IS IT POSSIBLE TO INFLUENCE METABOLIC PROCESS OF EUROPEAN SEA BASS JUVENILES BY A NUTRITIONAL CONDITIONING DURING LARVAL STAGE ?

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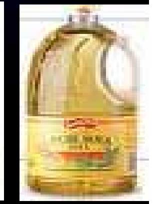
Natural fish stocks

Nowadays

Carnivorous marine fish are mainly fed with fish meals and oils

BUT fish aquaculture ↗ and natural fish stocks ↘

→ **Substitution of fish lipid sources with vegetable lipid sources**

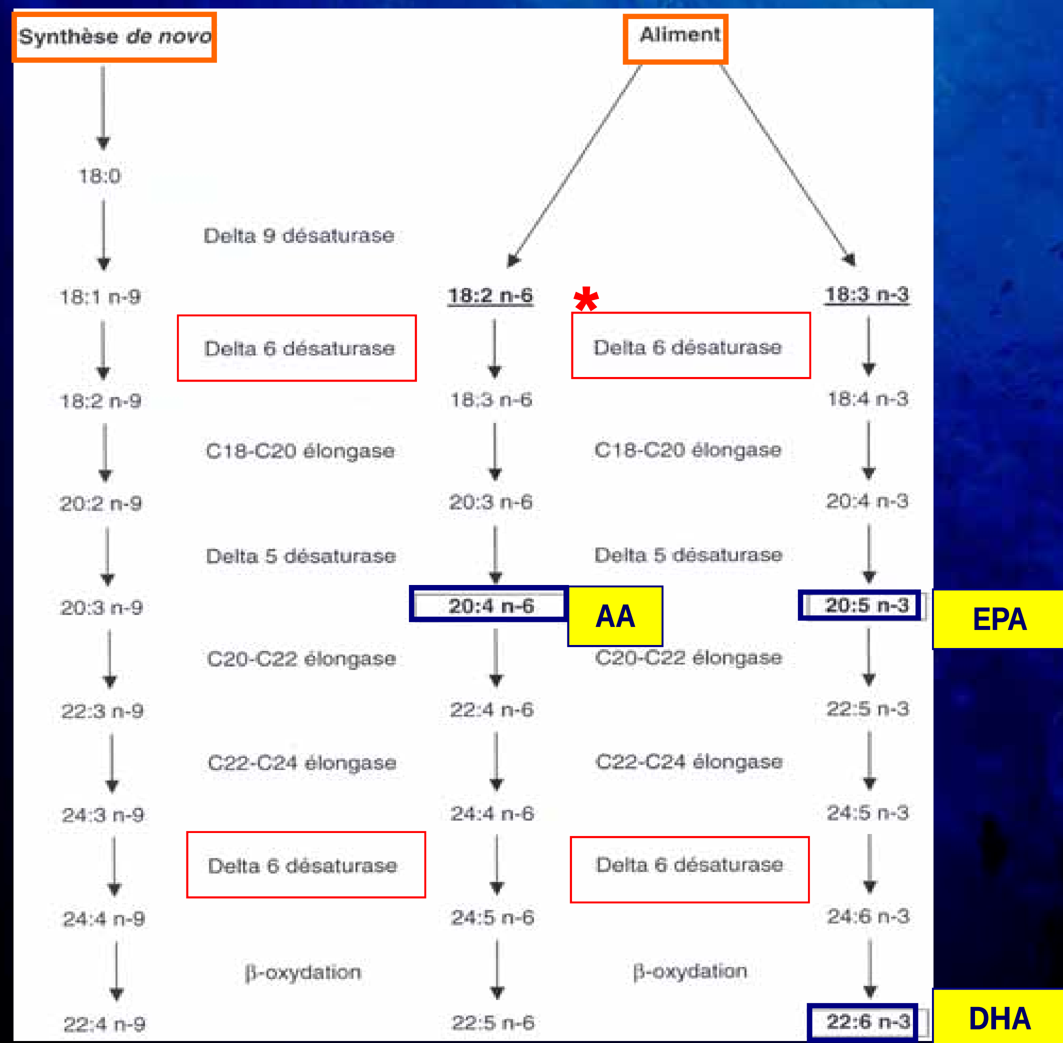


Disadvantages of vegetable products:

- **Not adapted to the requirement in essential fatty acids (HUFA):**

rich in C18 fatty acids (i.e. HUFA precursors) BUT poor in HUFA (EPA, DHA)

- **Low capacity for marine fish to convert C18 FA into HUFA, in particular because of the low expression of the Delta-6 desaturase (D6D)**



Synthèse *de novo*
Aliment
18:0
Delta 9 désaturase
18:1 n-9
18:2 n-6
*
18:3 n-3
Delta 6 désaturase
Delta 6 désaturase
18:2 n-9
18:3 n-6
18:4 n-3
C18-C20 élongase
C18-C20 élongase
20:2 n-9
20:3 n-6
20:4 n-3
Delta 5 désaturase
Delta 5 désaturase
20:3 n-9
20:4 n-6
AA
20:5 n-3
EPA
C20-C22 élongase
C20-C22 élongase
22:3 n-9
22:4 n-6
22:5 n-3
C22-C24 élongase
C22-C24 élongase
24:3 n-9
24:4 n-6
24:5 n-3
Delta 6 désaturase
Delta 6 désaturase
24:4 n-9
24:5 n-6
24:6 n-3
β-oxydation
β-oxydation
22:4 n-9
22:5 n-6
22:6 n-3
DHA

High interest in producing fish, which could better incorporate vegetable products.

BUT HOW ?

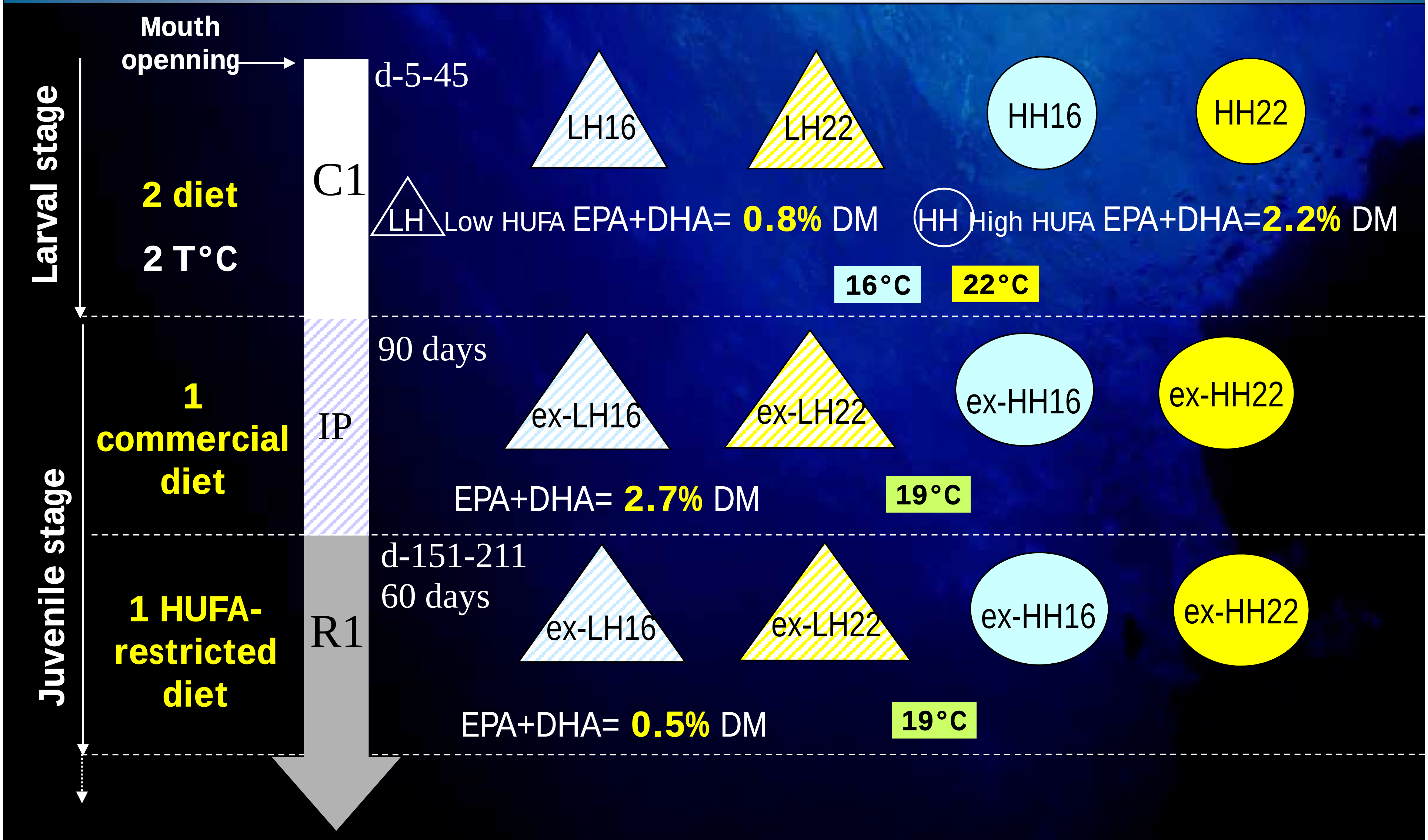
Could it be possible using the concept of metabolic programming ?

