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FRESHNESS AND HEALTHY EATING QUALITY OF FARMED FISH

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President of ASSOITTICA ITALIA and FEDERPESCA

Fish is perceived as healthy and nutritional food

its *freshness* and *price*

the most important reasons to buy it by European consumers.

Thanks to the natural abundance of LC n-3 highly unsaturated fatty acids (HUFA) C20:5 (EPA) and C22:6 (DHA), that characterise lipids, vitamin D, phospholipids, peptides, taurin, iodine, selenium…

Dietary Guidelines of International Committee
has recommended at least a twice-a week fish consumption
to prevent pathologies such as cardiovascular diseases,
colon cancer, psychiatric & psychological diseases,
neurodegenerative diseases, inflammatory bowel diseases….
However, to exert all the potential beneficial effects
without any risks for human health, it has to be

produced and maintained
safe, free from contaminants and
fresh up to the consumer.

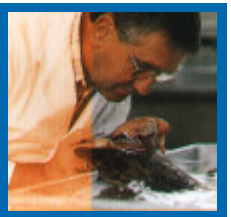
Freshness is more a concept than an entity

"Fish freshness means that fish is in its entire properties
not far away from those properties it had in the living state
or that only of short period of time has past
since the fish has been caught or harvested…
it is more a complex idea of an ideal state of wholesomeness,
soundness and perfection of a newly harvested fish"
"It is advisable to speak about
freshnesses,
where freshness $_{t=0}$ is the freshness at time of catch or harvest and
freshness $_{t>0}$ has to be differentiated from the initial one….

So it can be concluded that freshness is an attribute which changes continuously but comprises a certain time period"

(Oehlenschäger and Sörensen, 1997)

FISH QUALITY CHANGES



The high levels of non protein nitrogenous compounds and of n-3 HUFA, highly susceptible to oxidation, together with the low carbohydrate content and the consequent high final pH in muscle, not as efficient in microbial proliferation inhibition, contribute to fish trait of high perishable food in respect to other meats.

MOREOVER

All conditions affecting fish biochemical processes taking place at death and during *post mortem* period, can heavily influence the expression of its flesh quality and the subsequent changes during storage, including freshness loss and shelf life, all of them well indicated by the changes of the physical/ sensory/organoleptic attributes such as *rigor* status, general appearance and colour of skin/muscle/eye/gills, texture, and odour of the raw products.

COMPLEX SPOILAGE CHANGES IN FISH

Identify freshness/quality indicators

Fresh odor

Spoilage odor

Endogenous enzymes
lipoxygenase, cathepsin, calpain →

Oxidation
pro- and antioxidants →

Microbial growth
SSO
Specific Spoilage Organisms →

Glycolysis **pH↓, Lactic acid↑**

Rigor mortis, **ATP→ Inosine →Hx →Urea**

Autolysis

Protein / i.e. sarcoplasmic proteins
→ peptides→ amino acids

Lipids / i.e. phospholipids → PUFA

Soluble substances in the muscle
Nucleotides
NPN non protein nitrogenous components
TMAO → TMA/DMA pH ↑

Texture firm – soft

Colour changes

Fish Freshness

Even alone is a fundamental parameter of fish safety and quality.
It has been much work on developing scientific methods
for accurate, rapid, inexpensive fish freshness measure.

Sensory Evaluation

Microbial methods

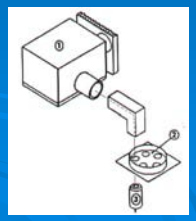
Volatile compounds

Protein and lipids

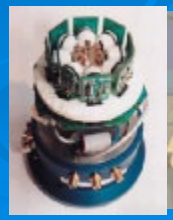
ATP Metabolites

Physical Measurements

For the present,
sensory assessment,
with all its disadvantages,
remains the favoured option and
the point of reference



Sensory evaluation (UE, QIM) the use of sensors (volatile compounds, electrical properties, ATP metabolites) , spectroscopic methods, can be considered as potential instruments for a rapid and not destructive freshness evaluation of fish products



FISH QUALITY EVALUATION BY SENSORIAL ANALYSIS EU SCHEME (Reg. 2406/96)

	Criteria			
	Freshness category			Not admitted (1)
	Extra	A	B	
Skin	Bright, iridescent pigment (save for redfish) or opalescent; no discolouration	Pigmentation bright but not lustrous	Pigmentation in the process of becoming discoloured and dull	Dull pigmentation (2)
Skin mucus	Aqueous, transparent	Slightly cloudy	Milky	Yellowish grey, opaque mucus
Eye	Convex (bulging); black, bright pupil; transparent cornea	Convex and slightly sunken; black dull pupil; slightly opalescent cornea	Flat; opalescent cornea; opaque pupil	Concave in the centre; grey pupil; milky cornea (2)
Gills	Bright colour; no mucus	Less coloured; transparent mucus	Brown/grey becoming discoloured; thick, opaque mucus	Yellowish; milky mucus (2)
Peritoneum (in gutted fish)	Smooth; bright; difficult to detach from flesh	Slightly dull; can be detached from flesh	Speckled; comes away easily from flesh	Does not stick (2)
Smell of gills and abdominal cavity				(2)
— whitefish other than plaice	Seaweedy	No smell of seaweed; neutral smell	Fermented; slightly sour	Sour
— plaice	Fresh oily; peppery; earthy smell	Oily; seaweedy or slightly sweetish	Oily; fermented; stale, slightly rancid	Sour

RAW FISH

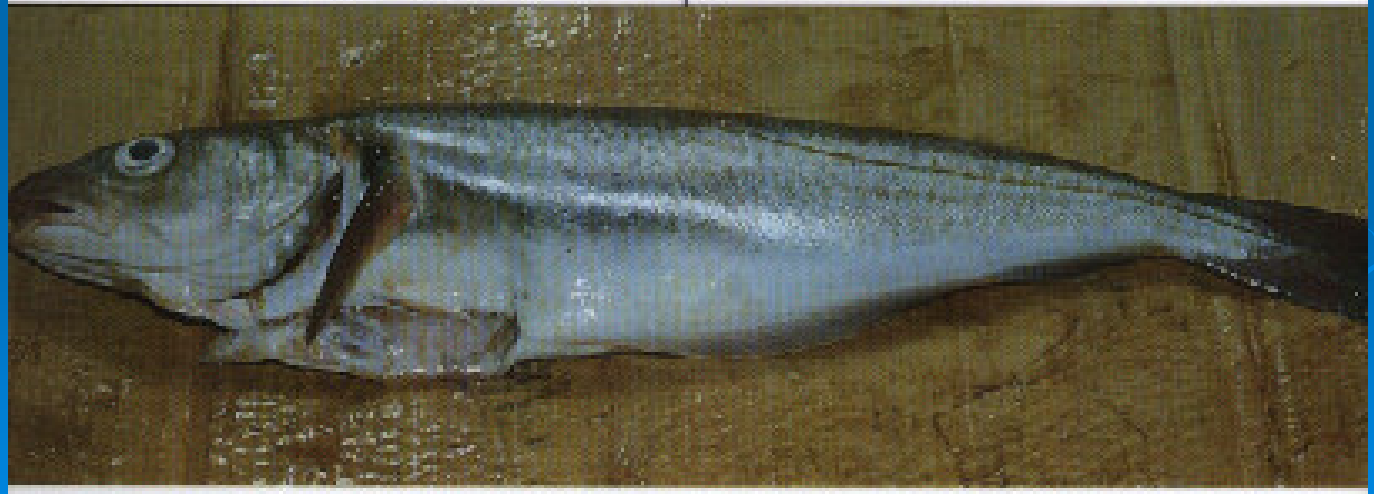
Criteria to evaluate freshness category

(Extra, A, B, not admitted) of fish

E CLASS QUALITA' EXTRA (POTASSOLO)

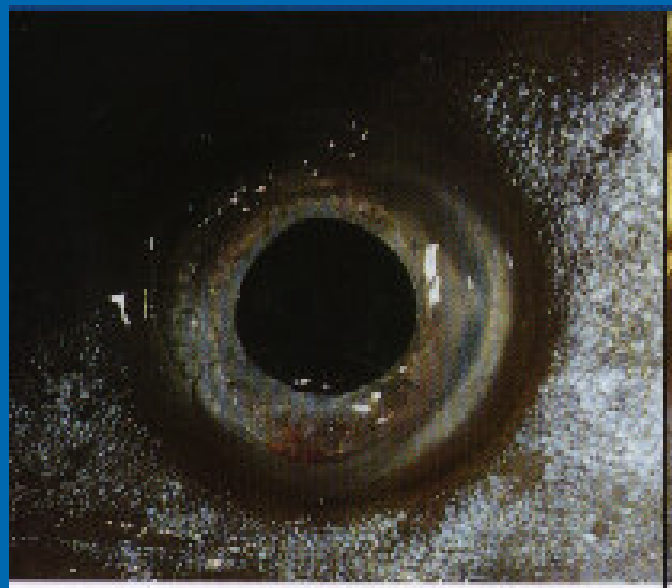
L'occhio è luminoso, chiaro e convesso

Le branchie sono di un rosso brillante e c'è poco o niente muco. Odorano di alghe.



