New species for EU aquaculture

Deliverable Report

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Deliverable Title	Effect of live prey enrichment products on wreckfish larval performance		
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Objective: To determine the quality of enrichment products and the effect on wreckfish larvae quality.

Description: The deliverable presents:

- Some new enrichment products for live food developed for larval wreckfish.
- The biochemical composition of live food enriched with different enrichment products.
- A comparison of wreckfish larvae fatty acid profile from the same spawning fed with rotifer and *Artemia* enriched with two different enrichments products.
- The fatty acid profile of larvae fed with the same live food enrichments from different spawnings.
- The fatty acid profile of eggs and wreckfish larvae through the larval development (until 58 dph).

1.-INTRODUCTION

The wreckfish, *Polyprion americanus*, is a good candidate for aquaculture due to its fast growth, good adaptation to captivity (Machias et al., 2003; Papandroulakis et al., 2004; Rodriguez et al., 2014), good flesh quality (Linares et al., 2015), high market value and consumer acceptance internationally. The major bottleneck to obtain success in the culture of wreckfish is the development of larval rearing.

Nutrition studies performed in the DIVERSIFY project were focused mainly on the development of adequate live prey enrichments for wreckfish larvae and broodstock feeds for enhancing fecundity and spawn quality. These are the first steps for the development of proper nutrition and culture of this polyprionidae species.

The quality of the first feeding regimes play an important role in the success of larval culture with dietary lipids being recognized as one of the most important nutritional factors that affect larval growth and survival (Watanabe et al., 1983).

The role of polyunsaturated fatty acids in broodstock nutrition which are involved in several metabolic pathways as energy production, membrane structure and function, eicosaenoids production and control of lipid homeostais has been documented (Watanabe, 1982; Tocher, 2003). Marine fish have restricted ability or are unable to synthetize n-3 and n-6 LC-PUFAs from their precursors, alpha linolenic acid (18:3n-3) and linoleic acid (18:2n-6) respectively (Tocher, 2003; Izquierdo and Koven, 2011; Oliva-Teles, 2012). Therefore, 22:6n-3(DHA), 20:5n-3(EPA) and 20:4n-6(ARA) are considered essential fatty acids for marine fish and must be included in the diets. An understanding of the PUFA requirements of marine fish larvae requires definition of optimal dietary ratio of DHA, EPA and ARA (Sargent et al., 1997).

Due to the lack of information about wreckfish larvae nutrition, studies done in DIVERSIFY about the composition of wild wreckfish gonads and eggs from reared females were very useful as the basis to formulate the enrichment products for live prey used as feeding of wreckfish larvae (data shown in D12.2). The development of enrichment products of live prey was very important to get success in the larval culture.

The aim of this study was to determine the quality of enrichment products and the effect on wreckfish larval quality.

2.-MATERIALS AND METHODS

Enrichment products

Some new enrichment products for live food were developed. Data of biochemical analyses performed at CIMA (CMRM) of gonads from wild wreckfish females and eggs and larvae obtained from reared fish were sent from CMRM to FCPCT (P2) to develop some live food enrichment products for larval wreckfish.

In 2017, three experimental enrichment products were formulated to meet the EPA&DHA&ARA levels obtained from tissues of wild-caught wreckfish. For experimental enrichment preparation a combination of different products based on microalgae were used: Chlorella meal powder (>1% Fat, > 12% Protein; Shaanxi Pioneer Biotech Co., Ltd, Xi'an, China), Microalgae DHA Powder (>40% DHA; Shaanxi Pioneer Biotech Co., Ltd, Xi'an, China), and ARA Powder (>40% ARA; Shaanxi Pioneer Biotech Co., Ltd, Xi'an, China)(**Table 1).**

Table 1. Experimental enrichment ingredients.

Experimental Enrichment	*DHA- Rot*	*ARA-Rot*	*ARA-Art*
	Ingredients (g kg^{-1}diet)		
Chlorella powder	*500*	*500*	*400*
Microalgae DHA Powder	*400*	*400*	*500*
ARA Powder	*20*	*100*	*100*

The experimental enrichments for rotifers were formulated using two different levels of ARA (3 and 10%) and in the case of *Artemia* only one level of ARA (9%) was used. The analyses of the experimental products reached higher levels of ARA & EPA & DHA than reference values from samples (**Table 2**).

Table 2. Proximate (% dry matter) and fatty acids composition (%TFA) of experimental enrichment.

	DHA- Rot	*ARA-Rot*	*ARA-Art*
	Proximate analysis (% dry matter)		
Lipids	9.34±0.14	9.34±0.24	9.23±0.34
Proteins	25.58±0.21	30.08±0.13	22.54±0.09
Ash	4.34±0.03	5.13±0.10	6.11±1.85
	Fatty acid content (%TFA)		
Saturated	19.39	17.61	21.93
Monoenoics	8.30	8.76	8.35
n-3	48.82	45.72	45.14
n-6	20.87	25.43	24.26
n-9	4.55	5.65	4.88
Total n-3HUFA	44.20	41.42	44.62
14:0	0.40	0.47	0.41
16:0	17.10	13.49	17.90
16:1 n-7	0.85	0.52	1.12
18:0	1.30	2.93	2.91
18:1 n-9	3.90	5.03	4.23
18:1 n-7	0.54	0.50	0.46
18:2 n-6	12.99	11.66	7.88
18:3 n-3	4.24	4.00	0.19
20:1 n-9	0.09	0.08	0.08
20:4n-6 (ARA)	3.10	9.86	9.03
20:5n-3 (EPA)	6.33	5.87	6.40
22:6n-3 (DHA)	33.06	31.16	33.55
EPA/ARA	2.04	0.60	0.71
DHA/EPA	5.22	5.31	5.24
DHA/ARA	10.66	3.16	3.71
Oleic/DHA	0.12	0.16	0.13
Oleic/n-3HUFA	0.09	0.12	0.09
n-3/n-6	2.34	1.80	1.86

HUFA. highly unsaturated fatty acid; ARA. arachidonic acid; DHA. docosahexaenoic acid; EPA. eicosapentaenoic acid.

In 2018 two new enrichment products were formulated (**Table 3**): Control enrichment product (CE) and ARA enrichment product (AE) with a higher level of proteins and lipids, 46.7 and 29.9% respectively in CE and 43 and 31.8% in AE, than the levels of proteins and lipids of the enrichement products formulated in 2017. Saturated fatty acid contents (SAFA) were 30 and 26% (TFA) and Monounsaturates (MUFA) were 7.4 and 10.4 in CE and AE respectively. Polyunsaturates fatty acids (PUFA) had values between 62-63%, being the n-3PUFA 41% in both enrichment products and n-6 PUFA 19 and 22% in CE and AE respectively. ARA content was 2.1% in CE and 5.4% in AE. DHA/EPA and EPA/ARA ratios were 8.3 and 2 in CE and 7.2 and 0.9 in AE.. These enrichment products were used as live food enrichments for wrechfish larvae experiments in 2018.

