Some insights in lipid metabolism of larvae from novel aquaculture candidates species

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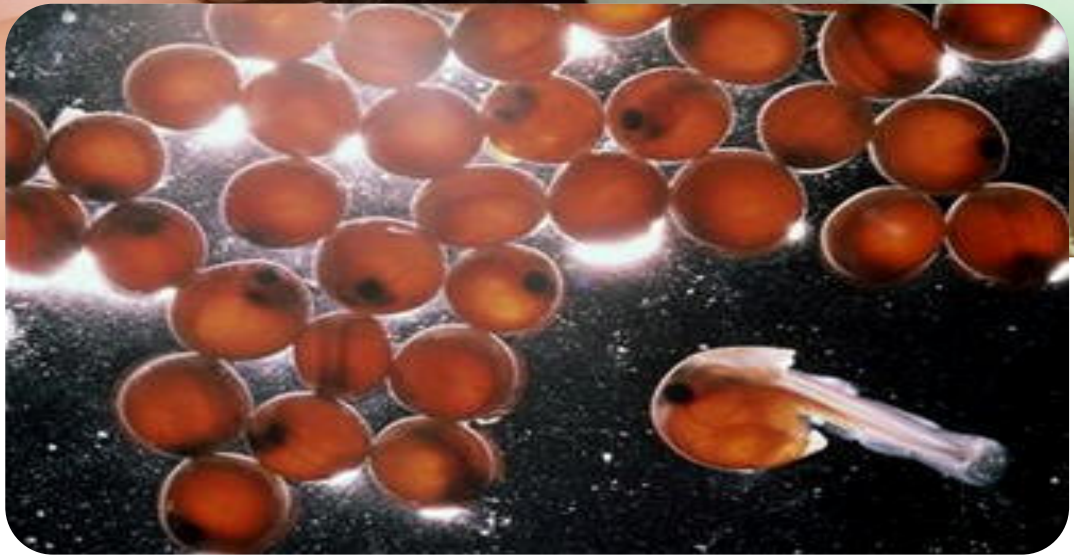
To satisfy global aquaculture fish demands, the introduction of new species must go much faster.

Novel and multidisciplinary approaches to understand larval nutritional physiology are still needed to improve formulas for live prey enrichment and microdiets

NUTRITION OF NEW SPECIES

When parental nutrition is correct **yolk provides all** the initial nutritional requirements.

WILD-REARED comparisons of **eggs**, larvae, **gonads**, liver, **muscle,... and its evolution are powerful tools in designing diets**

