Workshop on recent progress in pikeperch culture

Faculty of Sciences and Technologies

Nancy, France 27. June

# Studies on nutritional requirements and feed optimization for pikeperch larvae


Ivar Lund, DTU Aqua

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DTU Aqua
National Institute of Aquatic Resources

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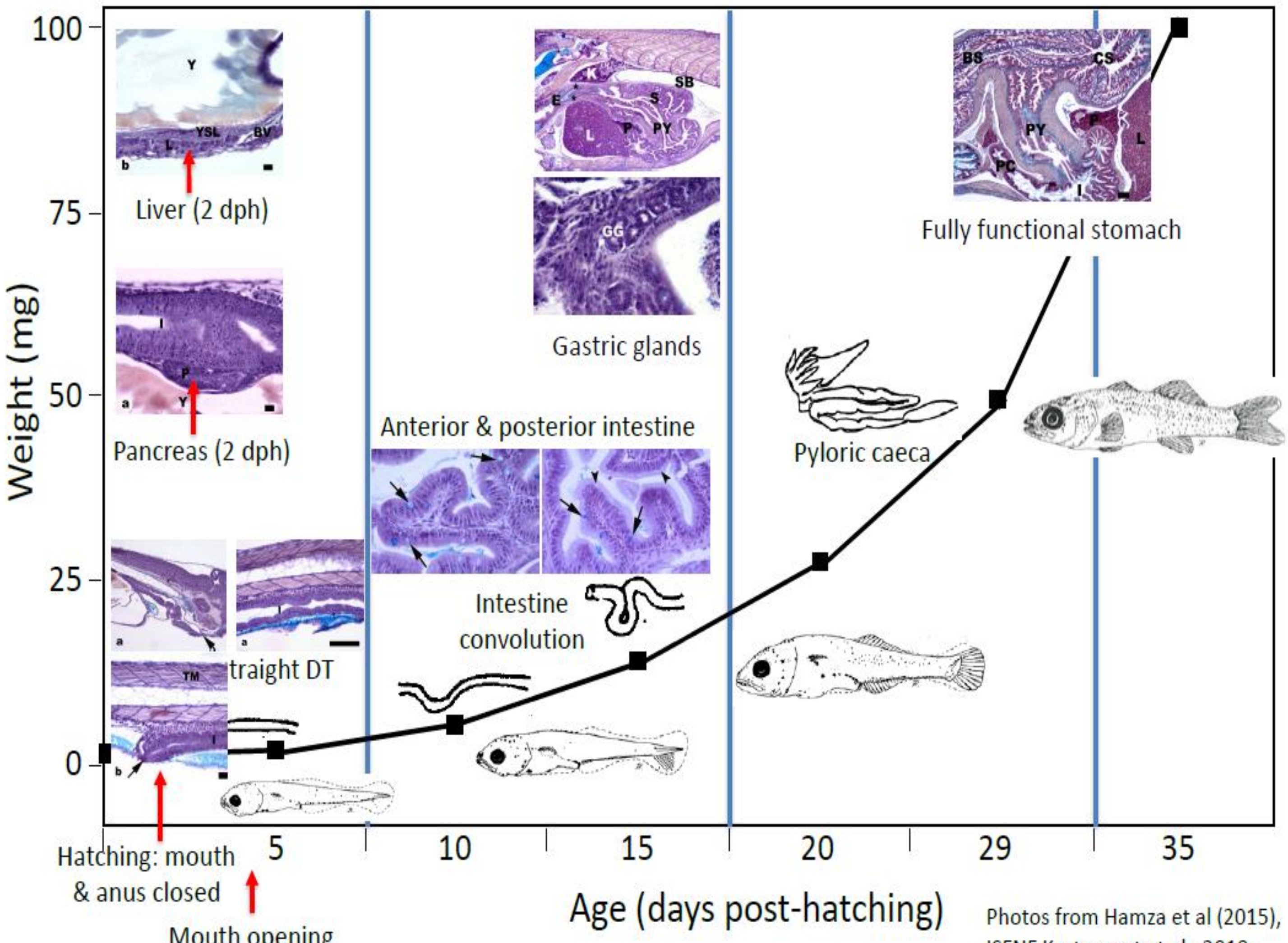
# Pike perch larval development




Photos from Hamza et al (2015), ISFNF Kestemont et al., 2018

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# Nutritional requirements of pike perch larvae, What do we know ?

- ✓ Nutritional deficiencies of artificial diets may hinder digestion and absorption
- ✓ Formulated feeds used from 15-20 dph
- ✓ Formulated feeds should have a relative high protein content of 530-580 g /kg and medium lipid content 170 g/kg
- ✓ Vitamin and mineral requirements may differ from marine fishes
- ✓ High requirement of phospholipids,- vegetable / marine origin ?
- ✓ Fatty acid requirements: Pike perch larvae are stress sensitive to lack of HUFAs causing neural deficiencies

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# What did we test ?

1. Influence of levels of phospholipids and EFA (essential fatty acids) in formulated diets on growth performances; stress sensitivity. gut maturation; liver function

2. Importance and the interaction of dietary levels of EFA, -vitamins (A,C, D, E) - and minerals (CA, P)

3. Influence of salinity on larval ability to utilize and metabolize EFA and physiological effects

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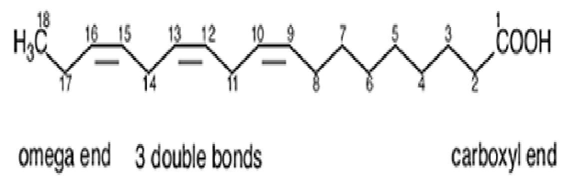
# What are oils, lipids and FA

The nutritionally important lipids are fats (solid) and oils (liquids) that consist of fatty acids with 12- 20 carbons. Most of the lipid found in food is in the form of triglycerides

Two major lipid groups:
Triacylglycerols (TAG, storage fat) & phospholipids (PL, membrane fat)

Figure 1b. Structures of Fatty Acids




The chemical structure of α-linolenic acid (ALA), 18:3n-3. ALA has 18 carbon atoms (C) and 3 double bonds, the first of which is located 3 carbon atoms from the terminal methyl group (omega [ω] end).

$CH_2$-OH + $R_1$
|
CH-OH + $R_2$
|
$CH_2$-OH + R3 + -P=OH

Glycerophospholipid = **Glycerol + fatty acid + polar phosphorous moity**

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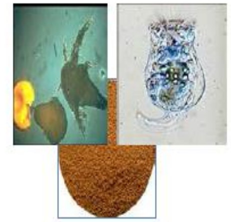
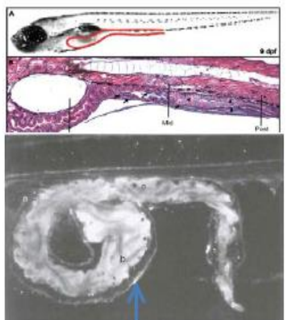
# Dietary uptake of TAG and PL

Natural intact phospholipids

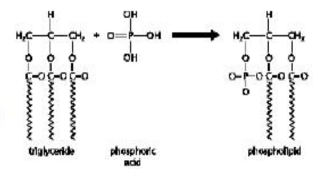
vs



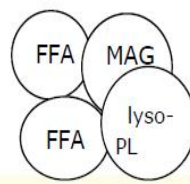
Supplemented lipids TAG or PL



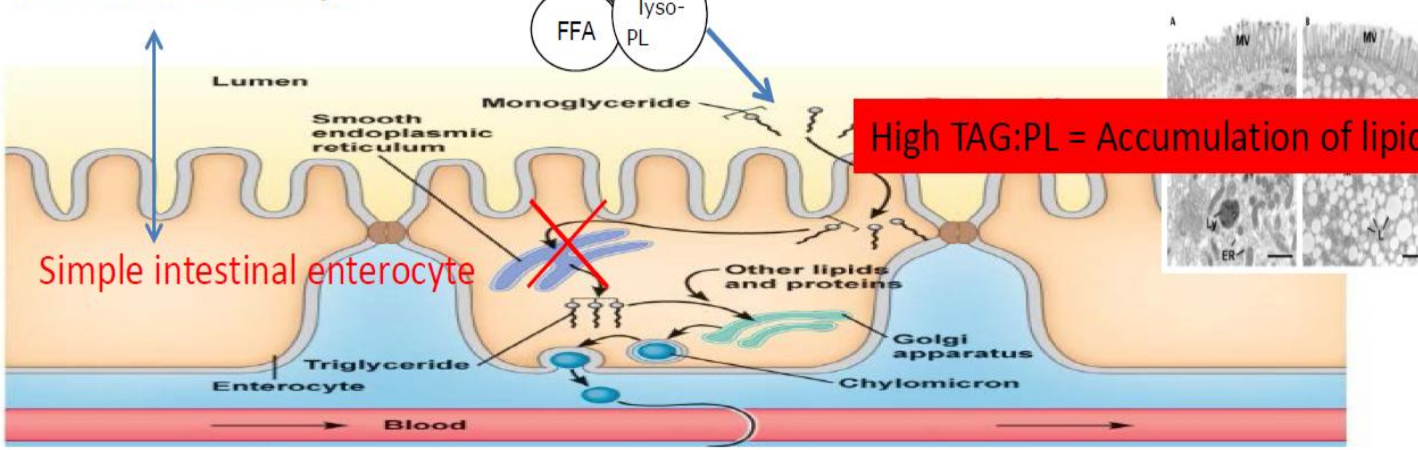
Bile salts hydrolysis




Marine PL vs vegetable PL ?




