# A NEW METHOD FOR THE STUDY OF ESSENTIAL FATTY ACID REQUIREMENTS IN FISH LARVAE

S. Morais [1]
D. Alves Martins [1]
F. Castanheira [1]
A. Mendes [2]
J. Coutinho [3]
N. M. Bandarra [3]
P. Pousão-Ferreira [2]
L.E.C. Conceição [1]

[1]CCMAR
Centro de Ciências do Mar do Algarve
Univ. Algarve, Campus de Gambelas
Faro, Portugal

[2]INRB I.P.-IPIMAR-EPPO
Av. 5 Outubro s/n 8700-305 Olhão, Portugal

[3]INRB I.P.-IPIMAR-UVPPA
Av. de Brasília 1449-006 Lisboa, Portugal

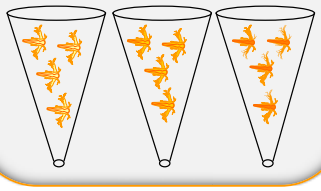
## INTRODUCTON

The provision of adequate dietary levels of essential fatty acids (EFA) for early developing fish is critical for the success of larval rearing. The recent development of new products and live food enrichment methodologies has enabled the use of dose-response designs to study EFA requirements in marine fish larvae. However, common indicators of larval performance and quality might not be the most sensitive to determine exact EFA requirements.

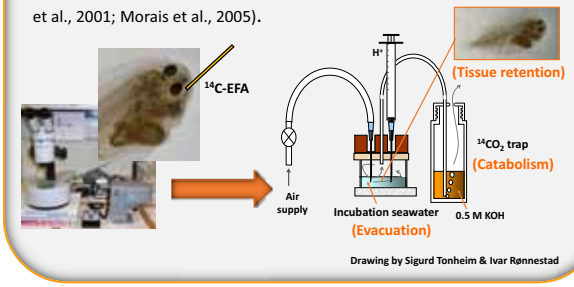
***OUR HYPOTHESIS:*** EFA, DHA in particular, are a poor substrate for β-oxidation and are mostly retained in body tissues (structural role). Dietary EFA supply above larval requirements should result in its increased oxidation, so that the requirement for a particular EFA might be determined as the level from which it becomes significantly more catabolised (Morais and Conceição, 2009).

## PROPOSED METHOD

**1. Dose-response dietary trial**
Supplying graded concentrations of EFA (microdiets or live preys enriched with oils rich in the EFA of interest (e.g., DHASCO, ARASCO, Martek; and EPA-Incromega, Croda).



**2. Metabolic study** (quantification of EFA metabolic utilization)
Tube feeding of $^{14}$C-labelled fatty acids and transfer into an incubation setup containing a metabolic chamber (Rønnestad et al., 2001; Morais et al., 2005).

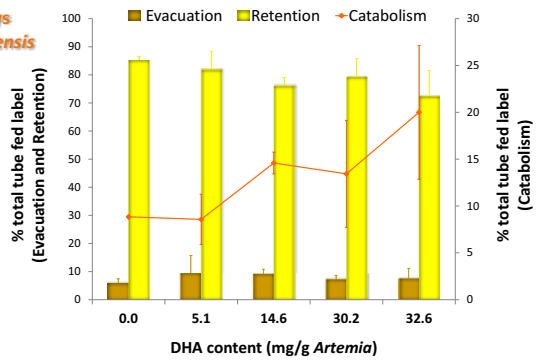



## SOME PRELIMINARY RESULTS

$^{14}$C-DHA tube feeding and metabolic trial with 33 days after hatch. *Solea senegalensis*

Fatty acid composition (mg/g *Artemia*) of *Artemia* enriched with oil emulsions containing increasing amounts of DHASCO (Martek Biosciences).

| | 0 | 5.1 | 14.6 | 30.2 | 32.6 |
|---|---|---|---|---|---|
| Tot. PUFA | 58.7 | 59.9 | 68.1 | 86.4 | 74.1 |
| ARA | 3.0 | 4.8 | 5.3 | 3.4 | 7.6 |
| EPA | 1.9 | 3.2 | 3.7 | 5.8 | 1.6 |
| DHA | 0.0 | 5.1 | 14.6 | 30.2 | 32.6 |
| DHA/EPA | 0.0 | 1.6 | 3.9 | 5.2 | 20.9 |
| EPA/ARA | 0.6 | 0.7 | 0.7 | 1.7 | 0.2 |




- It has been confirmed that the percentage of catabolised DHA increases with dietary supply, indicating that dietary DHA above larval requirements may result in its higher oxidation for energy purposes (as seen in Morais and Conceição, 2009).
- An experiment with a finer gradient of DHA levels (5-15 mg/g *Artemia*) should be conducted to determine more accurately the dietary requirement for DHA of Senegalese sole post-larvae.
- DHA/EPA ratio does not appear to affect substantially DHA catabolism (comparison diets 30.2 and 32.6) but variability of data was very high at high DHA inclusion levels; further studies are required to confirm this.

## CONCLUSIONS & PERSPECTIVES

The catabolism of a $^{14}$C-radiolabelled EFA tracer in response to graded dietary levels (dose-response studies) is a potentially reliable indicator for the study of EFA requirements (at least for DHA), as well as to investigate EFA interactions, which are difficult to address using classical methodologies. The anticipated advantages of this method are a finer discrimination between dietary EFA levels and the ability to screen a large number of dietary treatments in a shorter period of time, before other signs of EFA deficiency are visible, requiring only small-scale experimental facilities.

**References:**
•Rønnestad, I, Rojas-García, CR, Tonheim, SK, Conceição, LEC (2001) Aquaculture 201, 161-175.
•Morais, S, Koven, W, Rønnestad, I, Dinis, MT, Conceição, LEC (2005) Br. J. Nutr. 93, 813-820.
•Morais, S, Conceição, LEC (2009) Br. J. Nutr. 101, 1564–1568.

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