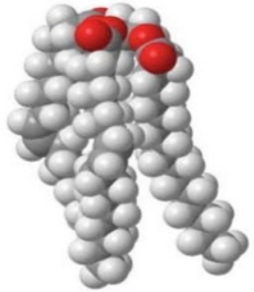


Biochemical composition of eggs and larvae from reared organisms

Differer from wild composition

↓Phospholipids PE; PC; PI & ↓ DHA-ARA-EPA

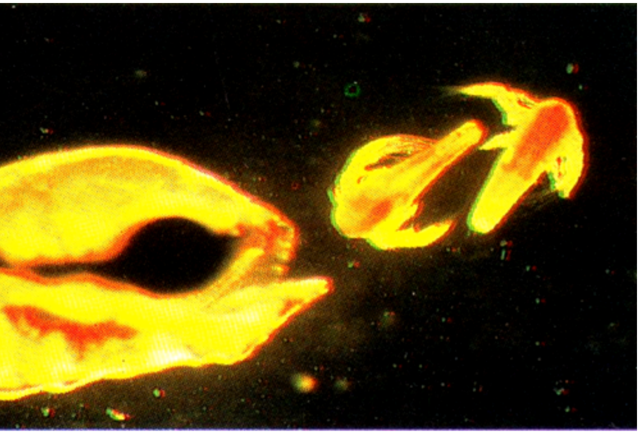
↑TAG & 18:3n-3-18:2n-6



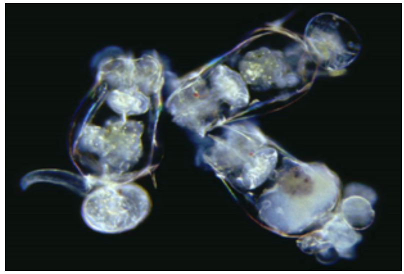
Origin

AVAILABILTY AND PRICES OF INGREDIENTS
Broodstock diets

Type of prey and enrichment protocols
Unknown requirements/DIGESTIVE ENZYMES



Poorer egg and larval quality and performance

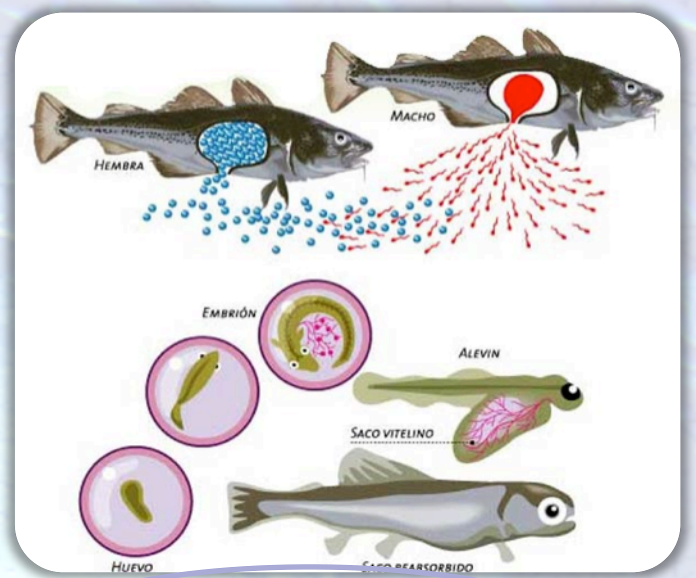


25% 18:3n-3
DHA into EPA

CHOLESTEROL

20:5n-3 EPA
22:6n-3 DHA
20:4n-6 ARA

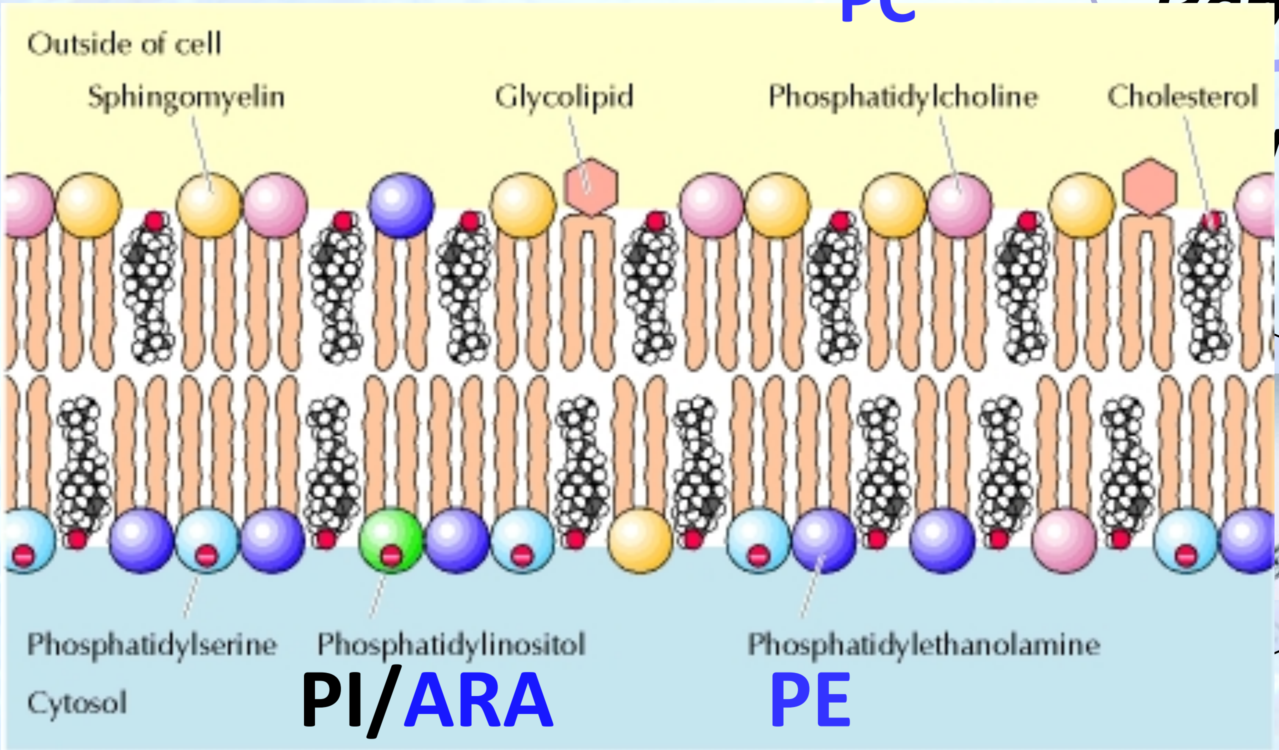
LC-PUFA or HUFA



Reproduction

PHOSPHOLIPIDS

PC

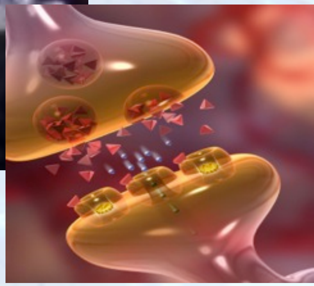


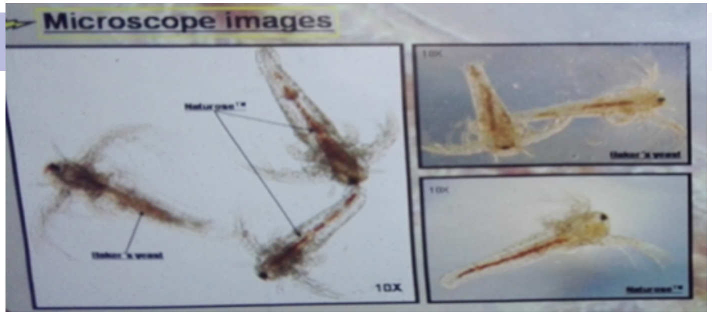
PI/ARA

PE

Yolk composition

LARVAL PERFORMANCE

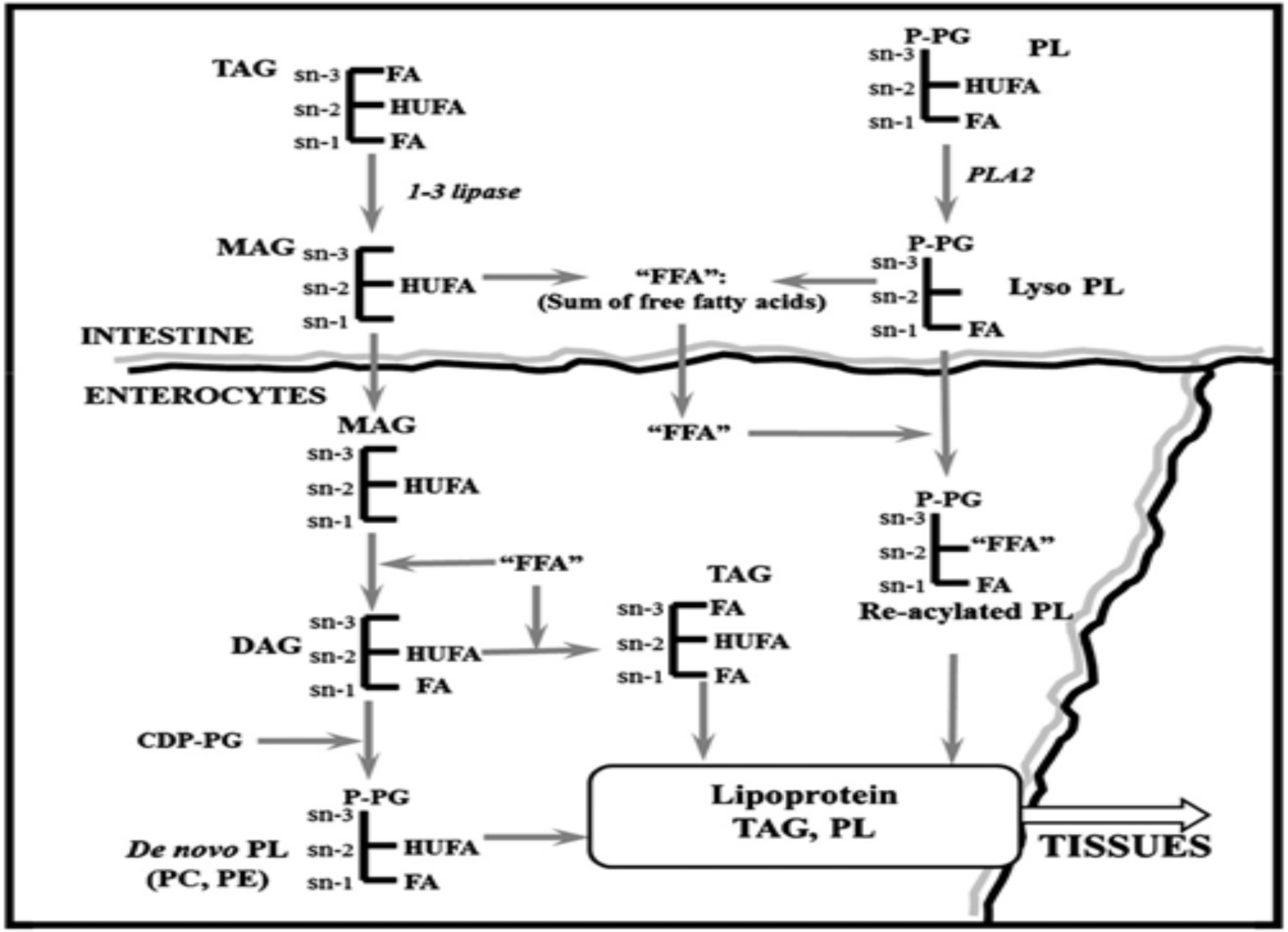




More Back ground

1. Most current enrichment protocols use triacylglycerols (TAG) whereas phospholipids (PL) are less used (Li et al. 2014) despite seems to be a more efficient source of LC-PUFA for larvae (Olsen et al. 2014).
2. Within DIVERSIFY, the use of phospholipids had a beneficial effect on greater amberjack and pikeperch growth and survival whereas in halibut it is not so clear…
3. Artemia differs from natural preys: converting DHA into EPA; tending to incorporate LC-PUFA into TAG, supplying as much as 25% 18:3n-3……