La pelle è chiara, lucente e colorata. Il muco è trasparente

A CLASS QUALITA' BUONA (POTASSOLO)



L'occhio è meno luminoso e leggermente infossato. La copertura dell'occhio è leggermente scura.

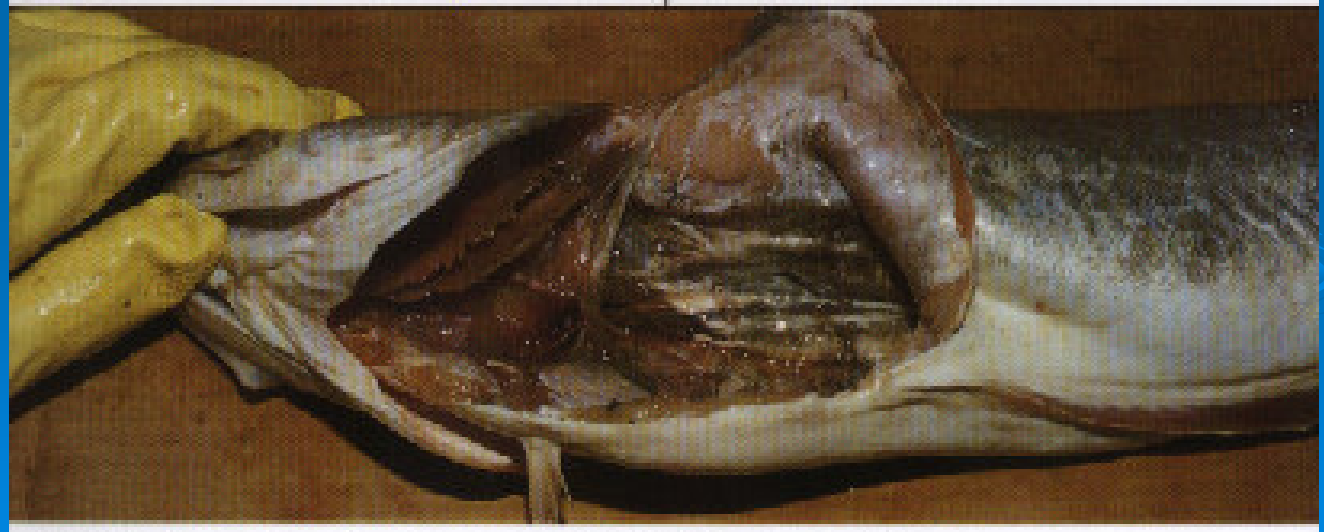
Le branchie sono un po' meno rosse e ci sono tracce di muco chiaro. Hanno poco o nessun odore.

La pelle è meno lucente e colorata. Il muco è leggermente opaco.

B CLASS
CATTIVA
QUALITA'
(POTASSOLO)

L'occhio è appannato e infossato. E' lattiginoso e vira verso il marrone

Le branchie sono pallide e diventano marroni. Il muco è opaco. Hanno un odore acre.



La pelle diventa decolorata e opaca. Il muco è lattiginoso.

Quality Index Method (QIM) s d salmon

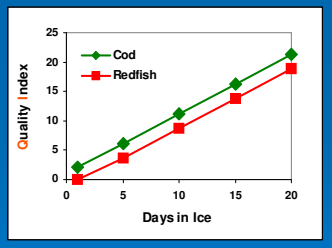
Quality parameter		Description	Score
Skin	Colour/ appearanc	Pearl-shiny all over the skin	0
		The skin is less pearl-shiny	1
		The fish is yellowish, mainly near the abdomen	2
	Mucus	Clear, not clotted	0
		Milky, clotted	1
		Yellow and clotted	2
	Odour	Fresh seaweedy, nutral	0
		Cucumber, metal, hay	1
		Sour, dish cloth	2
		Rotten	3
	Texture	In rigor	0
		Finger mark disappears rapidly	1
		Finger leaves mark over 3 seconds	2
Eyes	Pupils	Clear and black, metal shiny	0
		Dark grey	1
		Mat, grey	2
	Form	Convex	0
		Flat	1
		Sunken	2
Gills	Colour	Red/dark brown	0
		Pale red, pink/light brown	1
		Grey-brown, brown, grey, green	2
	Mucus	Transparent	0
		Milky, clotted	1
		Brown, clotted	2
	Odour	Fresh, seaweed	0
		Metal, cucumber	1
		Sour, mouldy	2
		Rotten	3
Abdomen	Blood in abdomen	Blood red/not present	0
		Blood more brown, yellowish	1
	Odour	Neutral	0
		Cucumber, melon	1
		Sour, fermenting	2
		Rotten/rotten cabbage	3
Quality Index			0-24

Changes in outer appearance of skin, eyes, gills and odour and texture and score system from 0 to 3 demerit points to have the Quality Index.

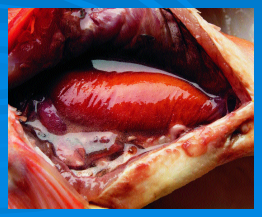
QIM Schemes:
brill (Rhombus laevis), cod (Gadus morhua), deep water shrimp (Pandalus borealis), farmed salmon (Salmo salar), fjord shrimp (Pandalus borealis), haddock (Melanogrammus aeglefinus), herring (Clupea harengus), peeled shrimp (Pandalus borealis), plaice (Pleuronectes platessa), pollock (Pollachius virens), redfish (Sebastes mentella/marinus), sole (Solea vulgaris) and turbot (Scophtalmus maximus).

Quality Index Method (QIM) scheme for farmed sea bass

Quality parameter		Description	Score
Skin	Colour/ appearanc	Bright, iridescent pigmentation	0
		Rather dull, becoming discoloured (head)	1
		Green, yellowish, mainly near the abdomen	2
	Odour	Fresh seaweedy, neutral	0
		Cucumber, metal, hey	1
		Sour, dish cloth	2
		Rotten	3
	Texture	In rigor	0
		Finger mark disappears rapidly	1
		Finger leaves mark over 3 seconds	2
Eyes	Pupils	Clear and black, metal shiny	0
		Grey	1
		Mat, grey	2
	Form	Convex	0
		Flat	1
		Sunken	2
Gills	Colour	Blood red/orange	0
		Pale red, pink/light brown	1
		Grey-brown, brown, grey	2
	Mucus	Transparent	0
		Milky, clotted	1
		Brown, clotted	2
	Odour	Fresh, seaweed, neutral	0
		Metal, grass	1
		Sour, mouldy,dish cloth	2
		Rotten	3
Flesh, fille	Colour	Translucent, bluish	0
		Waxy, milky	1
		Opaque, yellow, brown spots	2
Viscera	Solution	Whole	0
		Beginning to dissolve	1
		Viscera dissolved	2
Quality Index			0-22



Huidobro et al. 2001



Principal Components Analysis on

the sensorial scores and instrumental parameters
showed that the sensorial parameters, K_1, Rigor index,
dielectric properties were the most correlated
with the first factor.

