GWP Nutrition
WP 8,9,10,11,12 & 13

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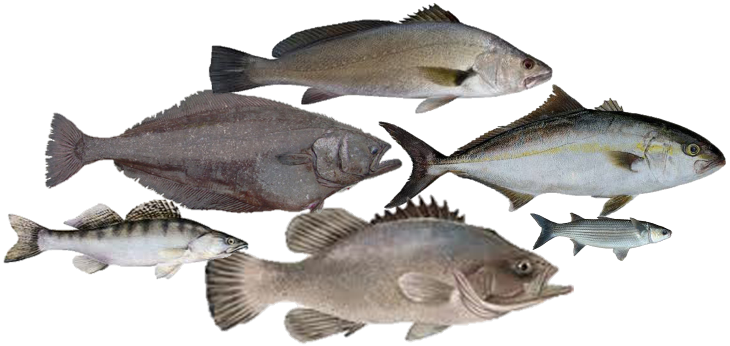
…The species were selected based both on their biological and economical potential…in order to overcome the documented bottlenecks in the production. The combination of biological, technological and socioeconomic research planned in DIVERSIFY are expected to support the diversification of aquaculture industry...

Nutrition as biological and economic factor

Structure of GWP
WP 8. Meagre
WP 9. Greater Amberjack
WP10. Pikeperch
WP11. Atlantic Halibut
WP12. Wreckfish
WP13. Grey Mullet



WP 8. Meagre

Objective: to better define the nutritional needs of meagre during both pre-growing and on-growing phases to improve growth consistency and fish health and welfare

Task 8.1. Improvement of larval weaning feeds (Led by FCPCT)

Task 8.2. Determination of nutritional requirements to promote feed utilization, growth and welfare (Led by FCPCT)

WP 8. Meagre

- Task 8.1. Improvement of larval weaning diets

1. Optimum essential fatty acids and related micronutrient levels in weaning diets for meagre

2. Importance of dietary vitamins A, K and D in weaning diets for meagre

WP 8. Meagre

- **Task 8.1**. Improvement of larval weaning diets.
  1. **essential fatty acids + micronutrients**

Table 1. Variable ingredients and proximate composition (g 100 g^{-1}dw) of early weaning diets containing several n-3 HUFA, vitamin E and vitamin C levels fed to meagre (*A. regius*) larvae from 14 to 28 dah.

	Diets					
	0.4/150/180	**0.4/300/180**	**0.4/300/360**	**3/150/180**	**3/300/180**	**3/300/360**
Ingredients						
Peruvian anchovy oil	0.00	0.00	0.00	10.00	10.00	10.00
Oleic acid[a]	10.00	10.00	10.00	0.00	0.00	0.00
Vitamin E*	150.00	300.00	300.00	150.00	300.00	300.00
Vitamin C*	180.00	180.00	360.00	180.00	180.00	360.00
Proximate composition						
Lipid	16.01	17.09	17.06	17.52	17.34	17.44
Protein	65.14	64.72	64.97	65.43	65.45	64.88
Moisture	10.32	10.59	9.38	9.67	9.39	9.35
Ash	5.47	5.55	5.70	5.88	5.73	5.81

WP 8. Meagre

- **Task 8.1. Improvement of larval weaning diets.**
  1. essential fatty acids + micronutrients

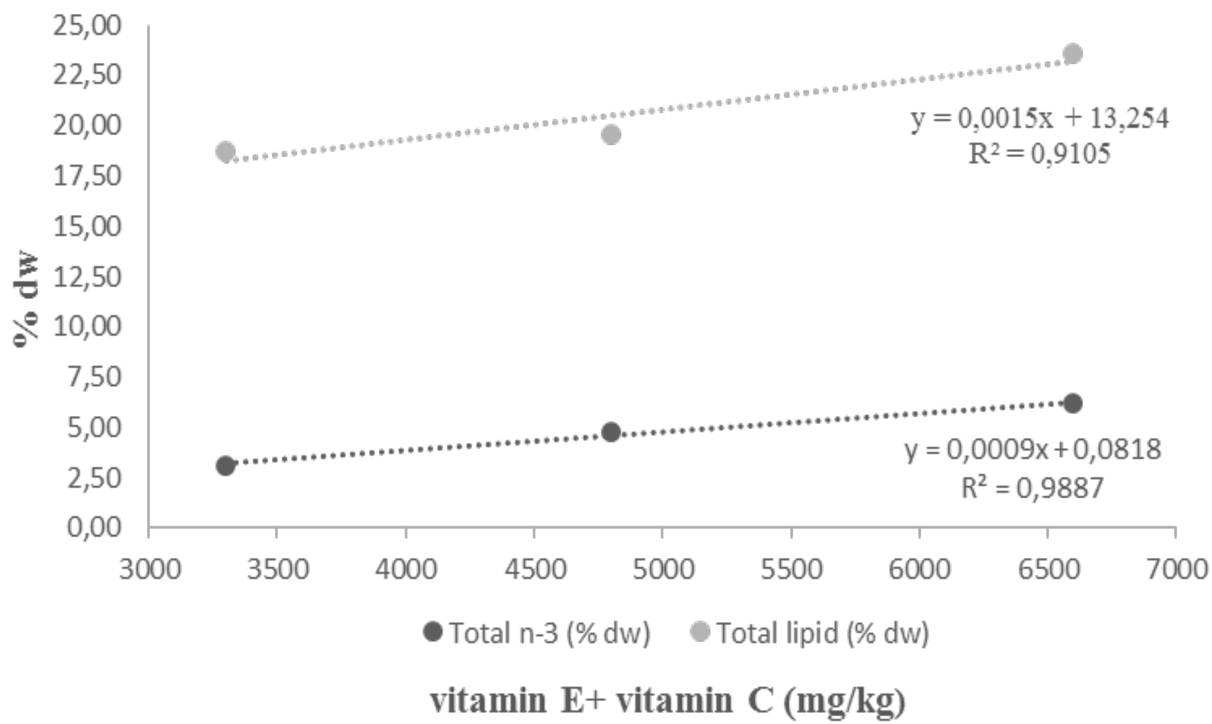
Table 2. Total length (mm), dry weight (mg), and survival of meagre larvae fed early weaning diets containing two levels of n-3 HUFA, vitamin E and vitamin C from 14 dah (initial total length 4.07±0.26 mm and dry body weight 0.06±0.01 mg).

	Diets					
	0.4/150/180	**0.4/300/180**	**0.4/300/360**	**3/150/180**	**3/300/180**	**3/300/360**
Total length						
24 dah	4.75±0.44^{b}	5.00±0.39^{a}	4.91±0.40^{ab}	4.96±0.45^{a}	4.96±0.48^{a}	5.06±0.38^{a}
28 dah	5.15±0.46^{ab}	5.20±0.43^{ab}	5.14±0.51^{ab}	5.29±0.44^{a}	4.97±0.31^{b}	5.34±0.59^{a}
Body weight						
24 dah	0.19±0.04^{c}	0.21±0.02^{bc}	0.20±0.03^{bc}	0.21±0.02^{bc}	0.22±0.02^{ab}	0.24±0.03^{a}
28 dah	0.23±0.02	0.21±0.04	0.21±0.03	0.27±0.05	0.23±0.05	0.24±0.04
Survival (%)	12.09±4.96	8.04±5.20	15.12±4.14	14.16±8.29	16.68±3.45	15.16±7.67

WP 8. Meagre

- **Task 8.1**. Improvement of larval weaning diets.
  1. essential fatty acids + micronutrients

Effect of dietary vitamin E and C on lipid and n-3 contents (dw) in meagre (*A. regius*) larvae after 14 days of feeding 3% HUFA diets.
Improved lipid absorption



WP 8. Meagre

- **Task 8.1**. Improvement of larval weaning diets.
  1. essential fatty acids + micronutrients

- 0.4% dietary HUFA is not enough to cover the essential fatty acid requirements of larval meagre and, since their elevation up to 3% markedly improved lipid absorption, essential fatty acids levels and growth, a high HUFA requirement in weaning diets is foreseen for this species.

- pointed out the importance of dietary vitamin E and vitamin C to protect these essential fatty acids from oxidation, increase their contents in larval tissues and promote growth, suggesting as well high vitamin E and vitamin C requirements in meagre larvae (higher than 1500 and 1800 mg kg^{-1} for vitamin E and vitamin C, respectively).

WP 8. Meagre

- **Task 8.1**. Improvement of larval weaning diets.
  2. **Vitamins A, K & D**

	Diets				
	C	**C+Taurine**	**C-Vit K**	**C-Vit D**	**C-Vit A**
Ingredients					
Taurine[i]	0.0	200.0	0.0	0.0	0.0
Vit K[j]	17.3	17.3	0.0	17.3	17.3
Vit D[k]	3.7	3.7	3.7	0.0	3.7
Vit A[l]	0.3	0.3	0.3	0.3	0.0
Proximate composition (%)					
Crude lipids	16.4	16.2	16.5	17.1	17.9
Crude protein	76.0	75.9	76.4	76.4	76.1
Moisture	13.7	13.6	13.6	13.8	13.8
Ash	6.5	6.5	6.5	6.6	6.5
Taurine[1]	4.0	5.8	4.0	4.0	4.0
Vitamin K[2]	2.4	2.4	0.0	2.6	2.2
Vitamin D[3]	28.9	29.0	30.4	2.3	27.4
Vitamin A[4]	4.2	4.3	4.2	4.3	4.1

WP 8. Meagre

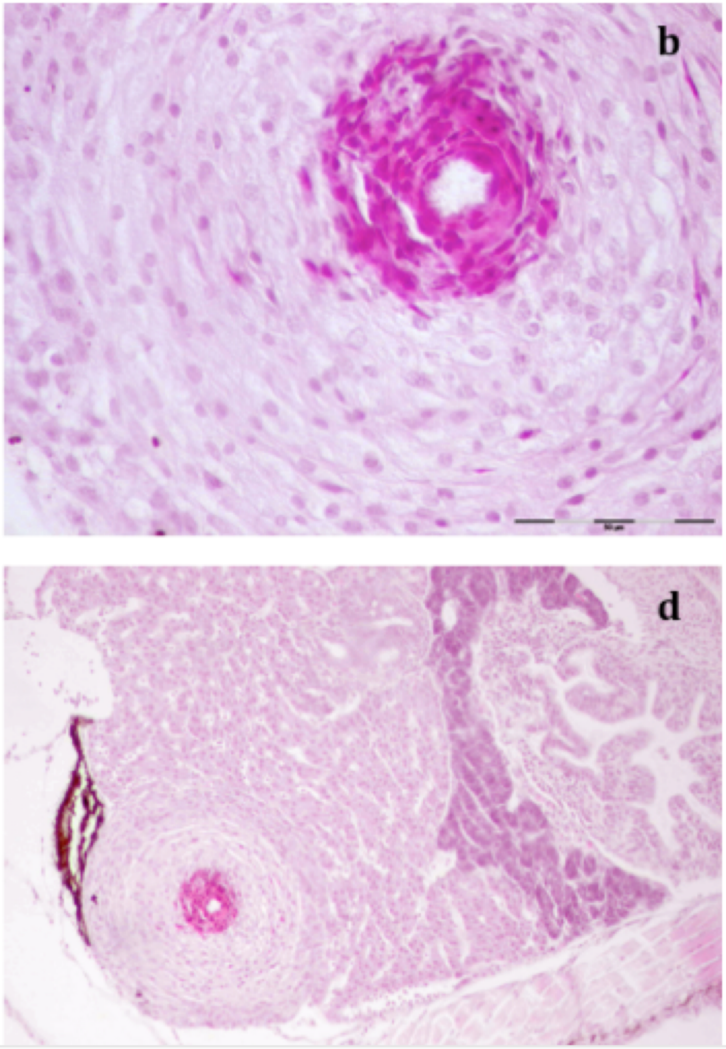
- Task 8.1. Improvement of larval weaning diets.
  2. Vitamins A, K & D

		Diets				
		C	**C+Taurine**	**C-Vit K**	**C-Vit D**	**C-Vit A**
Total length	26 dah	8.3±1.0 [a]	8.5±0.9 [a]	8.5±1.0 [a]	8.9±1.0 [b]	8.6±1.0 [a]
	33 dah	11.5±1.7 [a]	11.7±1.3 [a]	12.8±1.6 [b*]	12.6±1.3 [b]	12.2±1.7 [b]
Body weight	26 dah	0.7±0.1 [a]	0.8±0.1 [a]	0.7±0.1 [a]	0.9±0.2 [b]	0.8±0.2 [a]
	33 dah	2.4±0.6 [a]	2.3±0.4 [a]	3.2±0.2 [b*]	3.3±0.2 [b]	2.5±0.3 [a]
Survival (%)		16.7±6.5	12.9±1.2	7.1*	17.7±12.3	19.0±0.5

WP 8. Meagre

- Task 8.1. Improvement of larval weaning diets.
  2. Vitamins A, K & D

	33 dah		
Diets	Total	Stage I	Stage II
C	0.0±0.0	0.0±0.0	0.0±0.0
C+Taurine	0.0±0.0	0.0±0.0	0.0±0.0
C-Vit K	12.5±10.6	5.0±7.1	7.5±3.5
C-Vit D	3.3±2.9	1.7±2.9	1.7±2.9
C-Vit A	8.3±10.4	6.7±7.6	1.7±2.9



WP 8. Meagre

- Task 8.1. Improvement of larval weaning diets.
  2. Vitamins A, K & D

supplementation of meagre weaning diets with 2.4 mg/kg vit K, since the absence of this vitamin markedly reduced larval survival.

very sensitive to hypervitaminosis D and, in a lesser extent to hypervitaminosis A, since supplementation with these vitamins leaded to a growth reduction.

taurine supplementation did not have any effect in meagre larvae performance

DELIVERABLE 8.1. Delivered in time. Month 24

WP 8. Meagre

- **Task 8.2** Determination of nutritional requirements to promote feed utilization, consistent growth rates and fish welfare. The essential fatty acid requirements will be examined in grow out diets for meagre