Table 3. Protein, lipids and fatty acids of Enrichment Products (2018).

	Control Enrichment Product (CE)	ARA Enrichment Product (AE)
Proximate analysis (%dry matter)		
Proteins	46,73±0,18	43,00±0,16
Lipids	29,94±1,74	31,79±6,51
Fatty acids content (%TFA)		
14:0	1,08±0,02	0,96±0,13
16:0	24,66±2,70	20,92±1,36
17:0	0,42±0,39	0,72±0,00
18:0	2,08±0,17	2,41±0,21
Saturated (SAFAs)	29,73±2,94	26,01±1,95
16:1 n-9	0,23±0,00	0,19±0,00
16:1 n-7	1,79±0,02	1,75±0,21
18:1 n-9	2,85±0,66	4,84±1,16
18:1 n-7	0,69±0,18	1,07±0,05
20:1 n-9	0,72±0,28	0,49±0,02
Monoenoics (MUFAs)	7,37±0,54	10,41±2,29
18:2 n-6	5,49±0,18	5,26±0,36
20:4 n-6 (ARA)	2,13±0,10	5,44±0,21
20:5 n-3 (EPA)	4,33±0,53	4,75±0,02
22:6 n-3 (DHA)	35,84±4,04	34,19±1,58
Polyunsaturated (PUFAs)	62,48±4,69	62,59±2,27
Σn-3	41,26±6,47	41,37±1,21
Σn-6	19,14±0,32	21,95±1,23
n-3/n-6	2,24±0,26	1,89±0,16
DHA/EPA	8,30±0,06	7,20±0,37
EPA/ARA	2,03±0,16	0,87±0,04

HUFA, highly unsaturated fatty acid; ARA arachidonic acid; DHA docohexaenoic acid; EPA eicosapentaenoic acid

Enrichment standardized protocols

Rotifer enrichment:

L-type rotifer, *Brachionus plicatilis,* grown on commercial baker's yeast were used. Rotifers were enriched in conical 500 L tanks (water volume 400 L) at a density of 1000 rotifers mL^{-1} in an air conditioned illuminated room (12 L:12 D, from 8:00 am to 8:00 pm). The water was kept at a salinity of 37 g L^{-1} and 20 ± 0.01 ◦C. Rotifers were enriched twice at 6 and 3 hours prior to being collected (2:00 am and 5:00 am) with 0.3 g/million rotifers enriched. After 6h the rotifers were filtered cleaned and sampled for biochemical analysis.

Artemia Enrichment:

Artemia salina (EG Inve) was used . Newly hatched *Artemia* was enriched in 1 conical 150 L tanks (water volume 100 L) at a density of 500 Arte/mL^{-1} in an air conditioned illuminated room (12 L:12 D, from 8:00 am to 8:00 pm). The water was kept at a salinity of 37 g L^{-1} and 24 ± 0.01◦C. *Artemia* was enriched in a single dose 12 hours prior to being collected (20:00 pm) with 0.4 g/L. After this period *Artemia* was sampled for biochemical analysis.

Larval feeding experiments

Three different experiments of larval feeding were carried out in 2018:

1. *Effect of two different enriched live prey on larvae fatty acid composition*

The larval culture was performed at IEO facilities with larvae from one IEO broodstock spawning (24/18). The feeding sequence was: 0-9 dph, no feeding, 9-15 dph, rotifer enriched with control and ARA, 12-20 dph, nauplius of *Artemia* (A_0), 17-20 dph, enriched *Artemia* with ARA (A_1).

2. *Fatty acid composition of larvae from different spawnings*

Larvae from five different spawnings, four from IEO broodstock (14/18, 15/18, 24/18, 30/18) and another one from AF were analysed. All of them were fed with the same feeding (rotifer and *Artemia* enriched with ARA enrichment product).

The feeding sequence for each batch of larvae is shown in **Table 4.**

Table 4. Feeding sequence of larvae.

Spawns	0-5 dph	5-10 dph	10-15 dph	15-20 dph	20-25 dph	25-30 dph	30-40 dph	From 40- dph onwards
14/18 IEO	No feeding	No feeding	11 dph start feed Rot ARA	Rot ARA + Ao	Rot ARA+Ao End 24 dph			
15/18 IEO	No feeding	No feeding	13 dph start feed Rot ARA	Rot ARA + Ao	Rot ARA+Ao	Rot ARA+Ao End 28 dph		
24/18 IEO	No feeding	9 dph start feeding: Rot Control and Rot ARA	Rot Control and Rot ARA 12 dph Ao	Ao 17dph A_t enriched control and ARA	A_t enriched control and ARA	A_t enriched control and ARA End 21 dph		
30/18 IEO	No feeding	9 dph start feeding Rot ARA	Rot ARA	Rot ARA (15-17dph), Ao(15-18dph), A_t(18 dph)	A_t enriched ARA	A_t enriched ARA	A_t enriched ARA	A_t+ dry food 48dph
AF	No feeding	8 dph start feeding Rot ARA	Rot ARA	Rot ARA (15-19dph), Ao(18-20dph),	A_t(23 dph)	A_t	A_t	A_t+ dry food 48dph

3. *Evolution of fatty acid profile of eggs and wreckfish larvae through the larval development (until 58 dph)*

Some samples of eggs and larvae from a spawning of IEO (30/18) and cultured at IGAFA facilities from 1 dph to 58 dph were taken out to be analysed at CIMA facilities.

Sampling procedure

Samples of enrichment products, live food and larvae were taken out from the different experiments. All samples were stored at -80ºC and freeze dried prior to biochemical analyses. Some samples of larvae cultured at the IEO facilities from 30/18 spawning were collected along the larval culture to be measured.

Biochemical analyses

The analyses of enrichment products, enriched rotifers and *Artemia* were carried out by FCPCT (P2) and CIMA. Eggs and larvae of wreckfish were analysed at the CIMA.

Moisture was determined after drying the sample in an oven at 105 °C to constant weight; ash by combustion in a muffle furnace at 600 °C for 12 h; protein content (N×6.25) was determined by Kjeldahl method (AOAC, 1997).

The total lipids extraction was carried out following the Blight and Dyer method with chloroform:methanol (2:1) and gravimetric determination (Blight and Dyer (1959), modified by Fernandez-Reiriz et al. (1989)). Fatty acids methyl esters (FAME) were obtained by transesterification and methylation according to Lepage and Roy (1986).