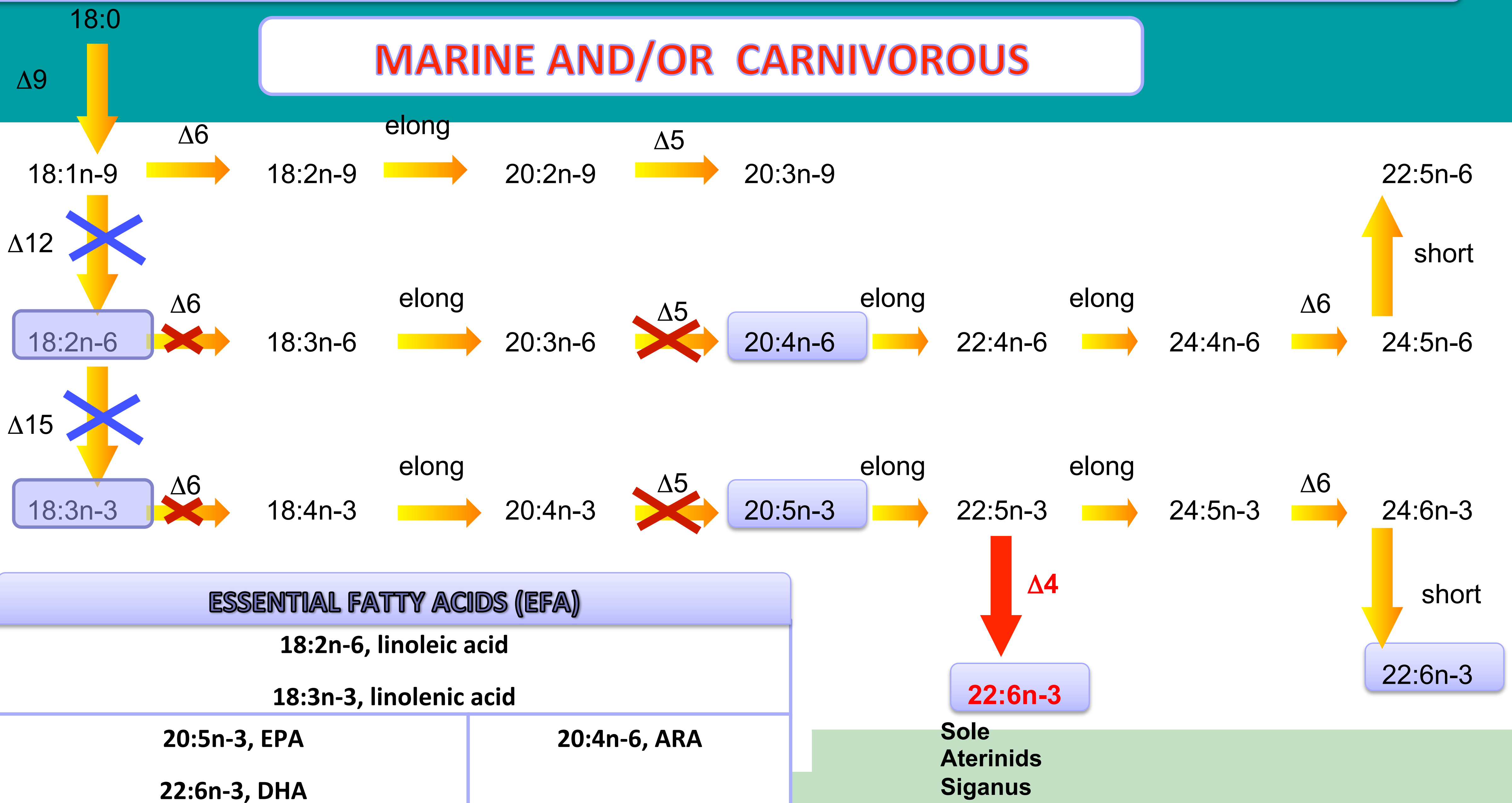
Olsen et al., 2014 – DHA content in dietary phospholipids affects DHA content in phospholipids of cod larvae and larval performance. Aquaculture 428-429, 203-214



Are these enzymes active or efficient enough in all our novel species?

VERTEBRATES PATHWAYS FOR LONG CHAIN PUFA (LC-PUFA)

MARINE AND/OR CARNIVOROUS



ESSENTIAL FATTY ACIDS (EFA)	
18:2n-6, linoleic acid **18:3n-3, linolenic acid**	
20:5n-3, EPA **22:6n-3, DHA**	**20:4n-6, ARA**

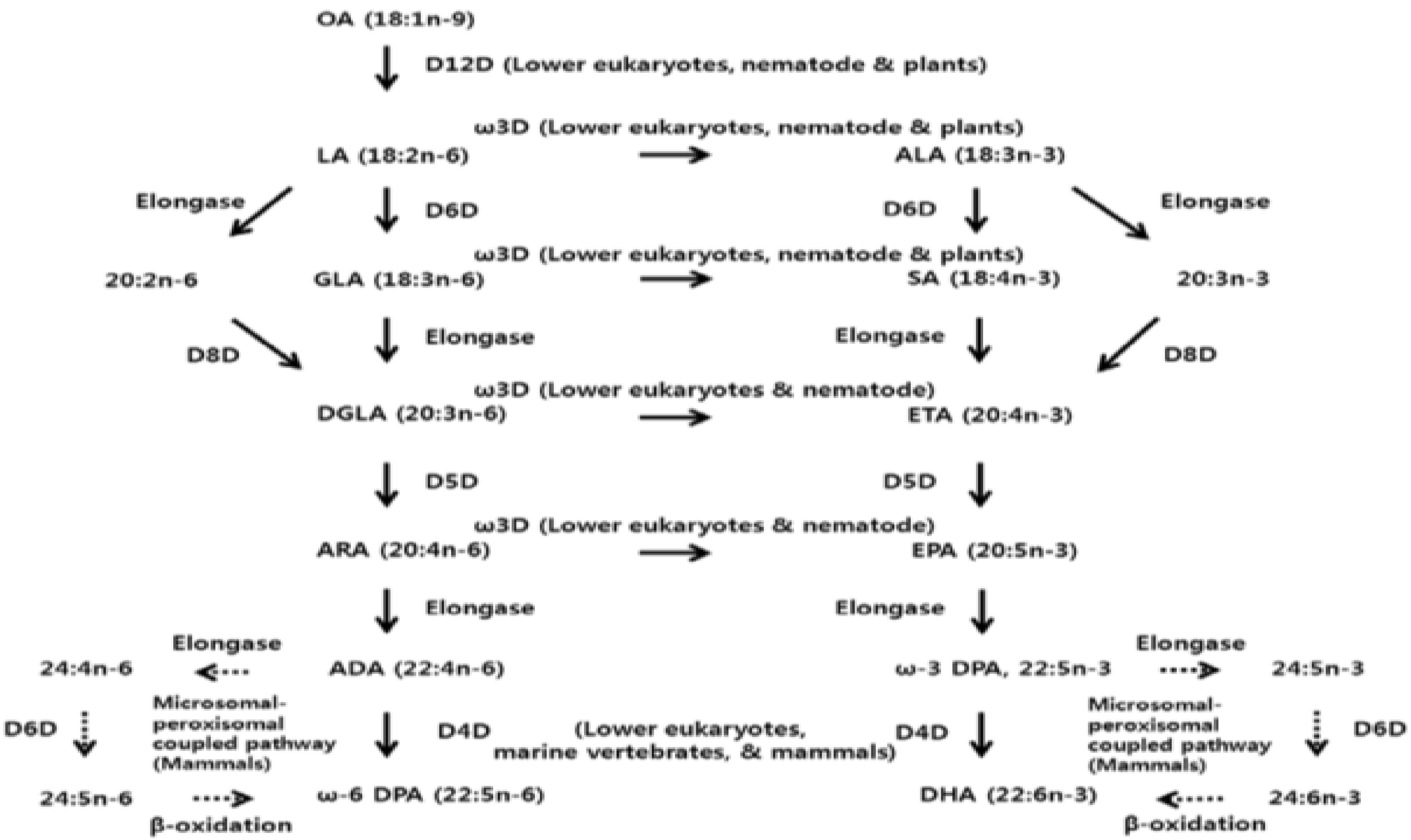


Figure 1. PUFA synthetic pathway to 22-carbon fatty acids from oleic acid in eukaryotic systems.