			Diet			
Raw Material (%)	AA	BB	CC	DD	EE	FF
Wheat	19,13	12,14	12,14	12,14	12,14	12,14
Corn gluten	5,00	10,00	10,00	10,00	10,00	10,00
Wheat gluten	8,11	15,89	15,89	15,89	15,89	15,89
Soya bean concentrate	17,00	25,00	25,00	25,00	25,00	25,00
Faba beans	5,00	10,00	10,00	10,00	10,00	10,00
Fish meal	35,00	15,00	15,00	15,00	15,00	15,00
Rapeseed oil	0,00	0,00	6,25	4,69	3,12	1,78
Fishoil	10,15	11,37	0,00	2,85	5,69	8,13
Palm oil	0,00	0,00	3,41	2,56	1,70	0,97
Linseed oil	0,00	0,00	1,71	1,28	0,85	0,49
Vit - Min premix	0,60	0,60	0,60	0,60	0,60	0,60
[VOLUME]	100,00	100,00	100,00	100,00	100,00	100,00
DRY_MAT	91,73	92,01	92,01	92,01	92,01	92,01
V MOIST	8,19	7,94	7,94	7,94	7,94	7,94
C PROT	50,00	50,00	50,00	50,00	50,00	50,00
C FAT	16,00	16,00	16,00	16,00	16,00	16,00
EPA+DHA	35,31	32,40	4,43	11,43	18,43	24,43

WP 8. Meagre

- **Task 8.2** Determination of nutritional requirements to promote feed utilization, consistent growth rates and fish welfare. Experimental design **in progress.**

To start in 2016

Implementation: FCPCT, ULL, SARC

Deliverable 8.2. Mont 48

WP 9. Greater Amberjack

Objective: to improve larval development and survival by adequate nutrient levels in enrichment products, effect nutritional enhanced grow out diets on juvenile performance and effective broodstock feeding regimes to boost reproduction

Task 9.1. Improvement of larval enrichement products to enhance production of larvae and juveniles (Led by FCPCT)

Task 9.2. Development of diets for grow-out amberjack to maximize growth (Led by HCMR)

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

WP 9. Greater Amberjack

- **Task 9.1.** Improvement of larval enrichement products to enhance production of larvae and juveniles
  - **Subtask 9.1.1.** Determination of optimum essential fatty acid in enriched products for live preys.

1. Optimum DHA in enrichment products for live preys for greater amberjack

2. Optimum EPA in enrichment products for live preys for greater amberjack

3. Combined effect of PUFA-rich lipids and carotenoids in enrichment products for live prey (rotifers) for greater amberjack

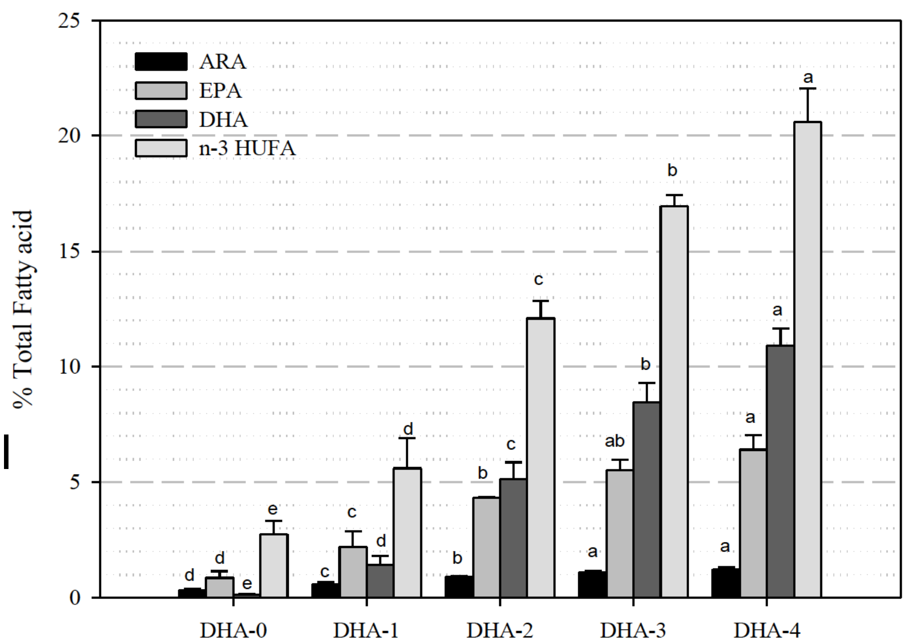
WP 9. Greater Amberjack

1. Optimum DHA in enrichment products for live preys for greater amberjack

Table 1. Emulsion ingredients, proximate and fatty acid composition of the resultant enriched *Artemia* containing increasing levels of DHA.

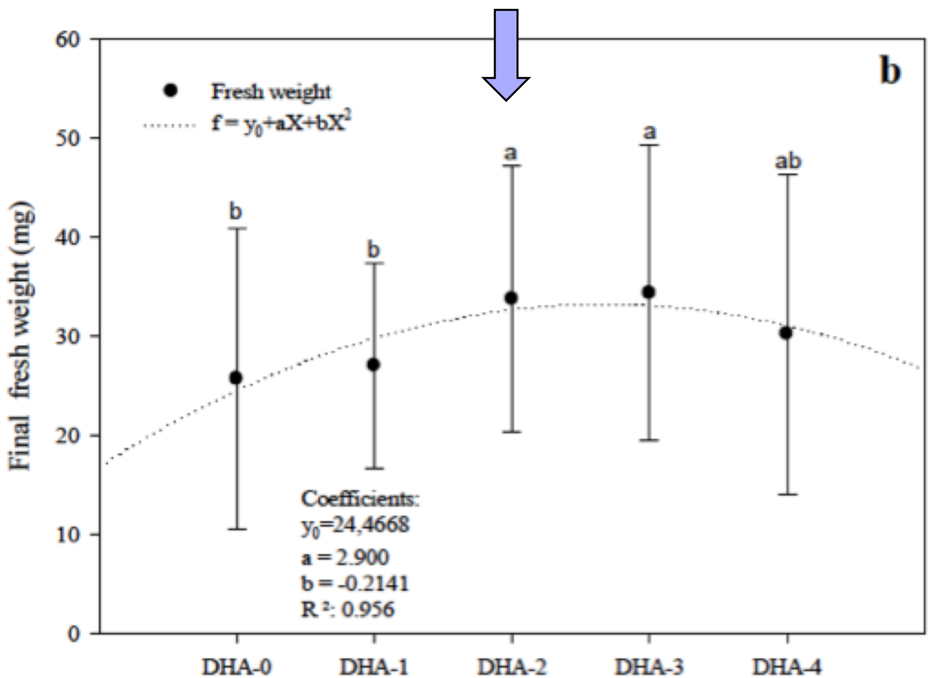
Experimental Emulsion	*DHA-0*	*DHA-1*	*DHA-2*	*DHA-3*	*DHA-4*
Ingredients (g kg^{-1} diet)					
DHA-7[a]	0	300	450	600	900
Oleic acid	900	600	450	300	0
Soy bean lecithin	100	100	100	100	100

Fatty acids content (percentage of total fatty acids) in the enriched *Artemia* experimental emulsions.

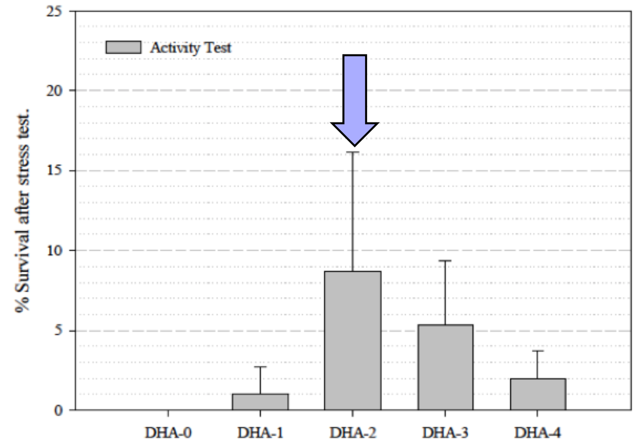


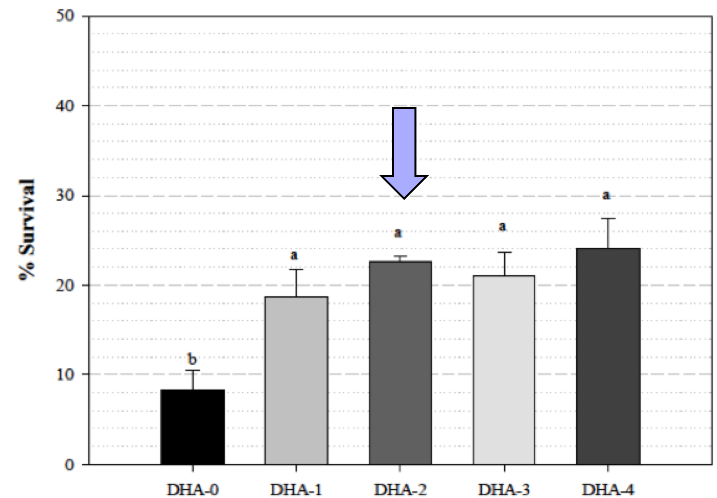
WP 9. Greater Amberjack

1. Optimum DHA in enrichment products for live preys for greater amberjack



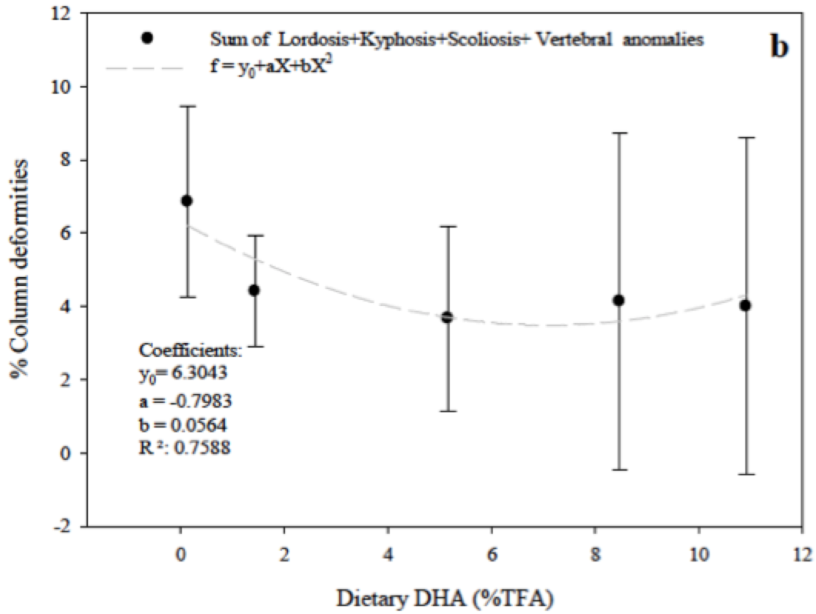
Relationship between fresh weight (mg) and dietary *Artemia* DHA (22:6n-3) content in greater amberjack larvae 35 days posthatch (mean ± S.D., n=3). Data are fitted to a quadratic regression analysis ($f=y_0+ax+bx^2$).

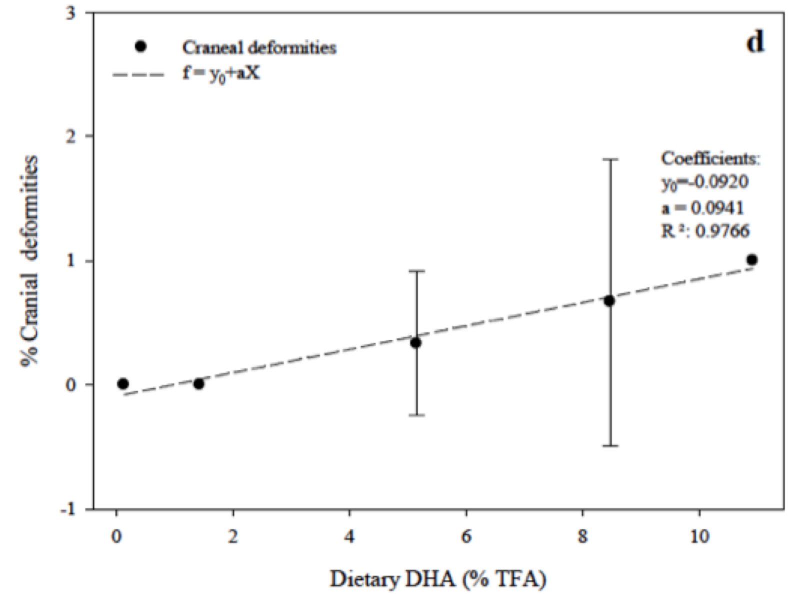




WP 9. Greater Amberjack

1. Optimum DHA in enrichment products for live preys for greater amberjack





Relationship between (b) sum of column deformities, (d) cranial malformation (%) to dietary *Artemia* DHA (22:6n-3) content (%TFA) in larval greater amberjack at 35 days post-hatch (mean + S.D., n=3)

Implementation: FCPCT

1.5 g 100 g^{-1} DHA DW is sufficient to promote fast growth in greater amberjack larvae, whereas increased levels were associated to skull anomalies

WP 9. Greater Amberjack

- **Task 9.1.** Improvement of larval enrichement products to enhance production of larvae and juveniles
  - **Subtask 9.1.2**. Combined effect of PUFA-rich lipids and carotenoids.

- Enrichment ingredients

	E1	E2	E3
Ingredients (mg L^{-1})			
Marine lecithin	78	23.6	0
Incromega DHA 500	0	39.3	47.2
Cod liver oil	0	15.7	31.5
Arachidonic acid	2	1.4	1.3

Implementation: IEO, ULL

WP 9. Greater Amberjack

- Task 9.1. Improvement of larval enrichement products to enhance production of larvae and juveniles
  - Subtask 9.1.2. Combined effect of PUFA-rich lipids and carotenoids.