FAME analyses were performed in a Clarus gas chromatograph (Perkin Elmer, Beaconsfield, UK) fitted with a flame ionization detector at 260 °C in triplicate. The separation was achieved using a capillary column SPTM-2330 fused silica (30 m length, 0.25 mm internal diameter and 0.2 µm film thickness). After holding at 140 °C for 5 min the temperature was raised at 1 °C/min to 177 °C, 0.50 °C/min to 180 °C and 2 °C/min to 210 °C and maintained for 7 min with the injector at 275 °C. Injection was made in a split ratio mode (ratio 10:1) and the quantification was done using the area of the internal standard 19:0 (nonadecanoic acid).

3.-RESULTS

Enrichment of live prey with two different enrichment products

The proximate composition of rotifer enriched with the different enrichment products (*Rot-DHA and Rot-ARA*) comparing with rotifer no enriched (*Rot-Yeast*) in the year 2017 is shown in **Table 5.**

Table 5. Proximate composition (% dry matter) of rotifer enriched with different experimental enrichments.

	Rot-Yeast	*Rot-DHA*	*Rot-ARA*
	Proximate analysis (% dry matter)		
Lipids	10.21±0.06	12.76±0.43	13.08±0.85
Proteins	58.20±0.37	60.66±0.03	60.91±0.41
Ash	11.94±0.71	10.24±0.69	9.34±0.30
Moisture	92.17±0.05	91.46±0.04	91.43±0.13

The proximate composition of *Artemia* enriched with the enrichment product (*Art-ARA*) comparing with *Art-non enriched* is shown in **Table 6.**

Table 6. Proximate composition (% dry matter) of *Artemia* enriched with different experimental enrichments.

	Art-Non enrich	*Art-ARA*
	Proximate analysis (%dry matter)	
Lipids	21.42±0.18	19.48±0.24
Proteins	54.59±0.87	68.53±2.21
Ash	7.19±0.15	10.03±0.02
Moisture	86.37±0.11	91.07±0.08

The influence of the enrichment products for live food on wreckfish larvae could not be tested because the amount and the survival of larvae obtained in 2017 was not sufficient to perform the experiments of larvae nutrition. The analyses (proteins, lipids and fatty acids) of live prey enriched with the enrichment products formulated in 2018 are shown in **Table 7.**

Table 7. Proteins, lipids and fatty acids of live prey enriched with the enrichment products (2018).

	Without Enrichment Rotifer (NoERot)	Control Enriched Rotifer (CERot)	ARA Enriched Rotifer (AERot)	Without Enrichment Artemia (NoEart)	ARA Enriched Artemia (AEArt)
			Proximate analysis (% dry matter)		
Proteins	62,33±1,11	60,45±0,58	59,41±2,79	61,64±0,41	69,48±1,96
Lipids	12,43±0,18	15,53±0,72	17,36±3,66	18,89±3,05	19,72±2,74
			Fatty acids content (% TFA)		
14:0	1,96±0,04	1,17±0,01	1,17±0,06	1,15±0,00	0,71±0,02
16:0	7,89±0,10	10,78±0,03	10,41±0,10	12,08±0,04	11,41±0,00
17:0	0,87±0,03	0,66±0,01	0,67±0,01	1,51±0,03	1,61±0,04
18:0	6,34±0,08	3,44±0,02	3,82±0,22	4,54±0,00	6,82±0,05
Saturated (SAFAs)	17,88±0,26	16,83±0,05	16,80±0,31	19,93±0,00	21,06±0,07
16:1 n-9	1,16±0,01	0,68±0,02	0,64±0,02	1,04±0,01	0,70±0,01
16:1 n-7	20,64±0,15	10,14±0,04	10,30±0,10	11,00±0,03	8,09±0,12
18:1 n-9	28,48±0,21	14,14±0,22	15,68±0,05	18,18±0,03	17,64±0,03
18:1 n-7	4,34±0,02	2,42±0,03	2,47±0,04	10,73±0,04	11,54±0,00
20:1 n-9	3,97±0,03	2,15±0,00	2,20±0,02	0,65±0,05	0,83±0,05
Monoenoics (MUFAs)	64,91±0,25	32,94±0,28	34,77±0,33	45,58±0,09	42,31±0,03
18:2 n-6	6,80±0,01	6,36±0,05	6,26±0,03	8,23±0,01	7,66±0,06
20:4 n-6 (ARA)	1,08±0,04	1,79±0,02	4,04±0,03	2,48±0,01	4,82±0,02
20:5 n-3 (EPA)	1,08±0,04	4,06±0,02	3,74±0,01	9,88±0,00	10,93±0,02
22:6 n-3 (DHA)	0,71±0,01	26,71±0,16	23,97±0,23	0,06±0,00	3,38±0,04
Polyunsaturated (PUFAs)	17,49±0,14	50,60±0,24	48,81±0,35	34,56±0,09	36,78±0,06
Σn-3	5,40±0,09	33,78±0,20	30,52±0,25	22,48±0,10	22,11±0,02
Σn-6	8,87±0,04	14,83±0,05	16,23±0,11	11,22±0,01	13,99±0,01
n-3/n-6	0,61±0,01	2,28±0,01	1,88±0,00	2,00±0,01	1,58±0,00
DHA/EPA	0,66±0,02	6,57±0,04	6,42±0,05	0,01±0,00	0,31±0,00
EPA/ARA	1,00±0,03	2,27±0,03	0,92±0,01	3,99±0,02	2,27±0,01

HUFA, highly unsaturated fatty acid; ARA arachidonic acid; DHA docohexaenoic acid; EPA eicosapentaenoic acid

The level of proteins in rotifer vary between 62.3% (DW) in no enriched rotifers (NoERot), 60.4 % in rotifer enriched with Control enrichment (CERot) and 59.4% in rotifers enriched with ARA enrichment (AERot). The lipid content was 12.4% (DW) in NoERot, 15.5% in CERot and 17.4% in AERot.

Regarding fatty acid content, SAFA vary between 17.9% of total fatty acids (TFA) in no enriched rotifers and 16.8% in rotifers enriched with both enrichment products. MUFA are higher in NoERot (64.9% TFA) than in CERot (32.9% TFA) and AERot (34.8%). On the contrary, the level of PUFA was much lower in the rotifer without enrichment (17.5%TFA) than in rotifer enriched with values of 50.6% when the rotifer was enriched with Control enrichment and 48.8% when the rotifer was enriched with ARA enrichment; n-3 PUFA level increased from 5.4% in NoERot to 33.8 and 30.5% in CERot and AERot respectively and n-6 PUFA from 8.9% TFA in NoERot to 14.8% and 16.2% in CERot and AERot. ARA, EPA and DHA were 1.1, 1.1 and 0.7 % TFA respectively in NoERot; 1.8, 4.1 and 26.7% in CERot and 4, 3.7 and 24% in AERot. DHA/EPA was lower in NoERot (0.7) than in CERot (6.6) and AERot (6.4), EPA/ARA ratio was 1 in NoERot, 2.3 in CERot and 0.9 in AERot.