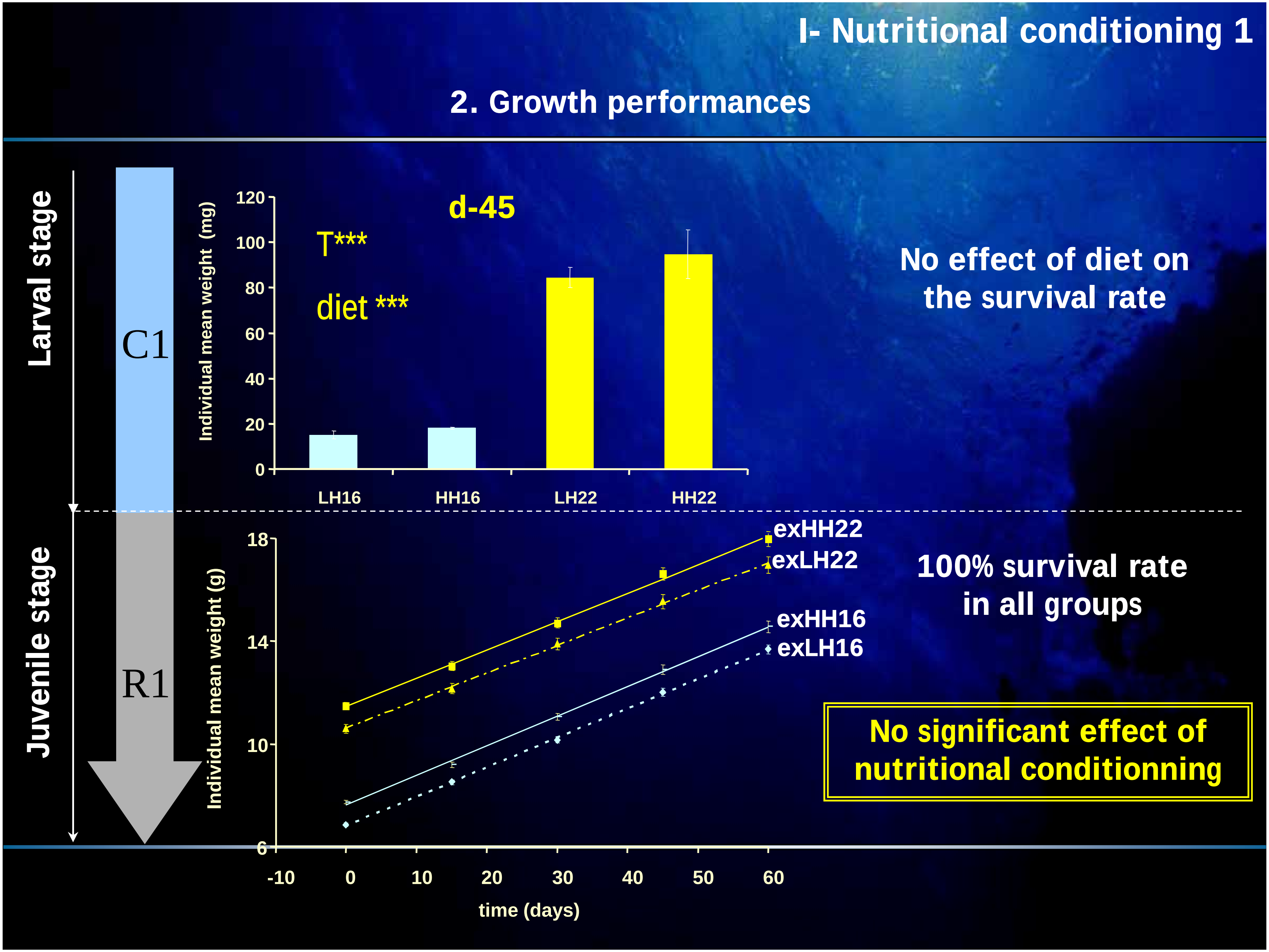
Adaptive process at a cellular, molecular and biochemical levels occuring during young stages, and then maintained in further developmental stages and potentially transmissible to the offspring (Lucas, 1998)

- **Is it possible in sea bass to orient physiological process of juveniles by a larval conditioning to dietary vegetable products, characterized by a low HUFA content?**

I- Nutritional conditioning 1

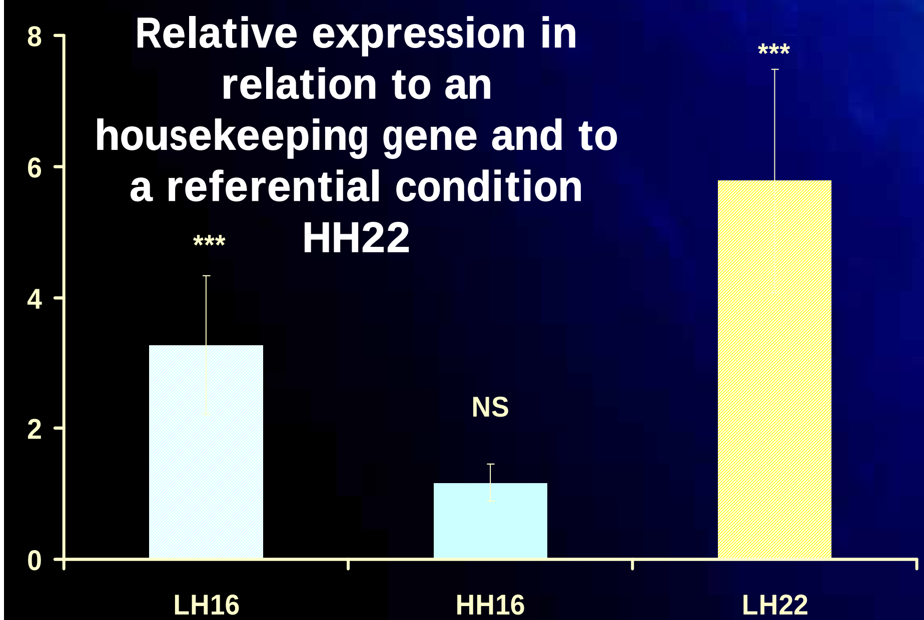
1. Experimental design



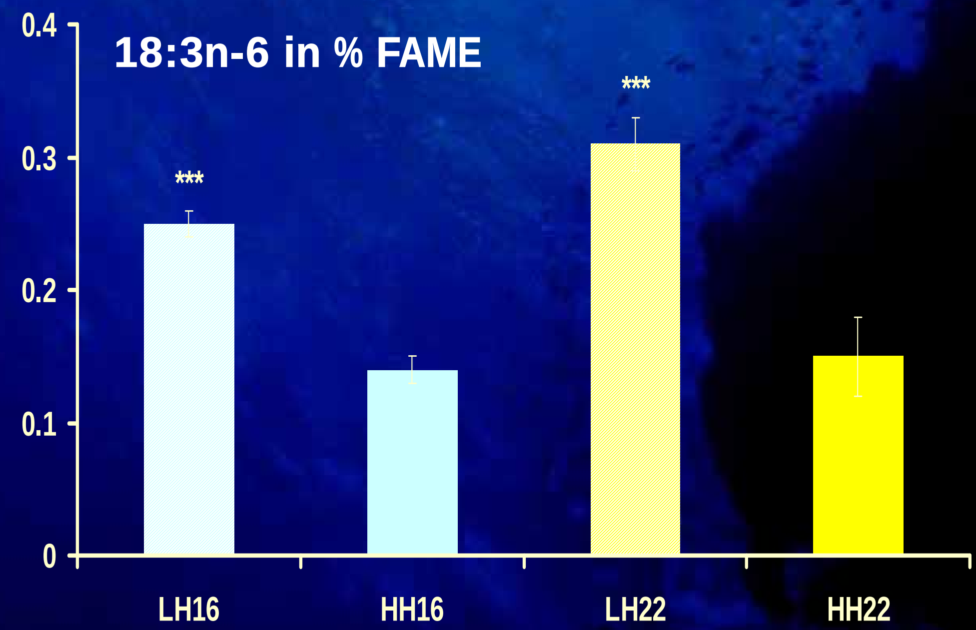

I- Nutritional conditioning 1
2. Growth performances
Larval stage
C1
Individual mean weight (mg)
d-45
T***
diet ***
0
20
40
60
80
100
120
LH16
HH16
LH22
HH22
No effect of diet on the survival rate
Juvenile stage
R1
Individual mean weight (g)
6
10
14
18
exHH22
exLH22
exHH16
exLH16
-10
0
10
20
30
40
50
60
time (days)
100% survival rate in all groups
No significant effect of nutritional conditionning