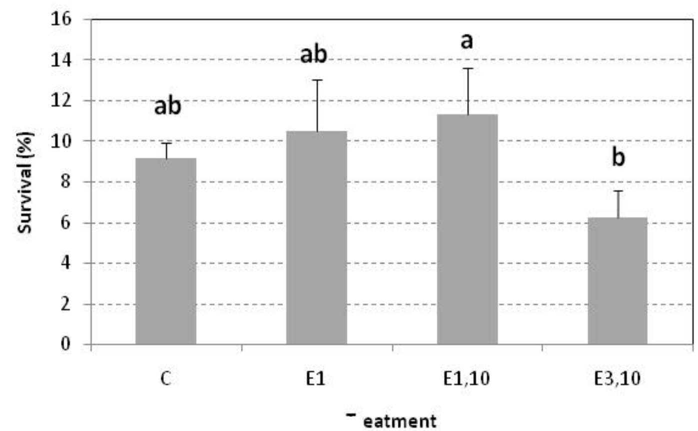
- **1.-** Enrichment protocol selection, 1st assay

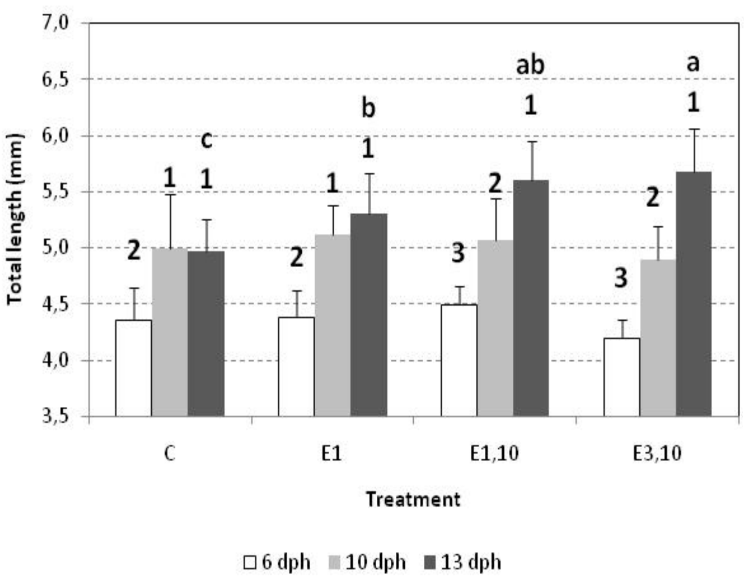
	Control			E1			E2			E3		
	3h	6h	10h	3h	6h	10h	3h	6h	10h	3h	6h	10h
TL	12.8±1.1^{a,B}	16.6±1.7^{b,B}	17.6±1.2^{b,A}	19.9±1.4^{b,A}	16.0±1.6^{a,B}	15.6±0.3^{a,A}	20.6±1.5^{A}	22.9±0.8^{A}	26.0±3.5^{B}	12.7±1.6^{a,B}	13.9±2.1^{ab,B}	16.5±1.0^{b,A}
TAG	33.5±0.7^{a,B}	39.8±1.8^{b,A}	46.1±1.9^{c,B}	24.5±0.9^{a,C}	33.5±0.3^{b,B}	36.1±1.3^{c,A}	43.4±0.8^{A}	42.2±2.4^{A}	42.5±0.4^{B}	35.9±2.5^{a,B}	41.6±2.4^{ab,A}	44.9±3.2^{b,B}
TPL	26.1±1.9^{A}	26.0±2.2^{A}	23.3±1.3^{B}	31.5±2.9^{A}	31.1±0.8^{A}	29.4±1.5^{C}	13.6±0.5^{C}	17.5±2.7^{C}	13.1±2.1^{A}	24.9±0.2^{B}	24.1±1.9^{B}	22.8±1.8^{B}
TPL-Fatty acids												
∑ SFA	14.3±0.9^{a,A}	16.2±0.3^{b,A}	17.0±0.5^{b,B}	22.5±0.3^{a,C}	19.7±1.2^{b,B}	19.8±0.2^{b,C}	18.5±0.9^{B}	18.4±1.8^{AB}	17.6±0.5^{B}	14.5±0.3^{A}	14.7±1.7^{A}	14.9±0.7^{A}
∑ MUFA	61.6±0.3^{c,D}	44.1±0.5^{b,B}	38.3±0.8^{a}	37.6±1.2^{a,A}	39.1± 0.4^{b,A}	36.9±0.4^{a}	42.1±0.8^{b,B}	38.7±0.8^{a,A}	35.8±2.9^{a}	49.8±0.6^{c,C}	46.3±0.3^{b,C}	41.6±2.3^{a}
∑ n-6 PUFA	8.9±0.1^{a,A}	15.7±0.3^{b,B}	16.4±0.3^{b,C}	9.7±0.0^{a,B}	1.3±0.4^{b,A}	11.0±0.3^{b,B}	10.7±0.4^{b,C}	11.3±0.2^{b,A}	9.1±0.9^{a,A}	15.6±0.6^{D}	16.1±0.3^{B}	14.8±1.1^{C}
20:4	0.8±0.1^{a,B}	1.6±0.0^{b,C}	1.7±0.1^{b,A}	4.0±0.0^{A}	4.6±0.5^{A}	4.8±0.1^{C}	3.7±0.3^{ab,A}	4.4±0.3^{b,A}	3.5±0.3^{a,B}	3.7±0.2^{A}	3.6±0.0^{B}	3.6±0.1^{B}
∑ n-3 HUFA	4.3±0.3^{a,C}	16.0±0.7^{b,B}	16.8±0.9^{b,A}	23.2±0.5^{A}	23.7±2.4^{A}	22.2±0.5^{B}	21.0±1.7^{a,A}	25.6±2.2^{b,A}	26.9±0.6^{b,C}	11.2±0.4^{a,B}	14.9±0.5^{b,B}	19.0±2.9^{b,AB}
20:5	1.1±0.1^{a,D}	3.3±0.2^{b,B}	3.3±0.2^{b,A}	6.4±0.2^{A}	6.5±0.7^{A}	6.3±0.3^{C}	5.0±0.5^{a,B}	6.4±0.6^{b,A}	5.5±0.4^{ab,BC}	3.1±0.3^{C}	4.1±0.6^{B}	4.4±0.8^{AB}
22:6	2.4±0.2^{a,C}	11.4±0.4^{b,B}	11.5±0.6^{b,A}	16.3±0.5^{b,A}	16.5±2.8^{ab,A}	14.4±0.2^{a,A}	15.3±1.1^{A}	18.1±2.8^{A}	19.7±0.6^{B}	7.4±0.1^{a,B}	9.5±0.1^{ab,B}	12.2±2.1^{b,A}

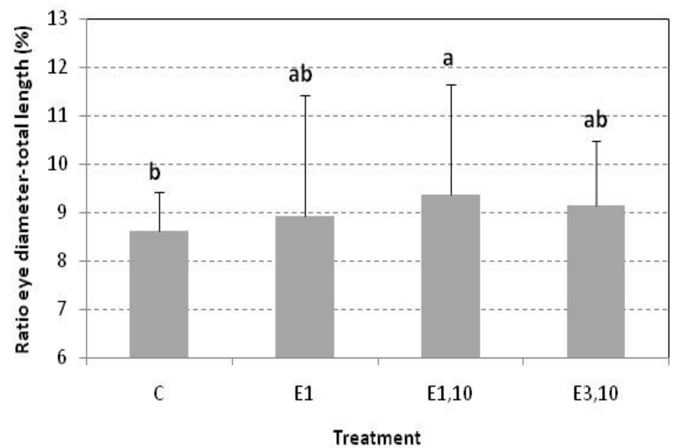
WP 9. Greater Amberjack

- Task 9.1. Improvement of larval enrichement products to enhance production of larvae and juveniles
  - Subtask 9.1.2. Combined effect of PUFA-rich lipids and carotenoids.

- Larval performance
  - Higher total length of E1,10 than Commercial and E1emulsic
  - Higher eye diameter of E1,10 than Commercial emulsion
  - Higher survival of E1,10 than E3,10

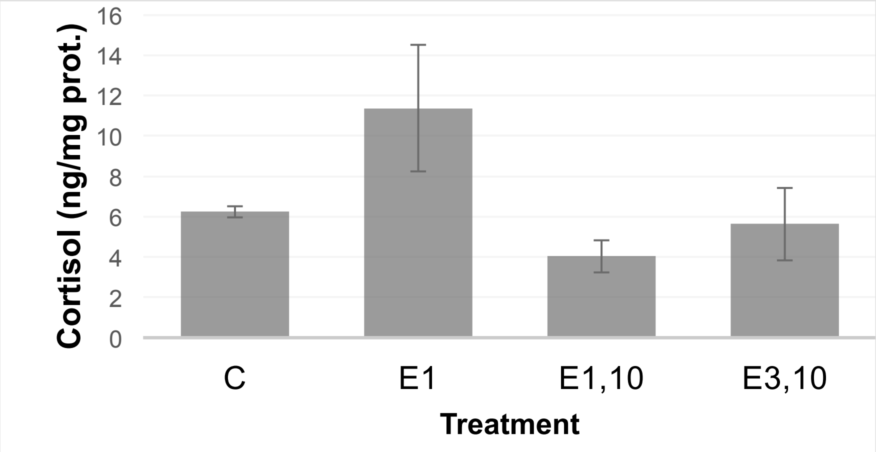






WP 9. Greater Amberjack

- Task 9.1. Improvement of larval enrichement products to enhance production of larvae and juveniles
  - Subtask 9.1.2. Combined effect of PUFA-rich lipids and carotenoids.

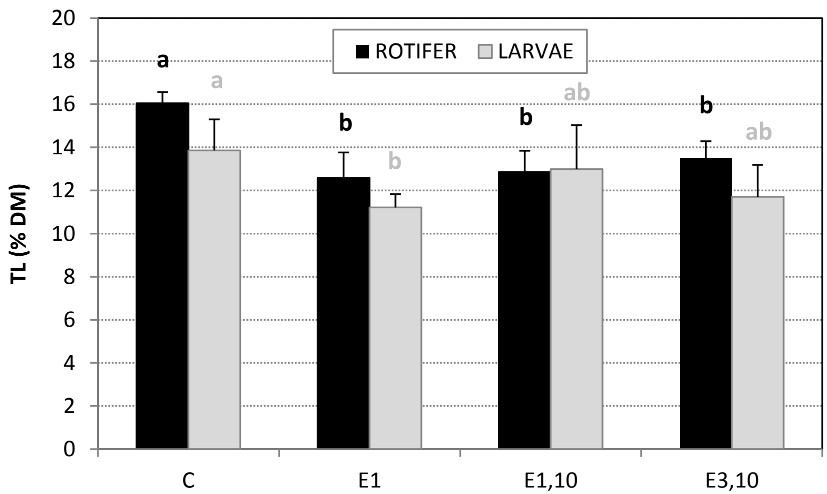


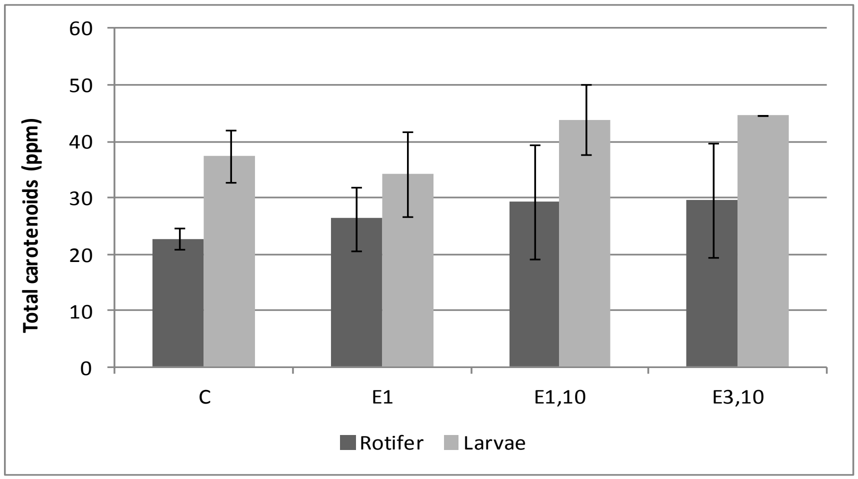
- **Welfare**
  - Lower body cortisol for E1,10.
  - Whole body lactate higher for commercial

	C	E1	E1,10	E3,10
Glucose	0.14 ± 0.05	0.10 ± 0.04	0.09 ± 0.05	0.15 ± 0.06
Lactate	1.76 ± 0.06 a	0.68 ± 0.13 b	0.65 ± 0.35 b	0.75 ± 0.14 b
Na^+	250.22 ± 26.05	160.12 ± 124.18	104.46 ± 6.09	261.78 ± 74.17
K^+	17.22 ± 3.77	11.36 ± 4.51	8.83 ± 1.41	7.69 ± 2.23

WP 9. Greater Amberjack

- Task 9.1. Improvement of larval enrichement products to enhance production of larvae and juveniles
  - Subtask 9.1.2. Combined effect of PUFA-rich lipids and carotenoids.
- Lipid, carotenoid and fatty acid contents





<u>Higher contents of DHA in E1,10 larval TL and PL than E1 and E3. E1,10, better resembling wild egg DHA/ARA/EPA ratios</u>

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

WP 9. Greater Amberjack

- **Task 9.1**. Improvement of larval enrichement products to enhance production of larvae and juveniles

.