On the other hand, *Artemia* without enrichment (NoEArt) had 61.6 % (DW) of proteins and 18.9 % of lipids and *Artemia* enriched with ARA enrichment product (AEArt) had 69.5% of proteins and 19.7% of lipids. SAFA content was 19.9 and 21.1% and MUFA 45.6 and 42.3, in NoEArt and AEArt, respectively. PUFA content reached the 34.6 and 36.8% of total fatty acids in NoEArt and AEArt. n-3 PUFA represent the 22% of TFA in both types of *Artemia*, no enriched and enriched and n-6 were 11.2 and 14% of TFA in NoEart and AEArt respectively. ARA, EPA and DHA levels were 2.5, 9.9 and 0.1 in NoEArt and 4.8, 10.9 and 3.4 % TFA in AEArt. DHA/EPA and EPA/ARA were 0.01 and 0.3 and 4 and 2.3 respectively in NoEArt and AEArt.

Wrechfish fatty acid profile.

Preliminary data of larvae fatty acid profile were obtained from different spawnings of IEO and AF(MC2) . The first data obtained in 2015 with larvae at 10dph are shown (**Fig. 1**) .

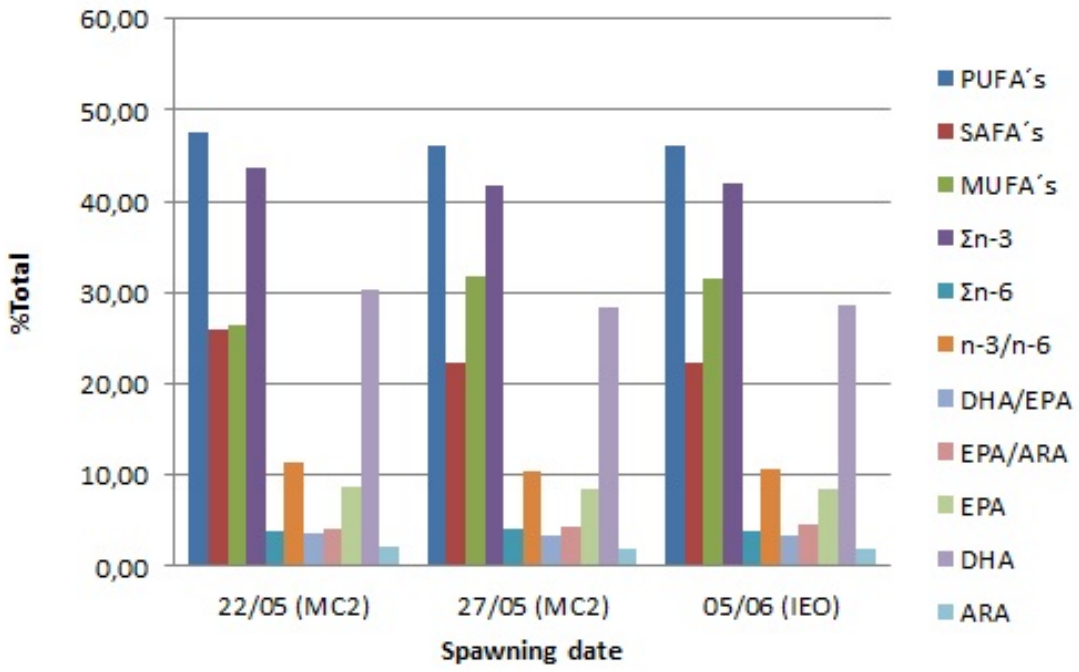

Figure 1. Fatty acids of wreckfish larvae at 10 dph from different spawnings.

First results show that PUFA, SAFA and MUFA values (% of total fatty acids) have a little variation in the first 10 days of life. PUFA values are between 44-49%, SAFA between 22-29% and MUFA 22-33%. n-3 PUFA represent 40-45% of the total fatty acids and n-6 PUFA 2-7% and EPA, DHA and ARA have values of 8- 9%, 26-31% and 2% respectively.

In 2016 and 2017 some culture of larvae were carried out but the survival was very low and the number of larvae was not sufficient to perform the experiments of larvae nutrition. These experiments were carried out in 2018.

***Nutrition experiments of wreckfish larvae* (2018)**

Effect of different enriched live prey on larvae fatty acid composition

Results of larvae fatty acid profile (%TFA) of wreckfish larvae from one IEO spawning (24/18) of eggs and larvae at different days of life: 1-6 and 8 dph (without feeding) and 11, 18 and 21 dph fed with live prey enriched with two different enrichments are shown in **Table 8.**

Table 8. Fatty acid profile of eggs and larvae fed with two different enriched live prey.