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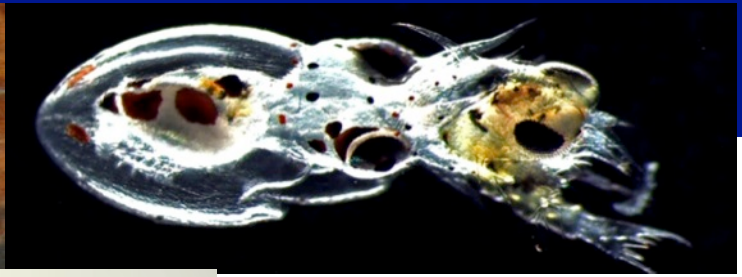
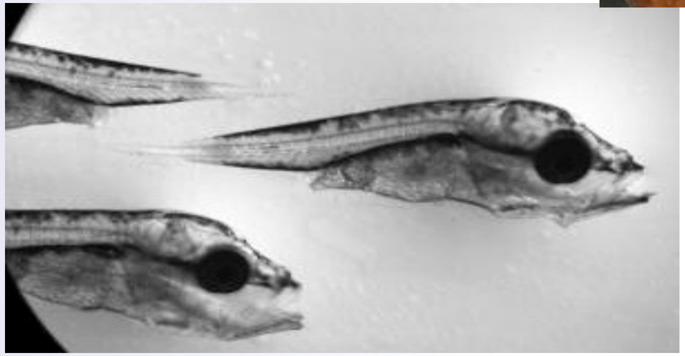
COOPERATION

DIVERSIFY

In vivo lipid metabolism of marine and freshwater larval species determined by incubation with ^{14}C-fatty acids labelled substrates directly added to the water

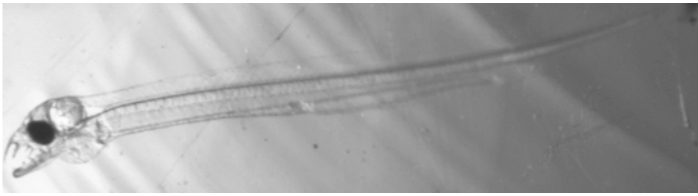
IEO-ULL-IATS-USt-UALg





WP10-DTU-ULL-FUNDP-FCPCT

WP11- NIFES-IMR-ULL



DTU-ULL

I.Lund/Jonna Tomkiewicz

Objective

Determine differences in the **lipid metabolism** between and within **cephalopods** and **marine** and **freshwater fish** species, providing a better knowledge on these species **lipid requirements** during **early life stages**, which should contribute to the **improvement** of live **preys enrichment protocols** and/or **formulated diets.**

MARKET AVAILABLE [1-^{14}C]Fatty acids or [1-^{14}C]Lipid classes

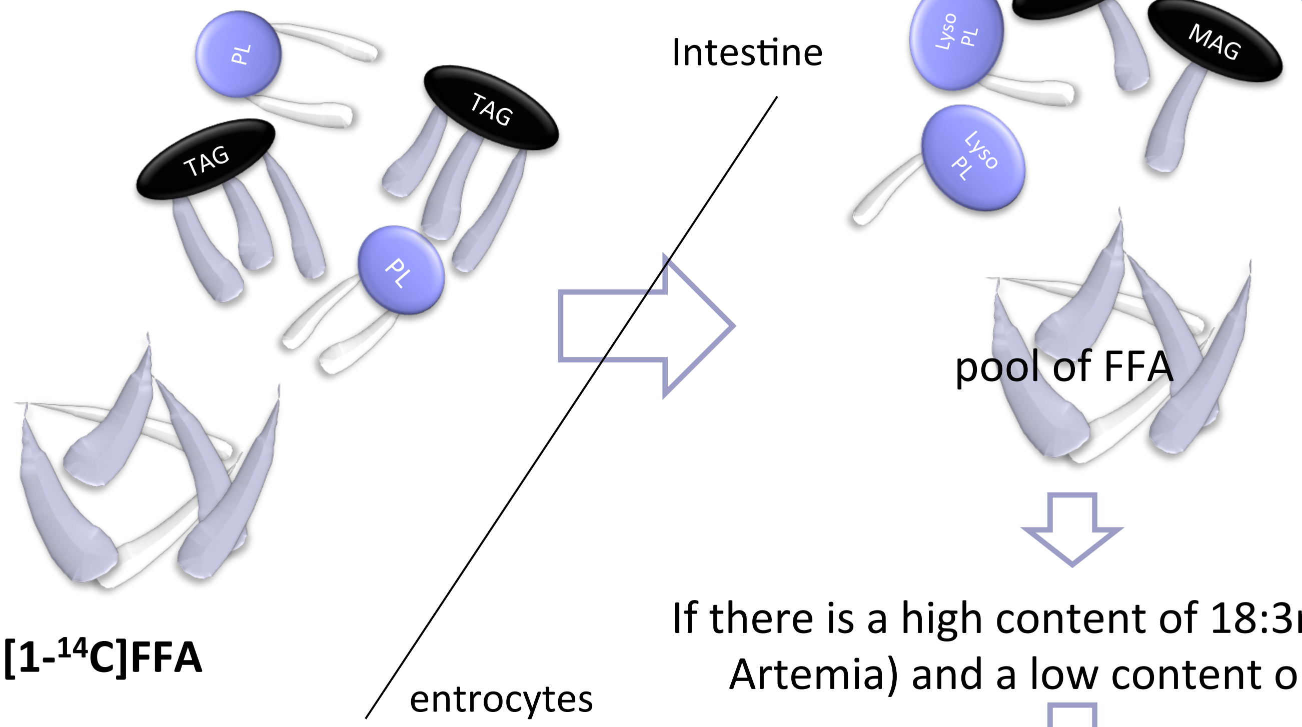
- [1-^{14}C]18:2n-6
- [1-^{14}C]18:3n-3
- [1-^{14}C]20:4n-6 (ARA)
- [1-^{14}C]20:5n-3 (EPA)
- [1-^{14}C]22:6n-3 (DHA)

- PC PHOSPHATIDYLCHOLINE L-∝-1-PALMOTOYL-2-ARACHIDONYL- **[ARACHIDONYL-1-^{14}C]**
- PE PHOSPHATIDYLETHANOLAMINE L-∝-1-PALMOTOYL-2-ARACHIDONYL- **[ARACHIDONYL-1-^{14}C]**

