Universidad de La Laguna

Facultad de Ciencias
Sección de Biología

Some approaches to improve the nutrition and husbandry of DIVERSIFY´s target species. A ULL collaborative contribution.

ACM 2017
Barcelona, 17-19 January 2017

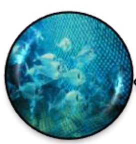
MAIN WORK PACKAGES (WP) & RESEARCH ACTIVITIES

Project Management & Dissemination

Reproduction & Genetics

Nutrition

Larval husbandry

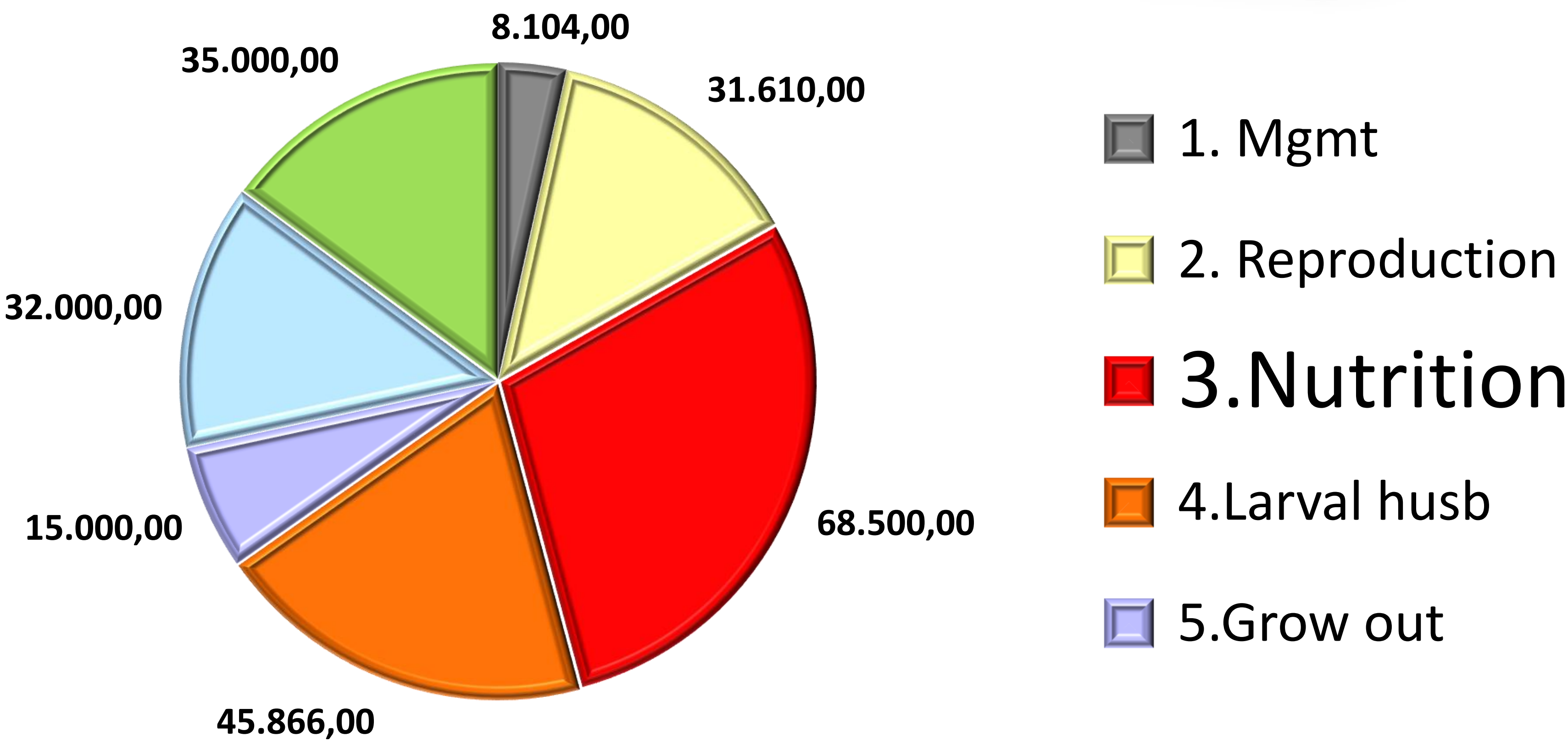


Grow out husbandry

Fish Health

Socioeconomics

Total EU Funded: 236.080,00 €



"To feed the nine billion people expected in 2050, agricultural production must increase by 70%."