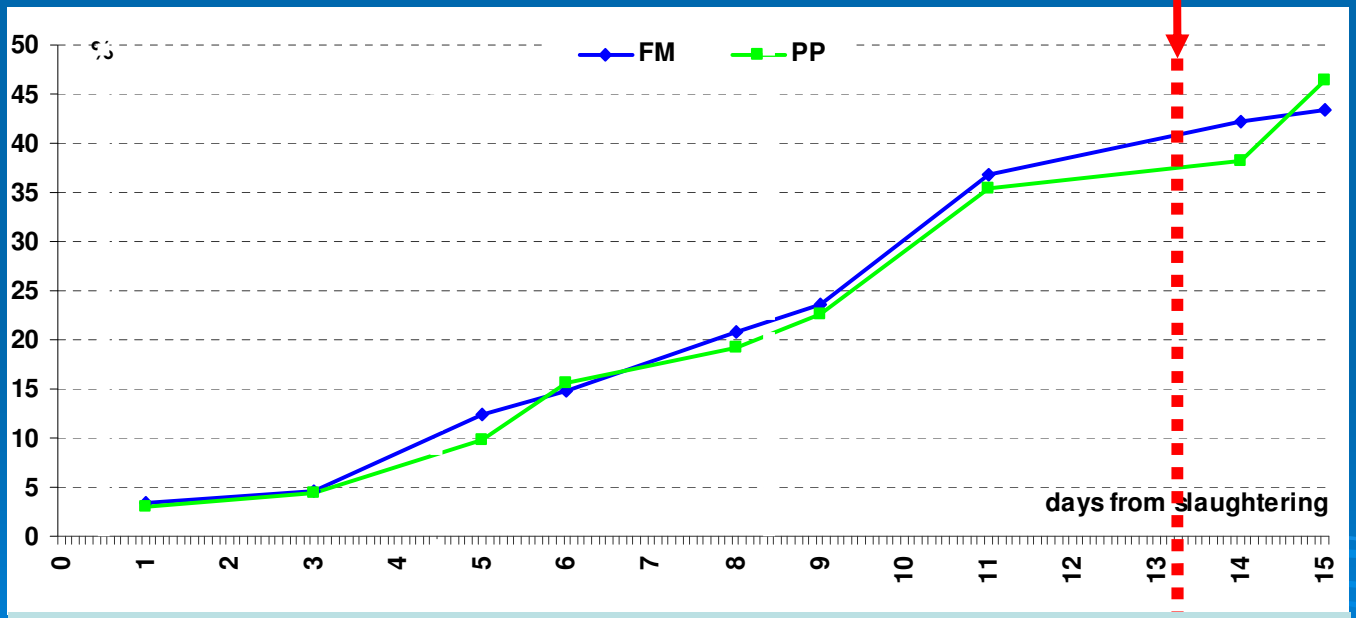
- The external sensorial parameters of fish alone
can be used to measure freshness.

- K_1, Rigor index and dielectric properties are particularly
sensitive to variations of the state of freshness
and could be considered valid alternative
to sensory analysis
in sea bass, sea bream, meagre and
pantex (Pagrus mayor x Dentex dentex).

Parisi et al. 2002, Aquaculture, 214: 153-166.

Changes during the shelf life in sea bream: application of UE scheme & K_1 value

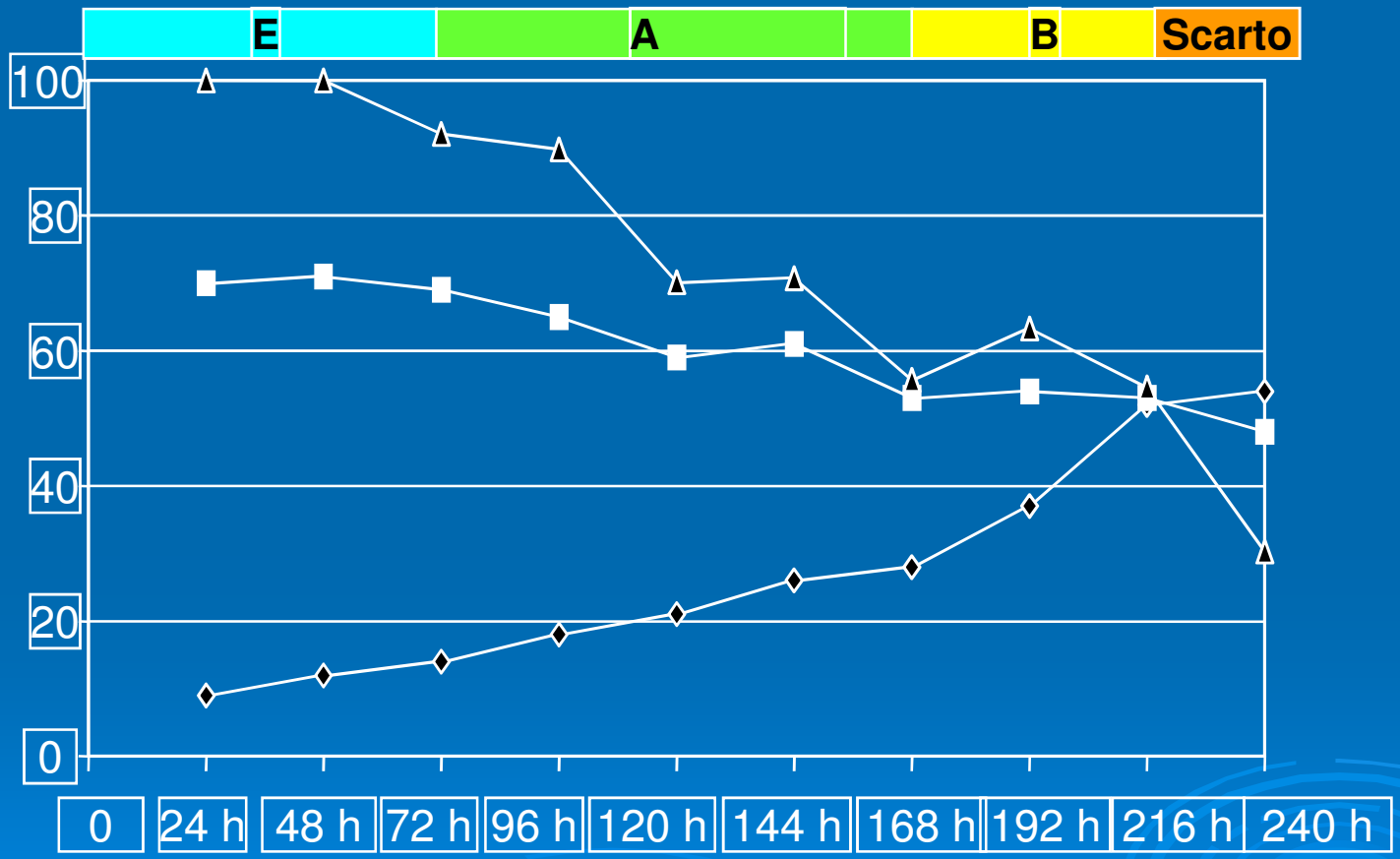
	1d 24h	*48h*	3d 72h	*96h*	5d 120h	6d 144h	*168h*	8d 192h	9d 216h	*240h*	11d 264h	12d 288h	*312h*	14d 336h	15d 360h
FM	E		E		A (2.2)	A (2.0)		A (1.9)	B (1.2)		B (0.9)	B (0.9)		NA (0.3)	NA (0.02)
PP	E		E		A (2.3)	A (2.1)		A (1.9)	B (1.2)		B (1.0)	B (0.9)		NA (0.3)	NA (0.06)



$$K_1 = ([HxR]+[Hx] / [IMP]+[HxR]+[Hx]) \times 100$$

Parisi et al., 2008

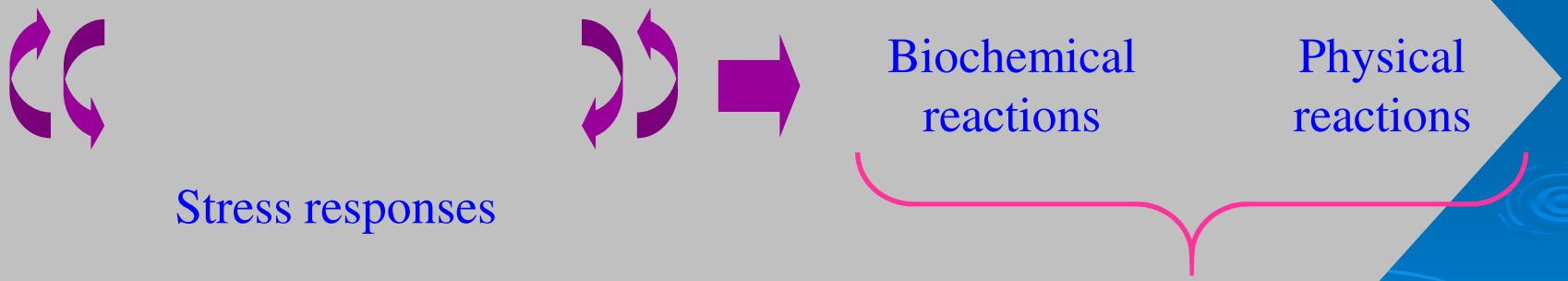
Shelf-life evolution, Rigor index, Impedance and K_1 index during 10 days after death (1 ℃ with ice cover) in meagre