LATER ON……ONLY IN HALIBUT

- MAG MONOACYGLYCERIDE **[2-MONO OLEOYL-1-^{14}C GLYCEROL]**
- TAG TRIACYGLYCERIDE **[1-2-3 TRIOLEOLEIN-1-^{14}C]**

What we want to demonstrate



PUFA (**18:2n-6;18:3n-3**)

LC-PUFA (**ARA; EPA; DHA**)

If there is a high content of 18:3n-3 (from Artemia) and a low content on DHA

High probability for re-acylation of 18:3n-3 over DHA in PL

An adequate and balanced dietary input of FA and PL might be crucial in these species development

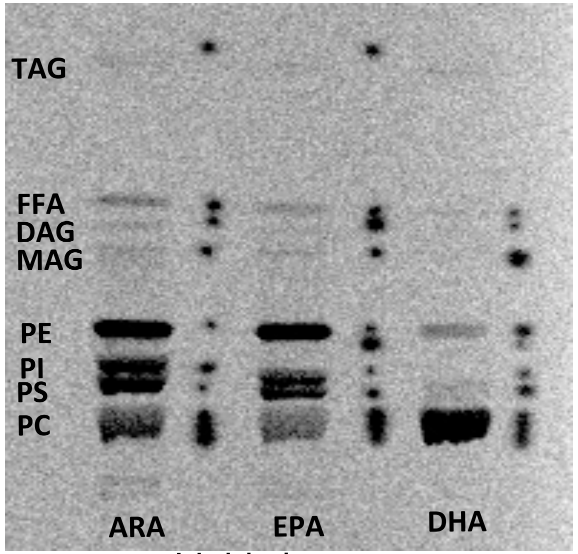
Material & Methods

1º [1-^{14}C]FA incorporation into TL

Rodríguez et al., 2002
ß-counter

2º [1-^{14}C]FA esterification into LC

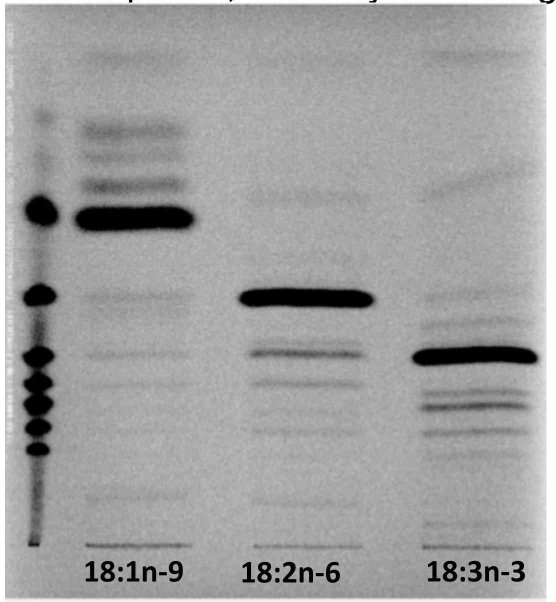
Tocher and Harvie 1988; Díaz-Lopez et al., 2010
Exposure Cassete-K, Image Screen-K, BioRad



Lipid classes
HPTLC plates, Quantity One image

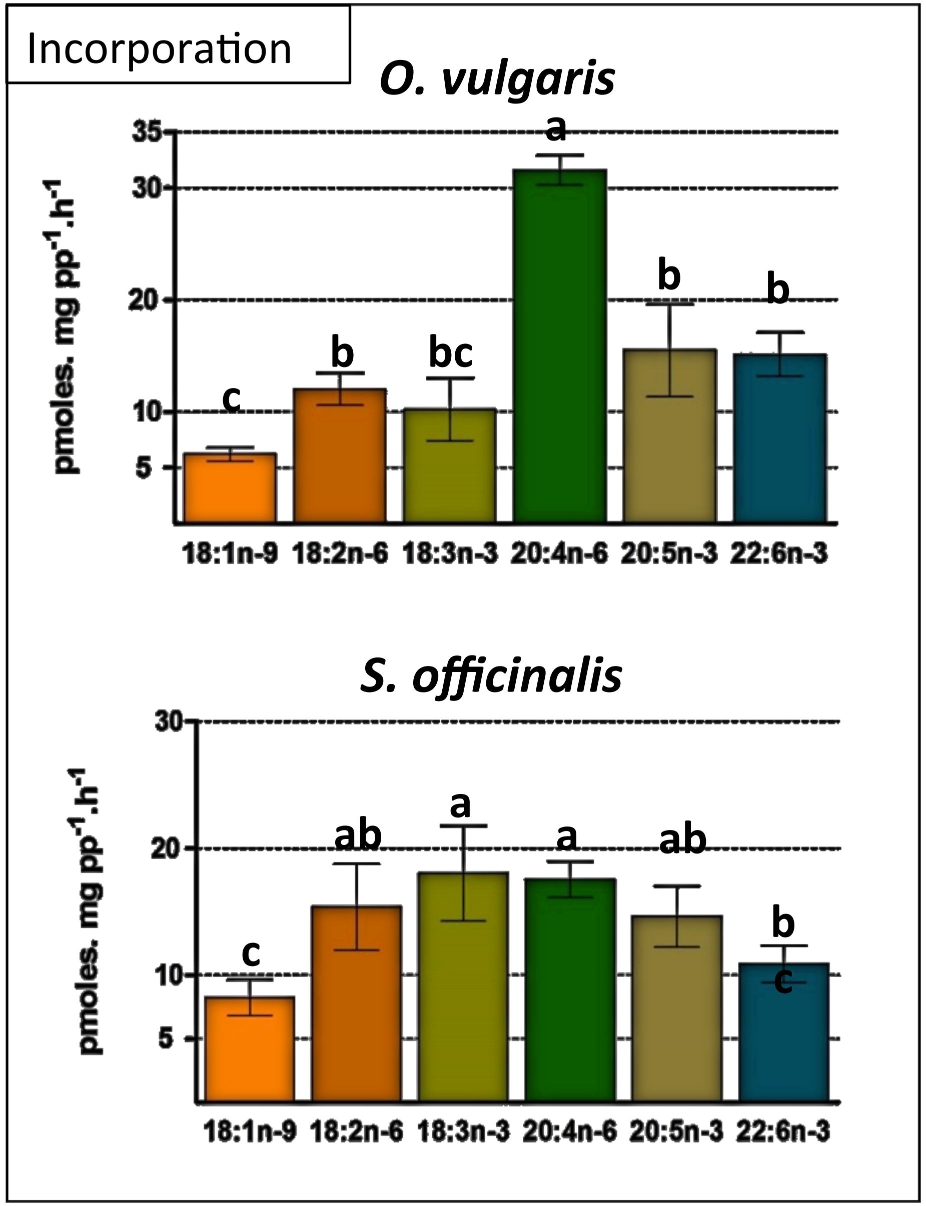
3º [1-^{14}C]FA transformation by elongation and desaturation. Complementary to gene cloning and expression.