Relevance of Aquaculture food production

APROMAR

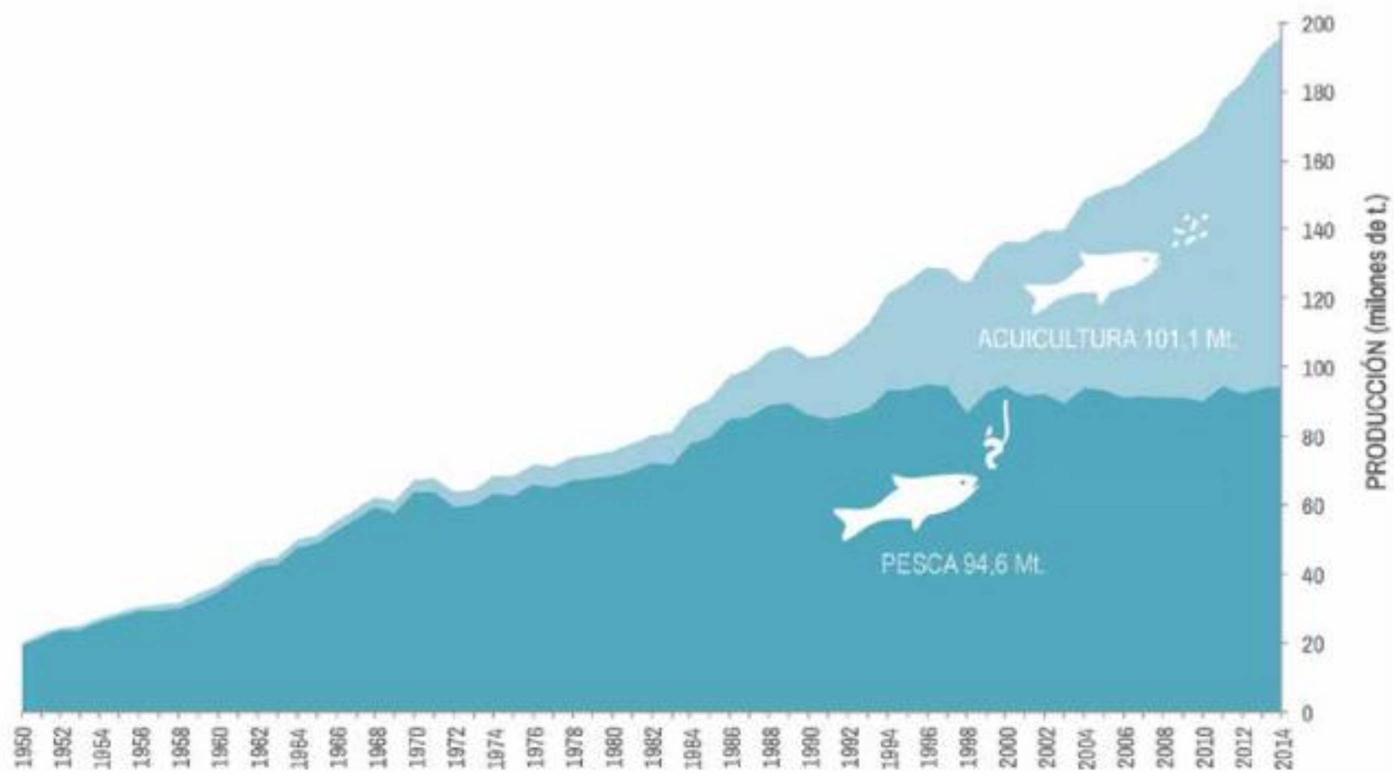


Figura 1. Evolución de la producción acuática mundial (acuicultura más pesca) en el periodo 1950-2014 (FAO).

Nowadays, aquaculture provides half of all fish supplies destined for direct human food consumption.

SOME CONTROVERSIAL ISSUES WITH CONSUMERS DEMANDS FOR HEALTHY AND ENVIRONMENTALLY FRIENDLY AQUACULTURE PRODUCTS

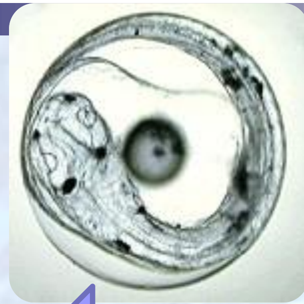
Although providing other important nutrients, fish products are the world's unique source of omega-3 long chain fatty acids (W-3 LCPUFA: EPA and DHA), which are incredibly important for our body and brain, and general health condition.

SUSTAINABILITY

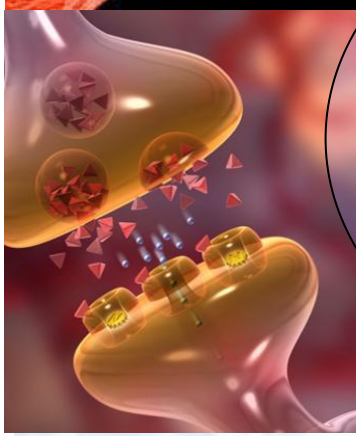
↓ FIFO RATIO

↓ Tissue w3 LC-PUFA

Fish Oil or VO??