Poli et al, 2002

Final product quality also depend to stress from rearing, handling, transport, and mostly to stunning/killing practices

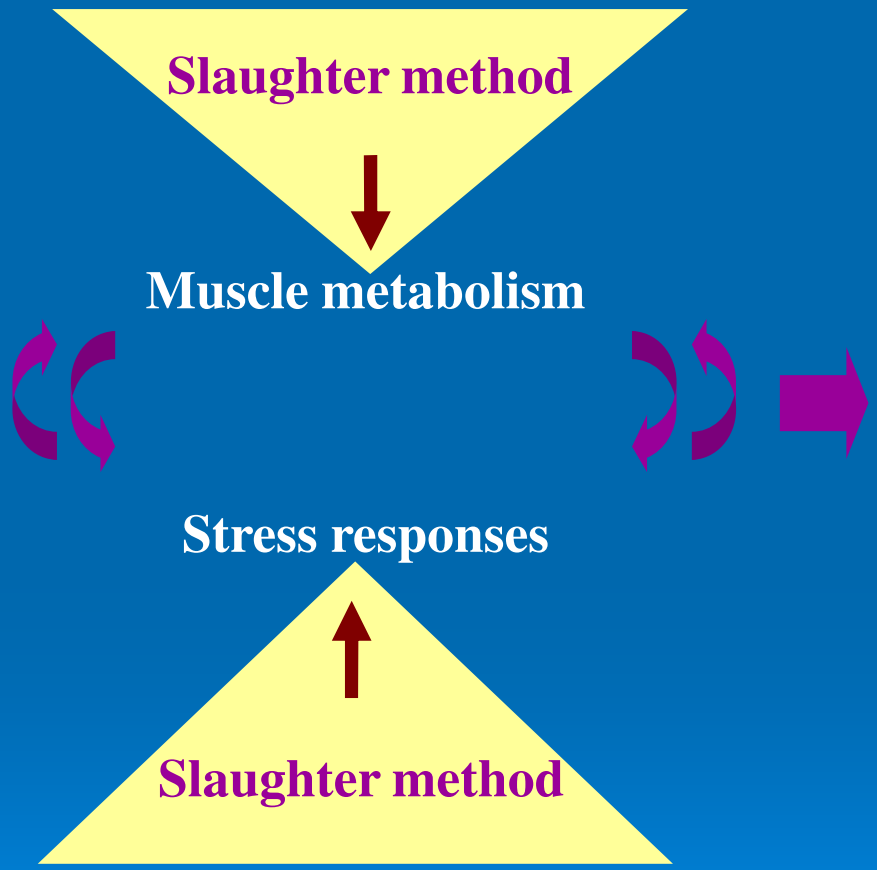
From a living fish into a food product



FISH

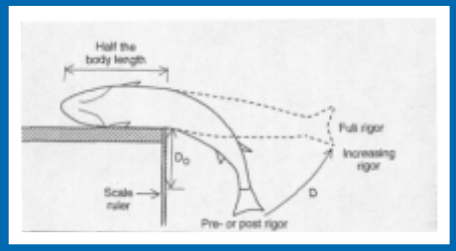
RAW MATERIAL FOR PROCESSING

FROM A LIVING FISH INTO A FOOD PRODUCT

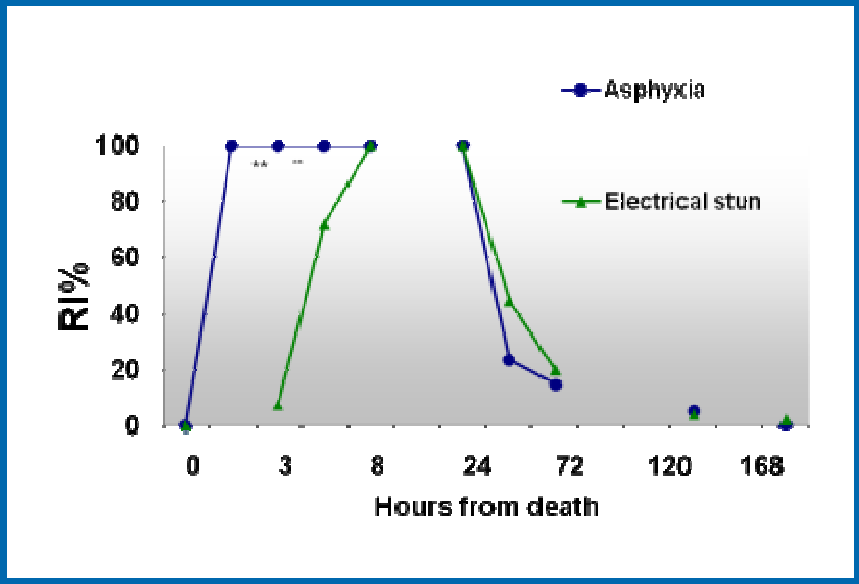


rigor development
muscle pH
muscle texture
water holding capacity
colour etc.

Freshness classes, rigor index and shelf life in trout



$RI\% = 100 \times (D - D_o) / D_o$



A =Asfixied fish earlier full rigor (RI 100% at 1 h)

E= Elettrically stunned fish reached 100% RI only at 8 h

		24h	72h	144h	192h
Portion size	A	E	A	B	B
	E	E	A	A	B

		24h	72h	144h	192h	240h
m	A	E	A	A	A	unfit
	E	E	A	A	A	B

Poli et al., 2007

Freshness classes and shelf life in sea bass

Jul	9d	10d	11d	12d	13d	14d	15d
IW		A (1.60)			B (0.92)	B- (0.54)	
IWN100		A (1.84)			B (1.08)	B- (0.63)	
IWN40		A (1.75)			B (1.00)	B- (0.62)	
IWN70		A (1.59)			B (0.92)	na (0.38)	
EL1p24	A (1.75)			B- (0.59)	B- (0.54)	na	
EL2p120	A (1.59)			na (0.33)	na (0)		

Nov		10d	11d	12d	13d	14d	15d
IW			A (1.73)	A (1.64)		B (0.92)	
IWN40			B (1.21)	B (1.45)		B (0.79)	
IWN70			B (1.19)	B (1.43)		B (0.96)	
EL2p40			A (1.61)	B (1.09)		B (0.75)	
EL1p40			A (1.64)	B (1.12)		B- (0.54)	
EL1p40N			A (1.77)	B (1.16)		B- (0.63)	
EL1p40N70			B (1.40)	B (1.03)		B/na (0.50)	

EU scheme (Rule 2406/96 EEC)