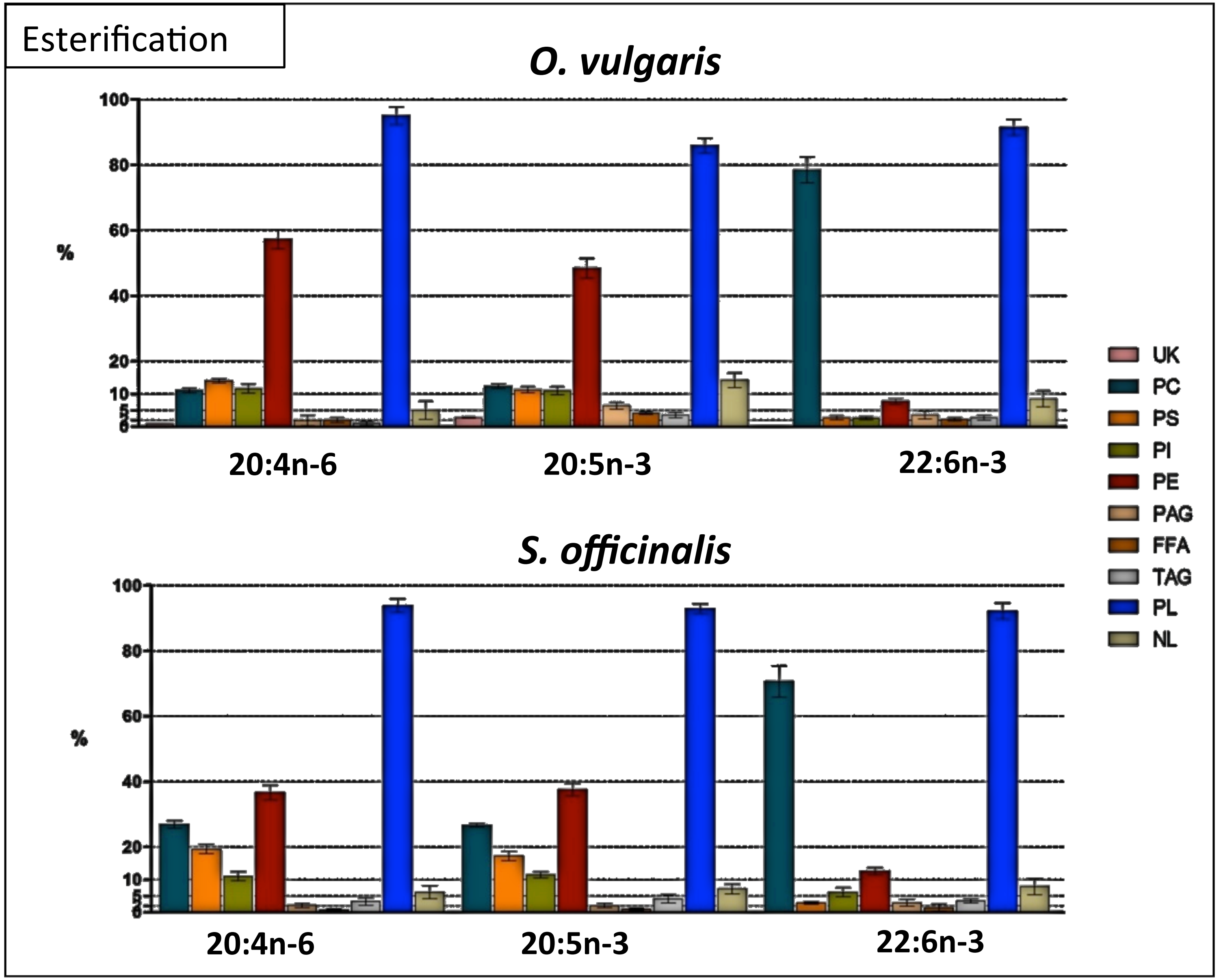
Rodríguez et al., 2002; Díaz-Lopez et al., 2010
Exposure Cassete-K, Image Screen-K, BioRad



Fatty acids
TLC plates, Quantity One image

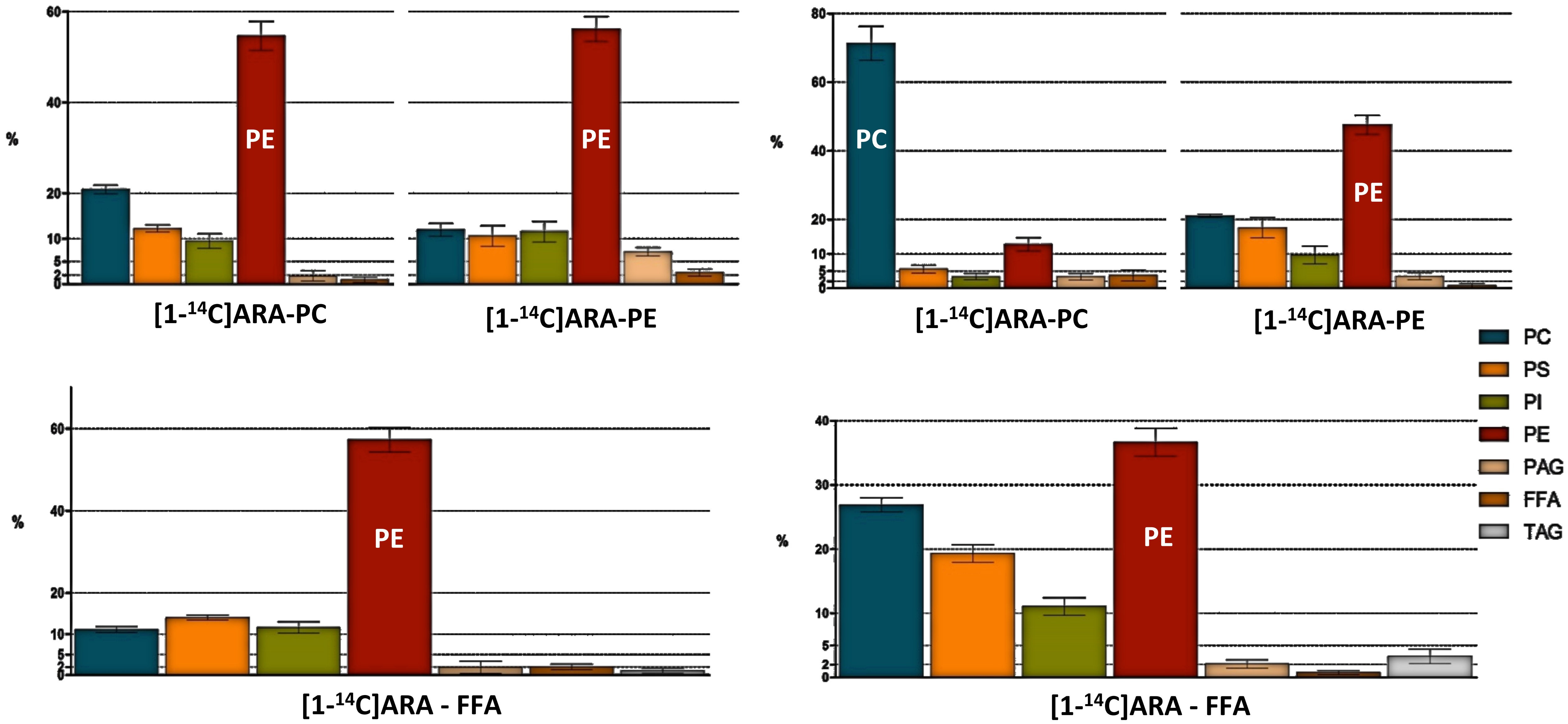
Results – Octopus vulgaris and Sepia officinalis