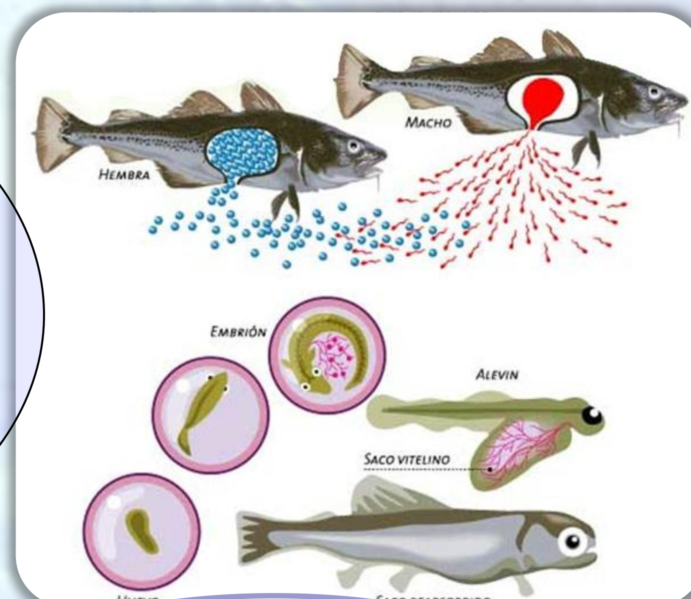


Lipid requirements change along development



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

CAROTENOIDS, FAT SOL. VITs



Reproduction

Regulation of reproductive proccesses and success

Yolk composition

NUTRITION OF NEW SPECIES

When vertebrates parental nutrition is correct, milk and **yolk**, **provides all the nutrients** to cover the initial nutritional requirements. **EXCEPTIONAL**

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

GWP Reproduction & Genetics WP3 Greater Amberjack

ULL, UNIBA, HCMR, IOLR

ACM 2017
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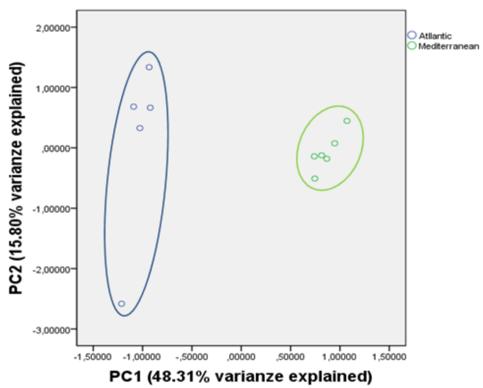
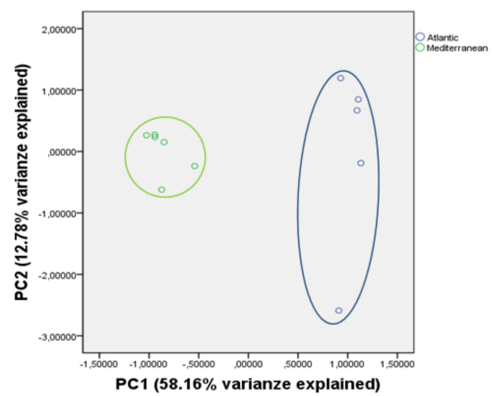
FATTY ACID PROFILE OF WILD GREATER AMBERJACK FEMALE GONADS FROM MEDITERRANEAN AND ATLANTIC AREAS

J. Herrera[1], D. Rodríguez-Barreto[1], N.G. Acosta[1], A. Corriero[3], S. Jerez[2], J.R. Cejas[2], A. Lorenzo[1] and C. Rodriguez[1*]

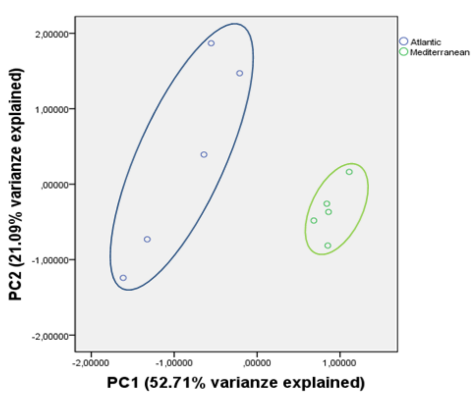
PC fatty acids



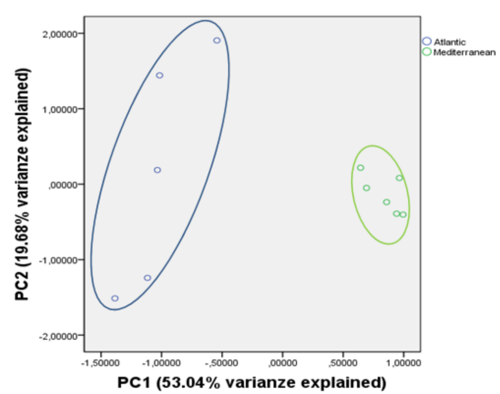
PE fatty acids



TG fatty acids



TL fatty acids



Task 3.1. **Description of the reproductive cycle of greater amberjack (led by UNIBA)**

D3.3. Identification of possible reproductive dysfunction of gametogenesis of greater amberjack reared in captivity based on the comparative evaluation of fish sampled in the wild (DELIVERED)

Study of fish reproductive status by comparison of female and male gonads from captive *vs*. wild broodstocks during three different phases of the reproductive cycle:

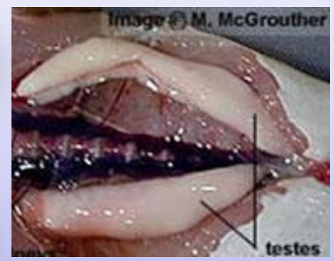
ULL/IEO Background

- Total Lipids from gonads of Atlantic cultured females displayed lower proportion of ARA (20:4n-6) and higher proportions of LA (18:2n-6) and EPA (20:5n-3) than wild specimens (**Rodríguez-Barreto et al., 2012, 2014**)

Task 3.1. Proximate and fatty acid composition and carotenoids from ovaries and testes of Mediterranean specimens.