E class(Extra): very fresh

A class: fresh

B class: stale

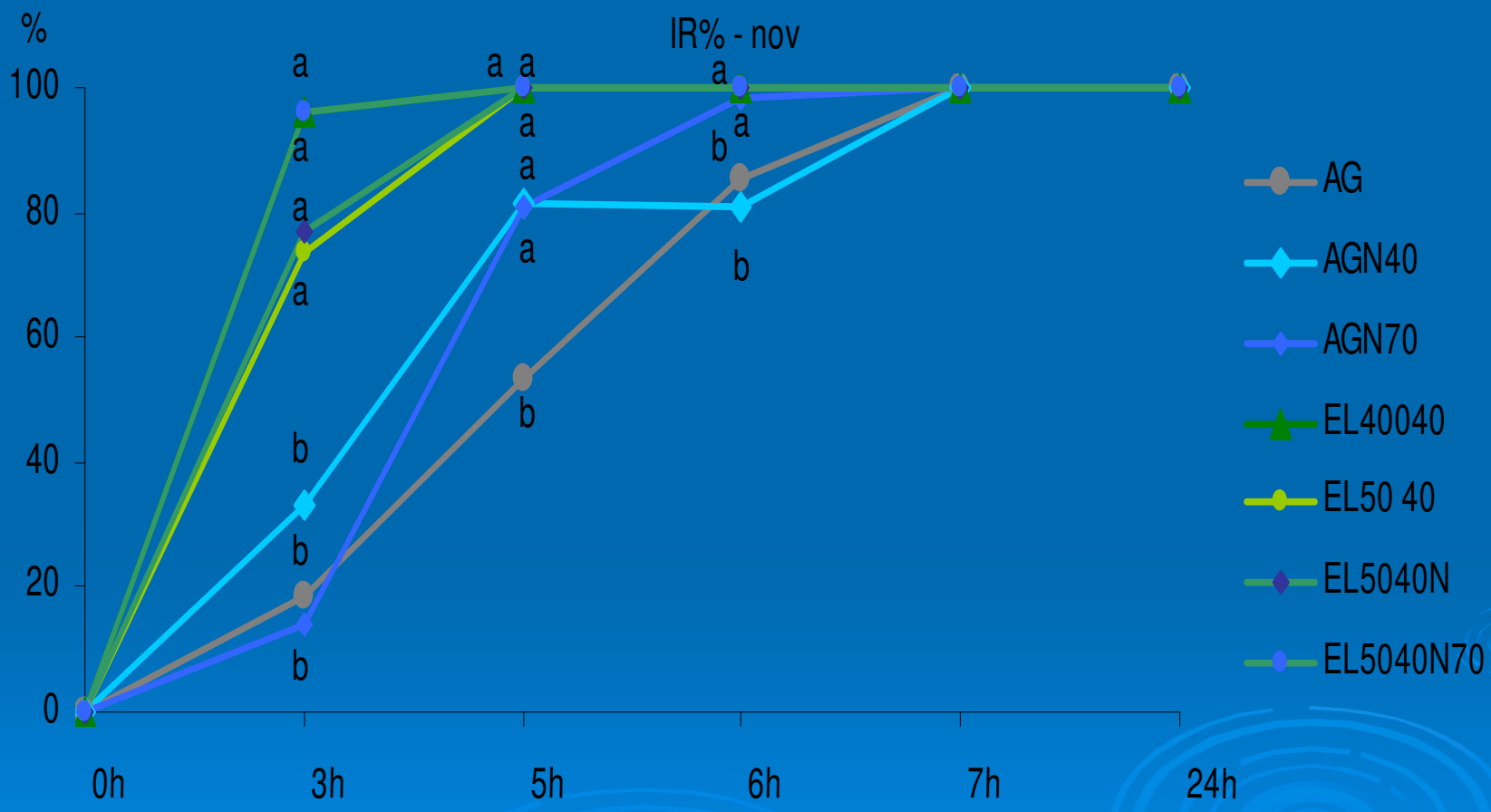
unfit : not edible

Zampacavallo et al., 2008

Electrical stunning: shorter shelf life

Ice Water (IW): more time in A class

gas: same shelf life than IW


IR% - nov
%
100
80
60
40
20
0
0h
3h
5h
6h
7h
24h
AG
AGN40
AGN70
EL40040
EL50 40
EL5040N
EL5040N70

Freshness classes and shelf life in sea bream reared in floating cage

	72h	*120h*	*168h*	*216h*	*240h*	*264h*
Ice/Water 2:1 (IW)	**E (2,7)**	**A (2,1)**	**A (1,9)**	**B (0,7)**	**B (0,9)**	**na (0)**
IW 70%N_2 30%$C0_2$	**E (2,7)**	**A (2,0)**	**A (1,6)**	**B/na (0,4)**	**B/na (0,5)**	**na (0,3)**
EL 50Hz 40V	**E (2,8)**	**A (1,9)**	**A/B (1,5)**	**B (0,9)**	**na (0)**	**na (0)**

Several stress and freshness/quality indicators

can be used to verify the fish stress condition at death and its reflexes on the product quality changes.

One of the most sensible and indicative parameters of the stress suffered and of the freshness/quality potential evolution is the early *full rigor mortis* onset and its rapid release

Other good stress /quality early indicators are:
ATP and its catabolites degradation rate
pH measured in muscle or, better, in eye liquor
Dielectric properties (Fish Tester)
Muscular lactic acid
Compactness

None of them exhaustive if used alone!

TRIALS OF SEAFOOD CONSUMPTION

Influence on biological markers related to atherothrombosis

as a measure of

seafood healthy eating quality

LC omega-3 PUFA most likely slow or limit atherosclerosis due to risk factor reduction but also reduce risk of coronary events in people with advanced atherosclerosis …

Three possible mechanisms:

1) **Decreased cardiac arrhythmias**
2) **Decreased thrombosis**
3) **Decreased inflammation**

Intake trial

Seabass fillet components mean intake in 10 WK trial (g) in dislipidemic* subjects

n.708

Orbetello sea bass

	Flesh	LIPIDS	C20:5	C22:6	SFA	MUFA	N-3 PUFA	N-6 PUFA
MEAN	8067	679.6	47.8	75.8	136.7	169.1	155.8	60.0
SD	± 226	± 47.9	± 2.6	± 2.6	± 6.6	± 8.1	± 7.6	± 5.9

Mean EPA + DHA = 12.4 g/wk
(minimum suggested 3.5 g/wk by the International Society for the Study of Fatty acids and Lipids)

Cooking guidelines:

Microwave or normal owen, with the addition of salt and olive oil

Poli et al., 2007
Sofi et al., 2009

*** Dyslipidemia was defined as:**

- Total cholesterol > 200 mg/dL
- LDL-cholesterol > 130 mg/dL
- Triglycerides > 150 mg/dL

Material and Methods

Lipid profile	**Total cholesterol** **LDL-cholesterol** **HDL-cholesterol** **Triglycerides**	**Conventional methods**
Inflammatory profile	**Interleukin-6** **Interleukin-8** **Interleukin-10** **TNF-alpha**	**Multiplex bead-based assay (Bioplex)**
Hemorheological profile	**Whole Blood Viscosity** **Erythrocytes' filtration rate** **Plasma viscosity**	**Rotational Viscosimeter LS30** **Filtrometer MF4**

Intake trial Blood biomarkers

Biomarker	T_0	T_{10}	Variation
N-3 PUFA EPA DHA in erythrocytes %	4.11 ± 4.5 0.26 ± 0.3 3.01 ± 3.4	10.48 ± 4.8 1.13 ± 0.62 7.67 ± 3.73	2.5 4.3 2.5 times
Triglycerides mg/dL	166.9 ± 81.2	127.8 ± 47.3	- 23%
Interleukin-6 pg/mL	3.13 ± 2.49	1.95 ± 1.27	- 38%
Interleukin-8 pg/mL	17.43 ± 11	12.56 ± 6.6	-28%
WBV 20.400 sec^{-1}	6.3 ± 0.39	6.3 ± 0.45	-5%
Erythrocytes' Filtration rate	5.33 ± 2.44	9.01 ± 2.75	+ 69%

P<0.05

SOFI et al. 2009. Int. J. Fd Sci. Nutr., 7:1-10

Intake trial

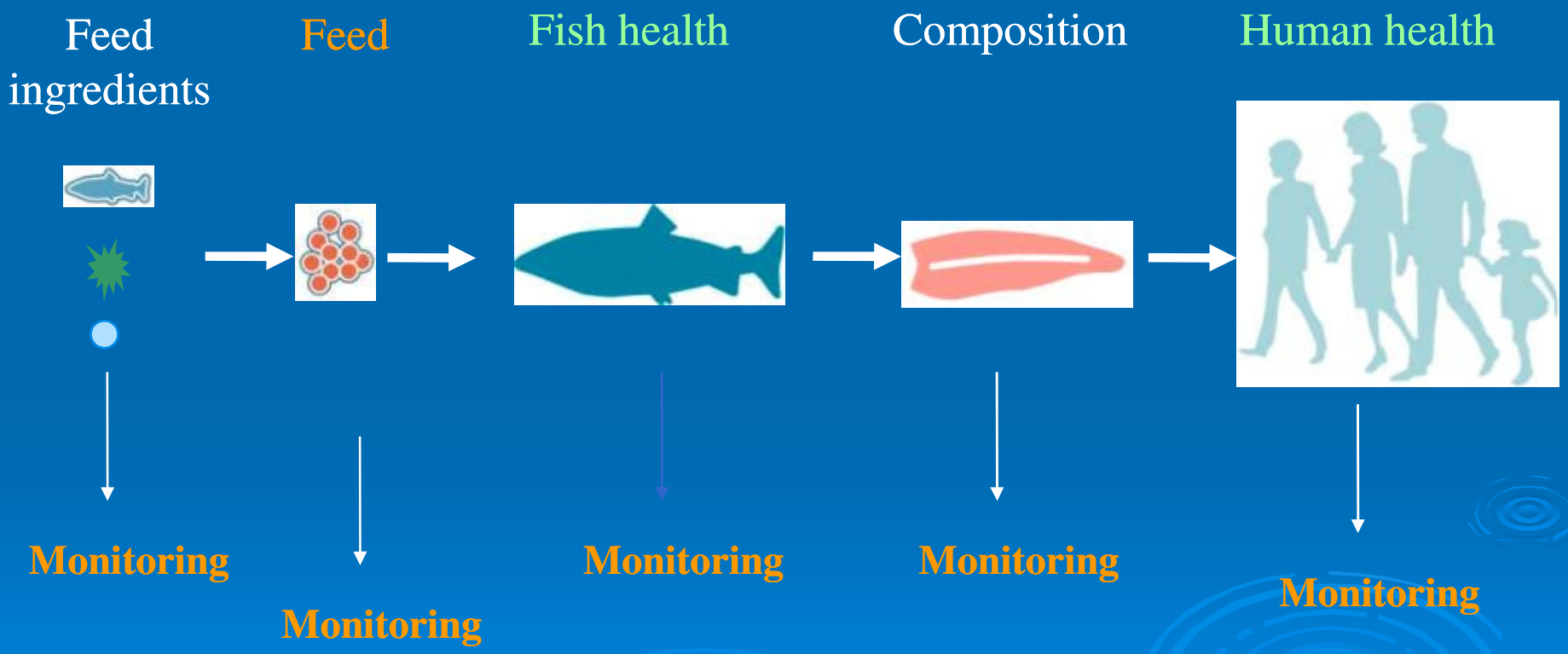
Sea bass dietary short-term intake

seems to set up favourable biochemical changes in dyslipidemic subjects, with regard to lower circulating levels of markers of atherosclerosis, such as