5. Lipid metabolism in larvae

D6D mRNA level at d-45

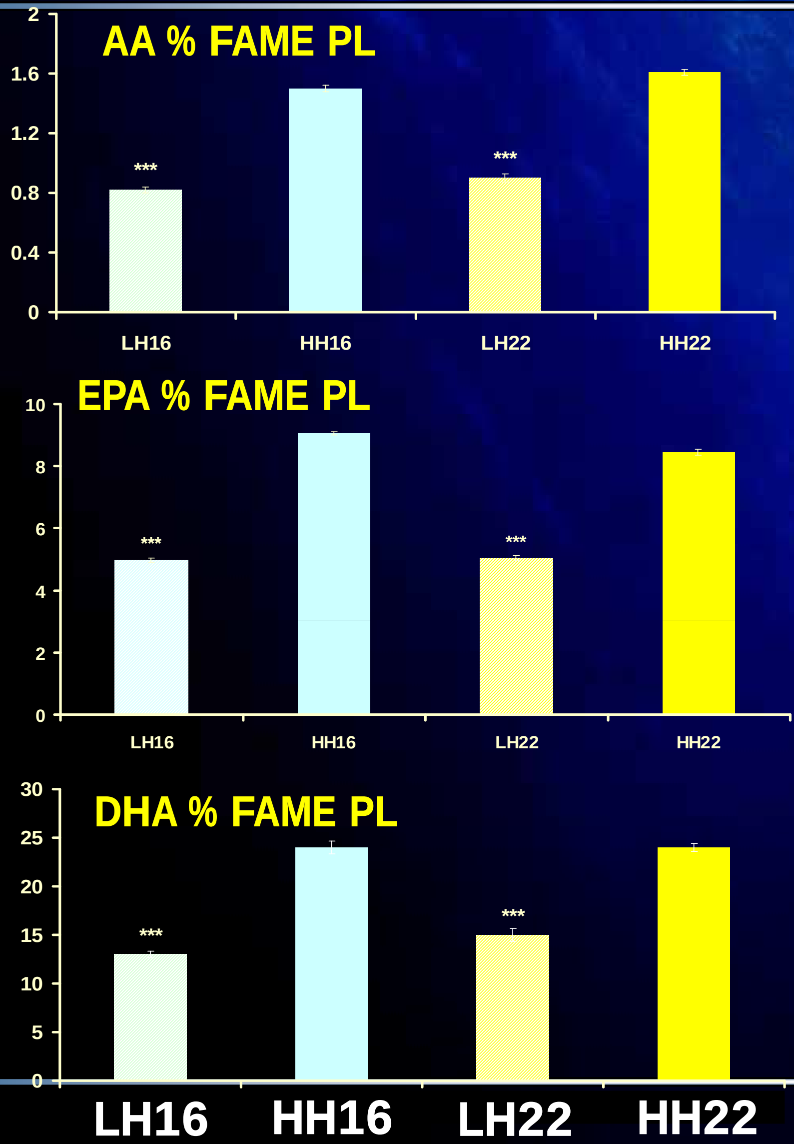


D6D Desaturation product at d-45 : 18:3n-6 in PL

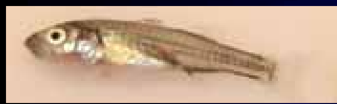


Stimulation of desaturation pathways for HUFA synthesis, without any effect of temperature

5. Lipid metabolism in larvae

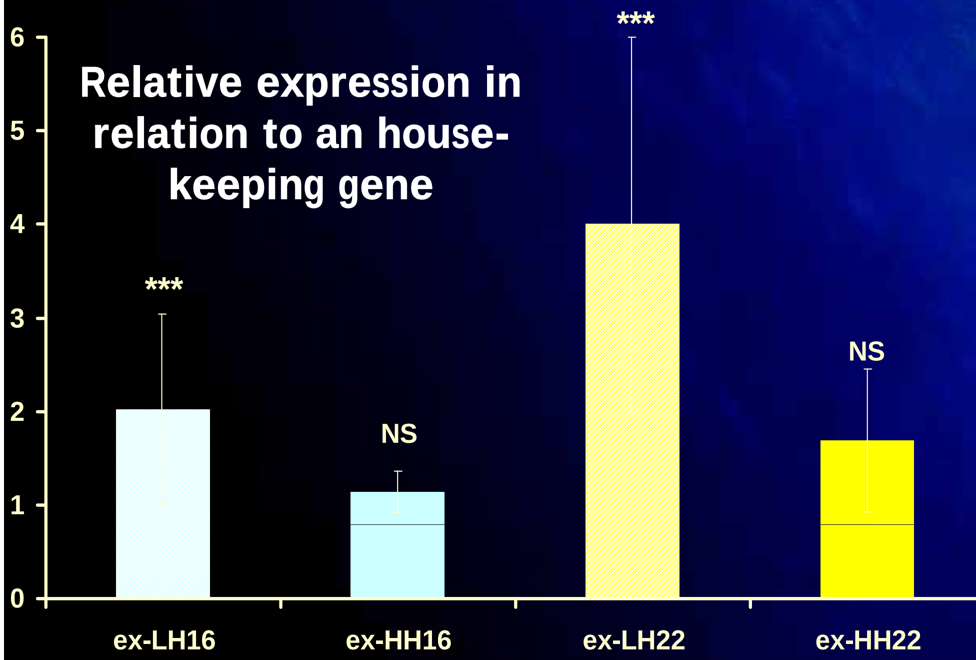


HUFA deficiency

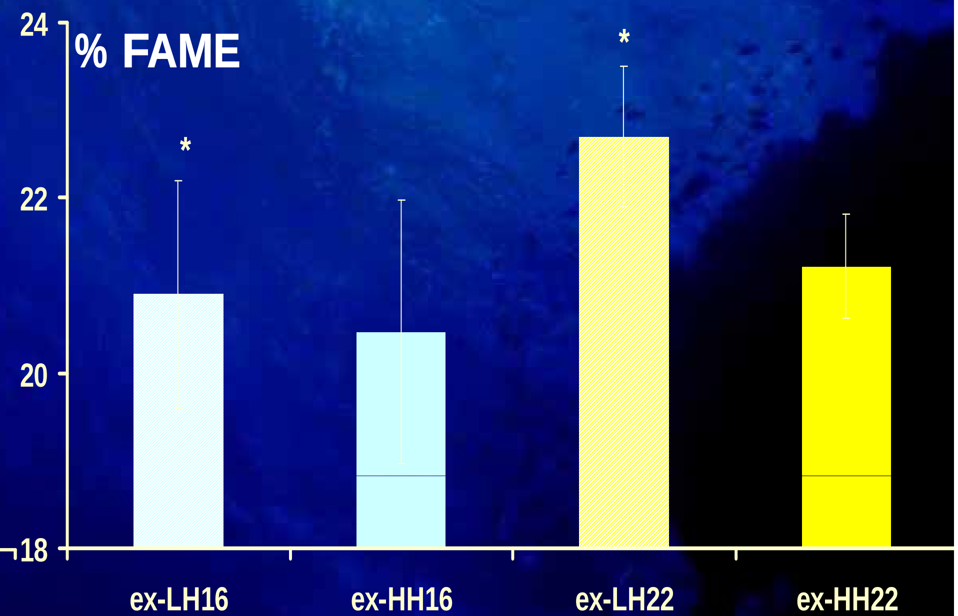


6. Lipid metabolism in juveniles

D6 mRNA level - 1st part of the challenge



PL final composition in DHA



- **Significant effect of larval conditionning**
- **BUT transient** (30 days)

- **Better capacity of ex-LH groups to adapt to a low-HUFA diet**
- **BUT with a low amplitude**
- **NS for EPA**

7. Conclusions

POSSIBLE to influence fatty acid desaturation pathways for HUFA synthesis in juveniles, using a nutritional conditioning during larval stage, without any effect of temperature on the capacity of FA desaturation **(- 30% of HUFA requirement)**

BUT this modulation seems to be limited

Is it possible to obtain a response with a larger magnitude?

