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

Differ from wild composition

\[ \downarrow \text{Phospholipids PE; PC; PI} \quad \downarrow \text{DHA-ARA-EPA} \]

\[ \uparrow \text{TAG} \quad \uparrow \text{18:3n-3-18:2n-6} \]

Origin

Availability and prices of ingredients
- Broodstock diets

Type of prey and enrichment protocols
- Unknown requirements/Digestive enzymes

25% 18:3n-3
- DHA into EPA

Poorer egg and larval quality and performance
Reproduction

Yolk composition

LARVAL PERFORMANCE

PHOSPHOLIPIDS

CHOLESTEROL

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

LC-PUFA
or HUFA

PHOSPHOLIPIDS

Outside of cell

Sphingomyelin
Glycolipid
Phosphatidylcholine
Cholesterol

Phosphatidylserine
Phosphatidylinositol
Phosphatidylethanolamine

Cytosol
PI/ARA
PE

PC

Reproduction

Yolk composition

LARVAL PERFORMANCE
More Background

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……
Are these enzymes active or efficient enough in all our novel species?
VERTEBRATES PATHWAYS FOR LONG CHAIN PUFA (LC-PUFA)

18:0

18:1n-9

Δ9

Δ6

18:2n-9

Δ6

18:3n-6

Δ12

Δ6

18:3n-6

Δ6

18:4n-3

Δ12

Δ6

18:4n-3

Δ6

18:5n-3

Δ12

Δ6

18:5n-3

Δ6

22:6n-3

Δ4

22:6n-3

Δ6

22:6n-3

Δ6

22:5n-6

Δ6

22:5n-6

Δ6

22:5n-6

Δ6

22:5n-6

ESSENTIAL FATTY ACIDS (EFA)

18:2n-6, linoleic acid
18:3n-3, linolenic acid
20:5n-3, EPA
20:4n-6, ARA
22:6n-3, DHA

Sole
Aterinids
Siganus

MARINE AND/OR CARNIVOROUS
Figure 1. PUFA synthetic pathway to 22-carbon fatty acids from oleic acid in eukaryotic systems.
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.
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.
Material & Methods

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

- [1-\(^{14}\text{C}\)]18:2n-6
- [1-\(^{14}\text{C}\)]18:3n-3
- [1-\(^{14}\text{C}\)]20:4n-6 (ARA)
- [1-\(^{14}\text{C}\)]20:5n-3 (EPA)
- [1-\(^{14}\text{C}\)]22:6n-3 (DHA)
- **PC** PHOSPHATIDYLCHOLINE L-\(\alpha\)-1-PALMOTOYL-2-ARACHIDONYL-[ARACHIDONYL-1-\(^{14}\text{C}\)]
- **PE** PHOSPHATIDYLETHANOLAMINE L-\(\alpha\)-1-PALMOTOYL-2-ARACHIDONYL-[ARACHIDONYL-1-\(^{14}\text{C}\)]

Later on......only in halibut

- **MAG** MONOACYGLYCYRIDE [2-MONO OLEOYL-1-\(^{14}\text{C}\) GLYCEROL]
- **TAG** TRIACYGLYCERIDE [1-2-3 TRIOLEOLEIN-1-\(^{14}\text{C}\)]
Results & Discussion

InteJne

entrocytes

Lyso

PL

to

pool of FFA

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-\textsuperscript{14}C]FA incorporation into TL
Rodríguez et al., 2002
β-counter

2º [1-\textsuperscript{14}C]FA esterification into LC
Tocher and Harvie 1988; Díaz-Lopez et al., 2010
Exposure Cassette-K, Image Screen-K, BioRad

3º [1-\textsuperscript{14}C]FA transformation by elongation and desaturation.
Complementary to gene cloning and expression.
Rodríguez et al., 2002; Díaz-Lopez et al., 2010
Exposure Cassette-K, Image Screen-K, BioRad
Results – Octopus vulgaris and Sepia officinalis

**Incorporation**

**O. vulgaris**

- 18:1n-9
- 18:2n-6
- 18:3n-3
- 20:4n-6
- 20:5n-3
- 22:6n-3

**S. officinalis**

- 18:1n-9
- 18:2n-6
- 18:3n-3
- 20:4n-6
- 20:5n-3
- 22:6n-3

**Esterification**

**O. vulgaris**

- 20:4n-6
- 20:5n-3
- 22:6n-3

**S. officinalis**

- 20:4n-6
- 20:5n-3
- 22:6n-3
Results – Re-esterification pattern into Octopus and Sepia LC

**O. vulgaris**

- [1-\(^{14}\)C]ARA-PC
- [1-\(^{14}\)C]ARA-PE

**S. officinalis**

- [1-\(^{14}\)C]ARA-PC
- [1-\(^{14}\)C]ARA-PE

**[1-\(^{14}\)C]ARA - FFA**
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
1. Can the substrates 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**
Background

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 6-7°C, 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.
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 [1-14C]FA, [1-14C]PC, [1-14C]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 [1-14C]FA, [1-14C]PC, [1-14C]PE, [1-14C]MAG, [1-14C]TAG
- n = 3
• 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