***Wild* ♀♂**

Gonads



***Captive* ♀♂**

- **Early Gamet. (EG)**
- **Advanced Gamet.(AG)**
- **Spawning (SP)**

Total Protein and Moisture
(AOAC, 2002)

Total Carotenoids
(Barua et al., 1993)

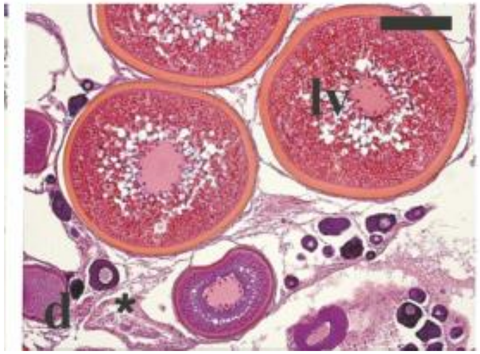
Total Lipid
(Christie, 1982)

Lipid Clases Profile
(Olsen y Henderson , 1984)

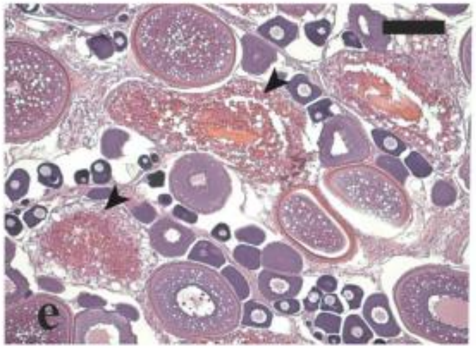
Fatty Acid Composition
(Christie, 1982)

- During the ADVANCED period, when the wild greater amberjack breeders were already in spawning condition, ovaries of captive-reared breeders showed extensive atresia of late vitellogenic oocytes and spermatogenic activity ceased in the testes of half of the examined males. GSI and plasma steroid hormones were lower in captive fish.

- During the SPAWNING period, all captive-reared fish had regressed gonads.



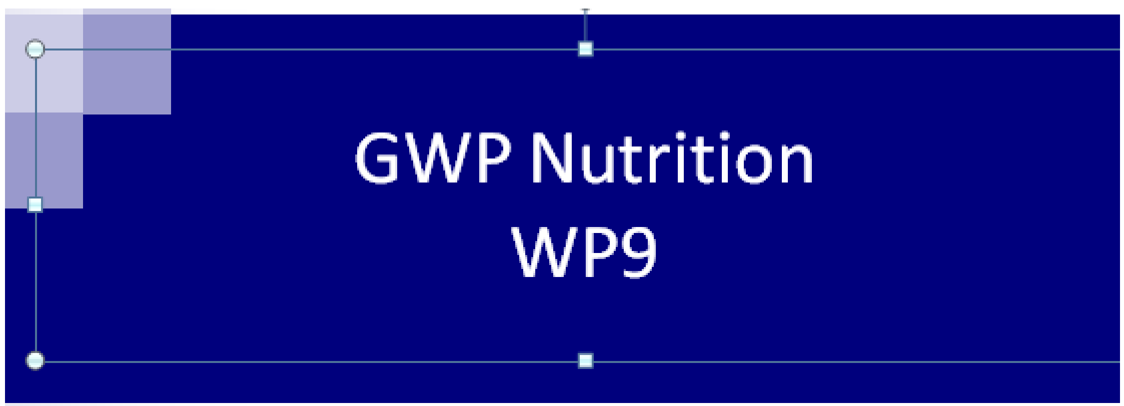
ULL



- During EARLY and ADVANCED period testes and ovaries of captive-reared fish presented ~30-40% less DHA and ARA, and higher LA (18:2n-6) (x5-6) and EPA (x1.2-1.7) contents

- DHA/EPA and ARA/EPA ratios were lower in the gonads of the captive fish.

- **This study underlines the need for an improvement in rearing technology for this species, which should include minimum handling during the reproductive season and the formulation of a specific diet to overcome the observed gonadal decrements of DHA (22:6n-3) and ARA (20:4n-6), compared to wild breeders.**



Task 9.3. **Design adequate feeding regimes for broodstock to optimize reproduction (led by IEO).**

SubTask 9.3.2 **Experimental diets with optimized EFA and carotenoid contents for amberjack broodstock (D 9.4, m58) IEO, ULL**

PLOS | ONE

RESEARCH ARTICLE

Comparative Study of Reproductive Development in Wild and Captive-Reared Greater Amberjack *Seriola dumerili* (Risso, 1810)

Rosa Zupa[1], Covadonga Rodríguez[2], Constantinos C. Mylonas[3], Hanna Rosenfeld[4], Ioannis Fakriadis[3], Maria Papadaki[3], José A. Pérez[2], Chrysovalentinos Pousis[1], Gualtiero Basilone[5], Aldo Corriero[1]*