Intestinal enterocyte



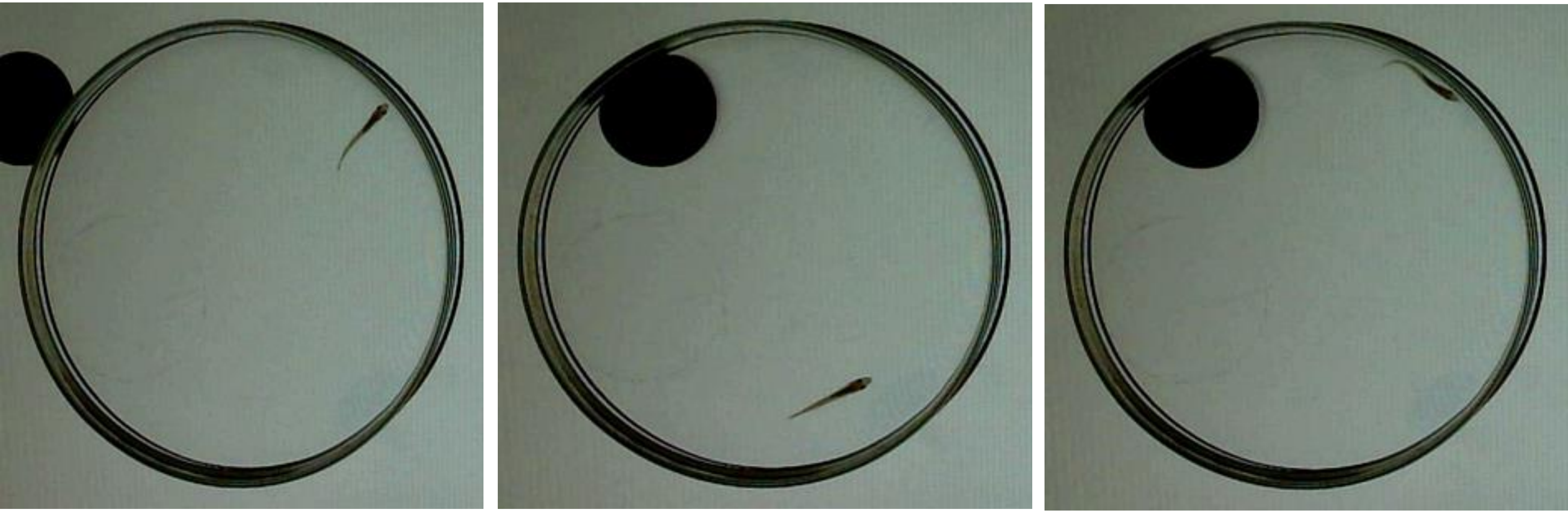

Simple intestinal enterocyte

High TAG:PL = Accumulation of lipid drop

# Previous studies

***Experiments shave shown  both short and long term consequences by lack of HUFAs on neural development and stress sensitivity***

Behavioural influence by lack of HUFAs in absence and presence of a simulated predator



# What did we test ?

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2. Importance and the interaction of dietary levels of EFA, - vitamins (A,C, D, E) - and minerals (CA, P)

3. Influence of salinity on larval ability to utilize and metabolize EFA and physiological effects

# Levels of phospholipids and influence of supplemented HUFA

| Analysed content (% DM) | PL1 | PL2 | PL3 | PL1H1 | PL1H2 | PL1H3 |
|---|---|---|---|---|---|---|
| Crude protein | 52,5 | 51,7 | 52,0 | 51,8 | 52,4 | 52,1 |
| Crude lipid | 27,0 | 27,0 | 27,0 | 27,0 | 26,9 | 27,0 |
| Gross Energy | 24,0 | 23,3 | 22,5 | 24,0 | 23,3 | 22,5 |
| | | | | Algatrium DHA70 | | |
| EPA (% ww, as fed) | 0,41 | 0,41 | 0,41 | **0,47** | **0,61** | **0,75** |
| DHA (% ww, as fed) | 0,66 | 0,66 | 0,66 | **1,04** | **2,06** | **3,04** |
| TPL (PC, PE, PI) (%) | **3,7** | **8,2** | **14,4** | **3,7** | **8,3** | **14,5** |

**Experimental prerequisites:**
Triplicate test. Use of vegetable oil
Exp. Duration 10 -30 DPH

3 dietary levels of phospholipids (i.e. soy bean lecithin)
+ additional 3 levels of EPA+DHA (Algatrium DHA, - TAG)

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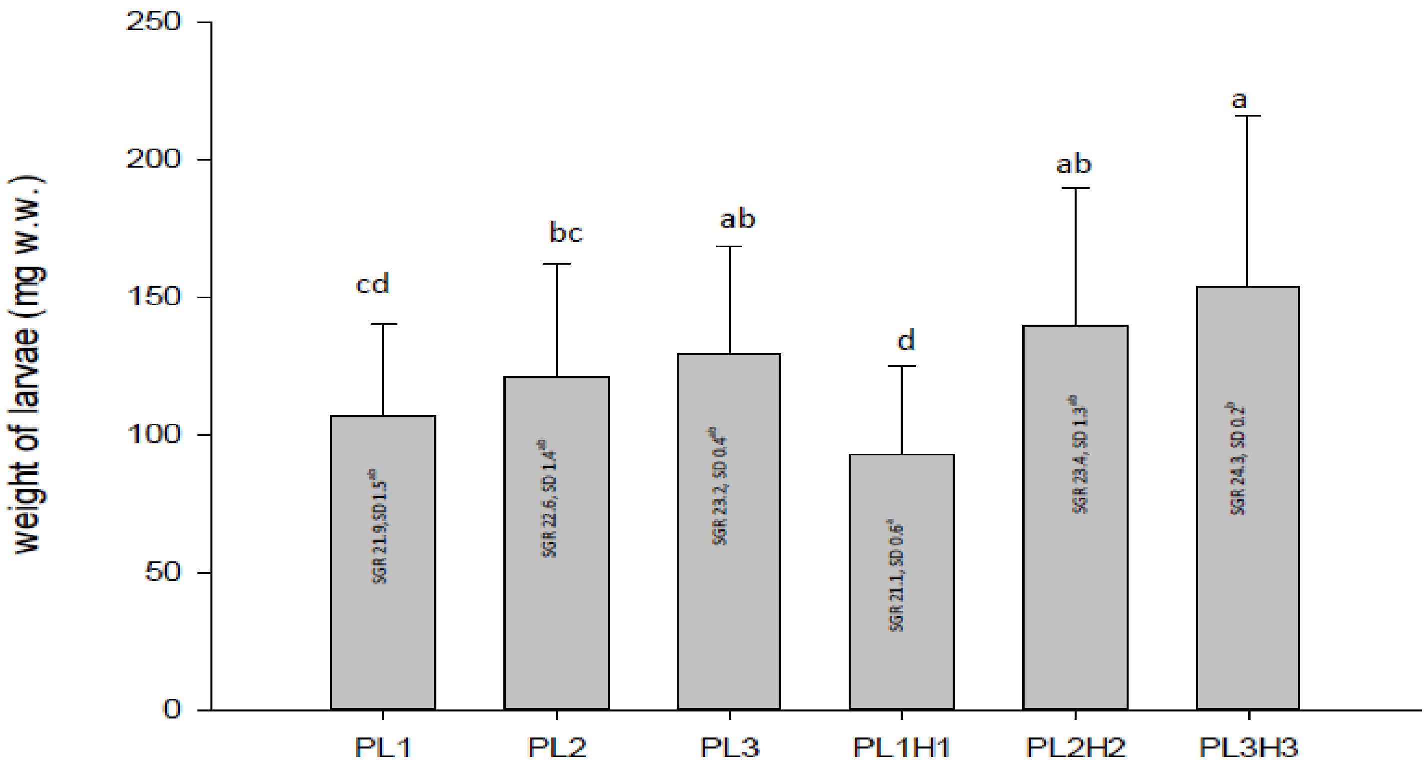
# Levels of phospholipids and influence of supplemented HUFAs : **Growth Results**