	Eggs Ch.24/18	Larvae 1dph	Larvae 6 dph	Larvae 8 dph	Larvae 11 dph Control Enrichment	Larvae 11 dph ARA Enrichment	Larvae 18 dph Control Enrichment	Larvae 18 dph ARA Enrichment	Larvae 21 dph Control Enrichment	Larvae 21 dph ARA Enrichment
					Fatty acids content (% TFA)					
14:0	1.66±0.04	1.67±0.12	1.39±0.09	1.40±0.03	1.24±0.08	1.22±0.03	1.16±0.13	1.18±0.04	0.91±0.12	1.08±0.06
16:0	14.81±0.23	15.08±0.72	14.74±1.50	15.06±0.44	15.27±0.72	14.61±0.23	13.43±0.15	13.89±0.27	12.38±0.96	14.06±0.20
17:0	0.83±0.07	0.60±0.00	0.59±0.06	0.61±0.03	0.84±0.04	0.77±0.05	0.90±0.06	0.88±0.07	0.85±0.09	0.88±0.02
18:0	4.62±0.13	2.99±2.55	4.95±0.42	5.46±0.09	6.29±0.04	6.11±0.26	6.42±0.21	6.59±0.07	6.32±0.35	6.41±0.06
Saturated (SAFAs)	22.20±0.34	20.59±1.73	21.91±2.05	22.78±0.53	23.89±0.72	22.96±0.46	22.13±0.16	22.73±0.33	21.61±1.43	22.68±0.18
16:1 n-9	1.10±0.21	1.22±0.04	0.99±0.05	1.04±0.05	1.03±0.03	1.07±0.09	1.00±0.06	0.98±0.06	0.88±0.18	1.06±0.14
16:1 n-7	5.22±0.46	5.22±0.47	3.93±0.54	3.97±0.13	4.57±0.23	4.50±0.17	3.98±0.22	4.41±0.50	3.91±0.46	4.22±0.25
18:1 n-9	13.50±0.51	12.75±6.81	14.28±1.58	14.75±0.30	10.59±2.67	11.75±1.34	11.47±0.18	12.16±1.96	10.06±0.92	10.49±1.12
18:1 n-7	8.99±0.53	11.09±5.79	6.47±0.98	7.13±0.12	10.37±3.46	9.44±0.81	9.45±1.09	9.46±2.69	10.27±1.14	11.04±0.82
20:1 n-9	1.60±0.12	1.45±0.17	1.09±0.15	1.41±0.26	1.42±0.18	1.35±0.13	1.60±0.03	1.55±0.31	1.32±0.28	1.67±0.19
Monoenoics (MUFAs)	33.15±0.99	34.68±0.82	29.53±1.35	30.86±1.07	30.30±1.07	30.43±0.50	30.18±0.79	30.71±0.95	28.64±2.35	30.79±0.65
18:2 n-6	5.97±0.41	7.49±0.37	6.37±1.41	6.66±0.74	7.90±0.94	6.89±0.48	9.92±0.60	8.05±2.34	14.85±6.86	8.63±0.57
20:4 n-6 (ARA)	5.79±0.63	5.46±0.31	7.73±2.66	6.37±0.19	6.29±0.03	6.44±0.34	6.45±0.12	6.52±0.16	5.91±0.10	6.55±0.42
20:5 n-3 (EPA)	6.60±0.40	5.68±0.29	5.24±0.85	5.38±0.07	4.69±0.21	5.14±0.11	5.25±0.33	4.86±0.15	4.95±1.03	5.54±0.44
22:6 n-3 (DHA)	21.76±0.07	21.74±1.37	22.42±2.18	23.72±0.24	23.69±1.43	24.55±0.51	22.56±0.55	23.52±1.30	20.76±1.70	22.26±0.21
Polyunsaturated (PUFAs)	44.65±0.66	44.73±2.11	48.56±3.39	46.36±0.64	45.81±1.78	46.61±0.51	47.70±0.72	46.56±1.17	49.75±3.75	46.53±0.48
Σn-3	32.55±0.28	31.65±1.81	34.32±1.98	33.12±0.35	31.44±1.78	33.06±0.15	31.07±0.61	31.76±1.28	28.75±3.11	31.14±0.48
Σn-6	11.76±0.92	12.95±0.56	14.09±1.62	13.03±0.89	14.19±0.95	13.33±0.60	16.37±0.53	14.57±2.34	20.76±6.83	15.18±0.89
n-3/n-6	2.78±0.24	2.45±0.14	2.45±0.21	2.55±0.19	2.22±0.23	2.48±0.12	1.90±0.08	2.22±0.41	1.50±0.54	2.06±0.15
DHA/EPA	3.31±0.19	3.83±0.09	4.32±0.33	4.41±0.04	5.05±0.20	4.78±0.20	4.31±0.36	4.85±0.36	4.29±0.66	4.04±0.28
EPA/ARA	1.15±0.13	1.04±0.02	0.75±0.31	0.84±0.04	0.75±0.03	0.80±0.04	0.81±0.05	0.75±0.01	0.84±0.17	0.85±0.11

HUFA, highly unsaturated fatty acid; ARA arachidonic acid; DHA docohexaenoic acid; EPAeicosapentaenoic acid

The SAFA, MUFA and PUFA content vary between 21-24 %, 29-35% and 45-50% of total fatty acids in eggs and larvae from 1 dph to 21 dph. **Figures 2** and **3** show the content in some fatty acids (PUFA, n-3, n-6, DHA, EPA and ARA) of larvae at 11, 18 and 21 dph from 24/18 spawning fed with two different enriched live food.

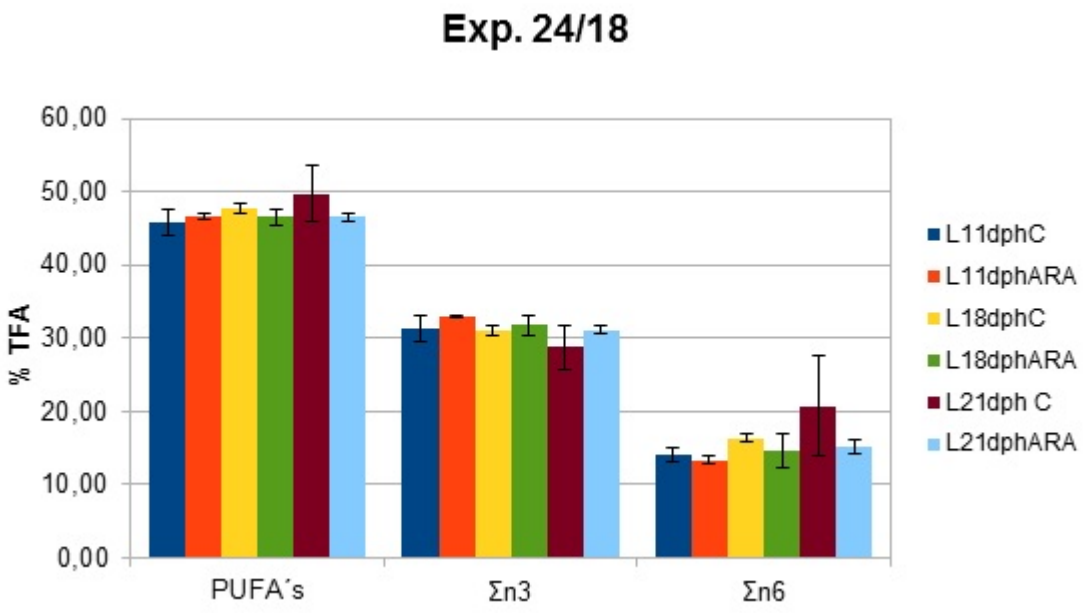

Figure 2. Fatty acid content (%TFA) in larvae at 11 dph, 18 dph and 21 dph fed with two different enriched live prey.

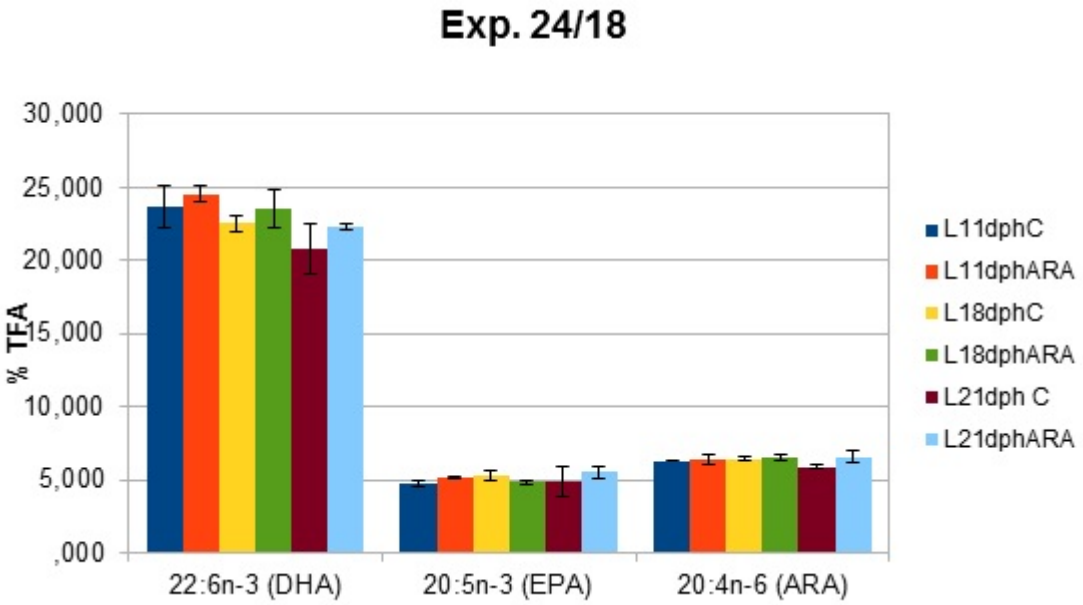

Figure 3. Fatty acid content (%TFA) in larvae at 11 dph, 18 dph and 21 dph fed with two different enriched live prey.