Published:January 5, 2017

Zupa et al., 2017. PLoSONE 12(1):e.0169645. DOI:10.1371/journal.pone.0169645)

GWPs
Nutrition and Larval Husbandry
WP9/WP15, WP10, WP11

IEO-ULL

HCMR-FCPCT-ULL

DTU-ULL-FUNDP-FCPCT

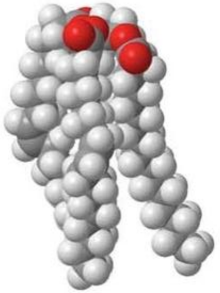
NIFES-IMR-ULL

ACM 2017
Barcelona, 17-19 January 2017

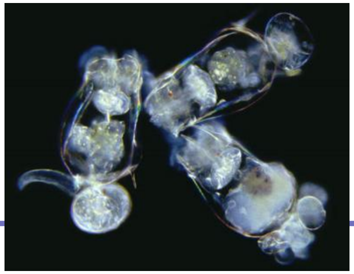
Biochemical composition of eggs and larvae from amberjack reared fish

Greatly differ from wild composition

- ↓Phosfolipids & DHA-ARA
- ↓astaxanthin ester
- ↑TAG & 18:3n-3-18:2n-6

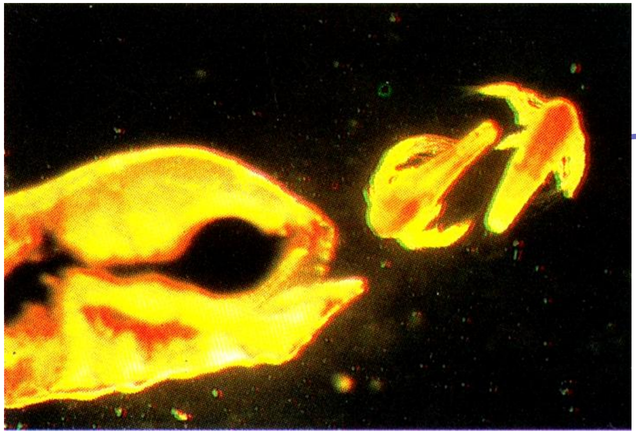


Origin



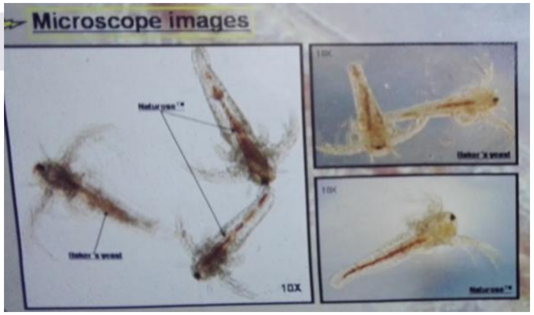
Broodstock diets

Type of prey and Enrichment protocols



Poorer egg and larval quality and performance

Back ground



1. **Most current enrichment protocols for live feed use triacylglycerols (TAG) or fatty acid ethyl esters as dietary lipids, whereas oils rich in phospholipids (PL) have occasionally been used (Li et al. 2014) despite dietary PL seem to be a more efficient source of LC-PUFA for larvae (Olsen et al. 2014).**

2. **Particularly Artemia sp. metanauplii differ from natural preys: converting DHA into EPA and cantaxanthin into astaxanthin; tending to incorporate LC-PUFA into TAG, supplying 25% 18:3n-3……**

SubTask 9.1.2 **Combined effect of PUFA-rich lipids and carotenoids**

(**D9.1, m24; DELIVERED**)

- **1. Preliminary rotifer enrichment assays.**
  - **1.-** Different lipid sources combined to have **LC-PUFA levels and DHA/EPA/ARA ratios to resemble amberjack eggs Polar Lipids (PL)**
  - **2.- The best** treatment **combined with three levels of carotenoids (astaxanthin-Naturose®)**
- **2. Effect on greater amberjack larval rearing.**
  - **Commercial enrichment** compared with **experimental emulsions**
    100-l triplicate tanks, 5000 larvae/tank, continuous water exchange and light
  - **13 days**
- Implementation: IEO, ULL

SubTask 9.1.2 **Effect on greater amberjack larval rearing**

CONCLUSION: Rotifers enriched for 3h with a polar rich emulsion containing a marine natural lecithin (LC60; PC+PE) a supplementacion of 20:4n-6, combined with 10 ppm of Naturose (E1,10), resulted in a significant increment of DHA in larval phospholipids and an advantage for seriola larval growth, survival and welfare.

GWP Larval Husbandry Greater Amberjack

IEO-ULL; HCMR-ULL; FCPCT-ULL

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Back ground

- ***Echium plantagineum*** seeds oil, a very balanced w3/w6 oil rich in SDA and GLA, has proved to retain tissue EPA and DHA, decrease fat deposition and stress symptoms and improve responses to a desease challenge in several fish species (Alhazzaa et al., 2013; Díaz López et al., 2009; Villalta et al., 2007).
- The antibacterial, anti-helmentic, anti-diabetic, antioxidant and anti-inflammatory effects of ***black cumine*** **(*N. sativa*)** seed oil (Atwa, 1997; Awad et al., 2013; John et al., 2007), has been mainly attributed to thymokinone.