II. Nutritional conditioning 2

2. Experimental design

Larval stage

Mouth opening →

4 diets

C2

d-5-45

= C1

XLH*1* LH*1* HH*1* XH*1*

XLH*1*= 0.5%

LH*1* = 0.7% HH*1* = 1.7%

XH*1* =3.7%

19°C

Juvenile stage

1 commercial diet

PI

30 days

≠ 90 (C1)

XLH*2* LH*2* HH*2* XH*2*

EPA+DHA= 2.7% DM

19°C

1 more HUFA restricted diet

R2

d-83- 118

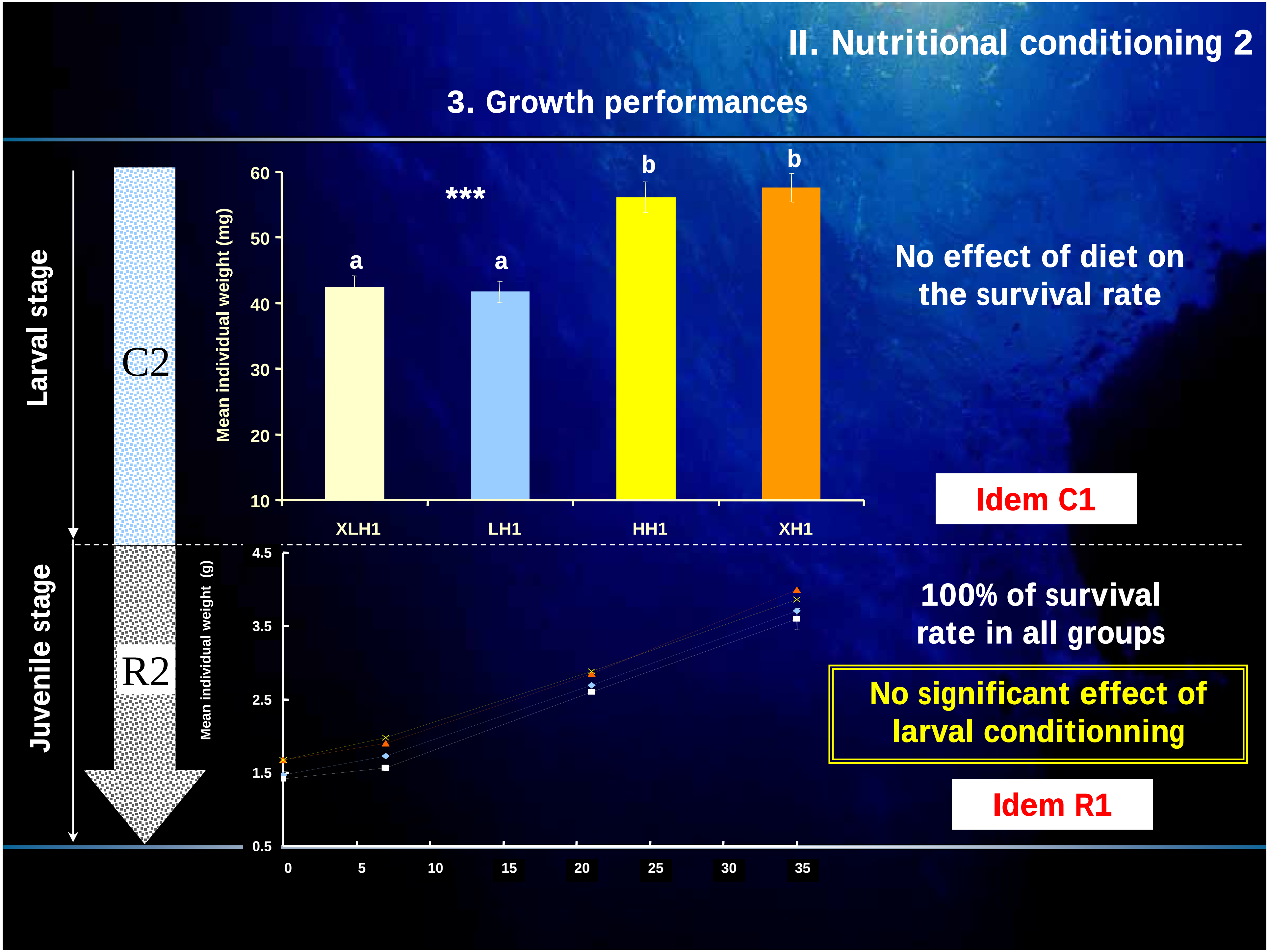
≠d151-211 (R1)

35 days

XLH*2* LH*2* HH*2* XH*2*

EPA+DHA= 0.3% DM

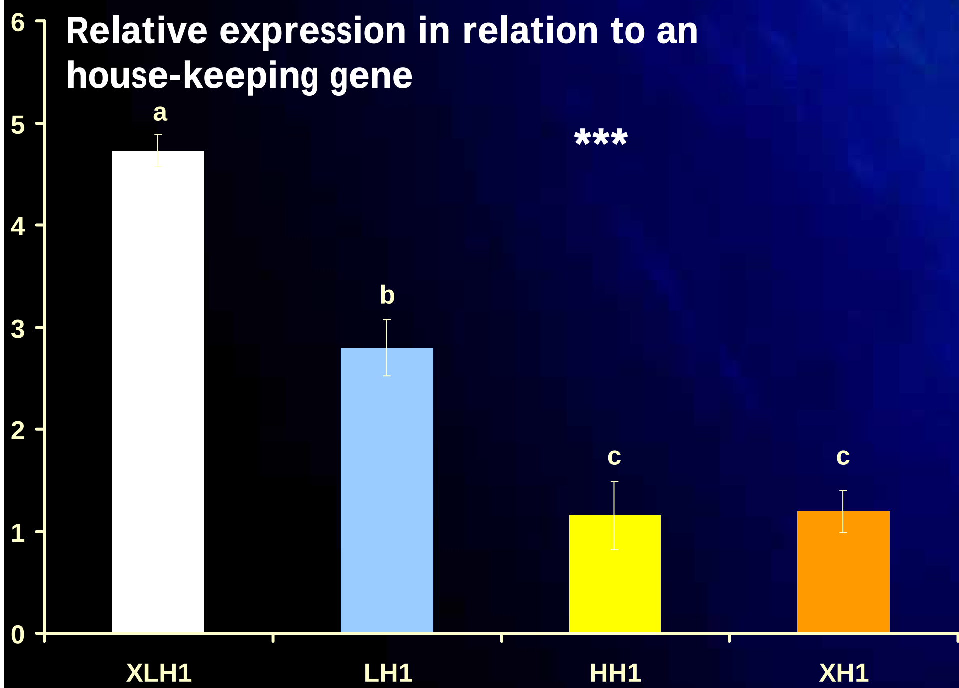
19°C


II. Nutritional conditioning 2
3. Growth performances
Larval stage
Juvenile stage
C2
R2
Mean individual weight (mg)
60
50
40
30
20
10
***
a
a
b
b
XLH1
LH1
HH1
XH1
Mean individual weight (g)
4.5
3.5
2.5
1.5
0.5
0
5
10
15
20
25
30
35
No effect of diet on the survival rate
Idem C1
100% of survival rate in all groups
No significant effect of larval conditionning
Idem R1