No differences were found between larvae fed with rotifer and *Artemia* enriched with the two different enrichments (Control and ARA) at 11dph, 18dph and 21dph. PUFA content varies between 46-50%, n-3 29-33%, n-6 14-17%. EPA, DHA and ARA content vary 5-5.5, 21-24 and 6-6.5%. The differences found in n-6 PUFA at 21 dph between larvae with ARA enrichment and Control are due to the high variability found in 18:2n-6 content in larvae fed with ARA enriched Artemia and they are not significant.

Fatty acid composition of larvae from different spawnings

Analysis of fatty acids from eggs and larvae from different spawnings at different days of life are shown at **Tables 9, 10, 11, 12** and **13**.

As it was said above, live preys (rotifer and *Artemia*) enriched with the same enrichment product were used to feed the larvae following the feeding sequence which was shown before (Material & Methods)

Total lipids reached 15-19% (DW) in eggs without signficant differences between the different spawnings and only little differences were found in SAFA and MUFA in eggs from four different spawnings representing between 20-23 and 33-37%TFA respectively. No significant differences were found in PUFA content between spawnings with values of 43-45%TFA (**Table 9**). Similar values were found in 1 to 10 dph larvae (no feeding) (**Table 10**). DHA vary between 20-22% with only little differences between spawnings in eggs and similar values were found in 1-10 dph larvae from 24/18 and 30/18 spawnings (23-24%TFA) and lower in 14/18 and 15/18 spawnings (18%). No differences were found in EPA and ARA in eggs and 1 to 10 dph larvae with values varying between 4-7% and 6% respectively. DHA/EPA and EPA/ARA ratios have similar values of 3-4 and 0.7-1.

Table 9. Lipid and fatty acid content of eggs (%TFA) from different spawnings.

Spawns	*14/18 IEO*	*15/18 IEO*	*24/18 IEO*	*30/18 IEO*
Total lipids	19.18±2.84	19.47±2.27	16.20±1.85	15.17±1.93
Saturated (SAFA´s)	22.58±0.14a	21.38±0.32b	22.20±0.34ab	19.91±0.54c
Monoenoics (MUFA´s)	34.83±0.41ab	34.37±1.56ab	33.15±0.99b	37.11±1.19a
Polyunsatwated (PUFA´s)	42.58±0.45	44.25±1.24	44.65±0.66	42.98±1.62
Σn-3	29.02±0.22 b	30.44±0.61 ab	32.55±0.28 a	31.00±2.21 ab
Σn-6	12.99±0.33	13.10±0.46	11.76±0.92	11.59±0.47
n-3/n-6	2.23±0.05a	2.33±0.09b	2.78±0.24b	2.68±0.27b
DHA	19.80±0.43b	21.14±0.46ab	21.76±0.07a	21.62±0.95a
EPA	5.71±0.13	5.73±0.16	6.60±0.40	5.87±1.20
ARA	6.16±0.22	6.38±0.24	5.79±0.63	5.74±0.12
DHA/EPA	3.47±0.06	3.69±0.06	3.31±0.19	3.78±0.77
EPA/ARA	0.93±0.01	0.90±0.03	1.15±0.13	1.03±0.22

Table 10. Fatty acid content of larvae (%TFA) from different spawnings 1-10 dph.

Spawns	*14/18 IEO*	*15/18 IEO*	*24/18 IEO*	*30/18 IEO*
Saturated (SAFA´s)	20.88±0.29ab	21.18±0.30ab	21.76±1.10a	19.14±0.69b
Monoenoics (MUFA´s)	36.10±0.50a	34.94±0.46a	31.69±2.67b	37.53±2.12a
Polyunsaturated (PUFA´s)	43.02±0.23b	43.88±0.35ab	46.55±1.92a	43.33±1.51b
Σn-3	28.10±0.24b	28.77±0.34b	33.03±1.34a	32.99±0.51a
Σn-6	14.52±0.16a	14.79±0.20a	13.36±0.64a	9.88±1.22b
n-3/n-6	1.93±0.03c	1.95±0.04c	2.48±0.06b	3.37±0.36a
DHA	17.60±0.23b	18.05±0.42b	22.63±1.01a	23.64±0.83a
EPA	4.28±0.03c	4.31±0.14c	5.43±0.22b	5.85±0.14a
ARA	6.07±0.14	6.19±0.13	6.52±1.14	5.57±0.26
DHA/EPA	4.11±0.04	4.19±0.04	4.19±0.31	4.05±0.21
EPA/ARA	0.71±0.02c	0.70±0.02c	0.88±0.15b	1.05±0.07a

The results of fatty acid content of larvae of 10-15 dph are shown in **Table 11.** Only little variations were observed in larvae (10-15 dph) from the different spawnings in SAFA, MUFA and PUFA with values of 22-23% , 31-32% and 46-47% respectively. n-3 PUFA are higher in AF larvae (39%) than in larvae from the rest of spawnings (31-33%TFA) and n-6 PUFA reached lower values (6.3 %) TFA in AF than in larvae from the rest of spawnings (13-14%) and as a consequence of this, n-3/n-6 is higher in AF (6.2) than in the rest of spawnings (2-2.5). DHA reached values of 28.5% in AF and 23-25% in the rest of spawnings, EPA vary between 4 and 6% in larvae from all the spawnings and ARA is lower in AF larvae(2.5) than in the rest of larvae (6-7.5% TFA). DHA/EPA ratio vary between 5-6 and EPA/ARA is higher in AF (2.3) than in the rest with values of 0.7-0.8.

Table 11. Fatty acid content of larvae (%TFA) from different spawnings 10-15 dph.