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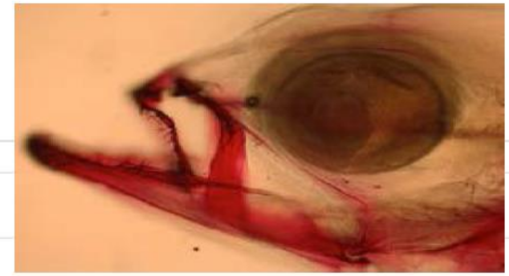
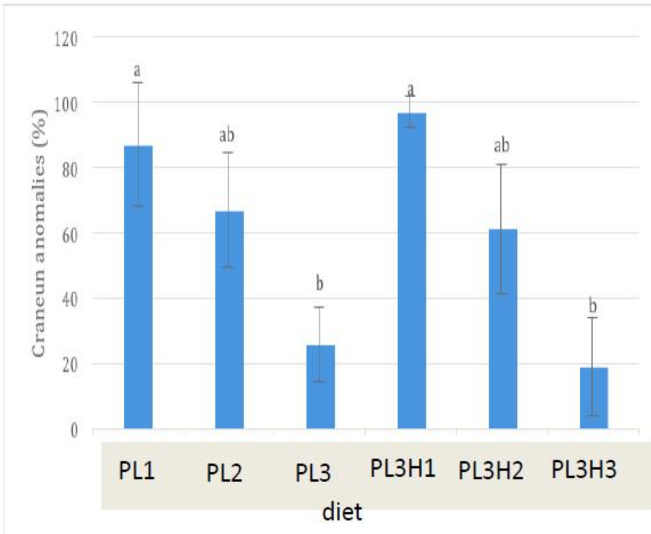
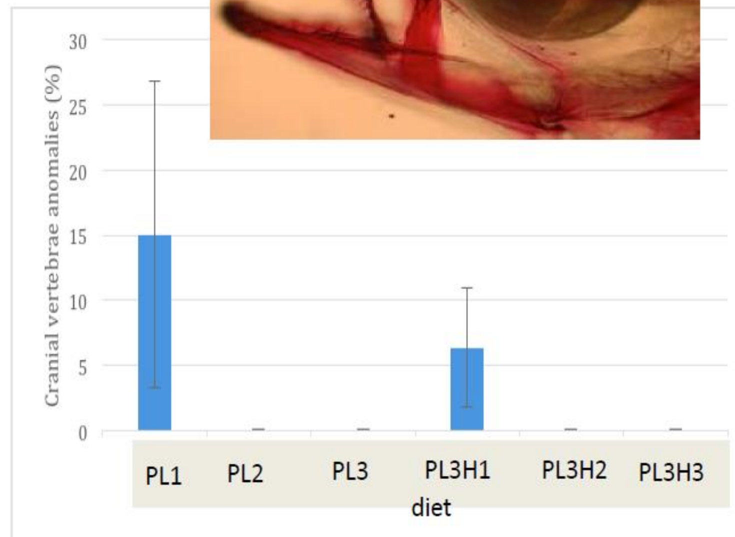
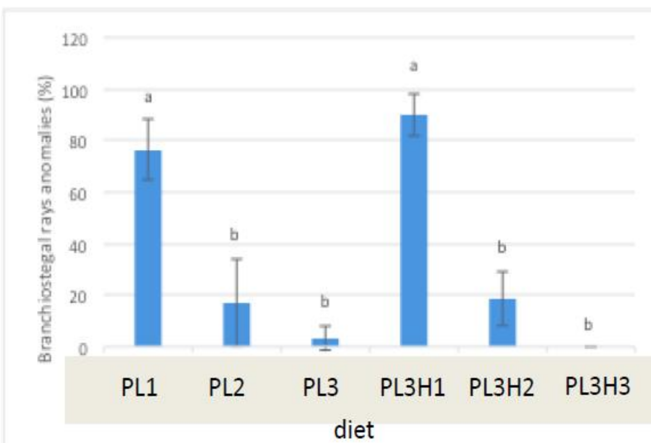
# Levels of phospholipids and influence of supplemented HUFAs