Task 15.1 **Effect of feeding regime and immunostimulants in greater amberjack (D15.2; m27)**

1- Product and enrichment time selection in rotifers

T1=commercial protocol (S.presso®);

T2=marine lecithin LC60+AA+10% ppm carotenoids

T3=T2+20% *Echium* oil

T4=T2+10% *Echium* oil

CONCLUSION

The enrichment protocol based on LC60+AA+10 ppm carot. supplemented with 20% *Echium* oil for 3 hours was selected

2- Effects of selected enrichment products supplemented with **immune modulators** substances and **rotifers density** in **larval** rearing of *S. dumerili*

- T1=commercial
- T2=LC60+AA+10 ppm carot.
- T3=T2+20% *Echium* oil
- T4=T2+20% black cumin oil
- Rotifers added at two prey density (5 and 10 rot ml^{-1}) twice a day (8:00 &16:00)

- The commercial treatment (T1) showed the worst results

- Rotifer's density (5-10 rot ml^{-1}) did not affect larval performance

3- Effects of selected enrichment products supplemented with **immune modulators** substances and rotifers supply frequency in **larval** rearing of *S. dumerili* (2 assays)

- ❑T1=commercial
- ❑ T2=LC60+AA+10 ppm carot.
- ❑ T3=T2+20% *Echium* oil
- ❑T4=T2+20% black cumin oil.
- ❑ Rotifers (5 rot ml^{-1}) two (10:30 & 20:30) or three times a day (10:30, 15:30 & 20:30)

- Feeding frequency neither affected larval growth, swim bladder and eye development, nor survival
- The protease alkaline and lipase activities were higher in black cumin oil treatment
- In the black cumin oil treatment, a surprising inhibition of peroxidase and bactericidal activities were observed at both 7 and 12 dph

Task 15.1 **Effect of feeding regime and probiotics (D15.2; m27)**

Conclusion: the results suggest the positive effect of experimental live prey enriching emulsions supplemented with immune-modulators such as *Echium* oil and particularly black cumin oil, on larval performance and welfare compared to the commercial emulsion.

Larval husbandry
Greater Amberjack
Task 15.2.3

WP 15	Larval husbandry - greater amberjack	RTD	2	79.50	5	48

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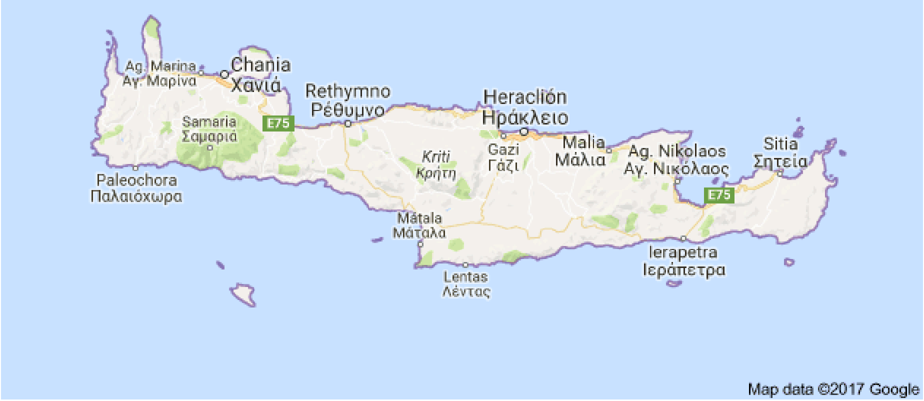
Ontogeny of the digestive system of greater amberjack larvae: amylase, lipase and protease activities, under different rearing conditions: intensive or mesocosm, and from different geographical origin



Sub-task 15.2.3 **(ULL)** Ontogeny of the digestive system of greater amberjack larvae

- The three pancreatic enzymes were more active in the youngest larvae compared to the 30 days-old larvae, whereas pepsin followed the opposite trend, displaying an almost null activity at 12-15 dph.

- The intensive rearing conditions seemed to favor amylase, alkaline protease and pepsin activities in the older larvae.

- Further studies are necessary to elucidate if larval rearing density has any influence in their digestive capacity development.

- At 30 dph, higher activities of pepsin and alkaline protease were found in Atlantic than in Mediterranean larvae.