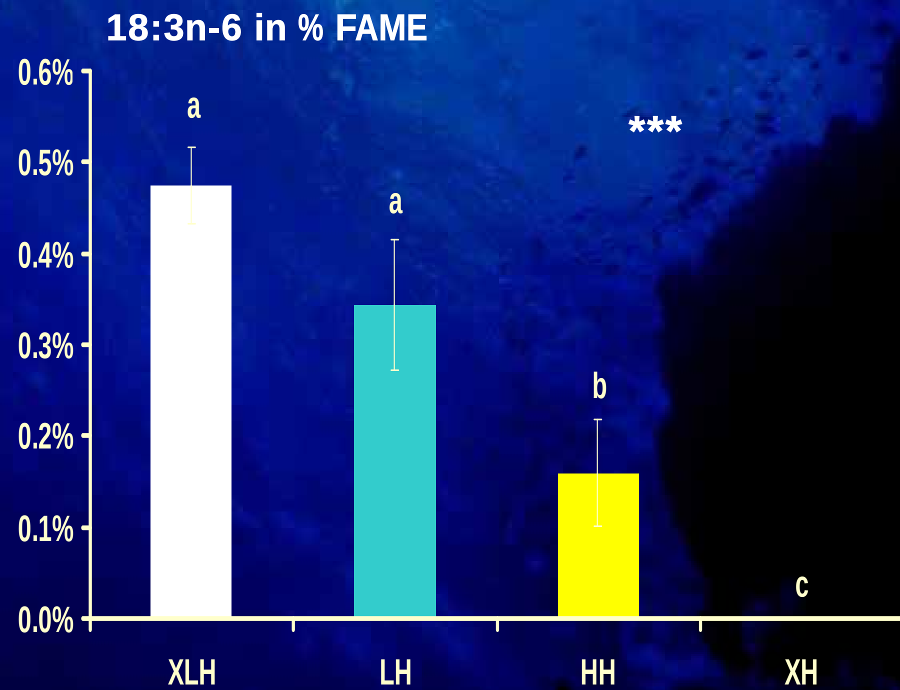
II. Nutritional conditioning 2

4. Lipid metabolism in larvae

D6D mRNA level at d-45



D6D desaturation product at d-45 in PL



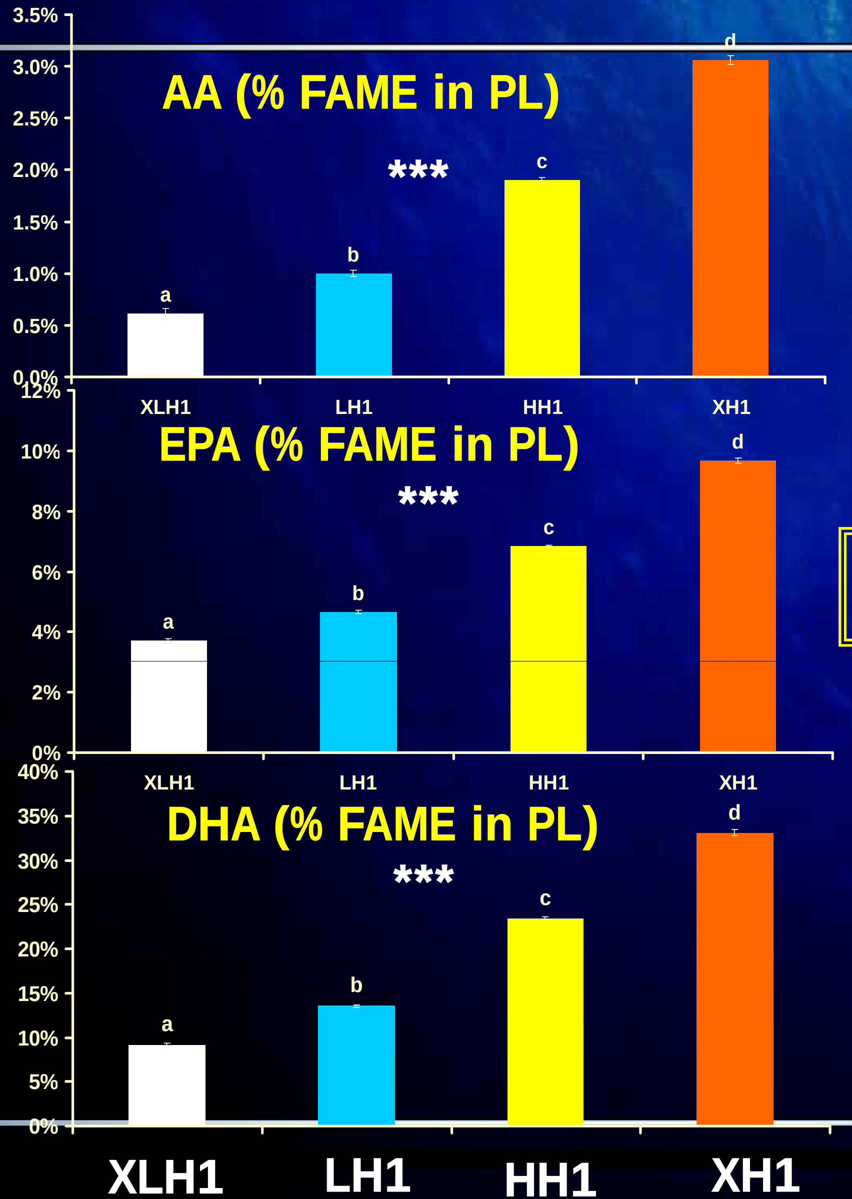
Stimulation of desaturation pathways for HUFA synthesis

Idem C1

II. Nutritional conditioning 2

4. Lipid metabolism in larvae

Idem C1

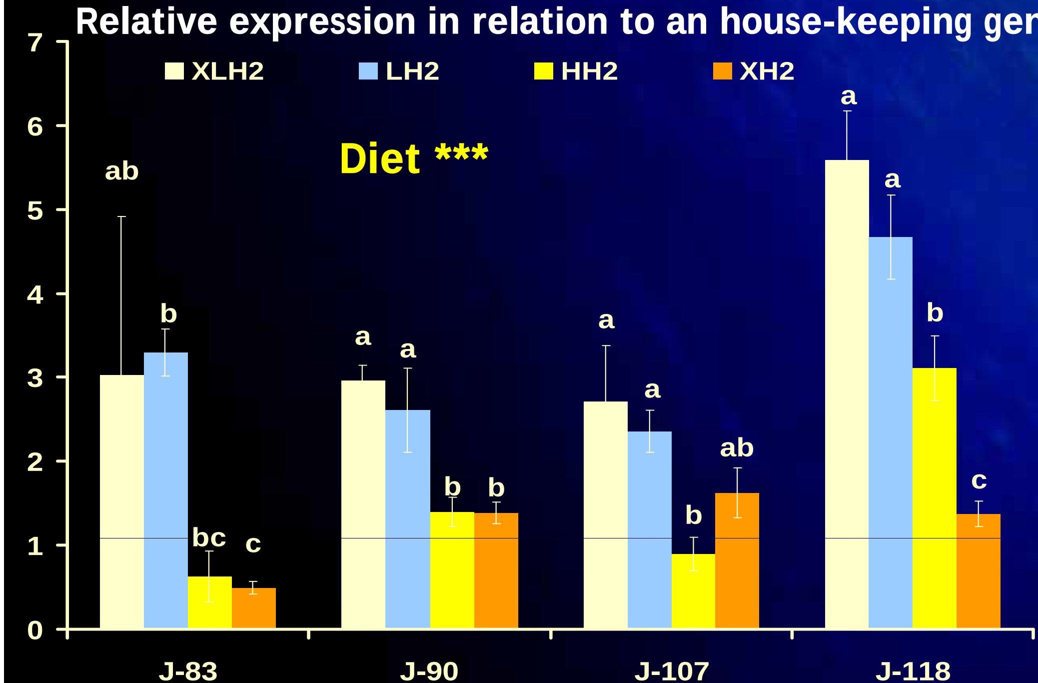


HUFA deficiency

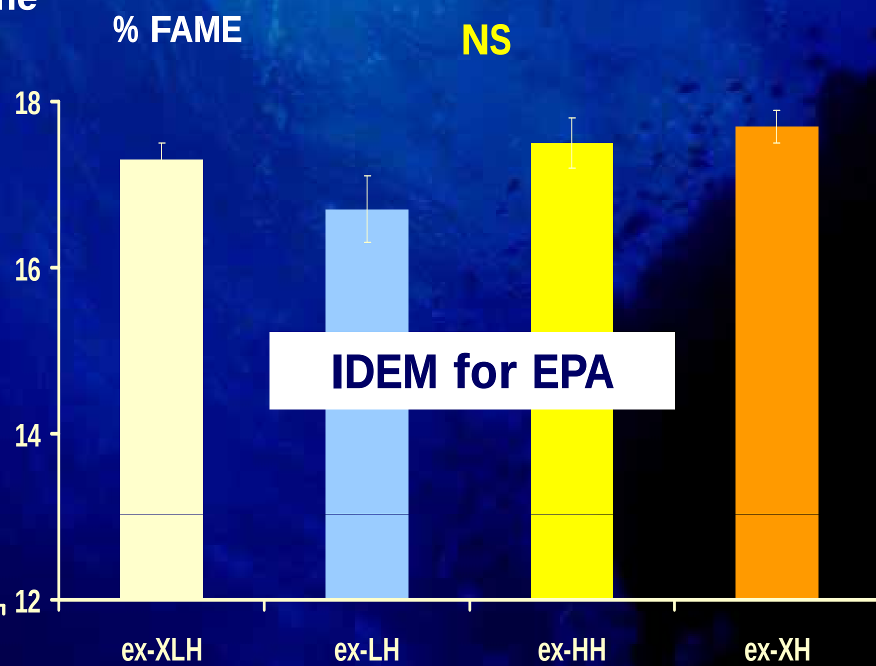
II. Nutritional conditioning 2

5. Lipid metabolism in juveniles

D6D mRNA level



PL final composition in DHA



- Significant effect of larval conditionning
- D6D stimulation NON transient

≠ R1

- Non significant effect of larval conditioning

≠ R1

Summary

Although no beneficial effect on growth performances and no clear higher HUFA content in PL, POSSIBLE to influence FA desaturation pathways for HUFA synthesis for a better incorporation of vegetable products, using a nutritional conditioning during larval stage (-60% of the HUFA requirement)