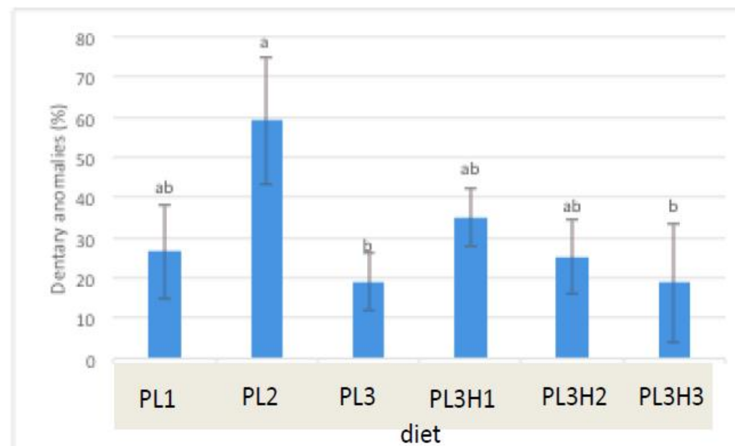
## Larval anomalies





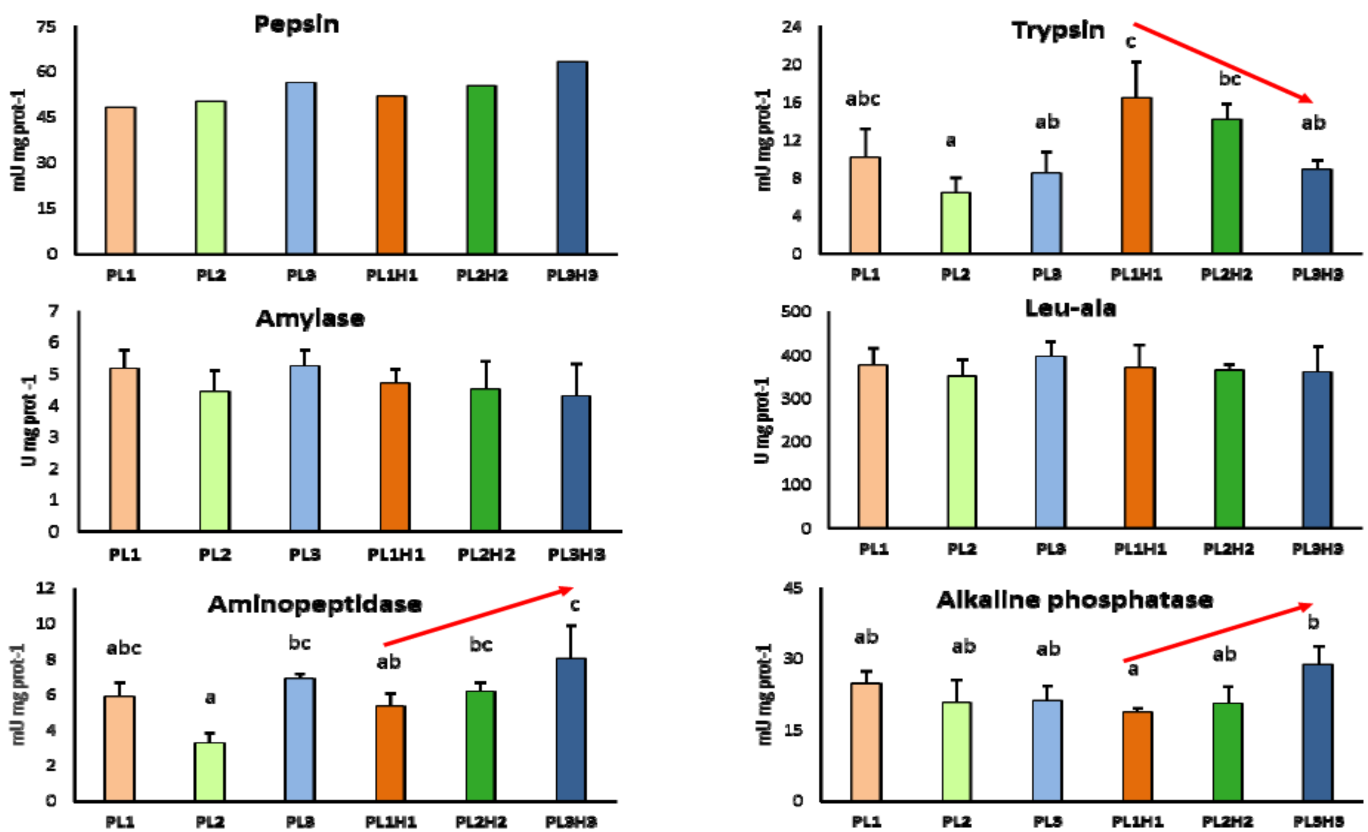



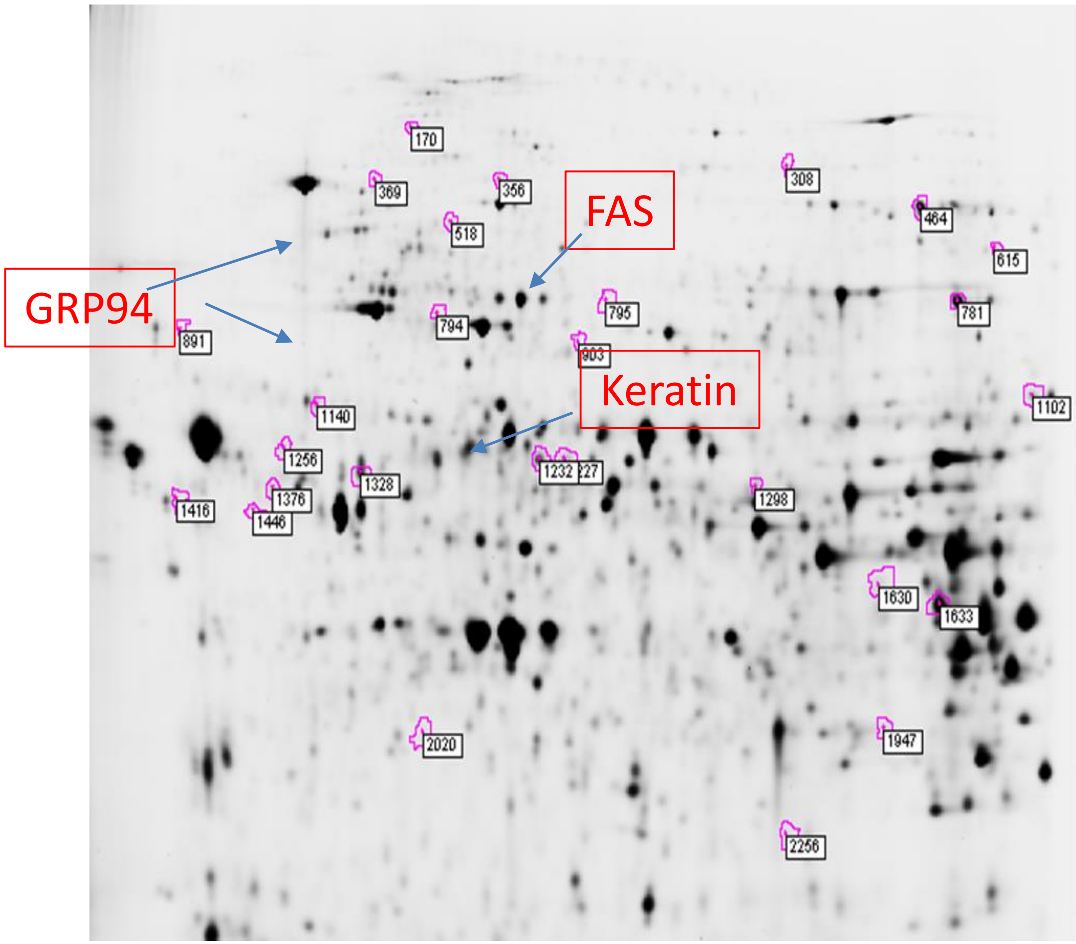



# Levels of phospholipids and influence of supplemented HUFAs: **Enzymatic activity**

- **Increased PL and n-3 LC-PUFA** enhanced the activities of the brush border membrane enzymes, alkaline phosphatase and aminopeptidase. This enhanced enzymatic activity is associated to a higher maturation of the gut followed by growth improvement



# Levels of phospholipids and influence of supplemented HUFAs: **Proteomics**




**Differential pattern proteins involved in:**

- ✓ Lipid metabolism
- ✓ Protein synthesis
- ✓ Endoplasmic reticulum (ER) stress
- ✓ Cytoskeletal and structural protein

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# Levels of phospholipids and influence of supplemented HUFAs: **Proteomics**

- 15 proteins identified by 2D-DIGE
- 8 proteins differentially expressed between treatments (*P*<0.05)

| Spot | accession | Protein identification | p | Fold change |
|---|---|---|---|---|
| 794 | A0A0F8AHC2 | Glucose-regulated | 0.007 | 1.70 in PL2/PL3H3 |
| 518 | A0A0F8AWU1 | Glucose-regulated protein (GRP94) | 0.031 | 1.48 in PL2/PL1H1<br>1.52 in PL2/PL3H3 |
| | UPI000557CE3B | Glucose-regulated protein (GRP94) | | |
| 795 | UPI000556131D | **fatty acid synthase-like** | 0.002 | **4.36 in PL1 vs PL3H3**<br>**3.65 in PL2 vs PL3H3**<br>**3.54 in PL3 vs PL3H3**<br>3.50 in PL1H1/PL3H3 |
| 1102 | G3P216 | ATP-citrate synthase | 0.036 | 2.60 in PL2/PL3H3 |
| 1633 | H2U634 | **non-specific lipid-transfer protein** | 0.042 | **2.03 in PL1H1 vs PL3H3** |
| | H2SWA2 | **hydroxysteroid dehydrogenase-like protein 2** | | |
| 1232 | G8G8Y1 | Keratin 8 (Fragment) n=2 | 0.035 | 2.27 in PL1/PL2H2<br>2.33 in PL1/PL3H3 |
| | G3NI19 | keratin, type II cytoskeletal 8-like | | |
| 1376 | UPI00054B498F | protein disulfide-isomerase | 0.047 | NS |
| 1947 | U3LRB6 | Protein disulfide-isomerase | 0.005 | 1.85 in PL1/PL2<br>1.99 in PL1/PL2H2<br>1.67 in PL2/PL1H1 |

| Spot | accession | Protein identification | p | Fold change |
|---|---|---|---|---|
| 794 | A0A0F8AHC2 | **Glucose-regulated** | 0.007 | **1.70 in PL2 vs PL3H3** |
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| 1376 | UPI00054B498F | protein disulfide-isomerase | 0.047 | NS |
| 1947 | U3LRB6 | **Protein disulfide-isomerase** | 0.005 | **- 1.85 in PL1 vs PL2**<br>**- 1.99 in PL1 vs PL2H2**<br>1.67 in PL2/PL1H1 |

FAS expression was down regulated in larvae fed PL3H3

FAS seemed to be more regulated by LC-PUFA content than by PL levels

Down-regulation of the expression of proteins involved in transfer and exchange of phospholipids and cholesterol

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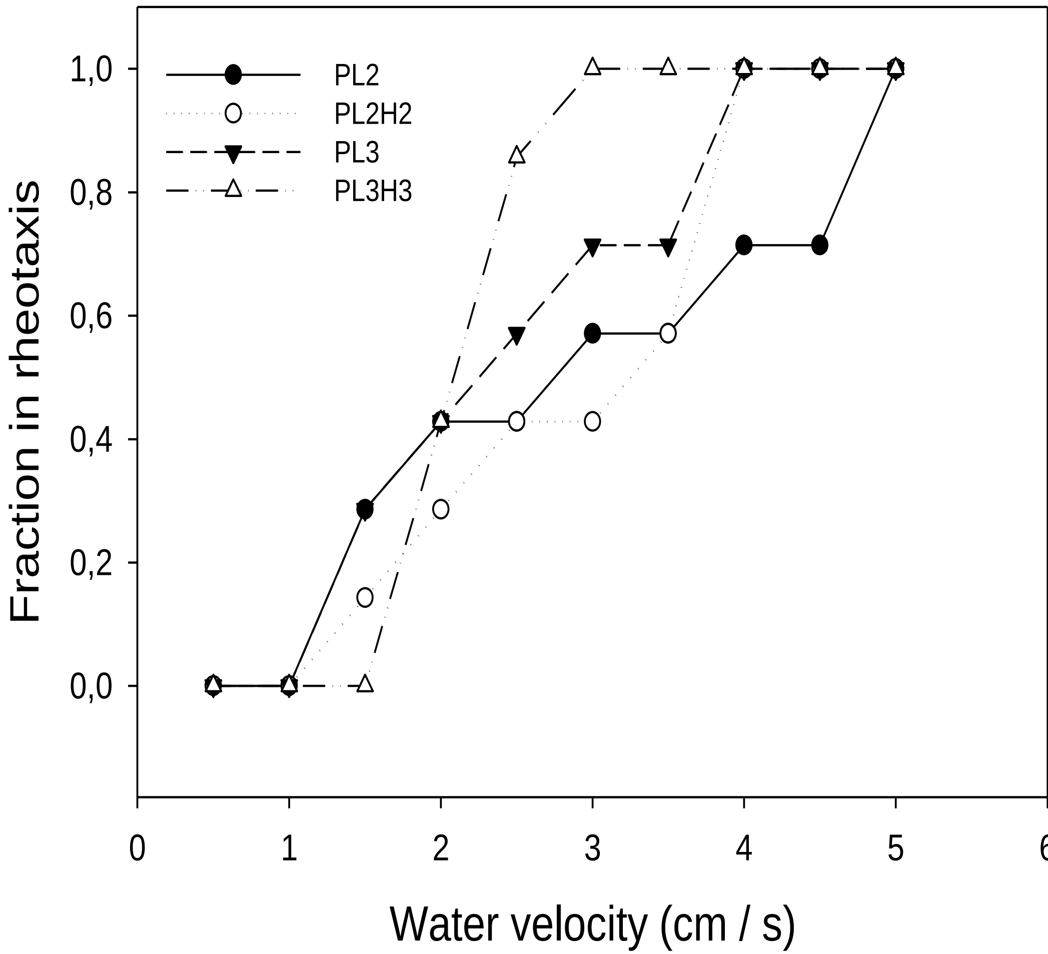
# Levels of phospholipids and influence of supplemented HUFAs:

## Rheotaxis

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2. **Importance and the interaction of dietary levels of EFA, - vitamins (A,C, D, E) - and minerals (CA, P)**

3. Influence of salinity on larval ability to utilize and metabolize EFA and physiological effects

# Search of key nutritional factors in pikeperch larvae

**Objective:** Screening of selected nutrients (fatty acids, vitamins and minerals) for development of specific formulated diets for pikeperch larvae

8 nutritional variables

2 modalities: high and low dietary levels

↓

8 factors * 2 modalities/factors

$2^8 = 256$ combinations

Factorial fractional design ↓

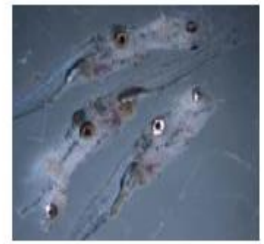
**16 different combinations**

| Dietary variables | Low level | High level |
|---|---|---|
| Ca/P | 0.6 | 1.2 |
| DHA+EPA | 1.25% | 3.5% |
| ARA | 0.8% | 1.6% |
| Vitamin E | 1000 mg/kg | 3000 mg/kg |
| Vitamin C | 2000 mg/kg | 3600 mg/kg |
| Vitamin A | 8000 IU/kg | 30000 IU/kg |
| Vitamin D | 2800 IU/kg | 28000 IU/kg |
| Se | 3 mg/kg | 12 mg/kg |