Implementation: HCMR, FCPCT, ULL

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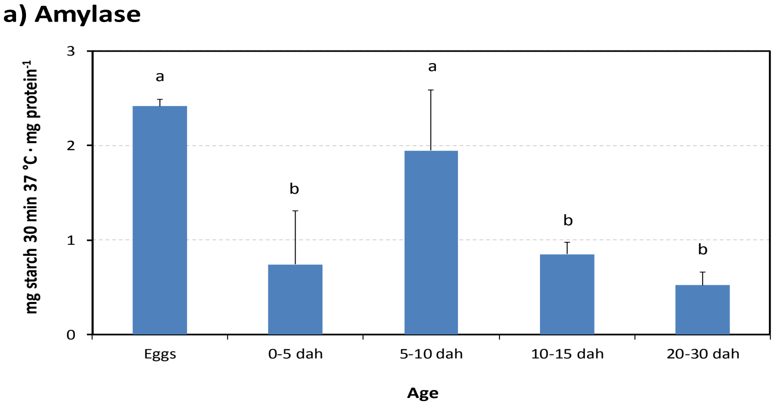
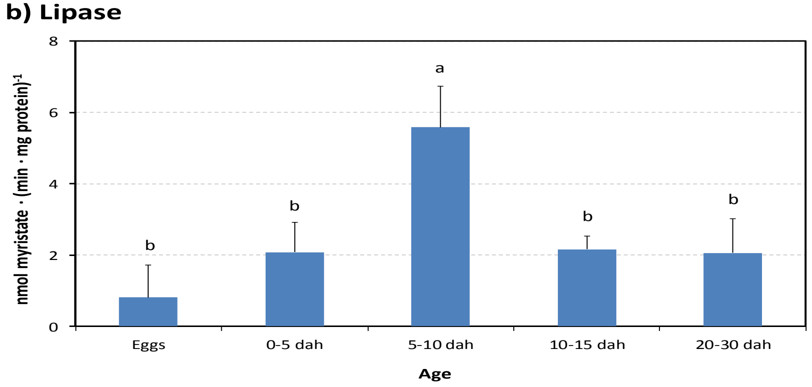
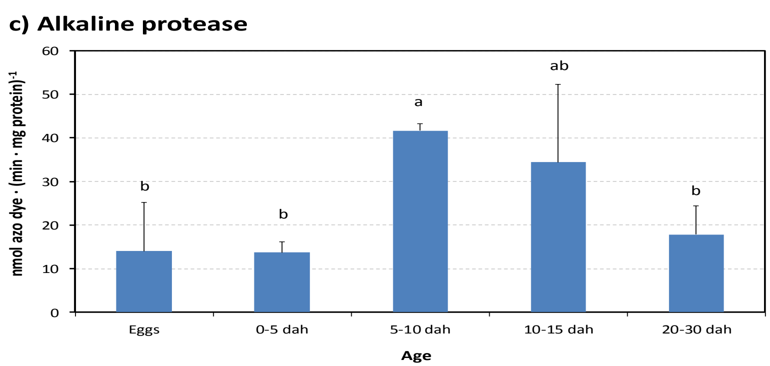
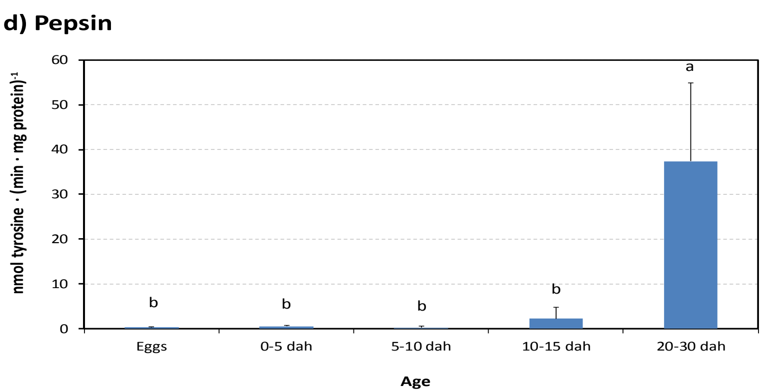
Sub-task 15.2.3 **(ULL)** Ontogeny of the digestive system of greater amberjack larvae. **Combined Mediterranean and Atlantic samples.**









- **Average values of total amylase (a), lipase (b), alkaline protease (c) and pepsin (d), measured in greater amberjack eggs and larvae sampled at different rearing protocols and geographical locations. Different letters denote significant differences (ANOVA, $P<0.05$)**

- **Implementation: ULL**

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Sub-task 15.2.3 **(ULL)** Ontogeny of the digestive system of greater amberjack larvae

Results 15.2.3 ULL. Summary of digestive enzyme activity

- Ontogeny of digestive enzymes clearly matches the histological description of digestive system development of amberjack (**See Deliverable 15.4**).

- Further studies are necessary to elucidate if initial larval quality differs among the two stocks and if this affects further digestive performance.

- **Implementation: ULL**

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GWP
Nutrition WP10, WP11
Pikeperch and Halibut

DTU-ULL-FUNDP-FCPCT
NIFES-IMR-ULL

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ULL
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"*In vivo*" unsaturated fatty acid metabolism of larvae determined by incubation with ^{14}C-labelled substrates added directly to the water

GWP Nutrition
WP10, Pikeperch

DTU-ULL-FUNDP-FCPCT

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Task 10.2 **Effects of pikeperch early fatty acid nutrition on long-term stress sensitivity** (D10.2, m36; DELAYED)

Background

- **Although pikeperch is a freshwater species, its larval development shares some characteristics in common with marine carnivorous fish larvae (e.g. size, mouth opening dph, EFA requirements, high demand for DHA).**

- **Dietary contents of n-3 and n-6 C18-PUFA precursors and also environmental salinities may influence pikeperch C14 fatty acid substrates metabolism (revised by Geay et al., 2015).**

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Task 10.2 **Effects of pikeperch early fatty acid nutrition on long-term stress sensitivity** (D10.2, m36; DELAYED)

"*In vivo* unsaturated fatty acid metabolism of larvae determined by incubation with ^{14}C-labelled substrates directly added to the water".