- **lipid parameters**
- **inflammatory markers**
- **haemorheological profile**

Poli et al., 2007
Sofi et al., 2009

AQUACULTURE
CONTROLLED PRODUCTION OF SEAFOOD

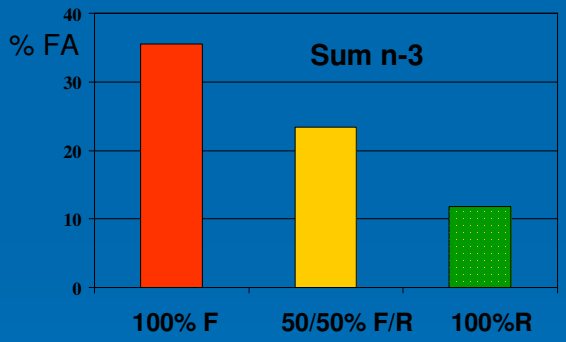


Intake trial

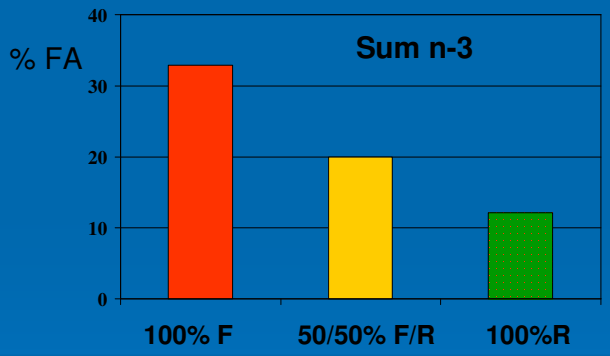
Seierstad et al.
Eur. J. Clin. Invest. 2005

Salmon **Man**

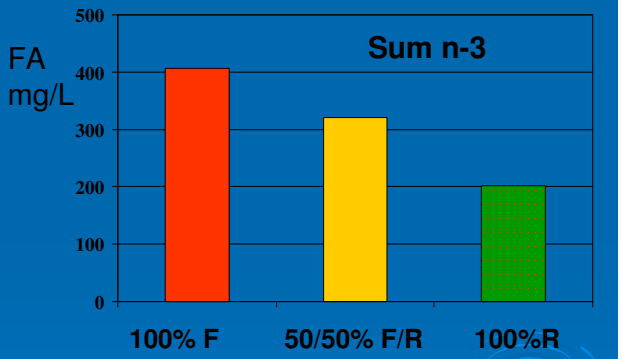
Feed



Fillet



Serum



100% Fish oil

50% Fish oil
50% Rapeseed oil

100% Rapeseed oil

The best results for CV system healthy !!

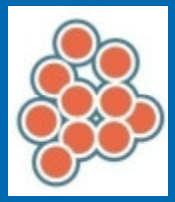
Intake trial

Sea bream dietary short-term intake

Fisr Project - responsable Poli B.M. research group prof. Abbate R.

n. 400 Fillets FM

100% Fish meal

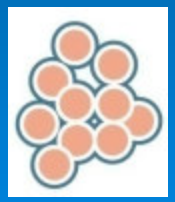


high omega-3

Man

50% Fish Meal
50% Plant mixture

n. 400 Fillets PP



Medium omega-3

Sofi et al., 2008

Intake trial FILLET COMPONENTS

%	FM	PP	Lev. Sig.
H_20	67.98	67,99	n.s.
Protein	18,27	18.51	n.s.
Lipid	12.00	11.85	n.s.
C18:1 n7,n9	21.62	22.77	**
C18:2 n6	11.26	20.16	**
C20:5 n3	8.62	6.25	**
C22:5 n3	3.63	2.92	**
C22:6 n3	14.18	12.60	**
SFA	23.40	25.80	**
MUFA	29.90	28.80	*
N-3 PUFA	29.5	25.1	**
N-6 PUFA	12.3	21.1	**

Sofi et al., 2008

Intake trial

INTERVENTION STUDY

20 healthy dyslipidemic subjects (12M; 8F)

[mean age: 48.6 ± 12.9 years (range: 23-67)]

Every subject was instructed to consume about 650 g/week of farmed sea bram (4 times per week), by maintaining a regular eating pattern

Dyslipidemia was defined as:

- Total cholesterol > 200 mg/dL
- LDL-cholesterol > 130 mg/dL
- Triglycerides > 150 mg/dL

Cooking guidelines:

Microwave or normal owen, with the addition of salt and olive oil

Sofi et al., 2008

Intake trial

Sea bream fillet components

mean intake in 10 WK trial (g) in dislipidemic subjects

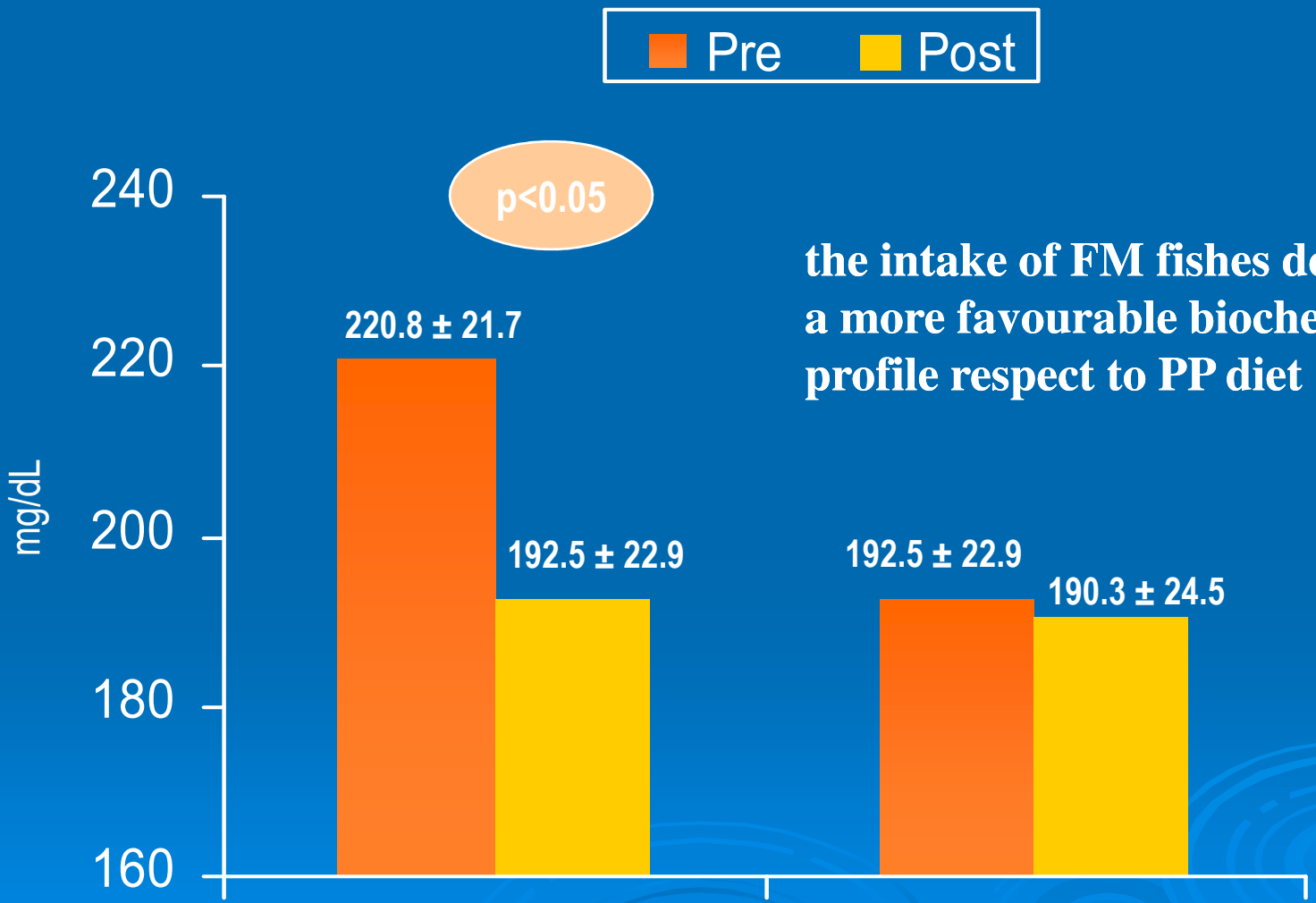
		FLESH	LIPIDS	C20:5	C22:6	SFA	MUFA	n-3 PUFA	n-6 PUFA
FM		5885	700.2	45.5	74.5	136.5	158.2	155.6	65.0
	± SD	265	22.1	1.5	32.	4.6	5.4	5.3	2.2
PP		6906	801	41.3	83.2	155.0	191.4	166.2	139.8
	± SD	463	52	1.8	3.7	6.8	8.4	7.3	6.2