*From Kestemont et al, ISFNF 3-7. June 2018*

# Experiment timeline

- 1st feeding with Artemia nauplii (enriched with HUFA)
- Co-feeding period from 18 to 24 dph using Artemia and mixture of the 16 diets



$D_{-24}$ — $D_0$ — $D_{14}$

**Daily mortality counting**

**Initial sampling**

25 dph larvae (9.44 mg) randomly distributed at a density of 770 larvae tank$^{-1}$

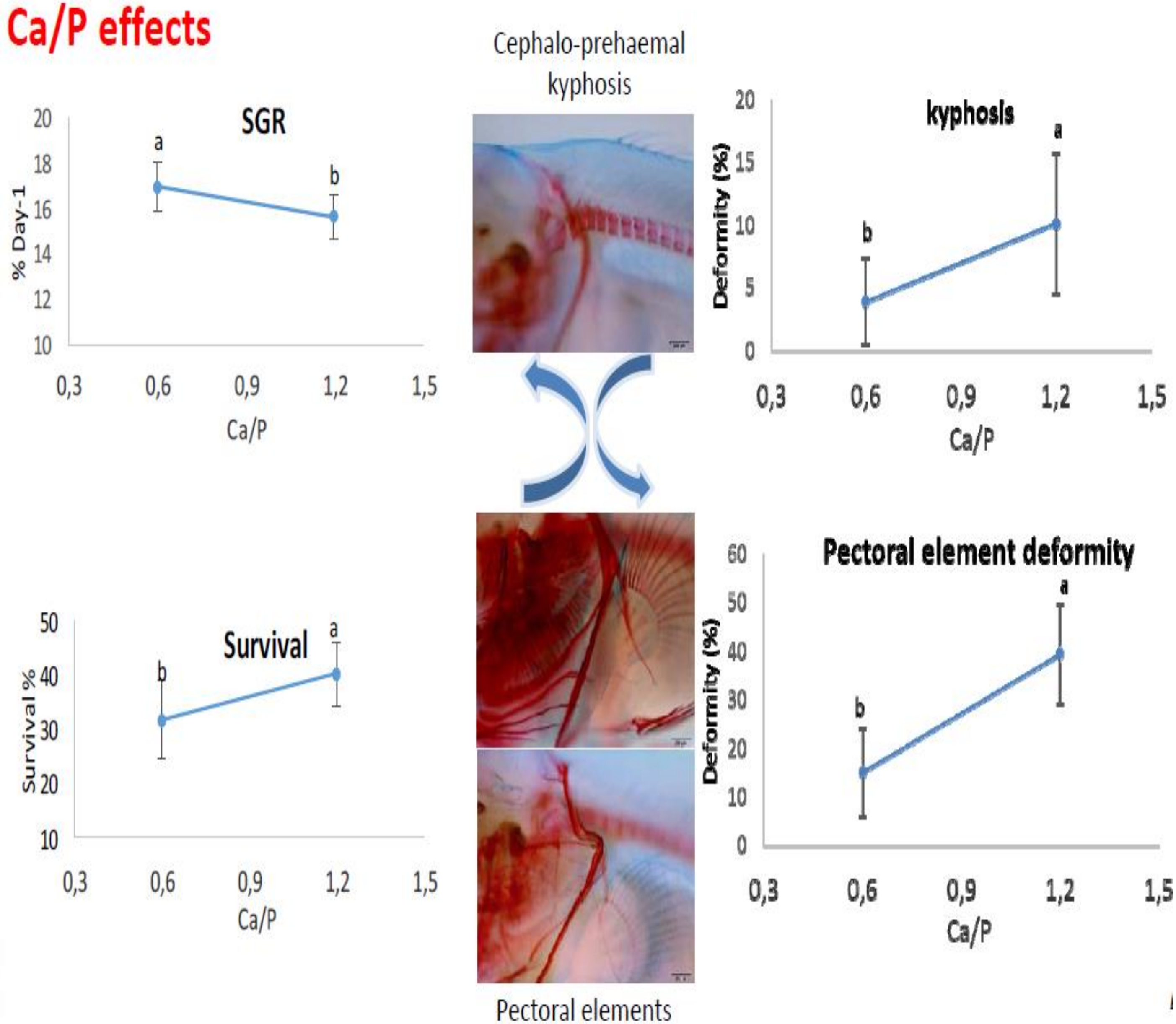
**Final sampling**

- Growth and survival
- Biochemical composition
- Digestive enzymatic assays
- Deformities evaluation
- Histology
- Gene expression

*Experimental set up at UNAMUR*
*16 independent 100L-aquariums*

*From Kestemont et al, ISFNF 3-7. June 2018*

# Ca/P effects

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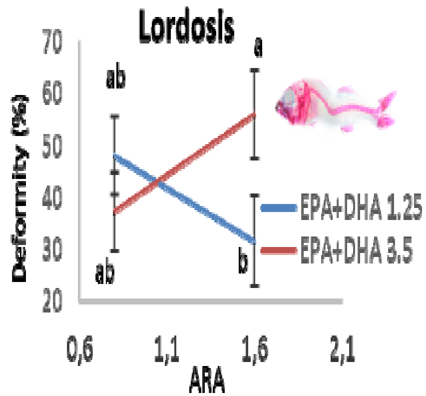
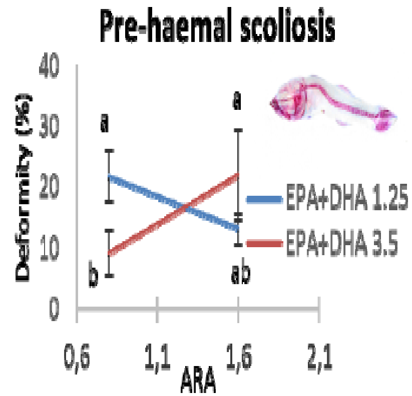
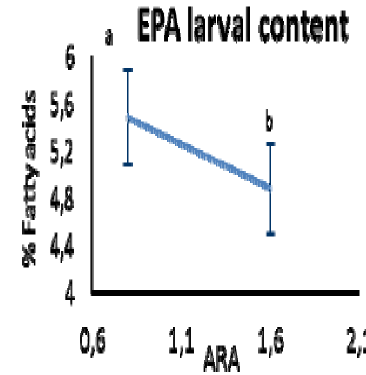
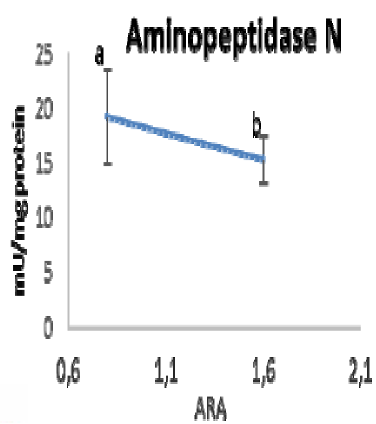
# LC-PUFA effects

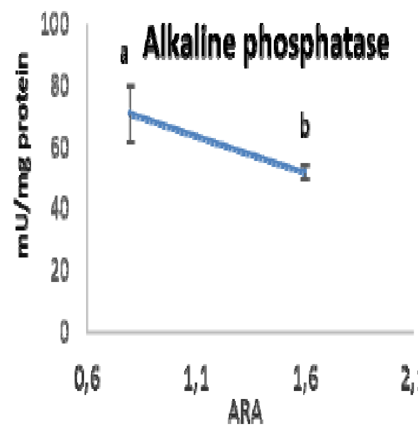


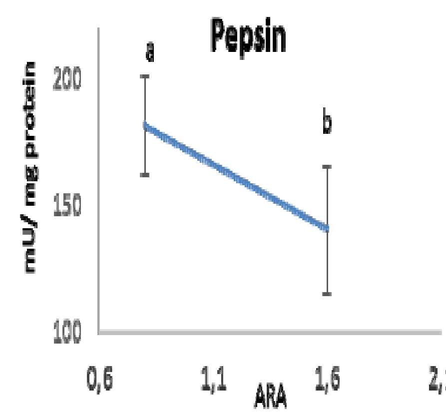




✓ **ARA:** potential involvement in the regulation of digestive tract development