- List of optimum levels and ratios of EFA and carotenoids in enrichment products

DHA in enrichment products for *Artemia* 10-17% TFA
EPA in enrichment products for *Artemia* 14-20% TFA
DHA/EPA in enrichment products for *Artemia* 1-5
DHA in enrichment products for rotifers 14% TFA
EPA in enrichment products for *Artemia* 6% TFA
DHA/EPA in enrichment products for rotifers 2.3
Carotenoids levels in enrichment products 10 ppm

DELIVERABLE 9.1. Delivered in time. Month 24

WP 9. Greater Amberjack

- **Task 9.2.** Development of diets for grow-out of amberjack to maximize growth
  - **Subtask 9.2.1.** To investigate the optimum levels of lysine in on-growing diets of amberjack juveniles, which will be based mainly on plant ingredients (low fishmeal inclusion) in favor of health, welfare status and growth of fish.

Materials & Methods

- Six iso-energetic (22.8 MJ/kg) extruded diets (2.5 mm pellets) containing 45% crude protein, 25% fish meal level and graded levels of crystalline lysine (1.85, 1.93, 2.01, 2.09, 2.17, and 2.25%, Table 1) were manufactured by Skretting and transferred to the experimental facilities of the Hellenic Centre for Marine Research (HCMR) in Ag. Kosmas, Athens, Greece.
- The diets were fed to juvenile amberjack (initial average weight 32.8 g ± 3.0) distributed in 18 experimental small cages (1.1 x 1.0 x 1.5 m), with 25 fish per cage, 3 cages per diet for a period of 60 days (started on October 21, 2015).

Table 1. Ingredients & chemical composition of the six experimental diets (%) .

	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6
Fish meal	25.00	25.00	25.00	25.00	25.00	25.00
Wheat	28.65	28.55	28.40	28.30	28.20	28.10
Corn gluten	10.00	10.00	10.00	10.00	10.00	10.00
Wheat gluten	21.95	21.95	21.95	21.95	21.95	21.95
Soya concentrate	1.01	1.01	1.01	1.01	1.01	1.01
Fish oil	12.33	12.33	12.33	12.33	12.33	12.33
L-Lysine	*0.00*	*0.10*	*0.21*	*0.31*	*0.41*	*0.52*
phopsphate	0.61	0.61	0.61	0.61	0.61	0.61
Mineral & Vit mix	0.50	0.50	0.50	0.50	0.50	0.50
(%) as fed						
MOIST	7.72	7.71	7.70	7.69	7.68	7.66
C PROT	45.00	45.08	45.17	45.25	45.34	45.42
C FAT	18.00	18.00	18.00	18.00	18.00	18.00
ASH	5.43	5.43	5.43	5.43	5.43	5.43
TOT Lys	*1.85*	*1.93*	*2.01*	*2.09*	*2.17*	*2.25*
CHO	29.28	29.21	29.13	29.06	28.99	28.91
Energy (MJ/kg)	22.81	22.82	22.83	22.83	22.84	22.85

WP 9. Greater Amberjack

- **Task 9.2.** Development of diets for grow-out of amberjack to maximize growth
  - **Subtask 9.2.1.** To investigate the optimum levels of lysine in on-growing diets of amberjack juveniles, which will be based mainly on plant ingredients (low fishmeal inclusion) in favor of health, welfare status and growth of fish.

Chemical analyses (*body proximate composition, blood analysis, etc.*) and evaluation of the amberjack growth performance is in progress.

D 9.2. month 36

Implementation: HMRC, SARC

WP 9. Greater Amberjack

- **Task 9.3.** Design of adequate feeding regimes for broodstock to optimize reproduction
    - **Subtask 9.3.1.** Optimum ARA, EPA and DHA for reproductive success of greater amberjack

Table 2.- Composition of experimental diets (Skretting, Burgos, España)

Tank/Diet	T3/PROTEIN	T4/TAURINE	T5/HISTIDINE
Raw material (%)			
Wheat	11.81	18.29	17.94
Wheat gluten	17.00	13.00	13.00
FM North-Atlantic	48.36	44.64	45.14
Squid meal	10.00	10.00	10.00
Fishoil North-Atlantic	12.18	12.50	12.47
Taurine	0.00	0.93	0.00
Histidine HCl	0.00	0.00	0.81
Premix vit. Min.	0.64	0.64	0.64
VOLUME	100.00	100.00	100.00
DRY MAT (%)	92.03	91.83	91.85
Moisture (%)	7.97	8.17	8.15
Crude protein (%)	56.00	52.00	52.00
Crude fat (%)	18.00	18.00	18.00
Ash (%)	8.63	8.12	8.19
Total histidine (g/kg)	12.10	11.07	17.00
Total lysine (g/kg)	32.71	30.42	30.68
Total methionine (g/kg)	13.44	12.38	12.47
Total taurine (g/kg)	3.30	12.30	3.08

WP 9. Greater Amberjack

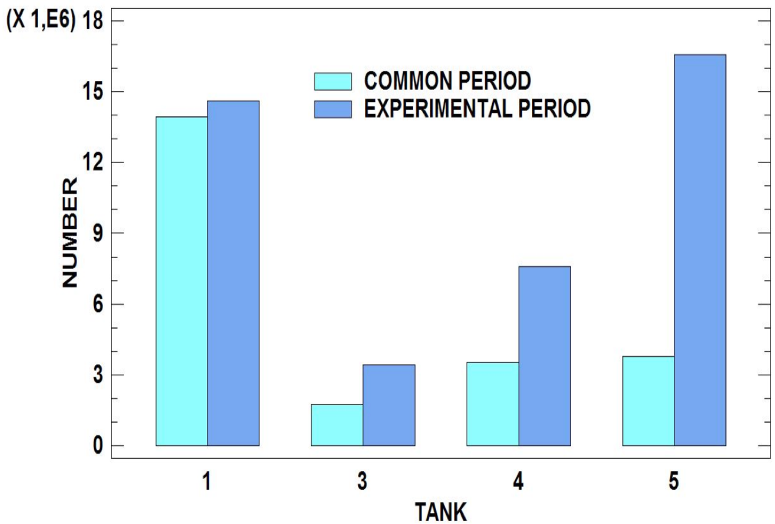
- Task 9.3. Design of adequate feeding regimes for broodstock to optimize reproduction
  - Subtask 9.3.1. Optimum ARA, EPA and DHA for reproductive success of greater amberjack

Tank/ Diet	**Spawn**	**% Fertilizatión** P < 0.01	**% Egg viability at 24h** P < 0.01	**% hatching** P < 0.01
T1 - GIA	Natural	81.22 ± 6.78 ^{a}	95.17 ± 2.60 ^{ab}	93.56 ± 3.35 ^{ab}
T3 - Proteína	induced	56.21 ± 14.58 ^{b}	90.00 ± 4.71 ^{b}	87.60 ± 5.57 ^{b}
T4 - Taurina	"	65.81 ± 16.49 ^{ab}	81.98 ± 6.59 ^{c}	77.92 ± 8.20 ^{c}
T5 - Histidina	"	77.85 ± 14.19 ^{a}	97.07 ± 3.57 ^{a}	96.12 ± 4.35 ^{a}
		Larval survival 1dph P < 0.01	**Larval survival 3 dph** P < 0.1	**Larval survival 5 dph** P < 0.01
T1 - GIA	Natural	91.78 ± 7.96 ^{a}	39.17 ± 17.67^{ab}	9.58 ± 5.48 ^{ab}
T3 - Proteína	induced	68.03 ± 8.93 ^{b}	46.85 ± 11.61^{a}	4.41 ± 1.83 ^{bc}
T4 - Taurina	"	53.23 ± 8.67 ^{c}	31.27 ± 18.29^{b}	0.11 ± 0.36 ^{c}
T5 - Histidina	"	87.72 ± 9.07 ^{a}	50.03 ± 15.88^{a}	11.49 ± 7.07 ^{a}

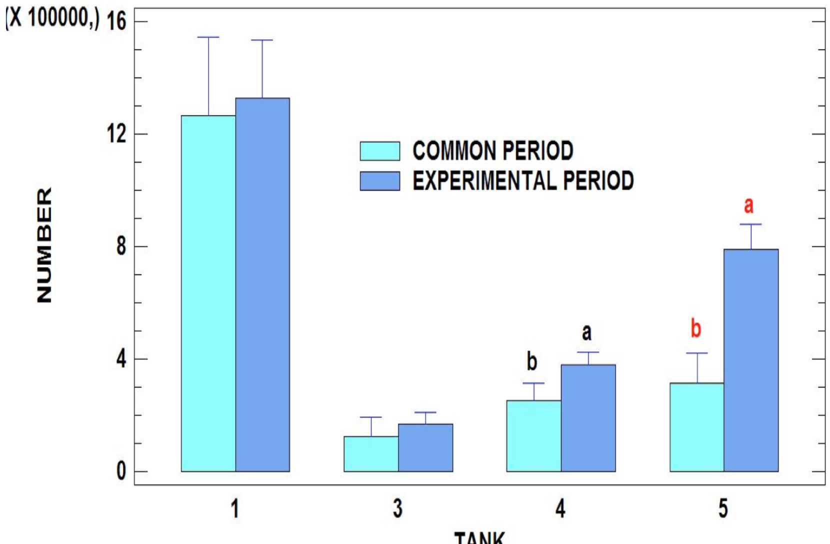
Spawning quality indexes, of broodstock fed with diet GIA and experimental diets

WP 9. Greater Amberjack

- Task 9.3. Design of adequate feeding regimes for broodstock to optimize reproduction
  - Subtask 9.3.1. Optimum ARA, EPA and DHA for reproductive success of greater amberjack



Production of total eggs



Number of eggs per spawn, obtained per broodstock group during the normal feeding period, compared with the experimental period

Implementation: FCPCT SARC

WP 9. Greater Amberjack

- **Task 9.3.** Design of adequate feeding regimes for broodstock to optimize reproduction
  - **Subtask 9.3.2.** Experimental diets with optimized EFA and carotenoid contents for amberjack broodstock
- Background
  - D3.3. Nutritional status of captive *vs.* wild broodstock (ULL)
- Determinations
  - Fecundity, egg quality and haematological and biochemical indicators of fish health
  - Sperm, eggs and larvae will be analyzed for lipid contents and lipid classes, EFA and carotenoids profiles

Maintenance of a new broodstock group during 2016

  - .
- Implementation: IEO, ULL

D 9.4. month 58

WP 10. Pikeperch

Objective: to study the influence of enrichment of live feeds with EFAs using a multifactorial approach. Enrichment of live preys with EFAs at different levels on larvae and juvenile physiology

Task 10.1. Effect of selected dietary nutrients on pickeperch larval development and performance (Led by DTU)

Task 10.2. Effects of pikeperch early fatty acid nutrition on long-term stress sensitivity (Led by DTU).

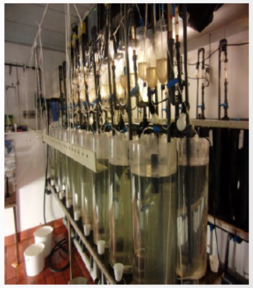
WP 10. Pikeperch

- **Task 10.1.** Effect of selected dietary nutrients on pikeperch larval development and performance

Objectives: The study investigated the effects of increasing inclusion of phospholipids +/- the additional effect of EPA and DHA on pike perch larval performance and larval development and composition. Further effects on digestive enzymatic activity, liver proteomics; skeleton morphogenesis and gene expression

M & M:

Eggs obtained from AquaPri Innovation, Denmark (F1-F2 domesticated generation). 18 tanks of 50 L, flow through, and heated water.



exp. set up at DTU Aqua

Feed: 6 dry feed diets with increasing phospholipid content (soy lecithin) combined with levels of EPA & DHA. Artemia dph 3-17. Weaning: dph 12-17. Only dry feed: dph 18 -30.

WP 10. Pikeperch

- **Task 10.1.** Effect of selected dietary nutrients on pikeperch larval development and performance

Table 1 Dietary composition and analytical content of the 6 experimental diets.

Diet Ingredients (%)	PL1	PL2	PL3	PL1H1	PL2H2	PL3H3
MicroNorse Fish Meal[a]	45	45	45	45	45	45
CPSP 90[b]	7	7	7	7	7	7
Squid meal[c]	13	13	13	13	13	13
Fish gelatin[d]	1	1	1	1	1	1
Wheat Gluten[e]	4.4	4.4	4.4	4.4	4.4	4.4
Wheat meal[f]	6.1	5.9	5.6	6.1	5.9	5.6
Algatrium DHA70[g]	0.0	0.0	0.0	0.6	2.0	3.4
Olive oil[h]	18.9	12.1	3.4	18.4	10.1	0.0
Vitamin & Mineral Premix PV01[i]	1.0	1.0	1.0	1.0	1.0	1.0
Soy lecithin powder[j]	3.0	10.0	19.0	3.0	10.0	19.0
Binder (guar gum)[k]	0.2	0.2	0.2	0.2	0.2	0.2
Antioxidant powder (Paramega)[l]	0.2	0.2	0.2	0.2	0.2	0.2
Antioxidant liquid (Naturox)[m]	0.2	0.2	0.2	0.2	0.2	0.2

WP 10. Pikeperch

- **Task 10.1.** Effect of selected dietary nutrients on pikeperch larval development and performance

Table 1… Analytical composition

Analysed content (% DM)	PL1	PL2	PL3	PL1H1	PL1H2	PL1H3
% dry matter (DM)						
Crude protein	52,7	52,7	52,7	52,7	52,7	52,7
Crude lipid	27,0	27,0	27,0	27,0	26,9	27,0
NFE + fibre (substracted)	4,0	3,9	3,7	4,0	3,9	3,7
Ash	8,1	8,1	8,1	8,1	8,1	8,1
Gross Energy	24,0	23,3	22,5	24,0	23,3	22,5
EPA	0,41	0,41	0,41	0,47	0,61	0,75
DHA	0,66	0,66	0,66	1,04	2,06	3,04
PC	1,40	2,61	4,31	1,42	2,68	4,29
PE	0,43	1,22	2,20	0,40	1,14	1,87
PI	0,44	1,28	2,44	0,43	1,28	2,48
TPL	**3,73**	**8,19**	**14,38**	**3,70**	**8,32**	**14,51**

WP 10. Pikeperch

- Task 10.1. Effect of selected dietary nutrients on pikeperch larval development and performance

Figure 1 Lipid class profile of larvae at 30 dph.