Results – Re-esterification pattern into Octopus and Sepia LC

O. vulgaris | *S. officinalis*



1. Why is ARA so specifically esterified into PE or PC and not into PI???

2. Efficient and consistent method for specific *in vivo* studies on lipid metabolism.

GWP
Nutrition WP10, WP11
Pikeperch and Halibut

DTU-ULL-FUNDP-FCPCT

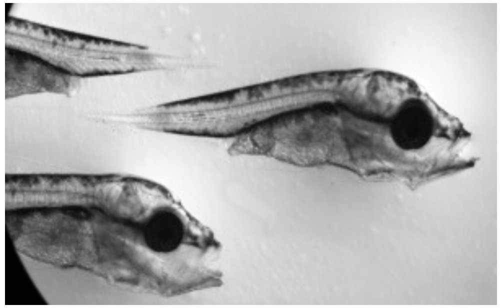
NIFES-IMR-ULL

SOME BACKGROUND ON RAINBOW TROUT



1. **Can the subtrates cross the integument?** **YES**
2. **Even being a freshwater fish, are they drinking actively so as to incorporate FFA, PC and PE substrates added to the culture water?** **YES**

Task 10.2; Task 11.4 **ARTEMIA FEEDING**



■ Pikeperch (*Sander lucioperca*)

- Freshwater species, with some characteristics in common with marine carnivorous fish larvae.
- 3-4 mm size, mouth opening 3-4dph, PLs high demand for **DHA.** Rotifer and Artemia.

■ Atlantic halibut (*Hippoglossus hippoglossus*)

- Marine species.
- The yolk sac stage is approximately of 230 daydegrees, taking around 30 days at 6-7ºC, to open the mouth.
- Larvae are approximately 12 mm in standard length (SL) at first-feeding (dpff) and, because of their relatively large larval size, they are first fed on Artemia.

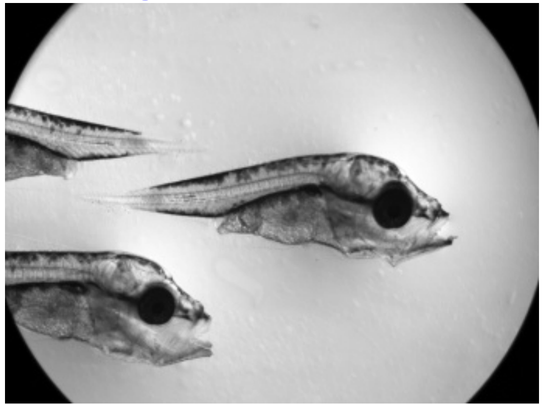
Task 10.2 ARTEMIA FEEDING Task 11.4

PIKEPERCH 20dph (Artemia)

- **2 diets** (18:2n-6 or 18:3n-3 rich diet)
- **3 salinities** (0, 5, 10 ppt)
- **1 control**, 0 ppt, LC-PUFA rich diet
- **10 pikeperch larvae** (20 dph) per well
- **10 ml of water** (0, 5, 10 ppt)
- **4 h** incubation
- 0.2 µCi (**0.3 µM**) of [1-^{14}C]FA, [1-^{14}C]PC, [1-^{14}C]PE
- n = 3

HALIBUT 30dpff (Artemia)

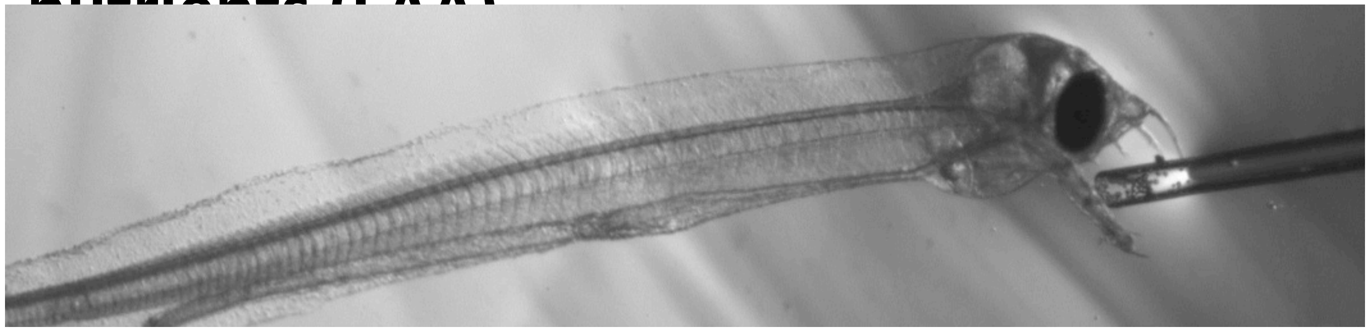
- **2 rearing systems** (flow-trough or raceway)
- **2 halibut larvae** (30 dpff; 65dph) per well
- **10 ml of water**
- **4 h** incubation
- 0.2 µCi (**0.3 µM**) of [1-^{14}C]FA, [1-^{14}C]PC, [1-^{14}C]PE, **[1-^{14}C]MAG, [1-^{14}C]TAG**
- n = 3



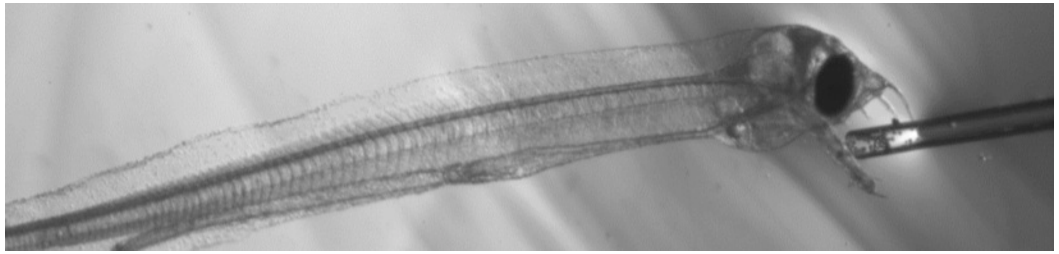
Eel (*Anguilla Anguilla*)

DTU I.Lund/Jonna Tomkiewicz

- Marine as a larvae.
- Opens the mouth 9-10dph (18-20ºC).
- 10-13dph normally yolk sac is absorbed.
- Larvae are not eating or a few are eating, but no improved survival.
- Force-feeding in which *in vivo* studies target digestibility and assimilation of key nutrients using radiolabeled dietary nutrients (FAA)



Eel (*Anguilla anguilla*)



NOT FEEDING

- **10 eel larvae** per well
- **10 ml of water**
- **4 h** incubation
- 0.2 µCi (**0.3 µM**) of [1-^{14}C]FA, [1-^{14}C]PC, [1-^{14}C]PE

DTU-ULL *in vivo* studies

4 dph

8 dph

12 dph

pmoles mg pp-1 h-1	4 DPH			8 DPH			12 DPH		
PC	9.8	±	3.8	13.2	±	1.9	1.5	±	0.2
PE	6.6	±	1.8	11.1	±	0.7	2.5	±	0.7