Mean EPA + DHA = 12 g/wk

C18:2n-6 = FM 6 g/wk ; PP 13 g/wk;

Mean n-3/n-6 = FM 2.4 ; PP 1.2;

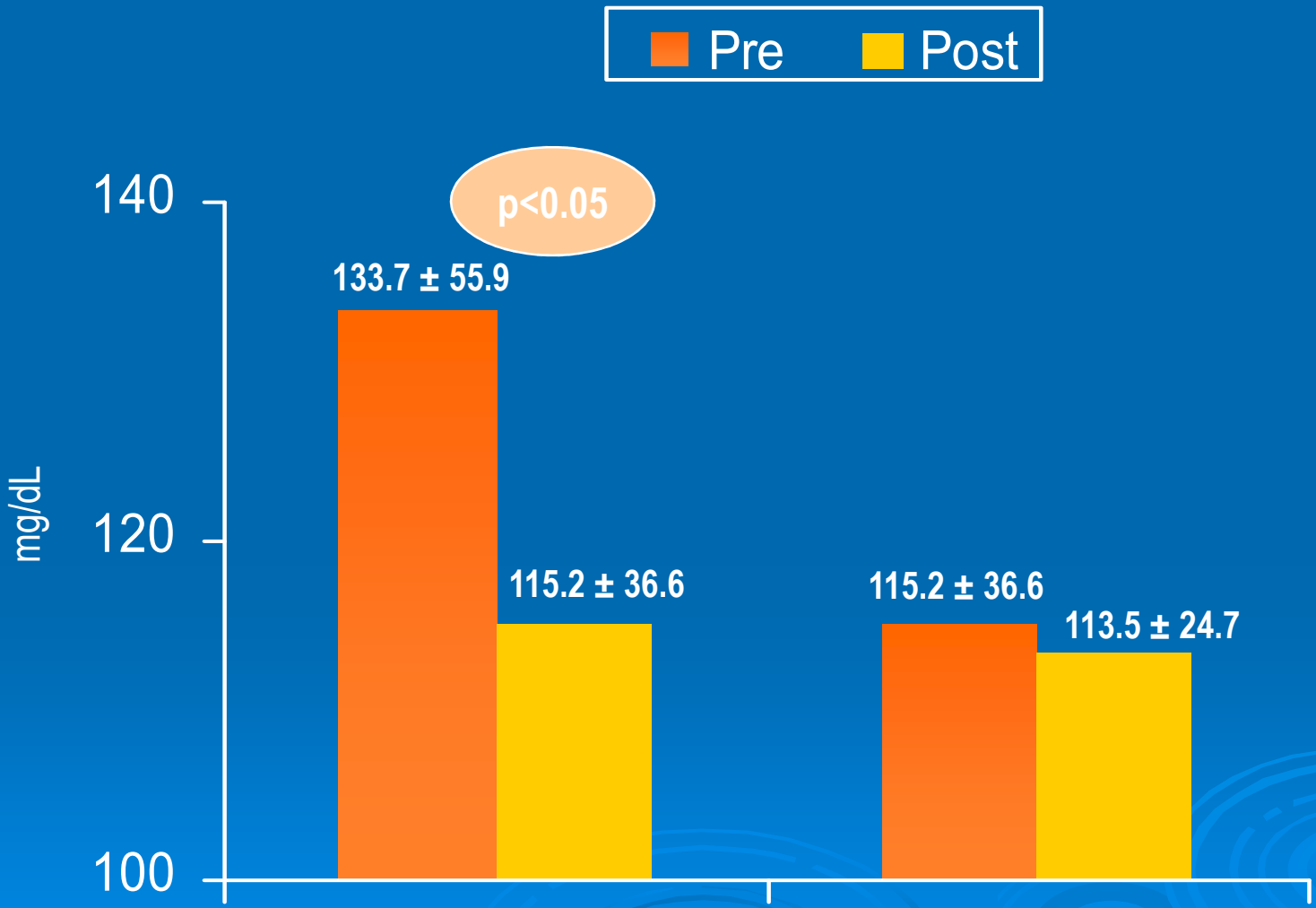
Results: Total cholesterol



the intake of FM fishes determined a more favourable biochemical profile respect to PP diet

General linear model for repeated measurements after adjustment for age, gender, and BMI change

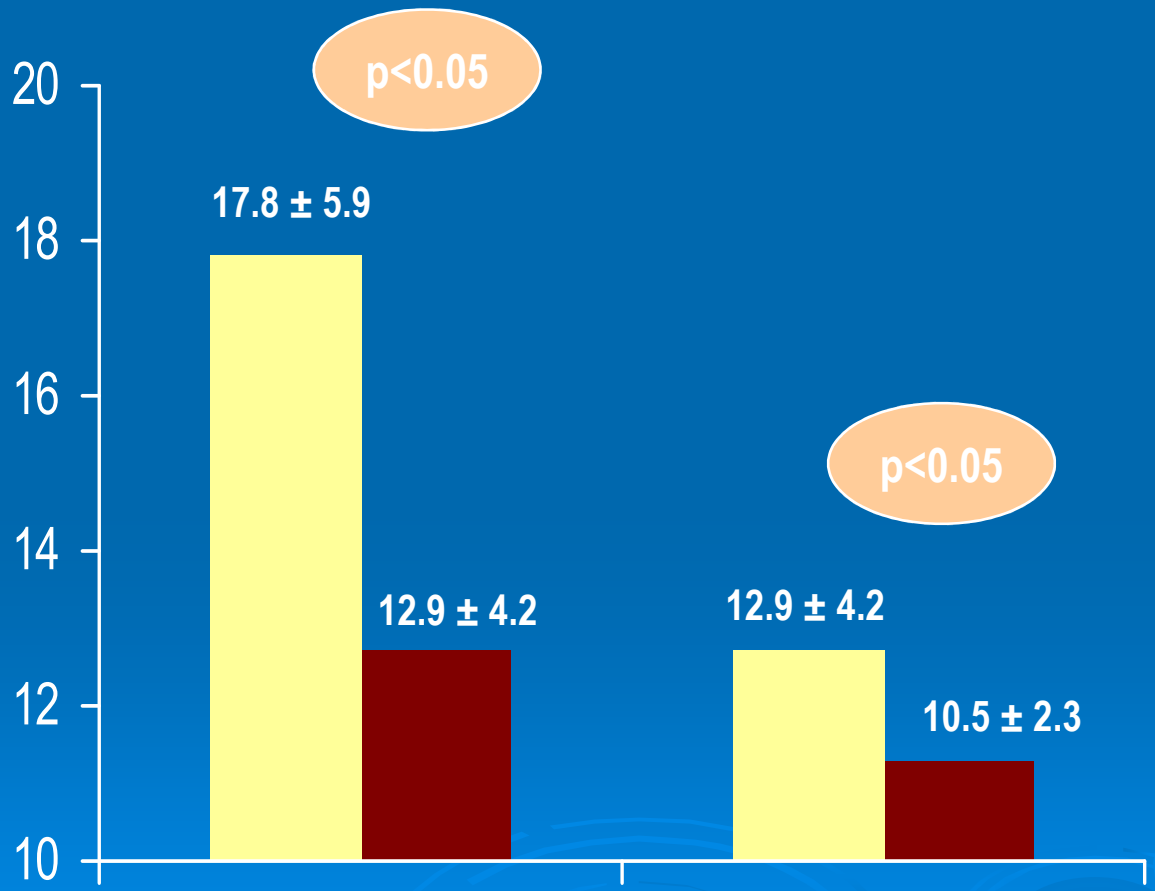
RESULTS: TRIGLYCERIDES



General linear model for repeated measurements after adjustment for age, gender, and BMI change