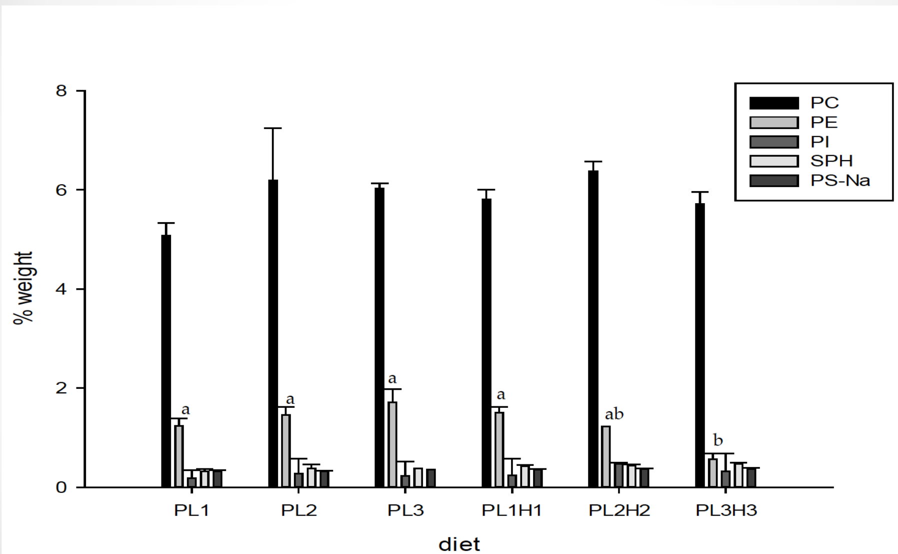
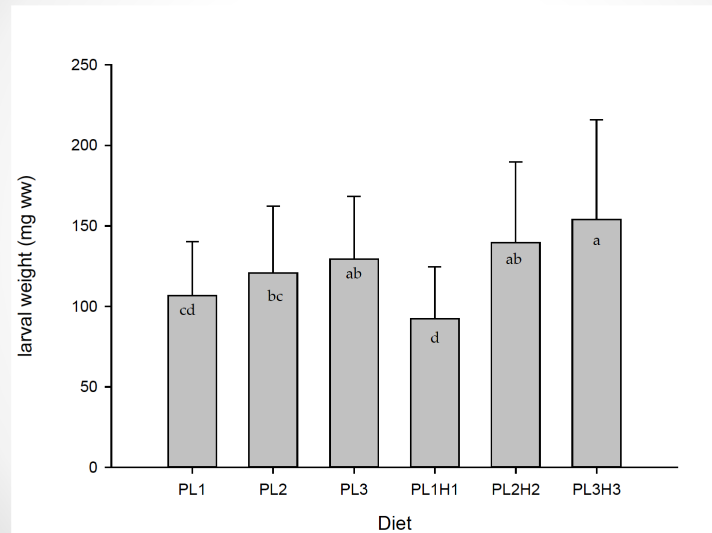


Figure 2 Growth performance larvae 30 dph.

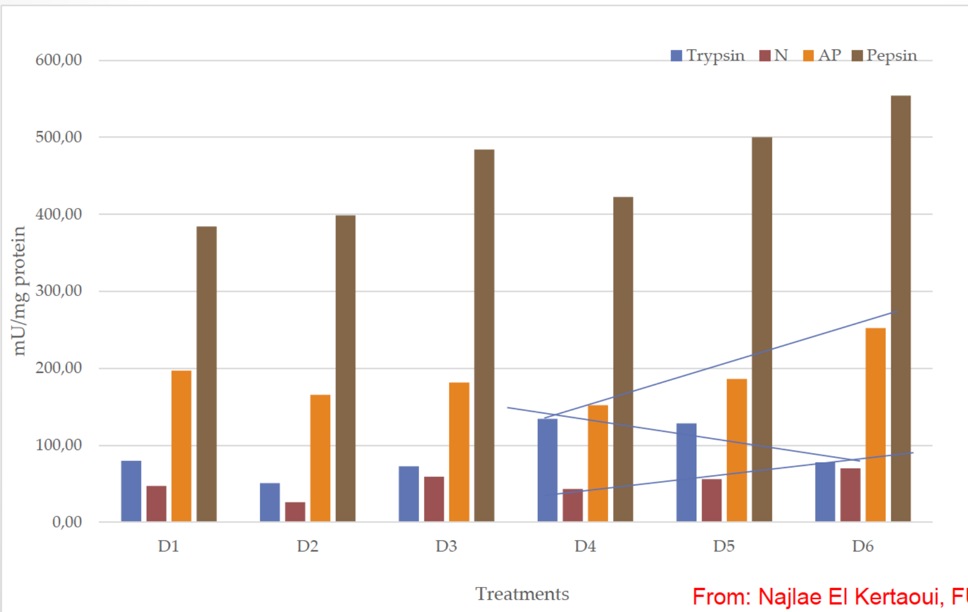


WP 10. Pikeperch

- Task 10.1. Effect of selected dietary nutrients on pikeperch larval development and performance

Figure 3 Larval enzymatic response 30 dph.

	D1	D2	D3	D4	D5	D6
Trypsin	79,57^{abc}	50,96^{c}	72,51^{bc}	134,35^{a}	128,37^{ab}	77,85^{abc}
Peptidase (N)	47,07^{bc}	25,97^{c}	59,01^{ab}	43,16^{bc}	55,76^{ab}	70,11^{a}
Phosphatase (AP)	196,83^{ab}	165,56^{b}	181,38^{ab}	152,11^{b}	186,13^{ab}	252,29^{a}
Pepsin	384,21^{b}	398,46^{b}	483,98^{a}	422,50^{ab}	499,89^{ab}	553,93^{a}
Amylase (u)	5,148	4,414	5,255	4,703	4,499	4,292
Leu-ala (U)	376,21	350,93	395,20	371,00	364,43	361,12



From: Najlae El Kertaoui, FUNDP

..larval FA composition, skeletal deformities, staining & gene expression, tissue proteomics… still awaiting lab analyses

D 10.1 month 36

Implementation: DTU, FUNDP

WP 10. Pikeperch

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

Background

- Dietary contents of n-3 and n-6 C18-PUFA precursors and also environmental salinities may influence pikeperch C14 fatty acid substrates metabolism

Objectives:

- To determine the pathways and activities of LC-PUFA biosynthesis in pikeperch larvae 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.

Materials & methods:

Two parameters tested in 18 tanks:
- High ALA (18:3n-3) vs. high LA (18:2n-6) (in Artemia)
- Three salinity levels (0-, 5-,10 ppt)

Analyses:
- Metabolism of lipid classes, esterification (HPTLC, TLC)
- Enzymatic activity, gene expression
- Lipid class composition and FA content
- Growth
- Deformities

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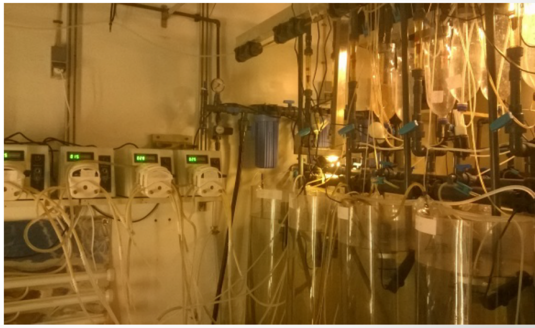
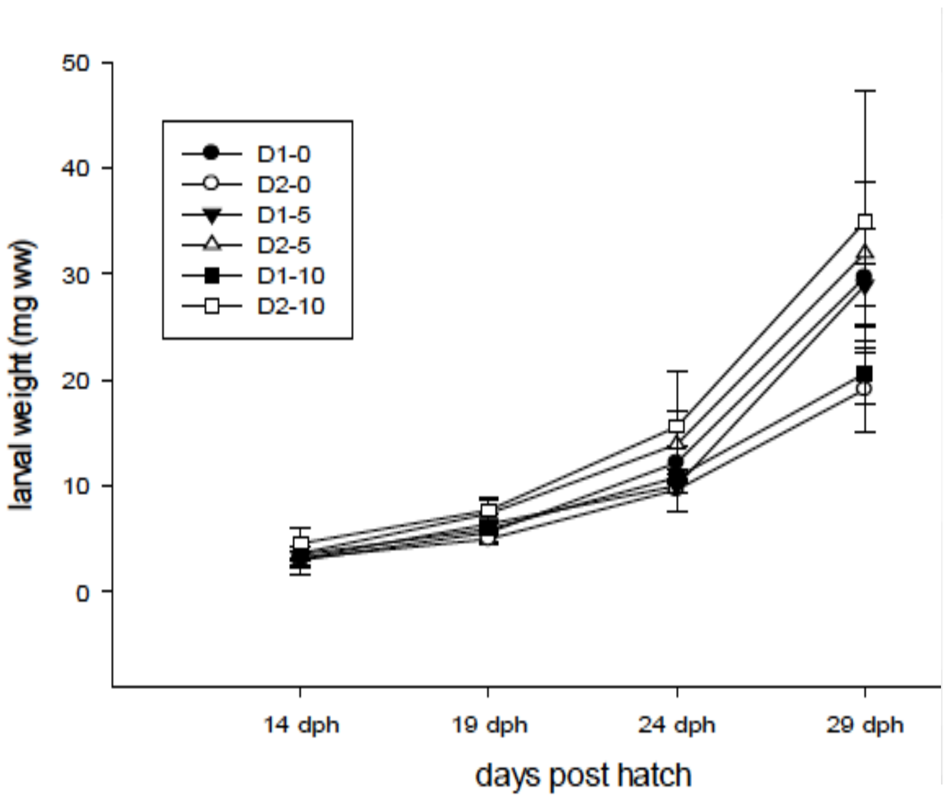
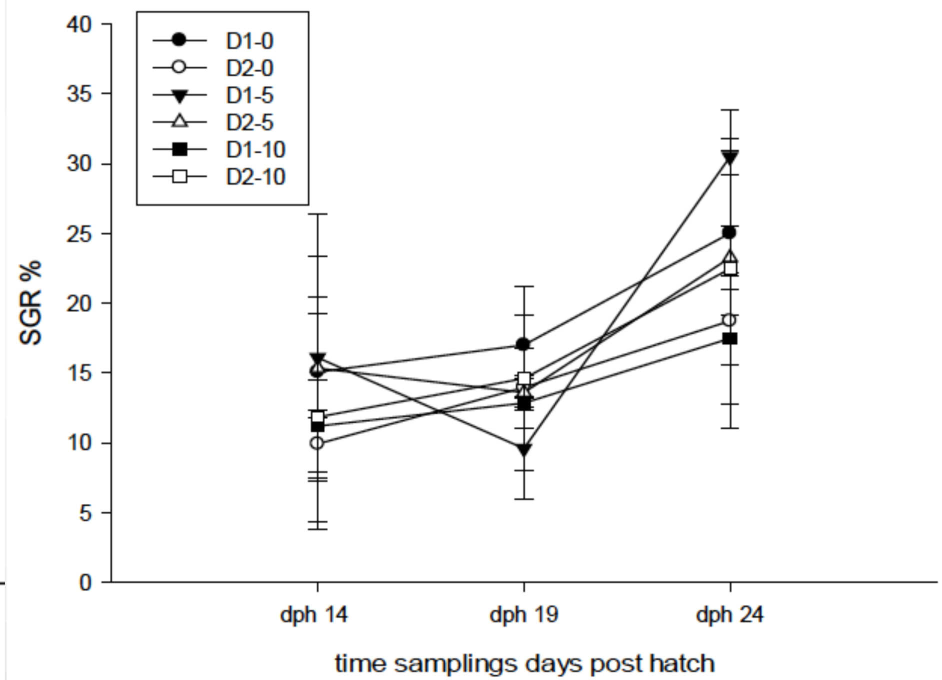


Fig. 1 Exp. setup, tanks and peristaltic pumps for accurate seawater flow

WP 10. Pikeperch

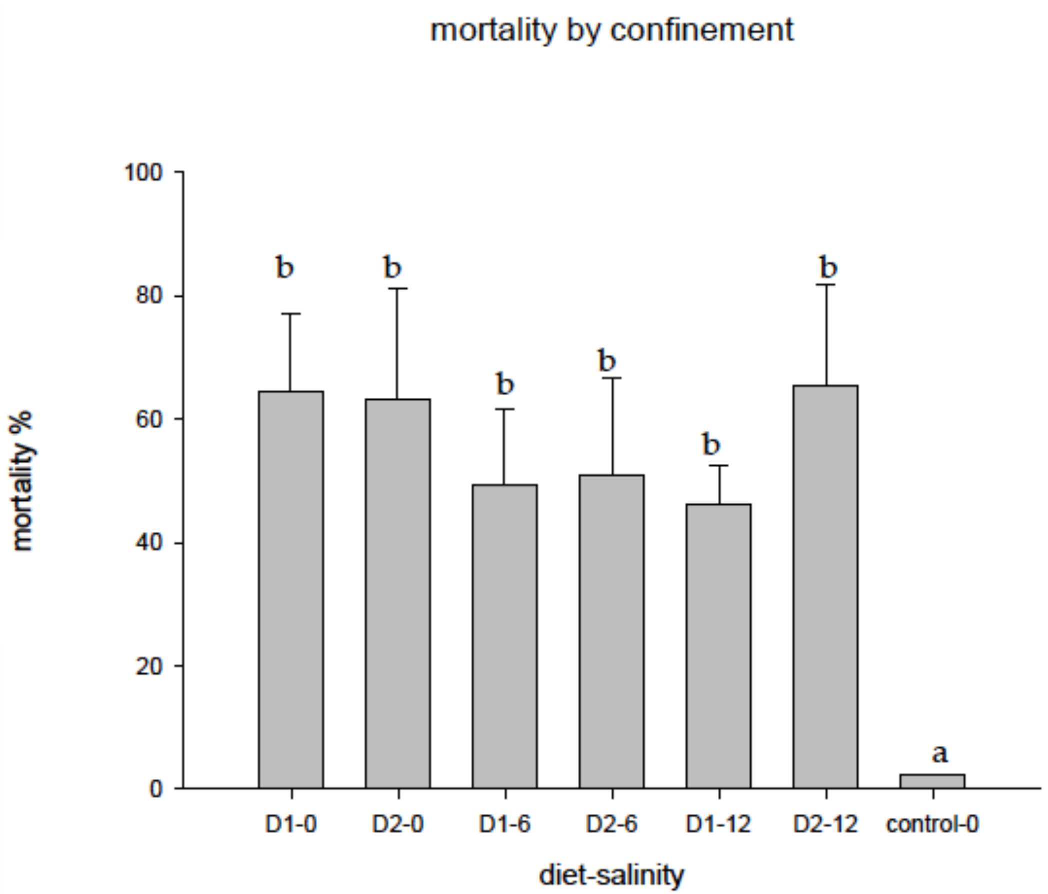
- Task 10.2. Effects of pikeperch early fatty acid nutrition on long-term stress sensitivity





