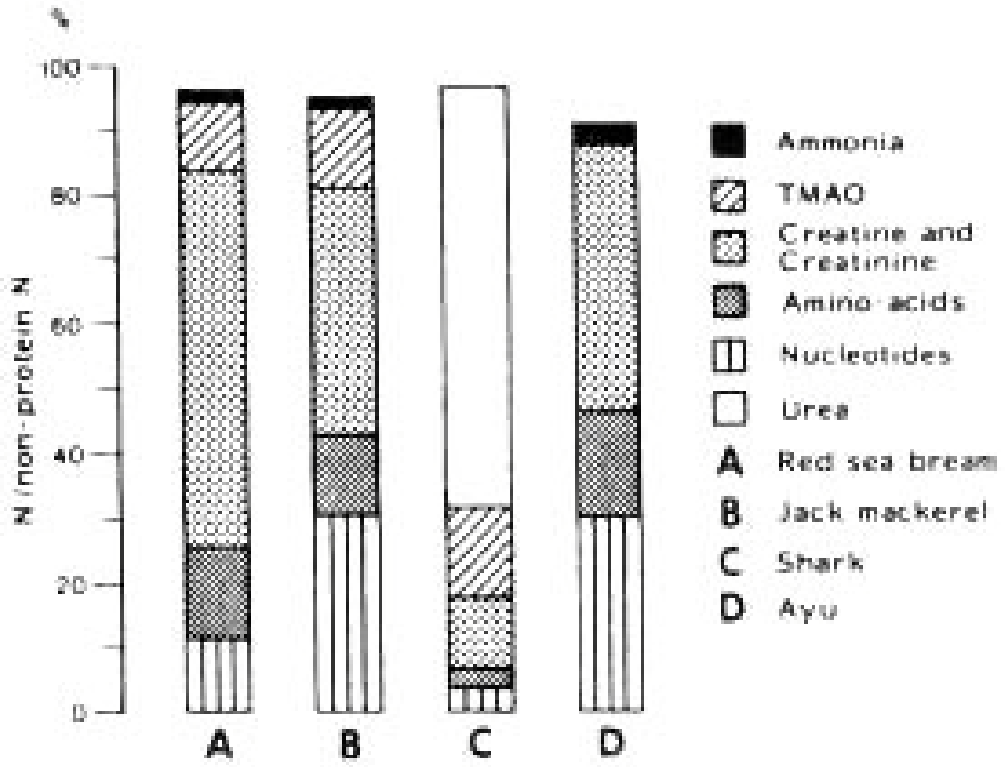
Bacterial spoilage of fish

Factors influencing bacterial growth and metabolism

Intrinsic factors = factors inherent to the fish species

→ **pH, aw, Eh, nutrient content of fish, antimicrobial systems**

NPN fraction



Distribution of non-protein nitrogen in fish muscles of two marine bonyfish (A,B), an elasmobranch (C), and a freshwater fish (D) (Konosu and Yamaguchi, 1982; Suyama *et al.,* 1977)

Bacterial spoilage of fish

Factors influencing bacterial growth and metabolism

Intrinsic factors = factors inherent to fish

→ pH, aw, Eh, nutrient content of fish, antimicrobial systems

Extrinsic factors

→ **temperature, RH, change of atmosphere (MAP)**

THANK YOU FOR YOUR ATTENTION!

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