Possible to use the concept of metabolic programming in sea bass

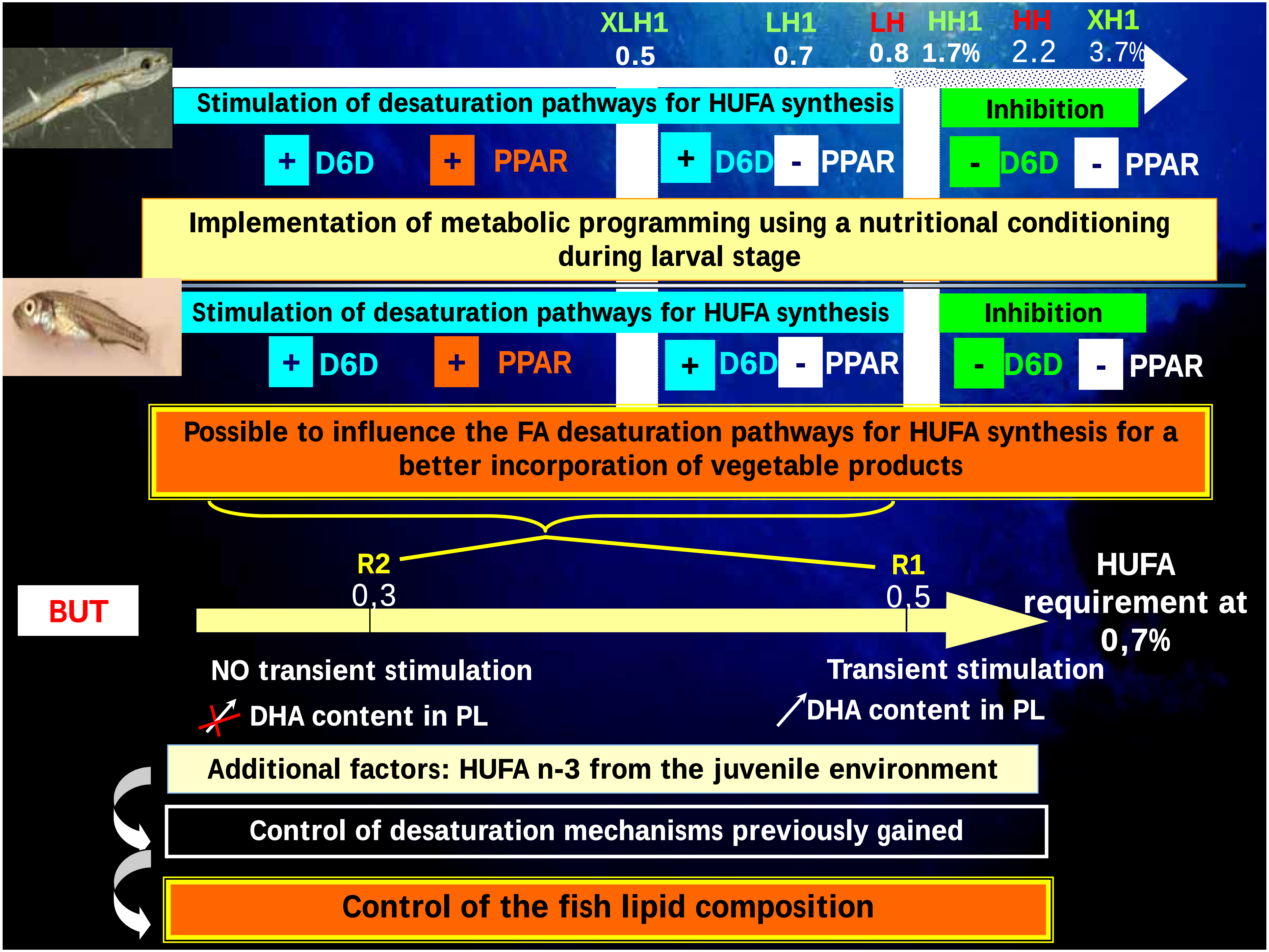
BUT is it a:

Long-lasting adaptation?

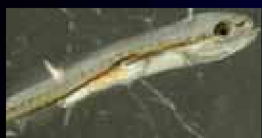
Transient acclimatation of juveniles to their nutritional environment ?

Need to better investigate mechanisms involved in desaturation pathways in response to a low HUFA dietary content in larvae and in juveniles

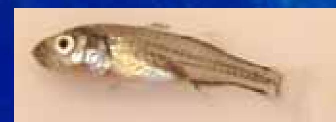
PPAR


XLH1 0.5
LH1 0.7
LH 0.8
HH1 1.7%
HH 2.2
XH1 3.7%
Stimulation of desaturation pathways for HUFA synthesis
Inhibition
+ D6D
+ PPAR
+ D6D
- PPAR
- D6D
- PPAR
Implementation of metabolic programming using a nutritional conditioning during larval stage
Stimulation of desaturation pathways for HUFA synthesis
Inhibition
+ D6D
+ PPAR
+ D6D
- PPAR
- D6D
- PPAR
Possible to influence the FA desaturation pathways for HUFA synthesis for a better incorporation of vegetable products
BUT
R2
0,3
R1
0,5
HUFA requirement at 0,7%
NO transient stimulation
DHA content in PL
Transient stimulation
DHA content in PL
Additional factors: HUFA n-3 from the juvenile environment
Control of desaturation mechanisms previously gained
Control of the fish lipid composition

Was the metabolic programming really present ?



Intermediate period: Stop or Non stop ?



Challenge extension: what will happen during adulthood ?

Transmission to the offspring ?

IF YES

Application to other carnivorous species with high commercial value in aquaculture

Thanks to the financially support: Ifremer, INRA, RAQ, Larvi.

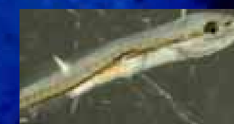
Thanks to the technical support: M.M Le Gall, A. Sévère, H. Le Delliou, P. Quazuguel, N. Le Bayon, J. Moriceau, C. Huelvan & E. Desbruyères.