WP 10. Pikeperch

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



Implementation: DTU, FUNDP, ULL, FCPCT

Larval fatty acid composition , enzymatic response, eicosanoid production, uptake and metabolism of lipid classes/ esterification deformities, staining & gene expression…still awaiting lab analyses

D 10.2 Month 36

WP 11. Atlantic Halibut

Objective: to develop a protocol for early weaning, to develop a production strategy for on-grow Artemia, to improve growth in late larval stages, and juvenile quality, through feeding witg on-grow Artemia, to better understand the effects of RAS vs FTS on larval nutrient utilization to investigate how dietary phospholipids after weaning affects growth and lipid metabolism.

Task 11.1. Early weaning of Atlantic Halibut (Led by IMR)
Task 11.2. Development of a production strategy for on-grown Artemia (Led by IMR).
Task 11.3. Nutrient retention and digestive physiology of juveniles fed Artemia nauplii or on-grown Artemia (Led by NIFES)
Task 11.4. Comparison of nutrient retention in larvae reared in RAS vs FTS (led by NIFES)
Task 11.5. Effect of dietary PL on digestion, absortion and metabolims of lipids in juveniles (Led by NIFES)

Task 11.1 Early weaning of Halibut larvae (Deliverable due in Dec. 2016)

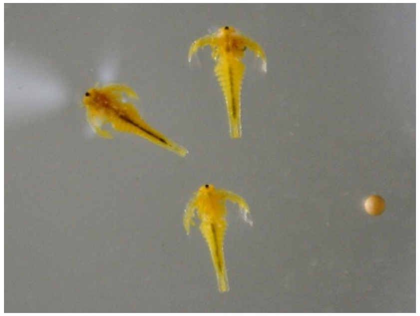
1. Survey of commercial feeds for marine fish larvae
2. Choice of experimental testdiets:
   - Otohime, AgloNorse, Gemma micro
3. Experimental design:
   - 9 tanks of 50L with similar light conditions, clay for turbidity, 12°C, relatively high water flow
   - Larvae (27 dpff), previously fed Artemia, were fed the three feeds by belt feeders and hand feeding, in triplicate
   - Feed intake was monitored twice a day for 4 days.
4. Preliminary results:
   - Larvae fed Otohime had high feed intake the first day. The feed was superior with respect to feed intake throughout the experiment and the tanks were cleaner.
   - Quantitative measures will be provided later.

Task 11.2 Nutrient profile of ongrown Artemia (Delivered in 2015)

1. Develoment of macronutrients, fatty acids and amino acids over a four days culture period
2. Nutrient profile of Artemia grown for three days compared to Artemia nauplii

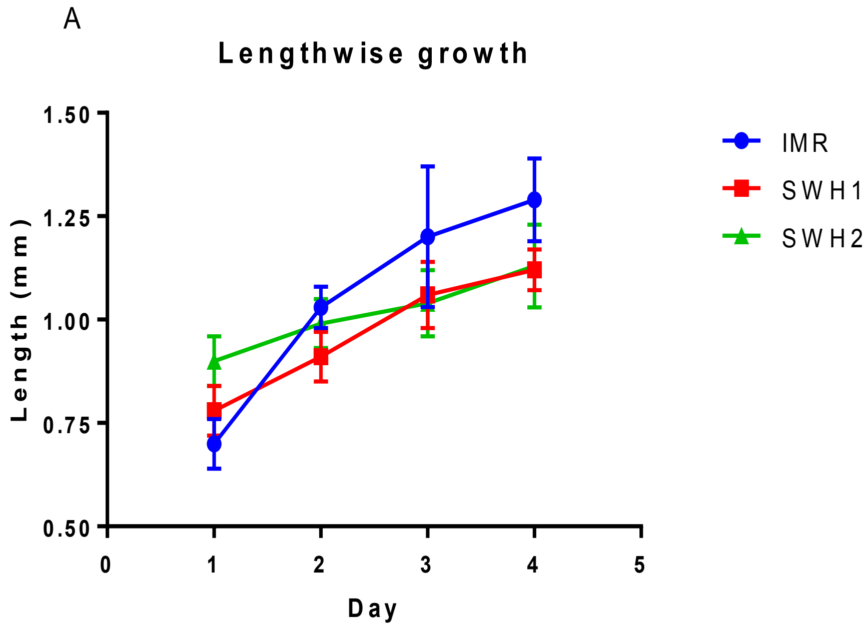
Artemia grown on

Oriculture from Skretting



Artemia grown for 3 days from nauplii compared to an *Artemia* cyst.

Growth and survival in Artemia grown for 4 days



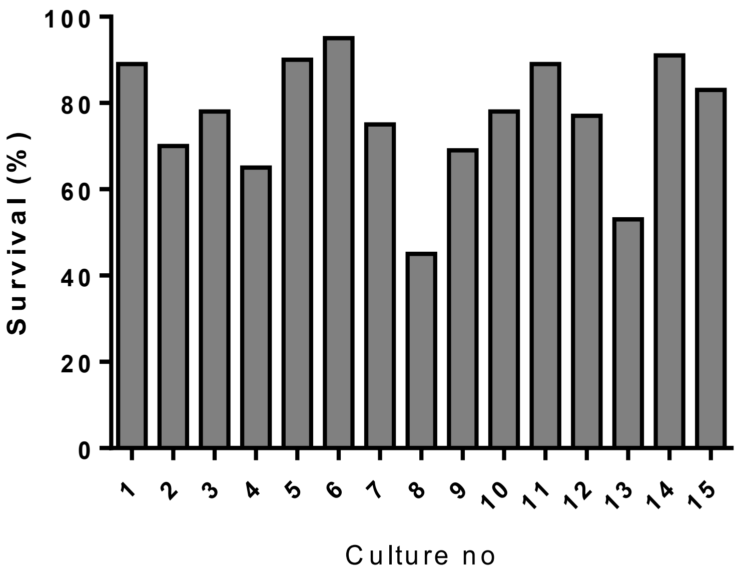
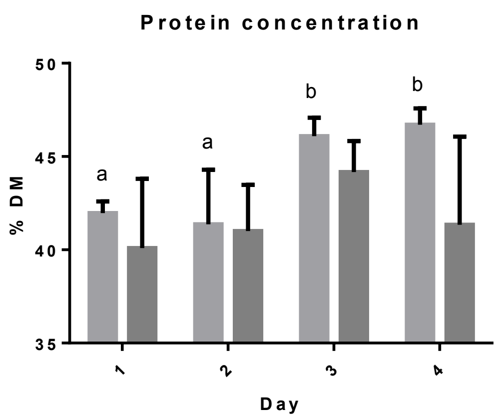


Figure 5. A. Growth of *Artemia* cultured for four days at IMR and SWH (mean ±SD). B. Survival in 15 batches of on-grown *Artemia* cultured for three days at IMR. Numbers are based on numbers of *Artemia* ml^{-1} at start and end of the 3-day culture period.

Development of macronutrients over 4 days of culture

A



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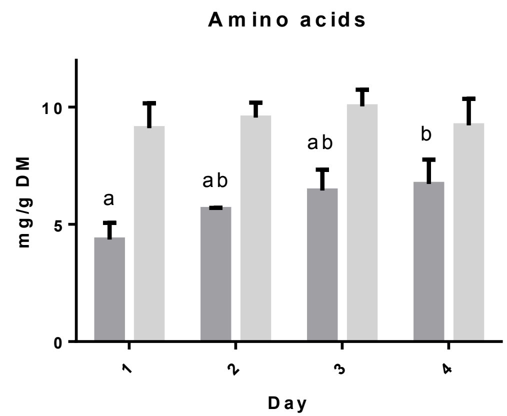
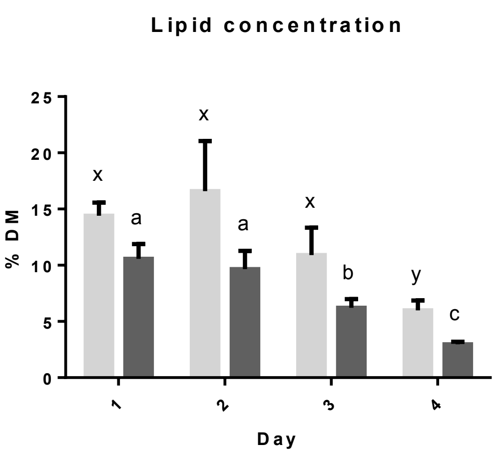


nauplie

C



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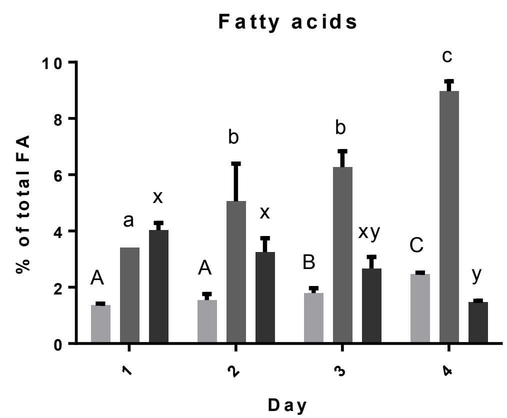
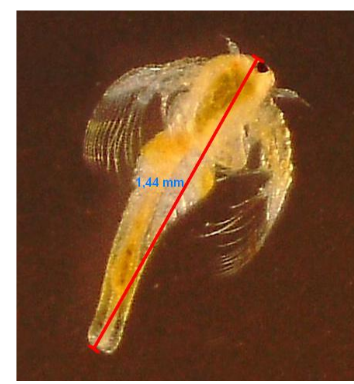
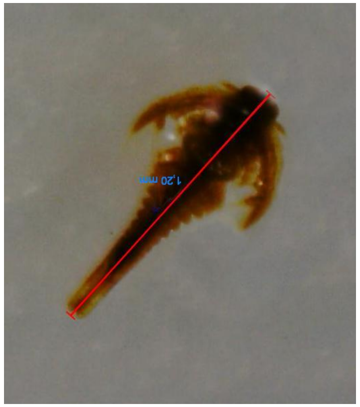




Day 4

Change in nutrient profile in Artemia grown for 3 days

	Unit	Nauplii enriched	On-grown enriched	*P* Day 1-3
Dry matter	g 100g^{-1}	8.4±0.6	8.3±0.4	0.884
Protein Nx5.30	g 100g^{-1}	46±1	51±2	0.026
TAA	g kg^{-1}	411±10	452±10	0.008
FAA	g kg^{-1}	70±4	92±13	0.044
Taurine	g kg^{-1}	4.4±0.2	5.5±0.6	0.040
Glycogen	g kg^{-1}	25±3	7.1±3.2	0.002
Lipid	g 100g^{-1}	17±1	11±1	0.004
TFA	g kg^{-1}	147±6	75±8	0.000
PL	% TL	24±3	34±3	0.013
ARA	% TFA	2.4±0.1	2.1±0.1	0.016
EPA	% TFA	4.1±0.2	6.0±0.7	0.010
DHA	% TFA	5.9±0.6	17±2	0.001
Thiamine	mg kg^{-1}	10.8±0.8	12.5±1.1	0.096
Vitamin C	mg kg^{-1}	1037±336	1401±|66	0.168
Vitamin D3	mg kg^{-1}	0.12±0.01	0.24±0.01	0.000
Vitamin E	mg kg^{-1}	580±27	890±224	0.076
MK4	µg kg^{-1}	1040±137	102±37	0.000
Phylloquinone (K1)	µg kg^{-1}	13±1	281±131	0.024
Total vitamin K	µg kg^{-1}	1073±124	778±340	0.231
Iodine	mg kg^{-1}	5.2±0.5	8.2±0.5	0.002
Selenium	mg kg^{-1}	1.7±0.1	1.06±0.17	0.000
Ca	g kg^{-1}	3.4±0.5	3.1±0.5	0.460
K	g kg^{-1}	15±1	14±0.1	0.152
Mg	g kg^{-1}	8.2±0.8	7.1±0.9	0.165
P	g kg^{-1}	11.1±0.9	10.9±0.4	0.420



Task 11.3 Nutrient retention and digestive physiology in response to on-grown Artemia
(Deliverable due in Dec. 2016)

- Atlantic halibut larvae fed Artemia nauplii until 14 dpff (days post firstfeeding)
- One group was fed nauplii, and the other ongrown Artemia until 28 dpff (2+ out of 3 meals)
- No difference in larval performance. 100% pigmentation and good eye migration (score: more than 2.5/3) in both groups.
- Samples taken for nutrient analyses (NIFES) and analyses of digestive capasity (ULL) after end of feeding ongrown Artemia