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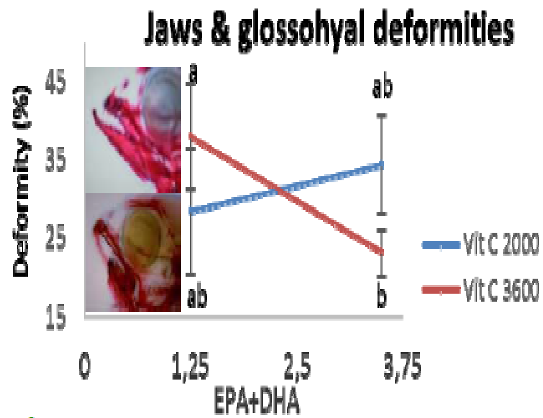
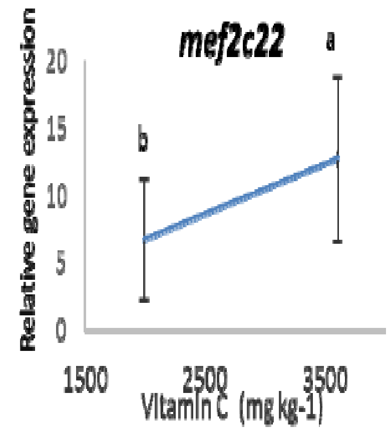
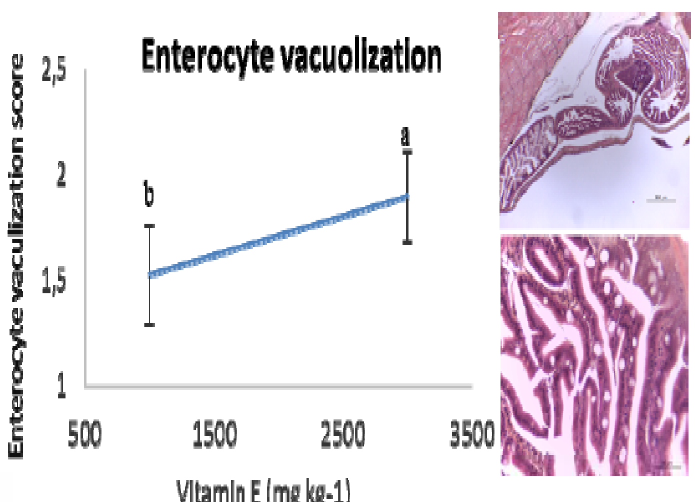
# Vitamin effects and interactions

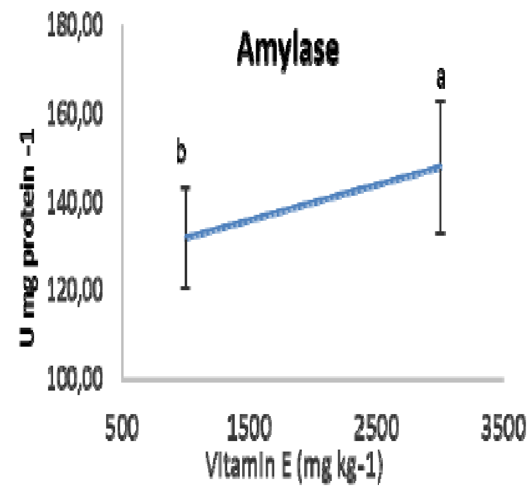
✓ **Vit C effects** on ossification of cartilaginous-origin bone process and its function as antioxidant




✓ **Vit E effects**

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# CA/P Confirmatory exp.

**The experiment investigated dietary Ca/P effect not only by varying one of the two minerals, but also varying both.**

| As fed basis | Diet 1 | Diet 2 | Diet 3 | Diet 4 | Diet 5 | Diet 6 |
|---|---|---|---|---|---|---|
| Crude protein | 51.16 | 51.15 | 51.14 | 51.14 | 51.16 | 51.17 |
| Crude fat | 18.46 | 18.46 | 18.46 | 18.46 | 18.46 | 18.46 |
| Fiber | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 | 0.16 |
| Starch | 9.97 | 8.02 | 4.20 | 4.21 | 11.48 | 15.17 |
| Ash | 9.04 | 10.96 | 14.72 | 12.95 | 8.46 | 6.18 |
| Total P | 2.68 | 2.68 | 2.68 | 3.97 | 2.01 | 1.01 |
| Ca | 0.80 | 1.61 | 3.21 | 1.20 | 1.20 | 1.20 |
| Ca/P | 0.30 | 0.60 | 1.20 | 0.30 | 0.60 | 1.19 |

*Najlae et al, 2018 in prep.*

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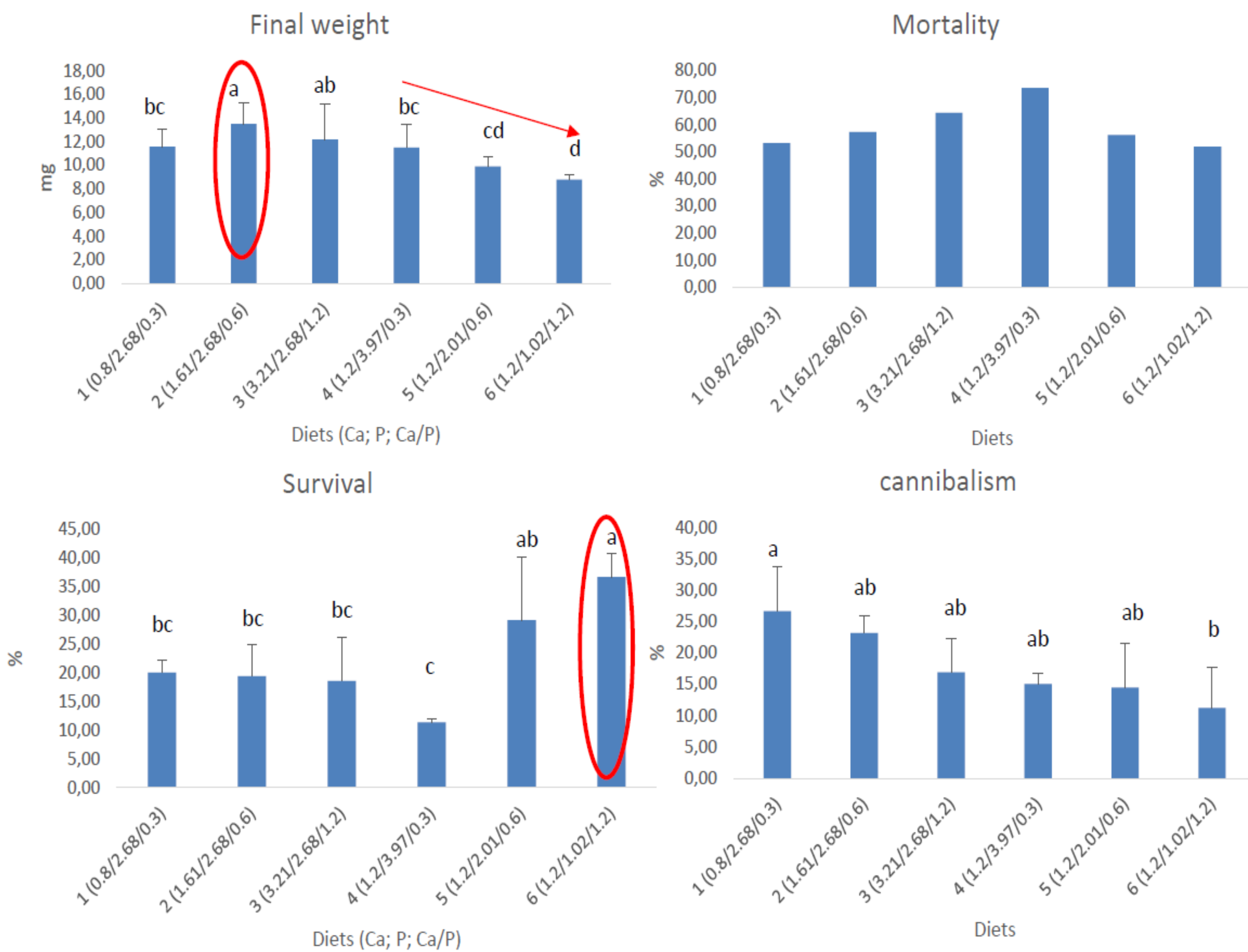
# Results

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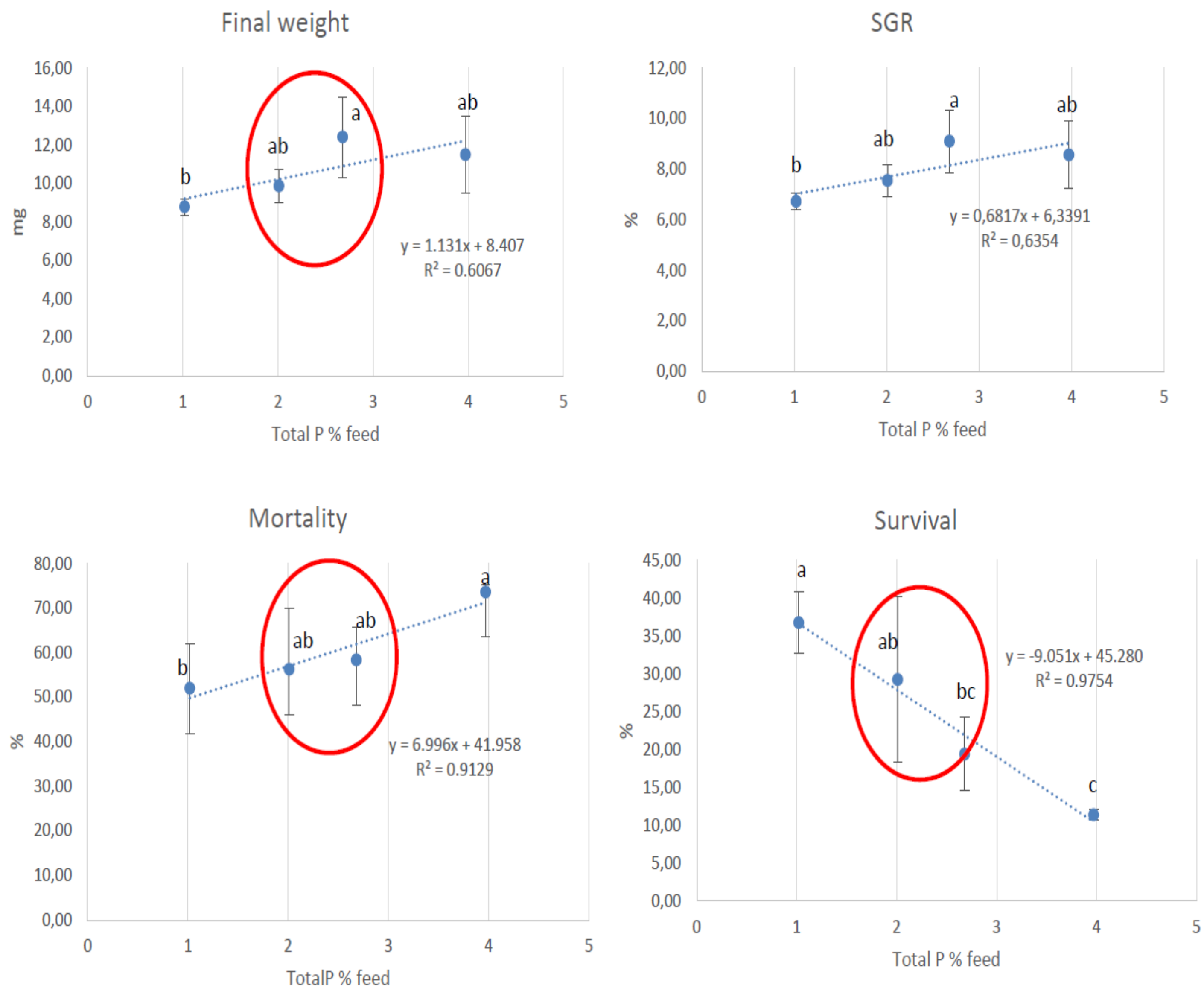