Control larvae composition, no added ^{14}C

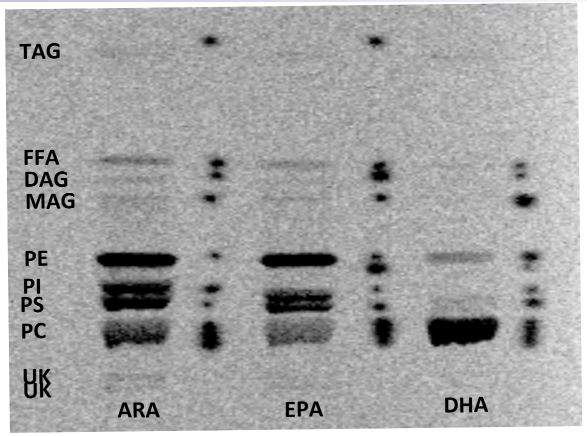
	Pikeperch 20dph	Halibut 30dpff	Eel 12dph
18:2n-6	6.13 ± 0.03	4.77 ± 0.05	2.30 ± 0.10
18:3n-3	12.44 ± 0.91	9.72 ± 0.78	**0.00** ± 0.00
20:4n-6	1.62 ± 0.04	4.25 ± 0.12	**7.50** ± 0.80
20:5n-3	5.30 ± 0.04	**7.37** ± 0.26	2.10 ± 0.30
22:6n-3	**10.39** ± 0.13	**11.45** ± 0.81	**9.80** ± 1.60
PC	**17.71** ± 0.93	**20.32** ± 1.26	**12.40** ± 1.60
PI	3.44 ± 0.20	**5.49** ± 0.15	2.50 ± 0.40
PE	11.27 ± 0.20	**19.18** ± 1.03	7.60 ± 0.00
TAG	**24.55** ± 1.42	7.44 ± 0.55	15.20 ± 0.20
MAG	3.76 ± 0.90	3.67 ± 0.23	5.00 ± 0.10

Total Incorporation of [1-^{14}C]FA or [1-^{14}C]LC (pmoles mg pp^{-1} h^{-1})

	Pikeperch 20dph	Halibut 30dpff	Eel 12dph
Substrate			
18:2n-6	7.5 ± 1.4	4.7 ± 1.0	15.0 ± 5.0
18:3n-3	9.4 ± 2.0	8.4 ± 5.5	**31.0 ± 10.5**
20:4n-6	**20.6 ± 4.3**	6.5 ± 3.1	11.2 ± 3.3
20:5n-3	**38.1 ± 13.3**	8.2 ± 0.3	**39.0 ± 7.9**
22:6n-3	6.2 ± 1.3	3.1 ± 2.6	-
PC	**8.9 ± 5.1**	0.9 ± 0.4	1.5 ± 0.2
PE	3.5 ± 1.2	1.0 ± 0.2	2.5 ± 0.7
TAG	-	0.5 ± 0.0	-
MAG	-	**13.6 ± 3.3**	-

Results – *Esterification patterns* [1-^{14}C]FFAs

- [1-^{14}C]18:2n-6
- [1-^{14}C]18:3n-3
- [1-^{14}C]20:4n-6 (ARA)
- [1-^{14}C]20:5n-3 (EPA)
- [1-^{14}C]22:6n-3 (DHA)



Lipid classes
HPTLC plates, Quantity One image

- In **Pikeperch** and **Halibut** larvae, **all [1-^{14}C]FFAs** mainly **esterified into PC** (≈ 50% for pikeperch and 35% in halibut), with the **exception of ARA**, with higher esterification **into PI** (35-40%), followed by PC.
- In **Eel** larvae **all [1-^{14}C]FFAs** were mainly **esterified into PC and interestingly only 9 % of ARA** was esterified **into PI.**

- The high content of 18:3n-3 naturally present in the Artemia may particularly compete with LC-PUFA for esterification into specific polar lipids.

- From the poor incorporation of DHA in pikeperch, it should be advisable a specific enrichment with DHA prior to other FAs enrichment.

Results – Re-esterification patterns of [1-^{14}C] from *lipid classes PC, PE, MAG, TAG*

Pikeperch –**ARA** bounded to **PC or PE** mainly goes **into PI**.

Habilut – **ARA** bounded to **PC**, **mainly goes** **into PI**

ARA bounded to **PE**, mainly goes into **PE or PC** and **only 16% as PI**.

Radioactivity in **oleic acid** provided as:

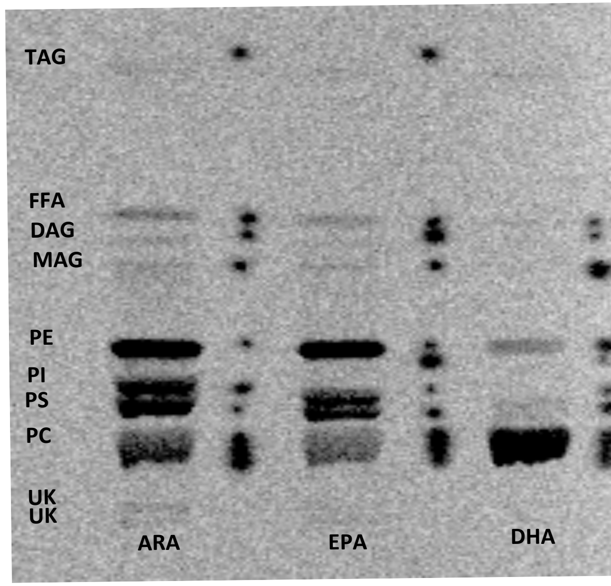
MAG –> PC (25%)

TAG –> **TAG (25%)** > PAG (20%)

Eel – **ARA** bounded to **PC**, **mainly goes** **into PC**

ARA bounded to **PE**, mainly goes into **PE or PC**,

less 10% in PI…….Why???



Results seems to be in agreement with DIVERSIFY results:

- Pikeperch larvae performs optimally with high dietary inclusion levels of phospholipids (in terms of soya lecithin).
- Halibut growth (from 0.92g), did not benefit from dietary increasing contents of PLs.

- **Although studied species have the capacity to remodelate dietary phospholipis, this capacity greatly varies among the species.**

- **It seems advisible to feed halibut larvae with pre-digested lipid molecules (MAG and FFA) specially to ensure for LC-PUFA incorporation in tissue PLs.**

Results – *Elongation-Desaturation patterns*

Halibut

Substrate	Product	Flow-through	Raceway
[1-^{14}C]18:2n-6			
	18:2n-6	57.8 ± 5.9	57.5 ± 9.2
	20:2n-6	7.8 ± 2.2	8.8 ± 0.3
	18:3n-6	9.3 ± 0.8	4.3 ± 1.9
	20:3n-6	3.5 ± 2.4	4.6 ± 2.0
	de novo	9.1 ± 1.8	10.0 ± 2.6
	UK	12.7 ± 2.2	14.9 ± 2.5
[1-^{14}C]18:3n-3			
	18:3n-3	74.5 ± 1.8	71.6 ± 4.8
	20:3n-3	17.4 ± 1.9	17.8 ± 3.9
	de novo	4.6 ± 1.0	5.3 ± 2.3
	UK	3.6 ± 1.4	5.3 ± 0.8
[1-^{14}C]20:5n-3			
	20:5n-3	93.5 ± 2.0	92.7 ± 1.8
	22:5n-3	6.5 ± 2.0	7.3 ± 1.8

MAINLY ELONGATION

1. **Accordingly to its carnivorous condition, a very poor capacity to produce ARA, EPA or DHA from dietary precursors, has been found in both pikeperch and halibut larvae.**
2. **Although some delta 6 activity is evident, it cannot compensate decrements of EFA caused by LC-PUFA deficient diets.**

Universidad de La Laguna

Facultad de Ciencias
Sección de Biología

COOPERATION

DIVERSIFY

THANK YOU VERY MUCH FOR YOUR ATTENTION