Only analyses of digestive capacity remains for this task

Nutrient profile of halibut larvae fed Artemia nauplii or ongrown Artemia from 15-25 dpff

On wet wt		Start	Nauplii	Ongrown
Protein	%	-	11.4±0.4	11.5±0.6
Sum FAA	mg/g	3.5	4.4±1.1	4.8±0.3
Taurine	mg/g	1.4	1.8±0.2	1.8±0.1
Glycogen	mg/g	0.95	1.6±0.3	1.4±0.3
20:4n-6 %	%TFA	5.2	6.2±0.1	6.2±0.2
20:5n-3 EPA %	%TFA	8.4	7.8±0.3	6.9±0.2
22:6n-3 DHA %	%TFA	14	9.6±0.9	8.4±0.7
Total FA	mg/g	16	15±3	19±2
Thiamin	mg/kg	2.6	2.1±0.2	2.2±0.2
Vitamin C	mg/kg	158	155±31	136±12
Vitamin-D3	mg/kg	0.02	0.01±0.00	0.01±0.00
Vitamin E	mg/kg	37	25±2	23±1
Vitamin A1	mg/kg	0.7	1.2±0.1	1.1±0.1
Iodine	mg/kg	0.26	0.28±0.02	0.26±0.01

Remaining tasks

- Task 11.4 Comparison of nutrient retention in Atlantic halibut larvae reared in RAS vs FTS.
  - Deliverable due December 2016. The experiment will be performed this spring/summer (March-July)
- Task 11.5 Effect of dietary PL on digestion, absorption and metabolism of lipids in Atlantic halibut juveniles.
  - Deliverable due December 2017. The experiment will be performed this Autumn (August-October)

Malpigmented

No eye migration

Normal

Implementation: IMR, NIFES, SWH, ULL, SARC

WP 12. Wreckfish

Objective: to test the effectiveness of live prey and influence of enrichment on larvae
To determine the influence of broodstock feeds on fecundity and spawning quality

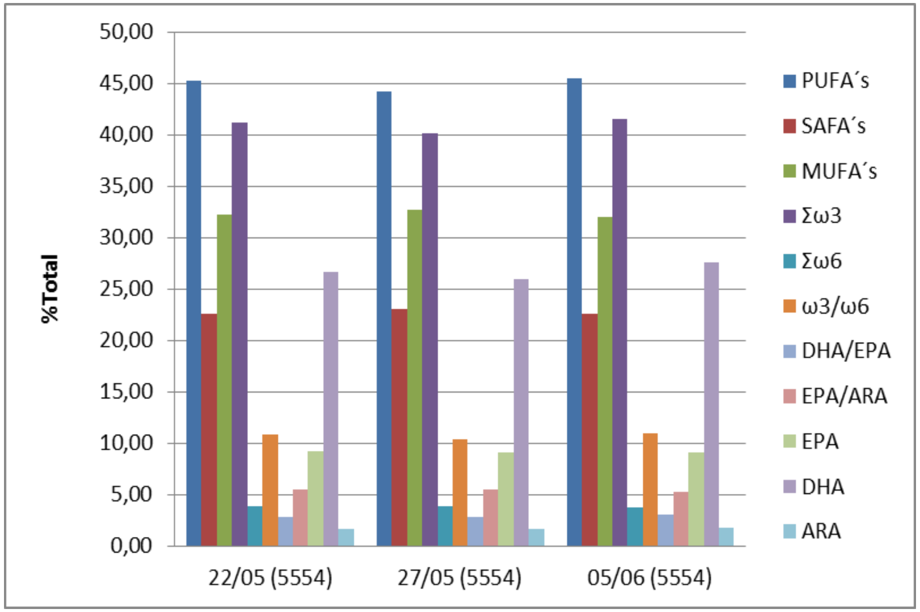
Task 12.1. Live preys and enrichments for larvae (Led by CMRM)
Task 12.2. Influence of broodstock feeding regimes for fecundity and spawn quality (led by IEO)

Task 12.1. Live preys and enrichments for wreckfish larvae

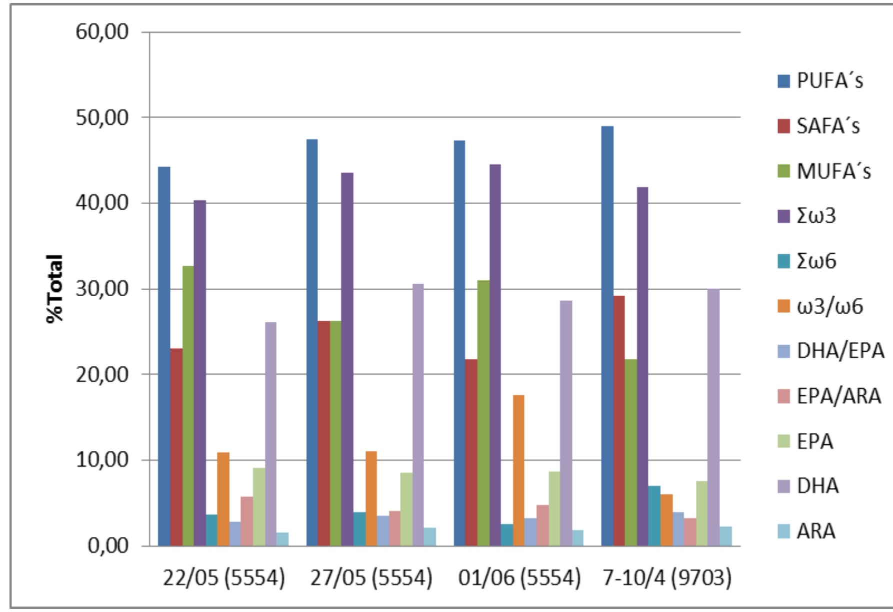
Some wreckfish larvae from different spawnings of IEO and AF broodstocks were obtained with a low survival rate at hatching and mortality was 100% at 20 days after hatch.
The enrichment of live food was based in commercial products and some samples of larvae were taken out at days 0, 5 and 10 of life, to obtain the fatty acid profile of wreckfish larvae.

The influence of different enrichments products for live food in wreckfish larvae could not be tested due to the amount of larvae obtained was not sufficient to perform the experiments.

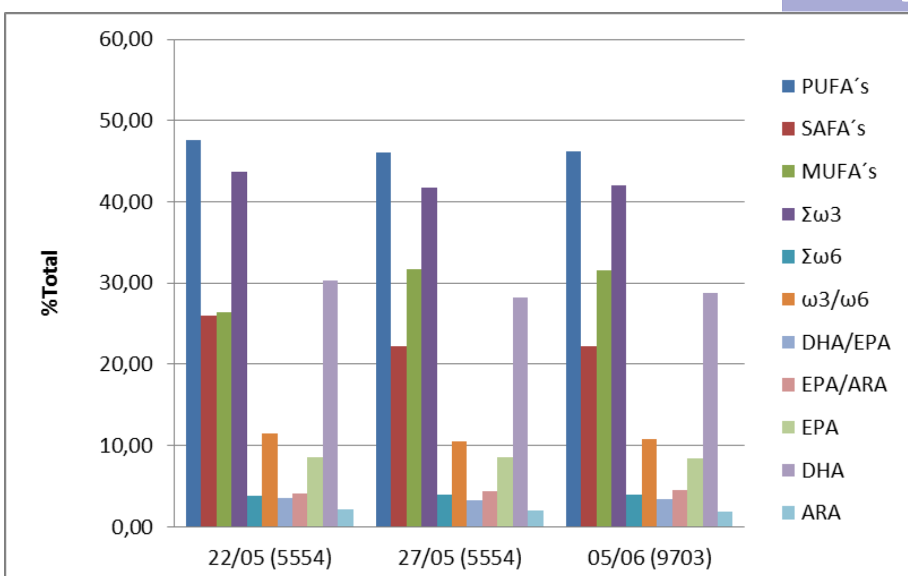
Hatching



5 DPH



10 DPH



Fatty acid profile of wreckfish larvae

Fatty acid profile have a little variation in the first 10 days of life

PUFA:44-48%; SAFA: 22-29%; MUFA:22-33%; ∑W3: 40-45%; ∑W6: 2-7%; EPA: 8-9%; DHA: 26-31%; ARA 2%

Task 12.2. Influence of broodstock feeding regimes on fecundity and spawn quality

The activities are related to the composition of wild fish and the feeding of wreckfish broodstock

Some information was obtained from:

✓Bibliographic information which is scarce

✓Biochemical composition of wild wreckfish tissues . A total of 86 fish were sampled from the Azores (North Atlantic, Portugal)

✓Analysis of tissues of cultured wreckfish and comparison with those obtained from wild fish. Data are obtained from 4 cultured fish.

✓Experiment of comparison of feeding of broodstock with semimoist diet and a "new dry food"

Objetive: To advance in the formulation of a specific "dry food" for wreckfish.

✓ Biochemical composition of wild wreckfish

	Muscle	Liver	Gonads
Proteins(%DW)	84,41±7,34	37,94±13,66	38,96±18,21
Lipids(%DW)	6,92±3,39	40,19±15,25	35,05±22,27
Fatty acids (% total)			
SAFA´s	28,83±1,28	26,11±3,51	28,12±2,54
MUFA´s	32,09±5,43	56,23±8,80	44,00±7,99
ARA	3,11±0,79	1,55±0,88	3,05±2,20
EPA	4,55±0,70	3,09±1,37	4,37±1,34
DHA	26,38±3,33	9,31±5,05	15,29±4,37
PUFA´s	39,08±4,41	17,66±8,19	27,87±7,58
Σω3	34,51±3,75	14,93±7,01	23,45±6,01
Σω6	4,08±0,81	2,55±1,23	4,07±2,18
n-3/n-6	8,50±1,18	5,79±1,42	6,52±2,58
DHA/EPA	5,69±1,23	2,99±0,91	3,49±0,77
EPA/ARA	1,54±0,37	2,13±0,60	1,95±1,04

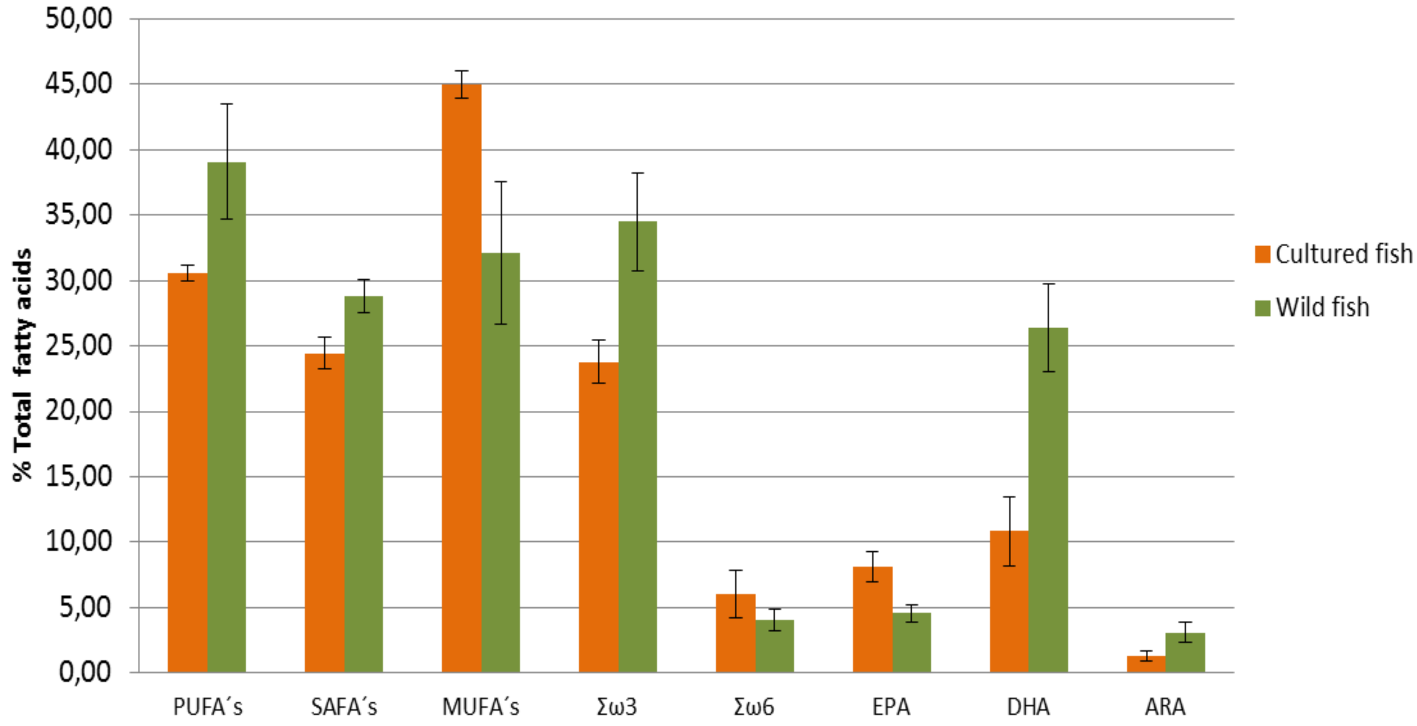
- ➢ Big amount of proteins in muscle (85% DW) and low lipid content (7% DW)
- ➢ In fatty acids 26% of DHA and 30% of EPA+ARA

✓ Biochemical composition of cultured wreckfish

	Muscle	Liver	Gonads
Proteins(%DW)	75,92±8,88	31,10±9,42	61,16±14,41
Lipids(%DW)	27,49±10,06	61,76±12,18	14,93±5,87
Fatty acids (% total)			
SAFA´s	24,46±1,25	22,44±2,27	26,35±0,46
MUFA´s	44,98±1,02	60,50±5,45	38,08±4,02
ARA	1,32±0,38	0,58±0,12	3,52±1,71
EPA	8,11±1,17	2,92±0,19	8,75±2,01
DHA	10,85±2,66	7,29±1,71	14,13±3,98
PUFA´s	30,57±0,58	17,07±3,32	35,57±3,60
Σω3	23,78±1,68	13,44±2,00	27,33±3,02
Σω6	6,02±1,85	3,48±1,99	7,61±1,46
n-3/n-6	4,51±2,39	4,97±3,31	3,68±0,80
DHA/EPA	1,38±0,49	2,48±0,47	1,71±0,72
EPA/ARA	6,58±2,15	5,16±1,00	3,27±2,45

- Cultured fish have a higher lipid content in muscle (27%DW) and liver (62%DW) than in muscle (7%DW) and liver of wild fish (40%DW) .