Objectives

- **To determine the pathways and activities of LC-PUFA biosynthesis in pikeperch in order to elucidate potential mechanisms underpinning its fatty acid profile.**

- **To assist the design of a good rearing protocol and a suitable diet for pikeperch larval rearing.**

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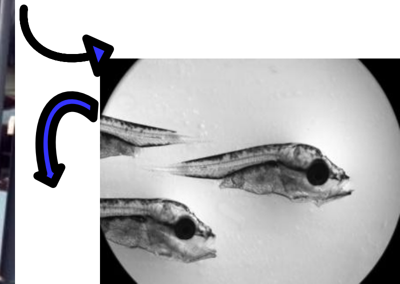
Material & Methods

- **2 diets (18:2n-6 or 18:3n-3 rich diet)**
- **3 salinities (0, 5, 10 ‰)**
- **1 control, 0 ‰, LC-PUFA rich diet**
- **10 pikeperch larvae** (25dph) per well

(SARSTEDT 6 well)

- 10 ml of water (0, 5, 10 ‰),
- Horizontal gentle stirring
- 4 h incubation
- 0.2 µCi (**0.3 µM**) of [1-^{14}C]FA, [1-^{14}C]PC or [1-^{14}C]PE: **n=3**



RADIOLABELLED 18:2n-6, 18:3n-3, ARA, EPA or **DHA**

PHOSPHATIDYLCHOLINE, L-α-1-PALMITOYL-2-ARACHIDONYL - **[ARACHIDONYL-1-^{14}C]**

PHOSPHATIDYLETHANOLAMINE, L-α-1-PALMITOYL-2-ARACHIDONYL - **[ARACHIDONYL**

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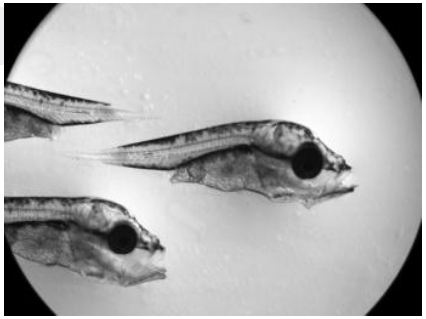
Material & Methods



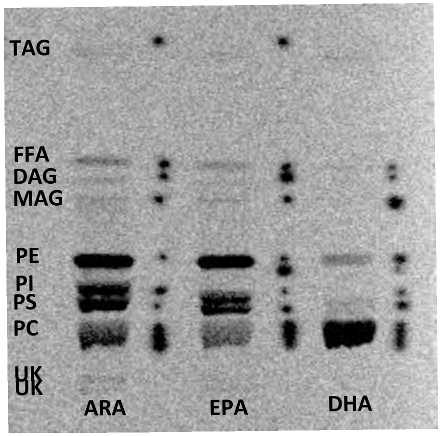
168 incubations

1.- Incorporation of [1-^{14}C]FA into TL

ß-counter. Rodríguez et al., 2002

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

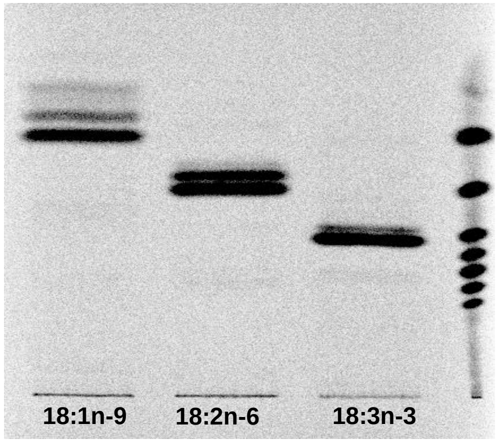
Exposure Cassete-K, Image Screen-K, BioRad
Tocher and Harvie 1988; Díaz-Lopez et al., 2010



Lipid classes
HPTLC plates, Quantity One image

3.-[1-^{14}C]FA transformation

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



Fatty acids
TLC plates, Quantity One image

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Task 10.2 **Effects of pikeperch early fatty acid nutrition on long-term stress sensitivity**

1. **Accordingly to its carnivorous condition, a very poor modulation capacity of dietary fatty acids or salinity to produce ARA, EPA or DHA from dietary precursors, has been found in pikeperch larvae,**
2. **Although some delta 6 activity is evident, it cannot compensate the strong decrement of EFA caused by the two LC-PUFA deficient diets.**
3. **The high content of 18:3n-3 naturally present in the Artemia and 18:2n-6 from VO particularly affects incorporation of DHA and its esterification into specific polar lipids.**
4. **According to the total incorporation (EPA 6x, ARA 3x > DHA) and esterification patterns into PC a good balance of DHA/EPA/ARA is also crucial for pikeperch larval development.**

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Facultad de Ciencias
Sección de Biología

Co-funded by the Seventh Framework Programme of the European Union

DIVERSIFY

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