Thanks for your attention

Récapitulatif

XLH1 0,5% — LH1 0,7% — LH 0,8% — HH 1,7% — HH1 2,2% — XH1 3,7%

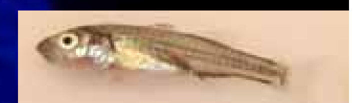
LARVES



Stimulation des mécanismes de désaturation

Adaptation à un régime carencé en HUFA n-3

JUVENILES



R2 0,3 — R1 0,5

↗ **Niveau ARNm D6 continue**

D6 fonctionnelle ? **D6 non fonctionnelle ?**

Synthèse d'HUFA

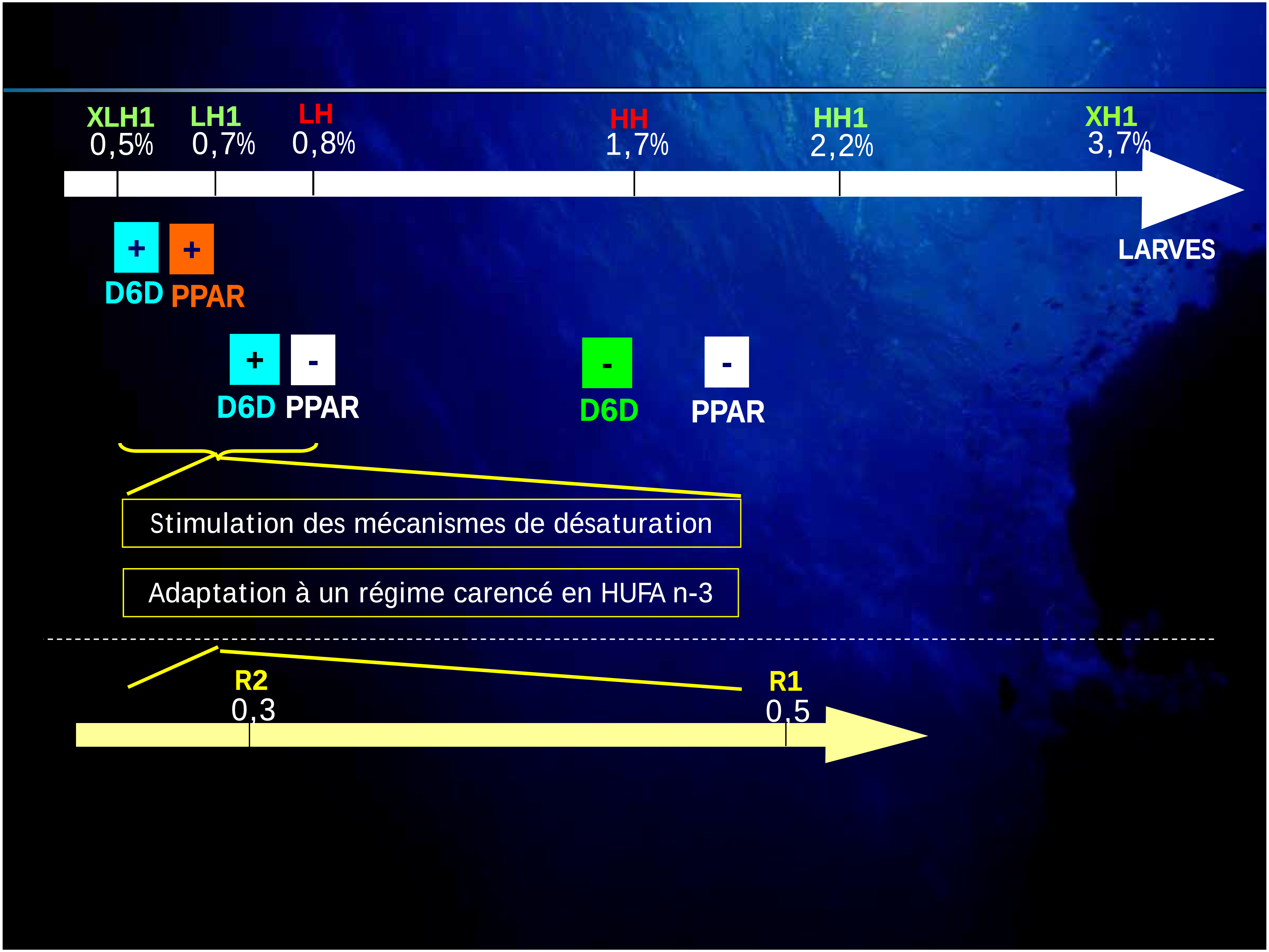
dérivées

teneur en DHA

↗ **Niveau ARNm D6 transitoire**

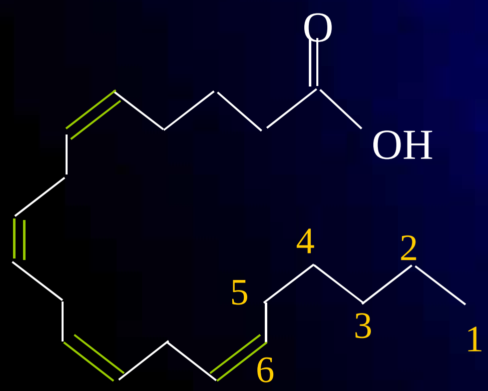
R1 trop proche du besoin (0,7% EPA+DHA)

↗ **Faible en DHA**


XLH1
0,5%
LH1
0,7%
LH
0,8%
HH
1,7%
HH1
2,2%
XH1
3,7%
LARVES
+
+
D6D
PPAR
+
-
D6D
PPAR
-
-
D6D
PPAR
Stimulation des mécanismes de désaturation
Adaptation à un régime carencé en HUFA n-3
R2
0,3
R1
0,5

HUFA = AGLPI (n-9, n-6 et n-3) = Acides gras insaturés à 20-22 atomes de carbone et au moins 4 doubles liaisons = Acides gras essentiels

C:X n-Y



Acide arachidonique 20:4n-6 (AA)

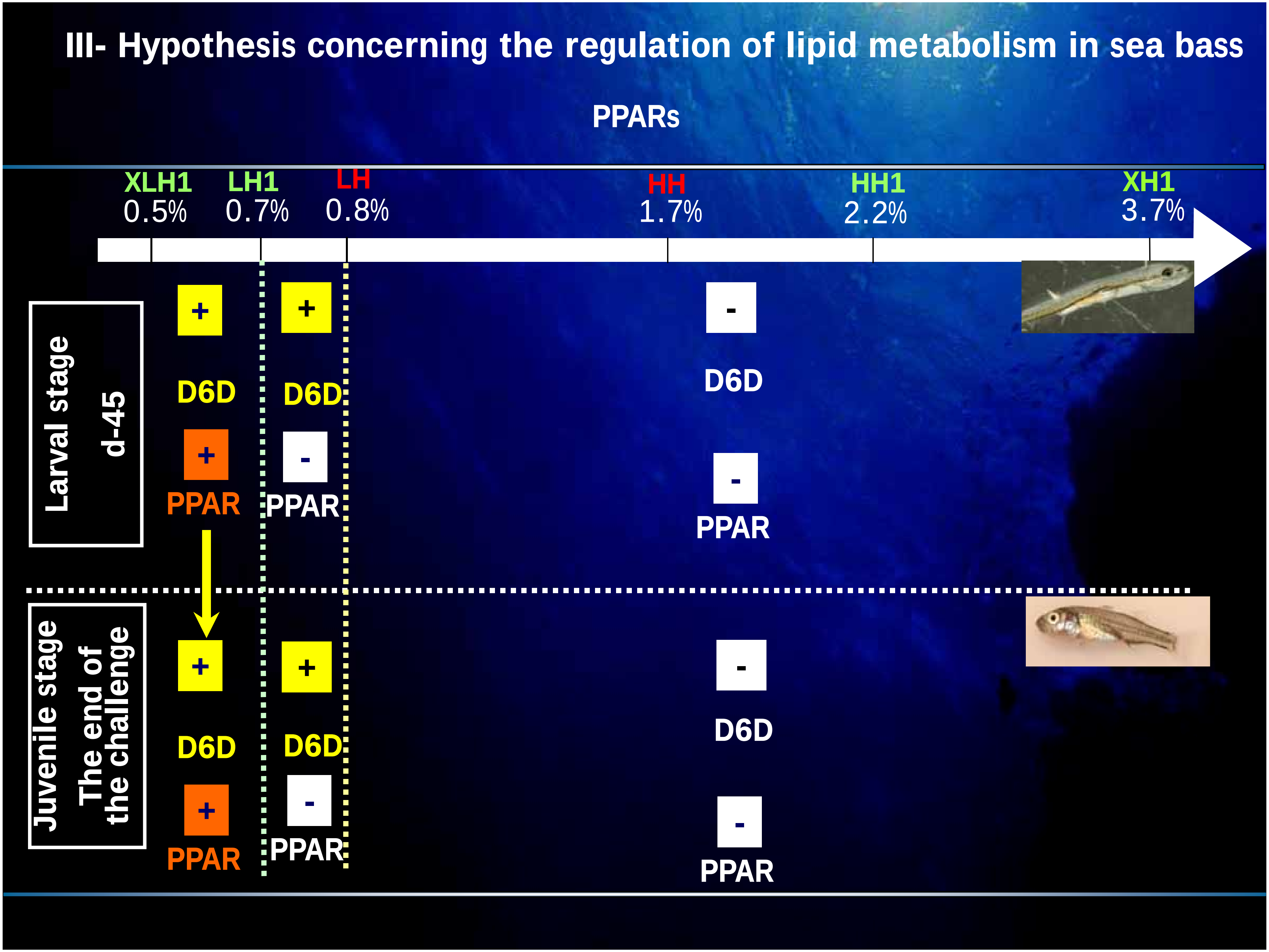
Constituants principaux des PL de la bi-couche membranaire

Chez les poissons, majoritairement les HUFA n-3:

-Acide docosahéxaénoique 22:6n-3 (DHA)

-Acide écosapentaénoique 20:5n-3 (EPA)

- **Croissance et survie larvaire**
- **Développement de la vision et du cerveau**
- **Résistance au stress et aux maladies**
- **Qualité de la chair, ...**


III- Hypothesis concerning the regulation of lipid metabolism in sea bass
PPARs
XLH1 0.5%
LH1 0.7%
LH 0.8%
HH 1.7%
HH1 2.2%
XH1 3.7%
Larval stage
d-45
+
D6D
+
PPAR
+
D6D
-
PPAR
-
D6D
-
PPAR
Juvenile stage
The end of the challenge
+
D6D
+
PPAR
+
D6D
-
PPAR
-
D6D
-
PPAR

III- Hypothèses de régulation du métabolisme lipidique du bar

Étude des PPAR

Peroxisome Proliferator-Activated Receptor, récepteurs nucléaires

Trois isoformes: α, β et γ

Décrits chez les mammifères et le bar

Facteur de transcription de la delta 6 chez les mammifères

Dans le contexte de programmation métabolique, les PPAR sont-ils impliqués dans la stimulation de la transcription du gène de la D6D chez le bar?