Fatty acid composition of muscle from wild and cultured wreckfish



- Values of PUFA and ∑W3 are higher in wild wreckfish than in cultured fish, however MUFA values are higher in cultured fish than in wild fish
- DHA values represent 14% in cultured fish and 26% in wild fish and ARA 1 % in cultured fish and 3% in wild fish.

✓ Experiment of comparison of feeding of broodstock with semimoist diet and a "new dry food"

Tanque S1: 5 fish (3♀ and 2♂)
Feed: semimoist diet
14,8% blue fish
14,8% white fish
18% mussel
17,6% squid
24,8% fish meal

Lipids (%DW)

17,35±2,45

17,31±0,33

Tanque S2: 6 fish (3♀, 1♂ and 2 undet)
Feed: Dry food (new formulation)

Ingredients	CTRL V2
	%
Fishmeal 70 LT FF Skagen	50,000
CPSP 90	7,500
Squid meal	12,500
Krill meal (Aker Biomarine)	6,000
Wheat Gluten	6,000
Wheat meal	4,940
Tuna oil	2,000
Incromega DHA 500TG	2,000
VEVODAR	3,000
Vit & Min Premix PV01	2,000
Lutavit E50	0,060
Soy lecithin - Powder	2,000
Macroalgae mix	1,000
Antioxidant powder (Paramega)	0,200
Antioxidant liquid (Naturox)	0,200
SelPlex - Se yeast	0,020
Carophyll Pink 10% - astaxanthin	0,050
Nucleotides (Nucleoforce)	0,030
L-Taurine	0,500
Total	**100,000**

Fatty acid profile (%total) of semimoisture diet and new dry food

	Semimoisture diet	New Dry food
14:0	4,81±0,59	3,92±0,10
16:0	19,51±0,93	14,88±0,10
17:0	1,01±0,18	0,78±0,05
18:0	4,05±0,30	4,47±0,04
ΣSFAs	29,96±1,19	24,44±0,22
16:1n-7	4,79±0,51	3,05±0,02
18:1n-9	12,21±1,46	14,48±0,09
18:1n-7	3,82±0,35	2,17±0,04
20:1n-9	3,55±0,64	3,79±0,03
22:1n-11	3,53±1,03	4,86±0,22
ΣMUFAs	31,55±0,97	30,83±0,15
18:2n-6	7,03±0,64	7,55±0,01
18:3n-3	1,16±0,14	1,23±0,02
18:4n-3	1,66±0,26	1,49±0,11
ARA	1,25±0,28	9,06±0,06
EPA	8,84±0,48	6,61±0,02
DPA	1,29±0,24	0,99±0,15
DHA	15,91±1,16	16,67±0,32
ΣPUFAs	38,491,65	44,72±0,37
Σn-3	29,40±1,57	27,61±0,43
Σn-6	8,28±0,56	16,62±0,07
n-3/n-6	3,56±0,23	1,66±0,03
DHA/EPA	1,43±0,66	2,52±0,04
EPA/ARA	7,45±1,86	0,73±0,01

- **The main differences are in PUFAn-6 content specially in ARA**

Fatty acids ≤ 1% are not included

Fatty acid profile of oocytes from two different females fed with semimoist diet (S1) and the new dry food (S2)

	Oocytes S1	Oocytes S2
14:0	1,47±0,07	1,42±0,02
16:0	15,35±0,65	13,750,55
17:0	1,02±0,20	0,91±0,19
18:0	4,92±0,28	4,70±0,19
ΣSFAs	23,12±0,86	21,09±0,76
16:1n-9	1,34±0,14	0,91±0,03
16:1n-7	4,11±0,13	3,45±0,17
18:1n-9	17,54±0,97	17,45±1,01
18:1n-7	4,94±0,46	4,64±0,51
20:1n-9	1,74±0,05	2,27±0,18
ΣMUFAs	32,41±0,86	31,69±0,46
18:2n-6	3,87±0,18	4,14±0,48
ARA	1,76±0,16	3,72±0,22
EPA	7,70±0,32	6,61±0,31
DPA	2,63±0,18	2,70±0,15
DHA	26,33±1,07	27,92±1,06
ΣPUFAs	44,47±1,62	47,23±1,14
Σn-3	38,62±1,57	39,18±1,16
Σn-6	5,63±0,20	7,96±0,59
n-3/n-6	6,86±0,32	5,01±0,44
DHA/EPA	3,42±0,08	4,23±0,27
EPA/ARA	4,40±0,44	1,78±0,11

- A clear relationship between fatty acid profile of broodstock food and oocytes was found

Fatty acids ≤ 1% are not included

- The main differences were in PUFA W6 which reached 8% in oocytes from ♀S2 and 5,6% in oocytes from ♀S1

Tasks 12.1 and 12.2 will result in deliverables:

D.12.1. Effect of live prey enrichment products on wreckfisl larval performance. (Month 54)

D12.2 Recommendations for wreckfish broodstock feeds.(Month 57)

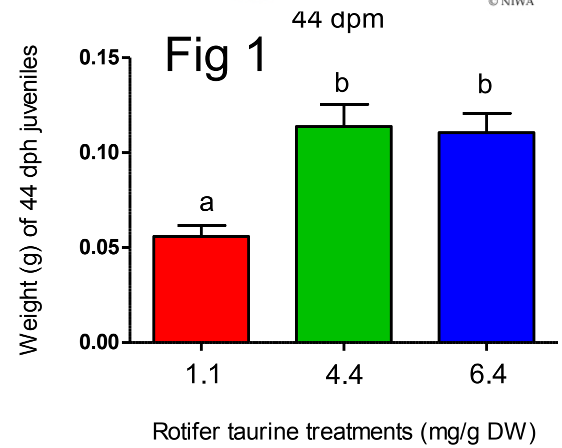
WP 13. Grey Mullet

Objective: to improve enrichment products, weaning, grow out and broodstock diets and to compare the effect of two types of potential soybean meals on growth, intestinal morphology and inflammation, peroxidation and antioxidant mechanisms and intestinal pathology

Task 13.1. Improvement of larval performance (Led by IOLR)
Task 13.2. Determining mullet nutritional needs for improved weaning to a dry diet (Led by IOLR).
Task 13.3. Determining nuritional needs for a more cost-effective production and digestive physiology of juveniles fed Artemia nauplii or on-grown Artemia (Led by IOLR)
Task 13.4. Design adequate feeding regimes for broodstock to optimize reproduction success (led by UNIBA)

Task 13.1 Dietary taurine on larval and juven performance

- Barie meeting in 2014:Lasting effect of rotifer taurine enrichment on growth ($P<0.05$) on 44 dph juveniles (32 days after rotifer feeding) (Fig. 1)



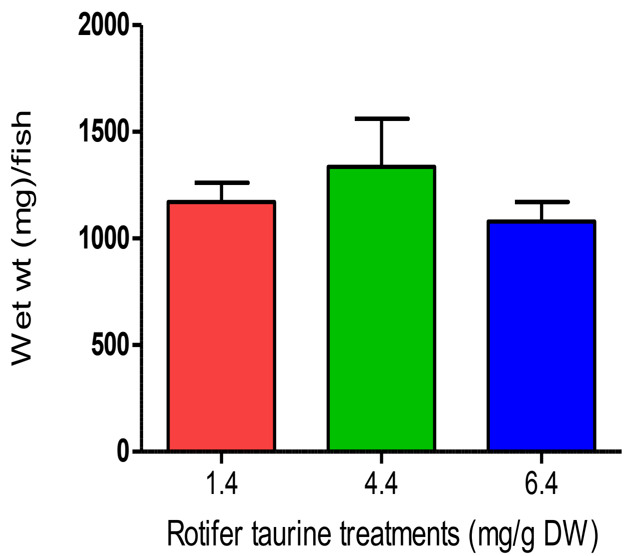
Aim: in 2015 tested if this taurine growth advantage continued after 44 dph.

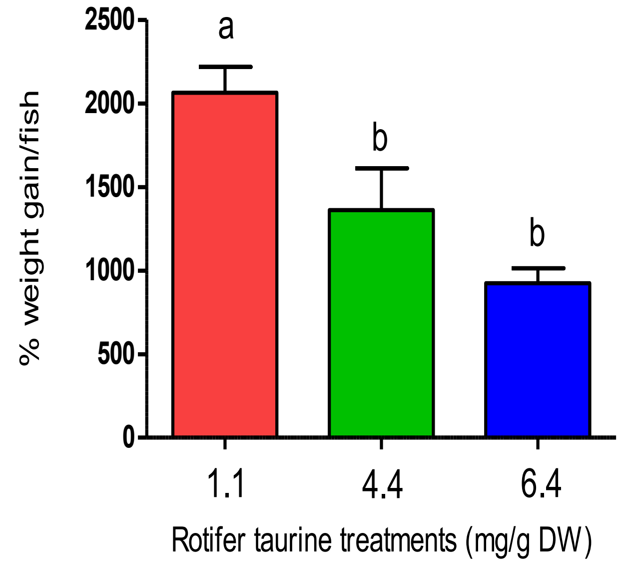
- 450 fish (44 dph) from each of the rotifer treatments (1.1, 4.4, 6.4 mg taurine/g DW) which differed ($P<0.05$) in average size (50.5, 113.84 and 125.42 mg wet weight, respectively were stocked in three replicate 20 l mesh containers (150 fish/container).

- Replicate containers from each taurine group floated in 1m^3 tanks fed by 40 ‰ , filtered (10 µm) and UV treated ambient sea water (22.5 to 24.5^{o}C).

- All fed same diet (Caviar Mem, Bernaqua, Belgium) from 44-80 dph and then weighed

Results and Conclusions





- Despite the significant (P<0.05) difference in weight among treatments at the beginning of trial, all treatments demonstrated similar wet weights at end of experiment.
- However, smaller fish fed low taurine rotifers grew significantly faster (P<0.05) than other treatments.
- Feeding taurine containing starter diets at the fry stage allowed growth compensation in the smaller fish.
- Importance for grading in fry management to grow more homogenous populations.
- Samples taken for gene expression for Pept1 in small and large fry populations.

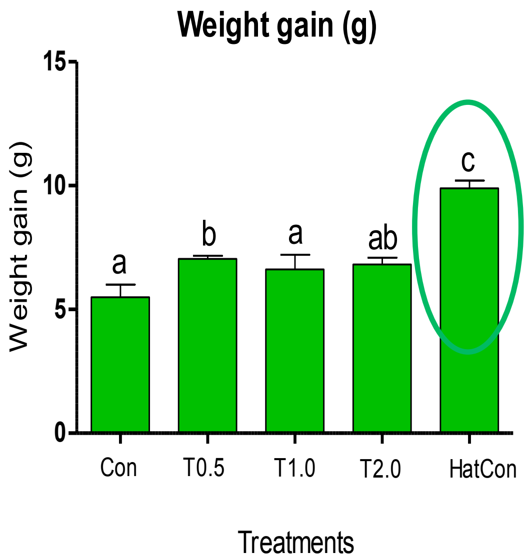
Task 13.1 Dietary taurine on larval and juvenile performance

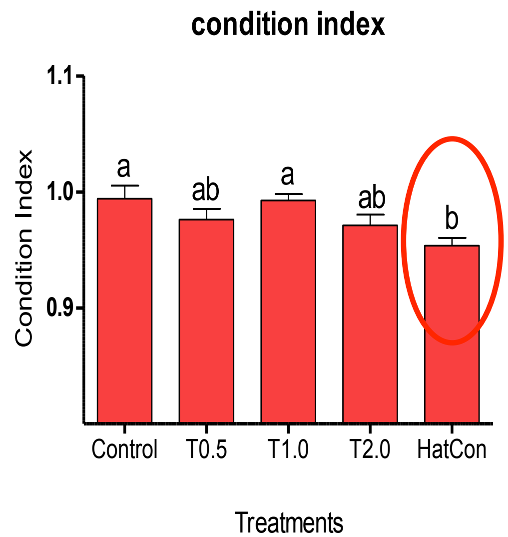
Aim: Effect of dietary taurine on growth in 126-185 dph juvenile grey mullet

- Individually weighed juvenile mullet (5.46 ± 0.04 g) were stocked in 400 l conical tanks (40 fish/tank) to test 5 diets in 4 tank replicates/ treatment.
- 7.5 tank exchanges/day of 40 ‰ temperature controlled (25 ^{o}C), ambient sea water.
- Fish were fed extruded taurine pelleted feed (Sparos Ltd., Portugal) for 58 days (126-185 dph)
- Taurine treatments tested:

1. Control-no taurine supplementation
2. 0.5 % DW diet
3. 1% DW diet
4. 2% DW diet
5. Hatchery control (starter diet:Ulva - 1:1, 35% protein)

Results and Conclusions





- Although fish are omnivorous at this stage, 0.5 % taurine DW diet gave significantly ($P<0.05$) better growth than the control where higher taurine levels (1 and 2%) did not improve fry performance
- However, hatchery control gave significantly ($P<0.05$) better growth than all other treatments and produced markedly ($P<0.05$) longer fish.
- Suggests nutrients in the hatchery control diet are lacking or deficient in the taurine diets.

Thank you for your attention