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# Larval FA metabolism by rearing in low salinity gradients ?

**Experimental design:**

Two factors tested in 18 tanks from 10 DPH:

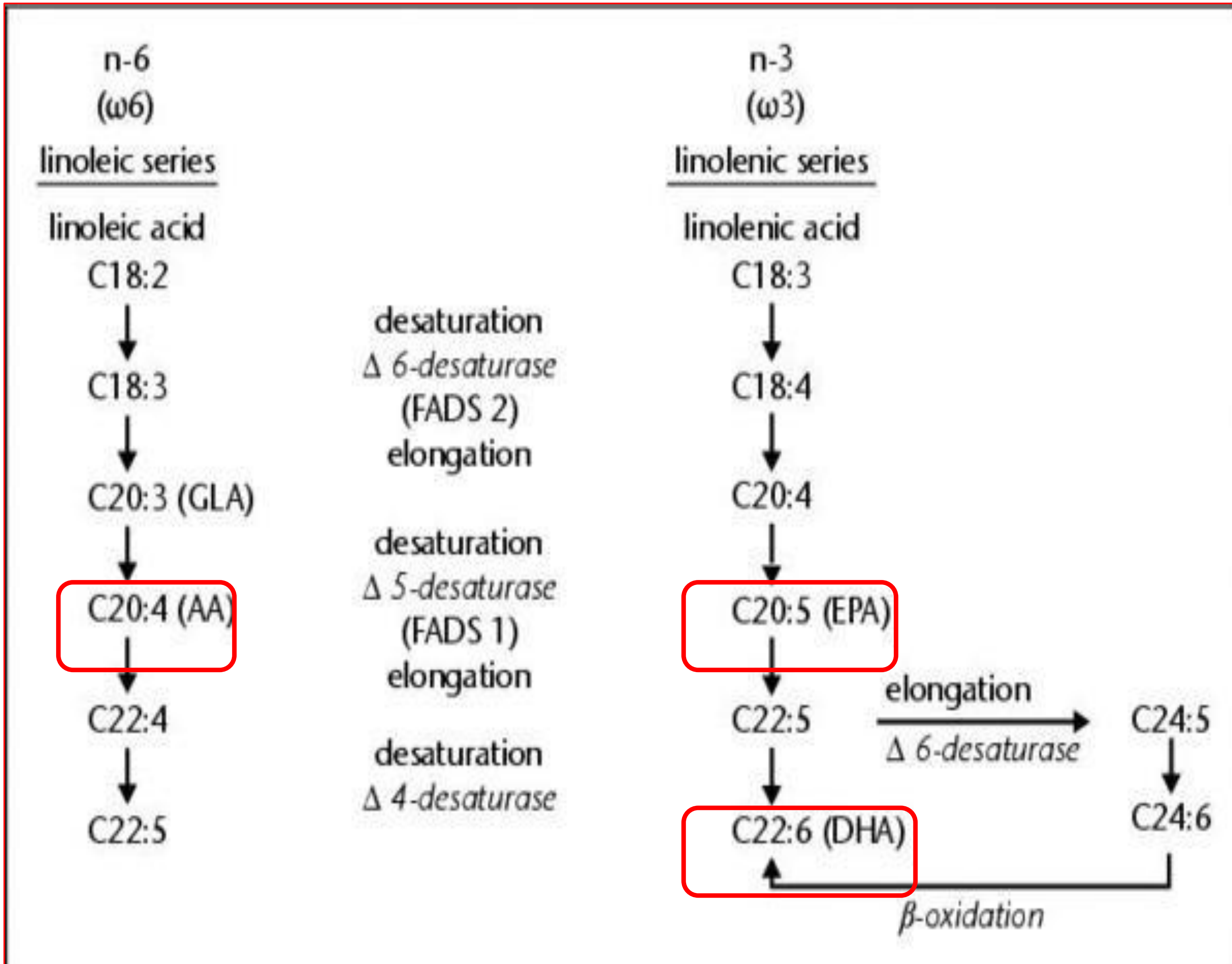
- High dietary ALA (18:3n-3) &. high LA (18:2n-6)
- Three salinity levels (0-, 5-, 11 ppt)

- In vivo incubation of larvae with labelled $^{14}C$ fatty acids (ALA, LA, EPA, DHA) (0, 5, 10 ‰)

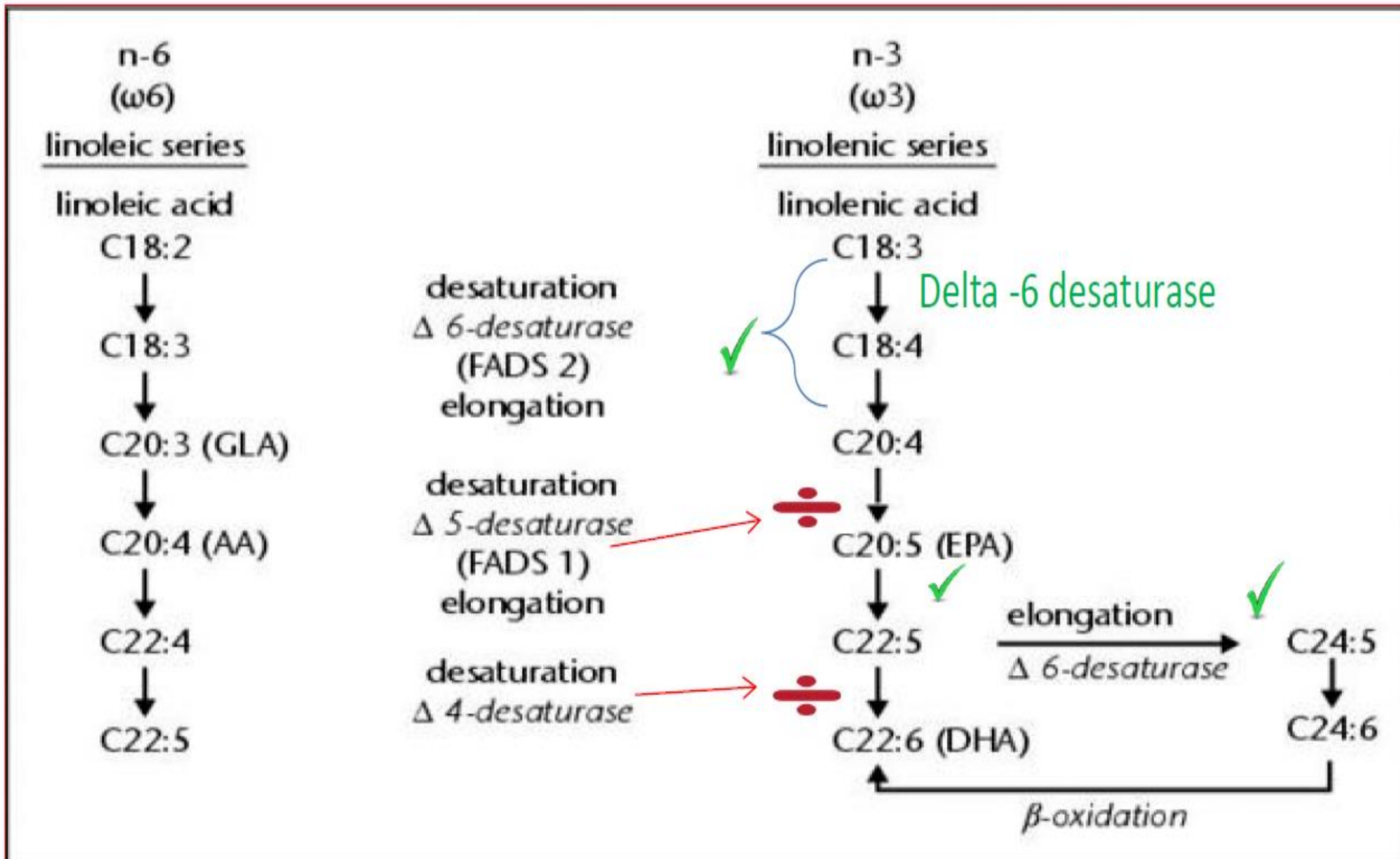
**Analyses:**

- Metabolism of lipid classes, esterification (HPTLC, TLC)
- Lipid class composition and FA content,
- Performance
- Eicosanoid activity
- Deformities, stress resistance