Sofi et al., 2008

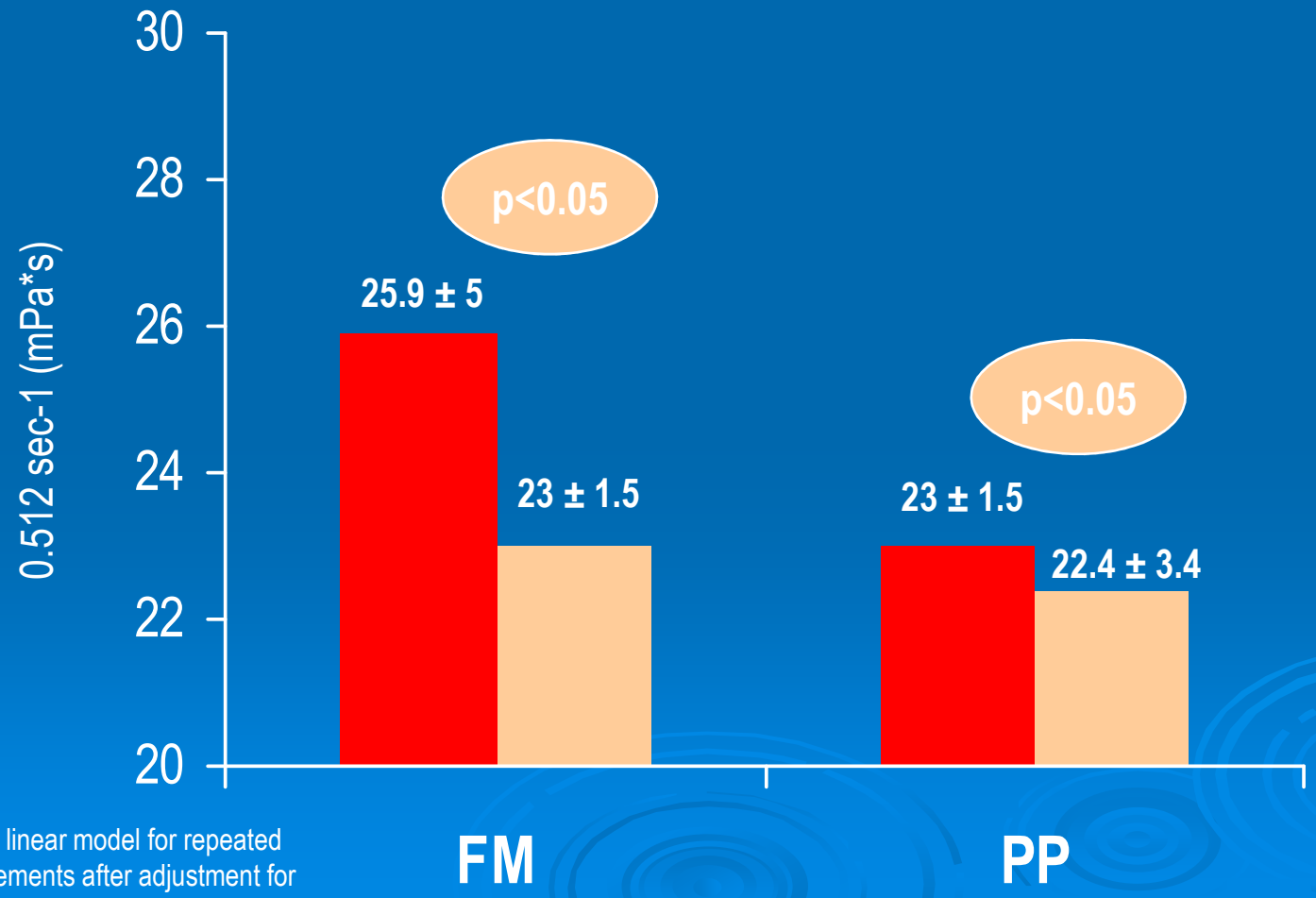
RESULTS: INTERLEUKIN-8



General linear model for repeated measurements after adjustment for age, gender, and BMI change

Sofi et al., 2008

Results: whole blood viscosity



General linear model for repeated measurements after adjustment for age, gender, and BMI change

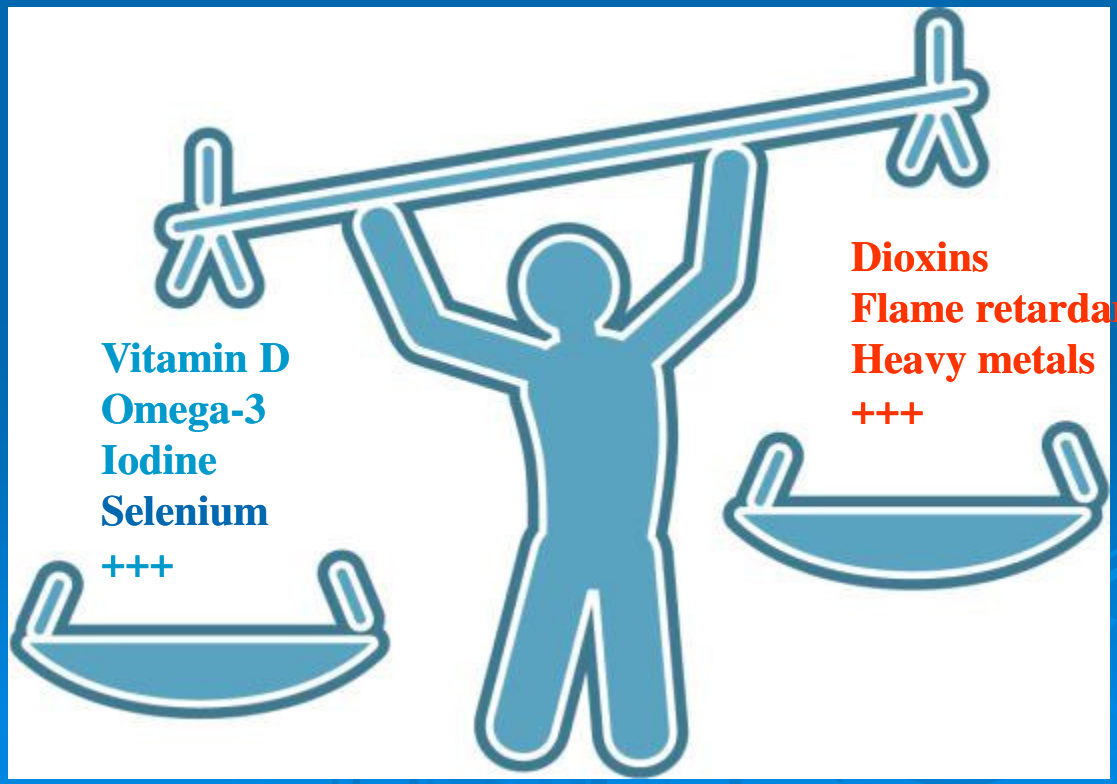
Researches on salmon, trout, seabass and seabream (RAFOA , Researching alternative to fish oil for aquaculture) indicated a good problem solution :

DHA and EPA levels were restored up to 90%
after feeding trout, seabass and seabream
by a finishing diet with fish oil for the last 14-24 weeks.

There is a "washing out" of fatty acid of vegetal oil
and a "washing in" of the fish oil fatty acids
restoring the most of the healthy
EPA + DHA content in fish muscle

However the ability to "wash-in" DHA & EPA was more easily achieved than "wash out" of 18:2n-6 and 18:3n-3!

It was aknowledged at European level that positive fish health effects generally outweights the potential negative effects (positive > negative) but the need to gradually change aquafeeds will also produce changes in fish healthy eating quality.
Monitoring these changes will become important to do the right choices for safe and healty seafood for consumer



Thank you for your attention!!