Spawns	*14/18 IEO*	*24/18 IEO*	*30/18 IEO*	*AF*
Saturated (SAFA´s)	22.15±0.03ab	22.96±0.46ab	21.69±0.20b	23.27±1.02a
Monoenoics (MUFA´s)	32.28±0.27a	30.43±0.50b	31.43±0.03ab	30.79±1.05ab
Polyunsaturated (PUFA´s)	45.57±0.26c	46.61±0.51ab	46.87±0.19a	45.94±0.17bc
Σn-3	30.57±0.35c	33.06±0.15b	32.71±0.47b	39.16±0.17a
Σn-6	14.20±0.29a	13.33±0.60a	13.79±0.13a	6.34±0.07b
n-3/n-6	2.15±0.07c	2.48±0.12b	2.37±0.06b	6.18±0.07a
DHA	22.62±0.13c	24.55±0.51b	25.35±0.52b	28.49±0.44a
EPA	4.91±0.29b	5.14±0.11ab	4.31±0.54b	5.89±0.16a
ARA	7.48±0.09a	6.44±0.34b	6.40±0.12b	2.55±0.12c
DHA/EPA	4.62±0.25b	4.78±0.20ab	5.95±0.91a	4.84±0.20ab
EPA/ARA	0.66±0.04b	0.80±0.04b	0.67±0.08b	2.31±0.17a

The comparison of fatty acid profile of 15-20 dph larvae (**Table 12)** shows a content in SAFA, MUFA and PUFA that represent 22-25%, 31-35% and 41-47% respectively, n-6 content is lower in AF larvae (10%) than in the rest of larvae (14-15%) similar to the one obtained for 10-15 dph larvae. DHA represents 15% TFA in AF and 21-23% in the rest, showing a decrease, specially in 30/18 and AF larvae from 10-15 dph to 15-20 dph. EPA has values of 10% TFA in AF and 4-6% in the rest of larvae and

ARA between 4-6% in all the larvae. DHA /EPA ratio is lower in AF (1.5) than in the rest of larvae (4-6) and EPA/ARA is higher in AF (2.1) than in the rest with values between 0.7-1. Only some significant differences were found in larvae from different spawnings at 20-25 dph (**Table 13**), SAFA and MUFA vary between 21-23% and 31-35% TFA. No significant differences were found in PUFA, n-3 and n-6 with values of 44-46%, 30-31% and 13-15 % of total fatty acids respectively. DHA, EPA and ARA reached values of 20-22%, 5-7% and 6% without significant differences between larvae from different spawnings, DHA/EPA is 4 in 14/18 and 24/18 larvae and 3 in 30/18 larvae and EPA/ARA ratio is around 1 in the three groups of larvae with very little differences between them.

Table 12. Fatty acid content of larvae (%TFA) from different spawnings 15-20 dph.

Spawns	*15/18 IEO*	*24/18 IEO*	*30/18 IEO*	*AF*
Saturated (SAFA´s)	21.64±0.07b	22.73±0.33b	21.33±0.51b	24.57±0.90a
Monoenoics (MUFA´s)	33.11±0.34ab	30.71±0.95b	35.25±1.12a	33.91±1.27a
Polyunsatwated (PUFA´s)	45.25±0.32 ab	46.56±1.17a	43.42±0.74bc	41.51±1.06c
Σn-3	30.27±0.18ab	31.76±1.28a	27.96±1.63b	31.28±1.29a
Σn-6	13.60±0.08a	14.71±2.34a	15.12±0.66a	9.82±0.39b
n-3/n-6	2.23±0.01b	2.22±0.41b	1.85±0.19b	3.19±0.23a
DHA	21.14±0.11a	23.52±1.30a	20.80±0.97a	15.15±2.05b
EPA	5.77±0.03b	4.86±0.15bc	3.81±0.76c	9.99±0.93a
ARA	5.86±0.10b	6.52±0.16a	5.79±0.07b	4.59±0.36c
DHA/EPA	3.66±0.04b	4.85±0.36ab	5.57±0.86a	1.54±0.36c
EPA/ARA	0.98±0.02a	0.75±0.01b	0.66±0.14c	2.18±0.08c

Table 13. Fatty acid content of larvae (%TFA) from different spawnings 20-25 dph (year 2018).

Spawns	*14/18 IEO*	*24/18 IEO*	*30/18 IEO*
Saturated (SAFA´s)	21.28±0.29b	22.68±0.18a	21.72±0.41b
Monoenoics (MUFA´s)	33.49±0.31a	30.79±0.65b	34.71±1.47a
Polyunsatwated (PUFA´s)	45.22±0.37	46.53±0.48	43.57±1.06
Σn-3	30.44±0.32	31.14±0.48	30.15±0.84
Σn-6	13.94±0.52	15.18±0.89	13.14±0.24
n-3/n-6	2.19±0.07	2.06±0.15	2.29±0.03
DHA	21.26±0.15	22.26±0.21	20.35±0.49
EPA	5.73±0.06	5.54±0.44	6.62±0.22
ARA	5.87±0.02	6.55±0.42	6.05±0.17
DHA/EPA	3.71±0.04a	4.04±0.28a	3.07±0.04b
EPA/ARA	0.98±0.01ab	0.85±0.11b	1.10±0.03a

Evolution of fatty acid profile through larval development of wreckfish larvae

For this experiment, all the samples of eggs and larvae came from 30/18 spawning and the evolution of the size along the larval development from 5 to 65 days of life (**Fig. 4**) shows that larvae at 5 dph has a

size of 6 mm and only a little increase was observed when reaching 22 dph (6.4 mm). Then, larvae show a higher increase in size having 7.75 mm at 39 dph , 11.16 mm at 50 dph and 12.56, 12.48 and 12.77 mm were reached at 50, 61 and 65 dph repectively.

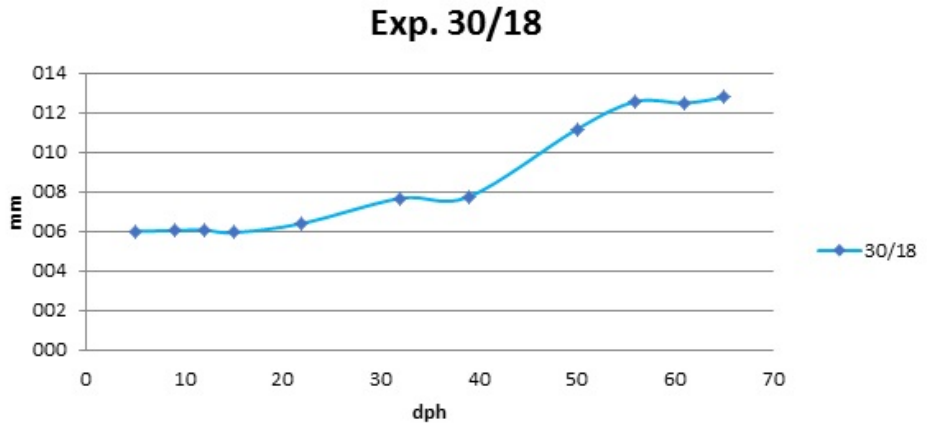

Figure 4. Evolution of size of larvae along the larval development.