<table>
<thead>
<tr>
<th>pmoles mg pp-1 h-1</th>
<th>4 DPH</th>
<th>8 DPH</th>
<th>12 DPH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PC</strong></td>
<td>9.8 ± 3.8</td>
<td>13.2 ± 1.9</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td><strong>PE</strong></td>
<td>6.6 ± 1.8</td>
<td>11.1 ± 0.7</td>
<td>2.5 ± 0.7</td>
</tr>
</tbody>
</table>
Control larvae composition, no added $^{14}$C

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

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

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Pikeperch 20dpf</th>
<th>Halibut 30dpff</th>
<th>Eel 12dpf</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2n-6</td>
<td>7.5 ± 1.4</td>
<td>4.7 ± 1.0</td>
<td>15.0 ± 5.0</td>
</tr>
<tr>
<td>18:3n-3</td>
<td>9.4 ± 2.0</td>
<td>8.4 ± 5.5</td>
<td><strong>31.0 ± 10.5</strong></td>
</tr>
<tr>
<td>20:4n-6</td>
<td><strong>20.6 ± 4.3</strong></td>
<td>6.5 ± 3.1</td>
<td>11.2 ± 3.3</td>
</tr>
<tr>
<td>20:5n-3</td>
<td><strong>38.1 ± 13.3</strong></td>
<td>8.2 ± 0.3</td>
<td><strong>39.0 ± 7.9</strong></td>
</tr>
<tr>
<td>22:6n-3</td>
<td>6.2 ± 1.3</td>
<td>3.1 ± 2.6</td>
<td>-</td>
</tr>
<tr>
<td>PC</td>
<td><strong>8.9 ± 5.1</strong></td>
<td>0.9 ± 0.4</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>PE</td>
<td>3.5 ± 1.2</td>
<td>1.0 ± 0.2</td>
<td>2.5 ± 0.7</td>
</tr>
<tr>
<td>TAG</td>
<td>-</td>
<td>0.5 ± 0.0</td>
<td>-</td>
</tr>
<tr>
<td>MAG</td>
<td>-</td>
<td><strong>13.6 ± 3.3</strong></td>
<td>-</td>
</tr>
</tbody>
</table>
Results – **Esterification patterns** [1-\(^{14}\)C]FFAs

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

In **Pikeperch** and **Halibut** larvae, all [1-\(^{14}\)C]FFAs mainly esterified into PC (\(\approx 50\%\) 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-\textsuperscript{14}C] from lipid classes \textit{PC, PE, MAG, TAG}

### Pikeperch
- ARA bounded to \textit{PC} or \textit{PE} mainly goes into \textit{PI}.

### Habilut
- ARA bounded to \textit{PC}, mainly goes into \textit{PI}
- ARA bounded to \textit{PE}, mainly goes into \textit{PE} or \textit{PC} and only 16\% as \textit{PI}.

Radioactivity in \textit{oleic acid} provided as:
- \textit{MAG} → \textit{PC} (25\%)
- \textit{TAG} → \textit{TAG} (25\%) > \textit{PAG} (20\%)

### Eel
- ARA bounded to \textit{PC}, mainly goes into \textit{PC}
- ARA bounded to \textit{PE}, mainly goes into \textit{PE} or \textit{PC}, less 10\% in \textit{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 phospholipids, this capacity greatly varies among the species.

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

### Results – Elongation-Desaturation patterns

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
<th>Flow-through</th>
<th>Raceway</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1-$^{14}$C]18:2n-6</td>
<td>18:2n-6</td>
<td>57.8 ± 5.9</td>
<td>57.5 ± 9.2</td>
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<tr>
<td></td>
<td>20:2n-6</td>
<td>7.8 ± 2.2</td>
<td>8.8 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>18:3n-6</td>
<td>9.3 ± 0.8</td>
<td>4.3 ± 1.9</td>
</tr>
<tr>
<td></td>
<td>20:3n-6</td>
<td>3.5 ± 2.4</td>
<td>4.6 ± 2.0</td>
</tr>
<tr>
<td>de novo</td>
<td>9.1 ± 1.8</td>
<td>10.0 ± 2.6</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>12.7 ± 2.2</td>
<td>14.9 ± 2.5</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Substrate</th>
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<th>Flow-through</th>
<th>Raceway</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1-$^{14}$C]18:3n-3</td>
<td>18:3n-3</td>
<td>74.5 ± 1.8</td>
<td>71.6 ± 4.8</td>
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<td></td>
<td>20:3n-3</td>
<td>17.4 ± 1.9</td>
<td>17.8 ± 3.9</td>
</tr>
<tr>
<td>de novo</td>
<td>4.6 ± 1.0</td>
<td>5.3 ± 2.3</td>
<td></td>
</tr>
<tr>
<td>UK</td>
<td>3.6 ± 1.4</td>
<td>5.3 ± 0.8</td>
<td></td>
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</table>

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Product</th>
<th>Flow-through</th>
<th>Raceway</th>
</tr>
</thead>
<tbody>
<tr>
<td>[1-$^{14}$C]20:5n-3</td>
<td>20:5n-3</td>
<td>93.5 ± 2.0</td>
<td>92.7 ± 1.8</td>
</tr>
<tr>
<td></td>
<td>22:5n-3</td>
<td>6.5 ± 2.0</td>
<td>7.3 ± 1.8</td>
</tr>
</tbody>
</table>
THANK YOU VERY MUCH FOR YOUR ATTENTION