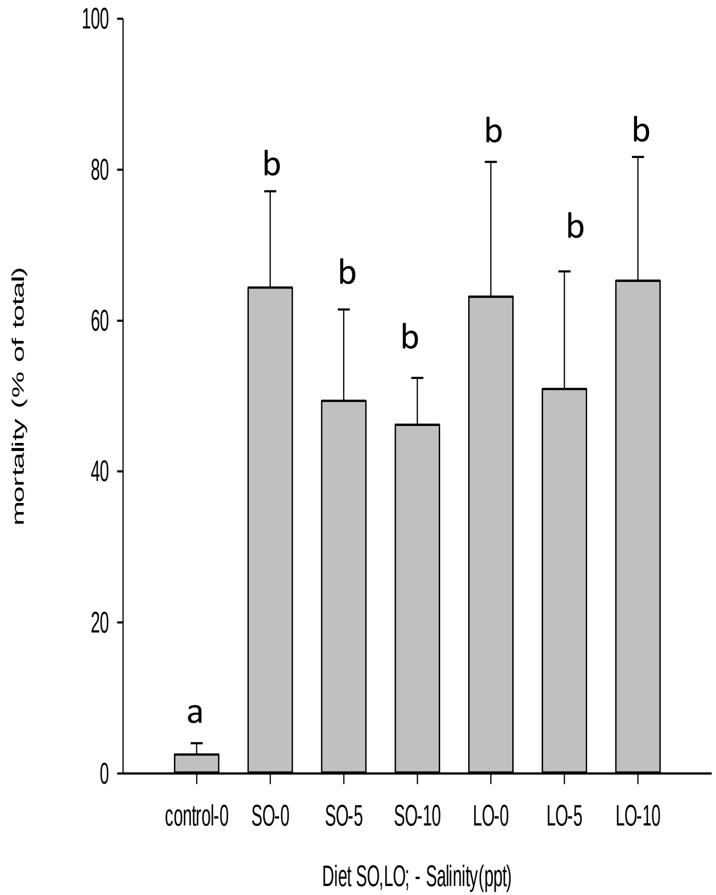
# FA elongation and desaturation capability



| Substrate | 18:2n-6 | 18:3n-3 | 20:4n-6 | 20:5n-3 | 22:6n-3 |
|---|---|---|---|---|---|
| Incorporation | 6.67 ± 3.22 [c] | 6.82 ± 2.52 [c] | 16.98 ± 5.99 [b] | 37.58 ± 14.33 [a] | 6.52 ± 2.72 [c] |



# Confinement stress and mortality



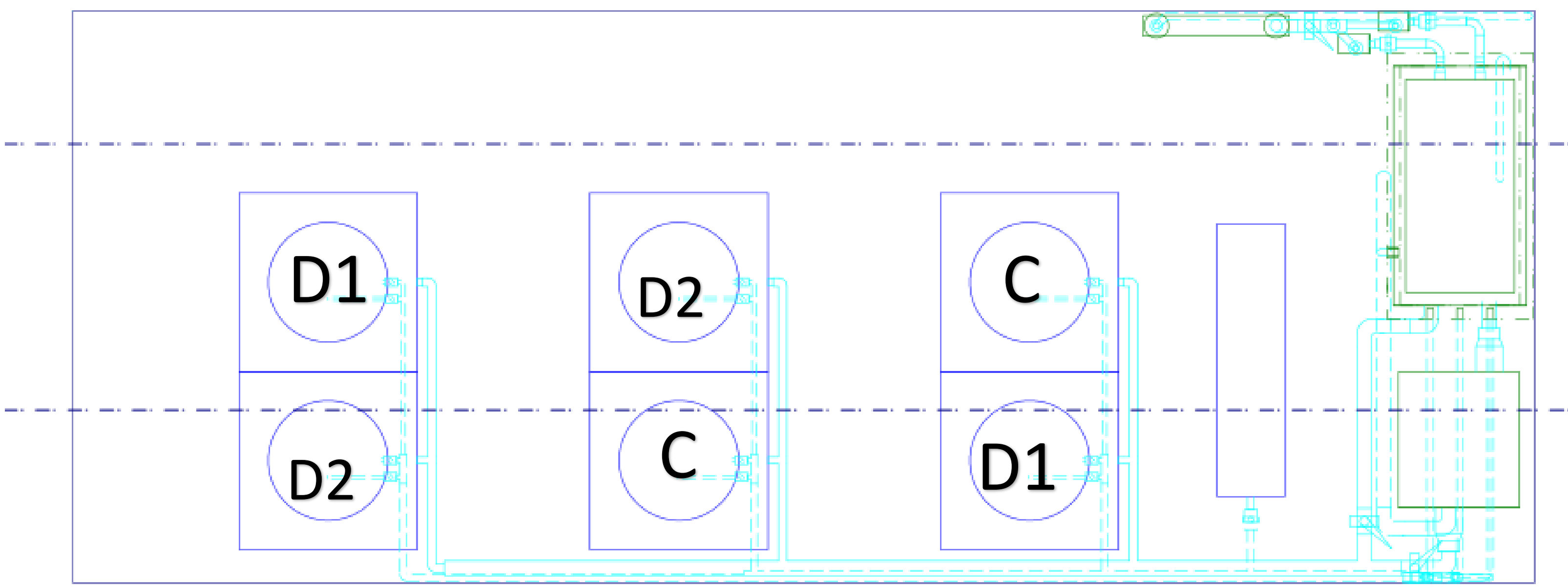

# Commercial test: Validation of optimised exp. diet

- We compared the efficiency of « exp. Diversify optimised diet » vs commercial diet (Otohime). Test: Fish2Be facilities
- 3 weeks of feeding trial
- Endpoints: Survival & cannibalism, growth & size heterogeneity

# Validation
# Preliminary results:

| Dietary composition % | D1 | D2 |
|---|---|---|
| | | |
| Crude protein | 53.6 | 53.6 |
| Crude fat | 24.1 | 24.1 |
| Fiber | 0.1 | 0.2 |
| Ash | 10.5 | 9.3 |
| Gross Energy. MJ/kg | 23.0 | 23.1 |
| **P** | **2.4** | **2.4** |
| **Ca** | **1.9** | **1.9** |
| **Ca/P** | **0.8** | **0.8** |
| **ARA** | **0.5** | **0.5** |
| **EPA** | **0.5** | **2.0** |
| **DHA** | **1.0** | **4.5** |
| **Total PL** | **8.6** | **8.6** |

# Experimental setup



# Setup

- 6 hatchery tanks, w automatic cleaning arm
- 6*35000 weaned larvae (25day old)
- Fed continuously with beltfeeders (12 hours/day)
- For around 40 days 26/04 – 05/6 or until some major concerns
- Cannibals were thrown out every 4 -6 days

Workshop on recent progress in pikeperch culture

Faculty of Sciences and Technologies

Nancy, France 27. June

# Preliminary results

- After a week major mortalities occurred in the groups fed D2
  - Larvae were floating on the surface with air in the stomach/intestine
  - effect was so severe that even after stopping with D2 it continued for days.
- D1 had slightly lower survival to the commercial diet (35% <> 45%)
- Deformaties were low (<5%) in both D1 and C
  - however in D1 was nearly all related to not inflating swimbladder
  - In C it there was some other deformaties as well
- No growth results yet

# Main conclusions and recommendations

- ✓ 8.2 % PL + supplementation with 1 % d.w. DHA and 0.17% d.w. EPA promote growth and digestive enzymatic activity, and reduce deformities and cellular stress.

- ✓ No effect on stress markers, escape response or metabolic respiration for larvae fed diets with ≥ 8 % phospholipids with or without n-3 HUFA supplementation

- ✓ Essential fatty acids (EFA) can be supplemented as TAG

- ✓ Several important enzyme proteins are affected by PL level and EFA level.

- ✓ Low Ca/P ratio induces similar effects as for high PL + EFA levels. P levels should also be considered

- ✓ Nutritional requirements must consider interactions between nutrients especially HUFA ratio (ARA/EPA/DHA and vitamin C and E (antioxidant effect).

# Main Conclusions and recommendations

- ✓ Rearing salinity up to 10 ppt has no growth promotory effect
- ✓ Salinity has no effect on the enzymatic capability of larvae to elongate shorter chain FA and thus biosynthesize lipid classes containing LC PUFAs /EFAs
- ✓ DHA (+ EPA) **must be** supplied in diets of pikeperch larvae for normal development and to reduce stress sensitivity

DTU

The presented work received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY)

***Thanks for your attention***