III- Hypothèses de régulation du métabolisme lipidique du bar

+ PPAR

D6D

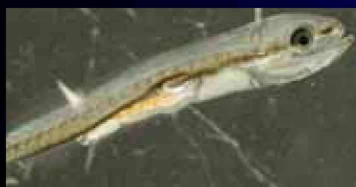
+ PPAR

D6D

Mise en place d'une mémorisation au niveau moléculaire des mécanismes de désaturation des AG

1. General conclusion

1



Nutritional environment

2

Nutritional conditionning during larval stage

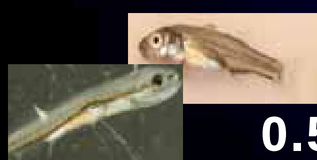
POSSIBLE to influence the fatty acid desaturation pathways for HUFA synthesis for a better incorporation of vegetable meals

Previously gained mechanisms

↓

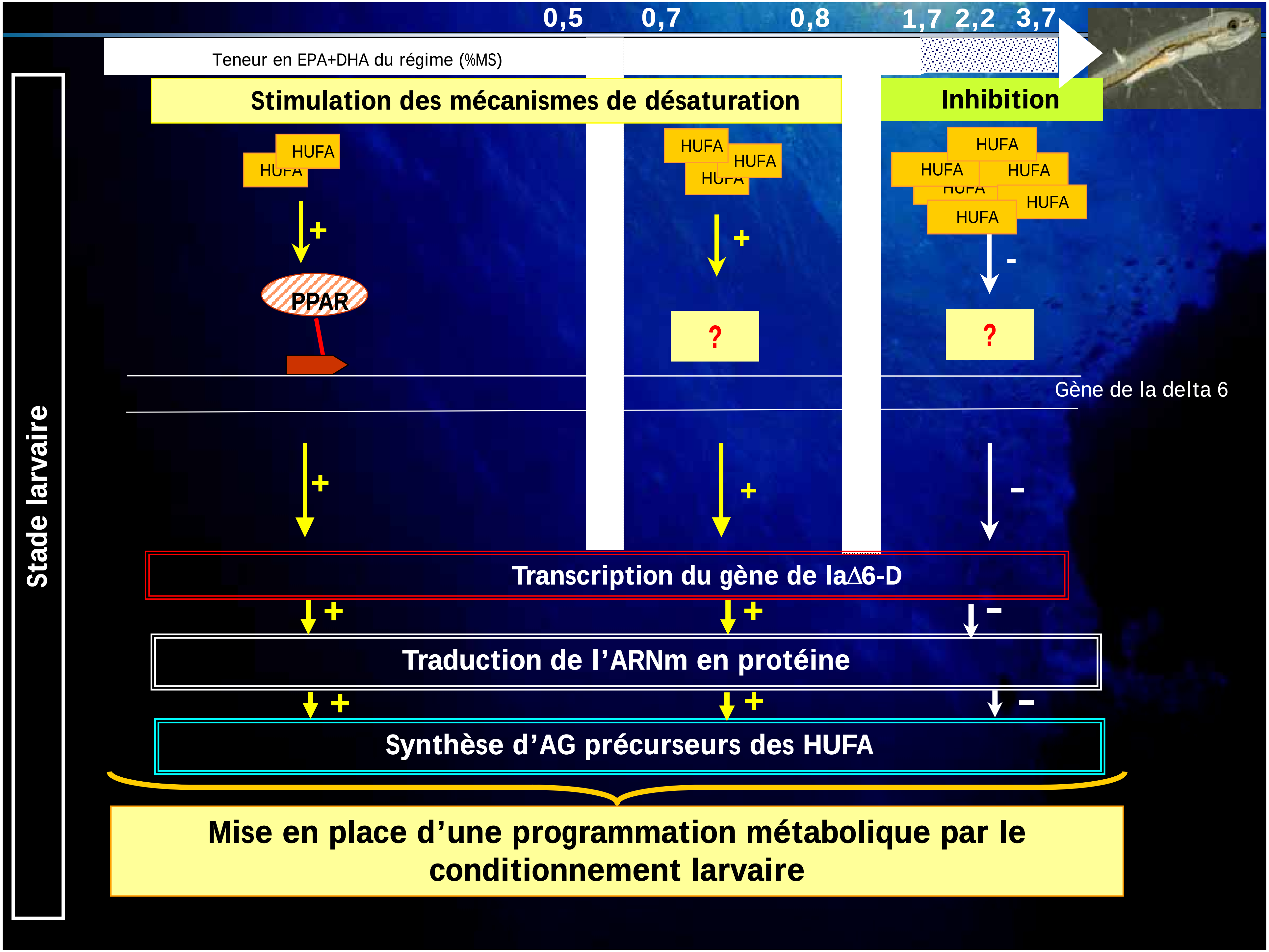
Fish lipid composition

3

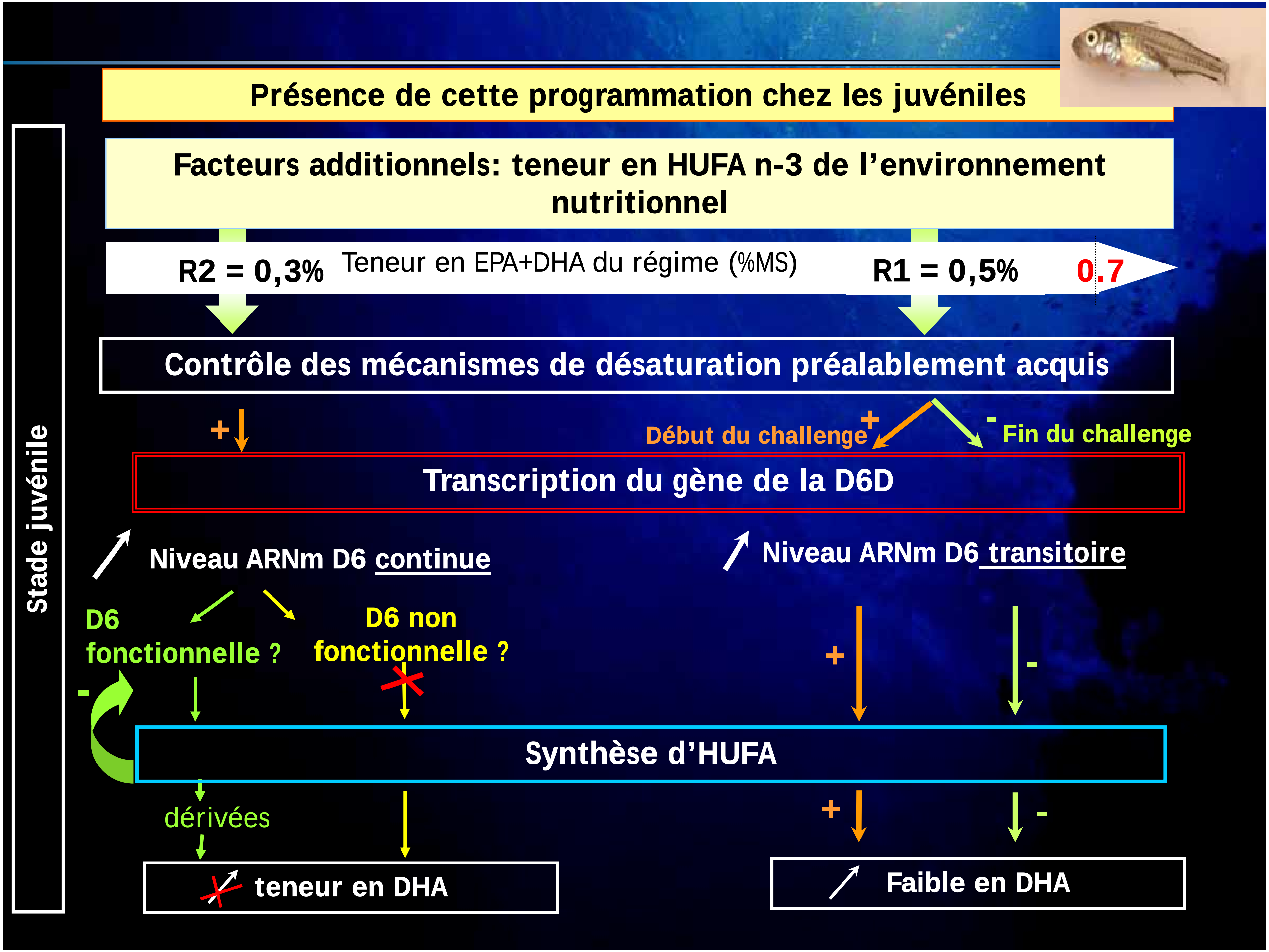


0.5 < HUFA dietary content < 0.7

PPAR —(+)→ **D6D transcription**



0,5
0,7
0,8
1,7
2,2
3,7
Teneur en EPA+DHA du régime (%MS)
Stimulation des mécanismes de désaturation
Inhibition
HUFA
HUFA
HUFA
HUFA
HUFA
HUFA
HUFA
HUFA
HUFA
HUFA
HUFA
+
+
-
PPAR
?
?
Gène de la delta 6
+
+
-
Transcription du gène de la∆6-D
+
+
-
Traduction de l'ARNm en protéine
+
+
-
Synthèse d'AG précurseurs des HUFA
Mise en place d'une programmation métabolique par le conditionnement larvaire
Stade larvaire


Présence de cette programmation chez les juvéniles
Facteurs additionnels: teneur en HUFA n-3 de l'environnement nutritionnel
R2 = 0,3%
Teneur en EPA+DHA du régime (%MS)
R1 = 0,5%
0.7
Contrôle des mécanismes de désaturation préalablement acquis
+
Début du challenge
+
-
Fin du challenge
Transcription du gène de la D6D
Niveau ARNm D6 continue
Niveau ARNm D6 transitoire
D6 fonctionnelle ?
D6 non fonctionnelle ?
-
+
-
Synthèse d'HUFA
dérivées
+
-
teneur en DHA
Faible en DHA
Stade juvénile