The fatty acid profile of eggs and wreckfish larvae trough the larval development is shown in **Table 14.**

Table 14. Fatty acid profile of eggs and larvae from 1 dph to 58 dph.

	Eggs Ch.30/18	Larvae 1dph	Larvae 12 dph	Larvae 18 dph	Larvae 21 dph L-1	Larvae 32 dph L-1	Larvae 56 dph L-1	Larvae 58 dph L-1
				Fatty acids content (% TFA)				
14:0	1.37±0.14a	1.30±0.08ab	1.05±0.06c	1.12±0.05bc	0.96±0.04cd	0.66±0.08d	0.79±0.05d	0.80±0.02d
16:0	13.65±0.43ab	12.97±0.39ab	13.61±0.18ab	13.03±0.31ab	12.50±0.24b	12.01±0.60b	14.55±0.65a	14.53±1.27a
17:0	0.24±0.12d	0.33±0.05d	0.27±0.06d	0.46±0.03cd	0.63±0.02c	1.06±0.07b	1.58±0.11a	1.66±0.12a
18:0	4.43±0.007d	4.35±0.41d	6.51±0.13c	6.49±0.15c	7.39±0.30c	8.89±0.34b	11.56±0.12a	11.64±0.91a
Saturated (SAFAs)	19.91±0.54c	19.14±0.69c	21.69±0.20bc	21.33±0.51bc	21.72±0.41bc	22.89±0.49b	28.92±0.69a	29.11±2.34a
16:1 n-9	1.32±0.23a	0.93±0.26abc	1.12±0.18ab	1.11±0.10ab	0.70±0.10bcd	0.74±0.14bcd	0.50±0.12cd	0.46±0.11d
16:1 n-7	4.98±0.09ab	5.21±0.58a	3.71±0.22c	4.71±0.17ab	4.45±0.59abc	4.15±0.26bc	2.25±0.19d	2.58±0.06d
18:1 n-9	17.99±0.35a	17.62±2.63a	13.42±3.43ab	15.77±2.11ab	15.75±2.09ab	13.58±1.38ab	11.39±1.19b	10.83±0.57b
18:1 n-7	8.06±1.13	9.29±0.91	9.76±3.34	9.67±2.56	10.72±2.25	11.49±0.72	6.83±0.14	7.46±0.09
20:1 n-9	1.51±0.06ab	1.57±0.16ab	1.46±0.04ab	1.63±0.09a	1.17±0.35b	0.62±0.08c	0.66±0.06c	0.67±0.03c
Monoenoics (MUFAs)	37.11±1.19a	37.53±2.12a	31.43±0.03c	35.25±1.12ab	34.17±1.47abc	33.00±0.72bc	24.19±1.44d	24.62±0.49d
18:2 n-6	5.86±0.37cd	4.32±0.99e	7.39±0.06b	9.32±0.59a	7.09±0.16bc	10.18±0.03a	4.05±0.42e	4.40±0.70e
20:4 n-6 (ARA)	5.74±0.12bc	5.57±0.26c	6.40±0.12bc	5.79±0.07bc	6.05±0.17bc	6.55±0.29b	7.53±0.71a	7.66±0.46a
20:5 n-3 (EPA)	5.87±1.20cd	5.85±0.14cd	4.31±0.54de	3.81±0.76e	6.62±0.22bc	8.89±0.69a	7.74±0.49ab	8.25±0.07ab
22:6 n-3 (DHA)	21.62±0.95bc	23.64±0.83ab	25.35±0.52a	20.80±0.97c	20.35±0.49c	15.34±1.40d	20.98±0.57bc	19.05±1.61c
Polyunsaturated (PUFAs)	42.98±1.62	43.33±1.51	46.87±0.19	43.42±0.74	43.57±1.06	44.11±0.94	46.90±2.09	46.28±2.26
Σn-3	31.00±2.21ab	32.99±0.51a	32.71±0.47a	27.96±1.63bc	30.15±0.84abc	27.02±1.40c	33.55±0.99a	32.68±1.29a
Σn-6	11.59±0.47de	9.88±1.22e	13.79±0.13bc	15.12±0.66ab	13.14±0.24bcd	16.74±0.29a	11.58±1.11de	12.07±1.11cde
n-3/n-6	2.68±0.27bc	3.37±0.36a	2.37±0.06bcd	1.85±0.19de	2.29±0.03cd	1.62±0.11e	2.91±0.20ab	2.72±0.17bc
DHA/EPA	3.78±0.77cd	4.05±0.21bc	5.95±0.91a	5.57±0.86ab	3.07±0.04cde	1.74±0.26e	2.72±0.15cde	2.31±0.21de
EPA/ARA	1.03±0.22b	1.05±0.07ab	0.67±0.08c	0.66±0.14c	1.10±0.03ab	1.36±0.12a	1.03±0.03b	1.08±0.08ab

HUFA, highly unsaturated fatty acid; ARA arachidonic acid; DHA docohexaenoic acid; EPA eico*sapentaenoic acid

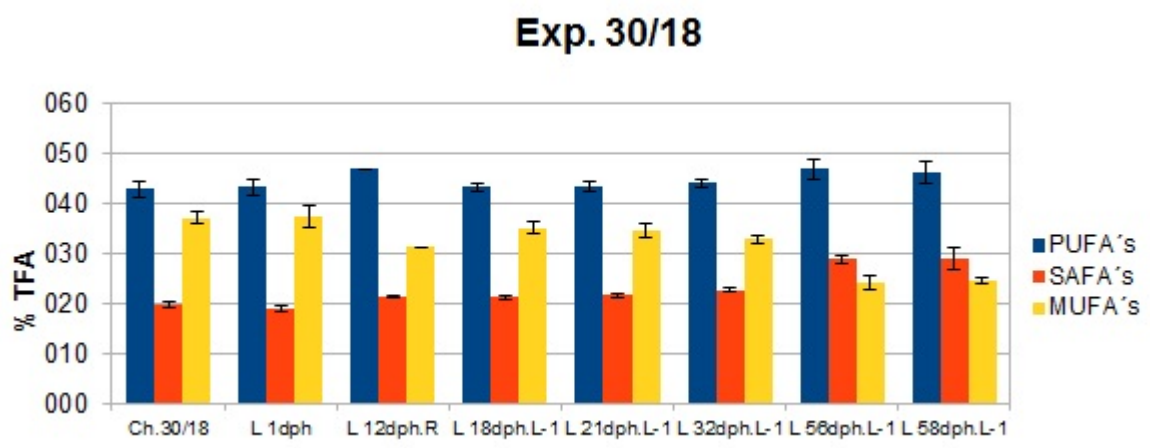

Figure 5. Evolution of PUFA, SAFA and MUFA of eggs and through wreckfish larval development (1-58dph).

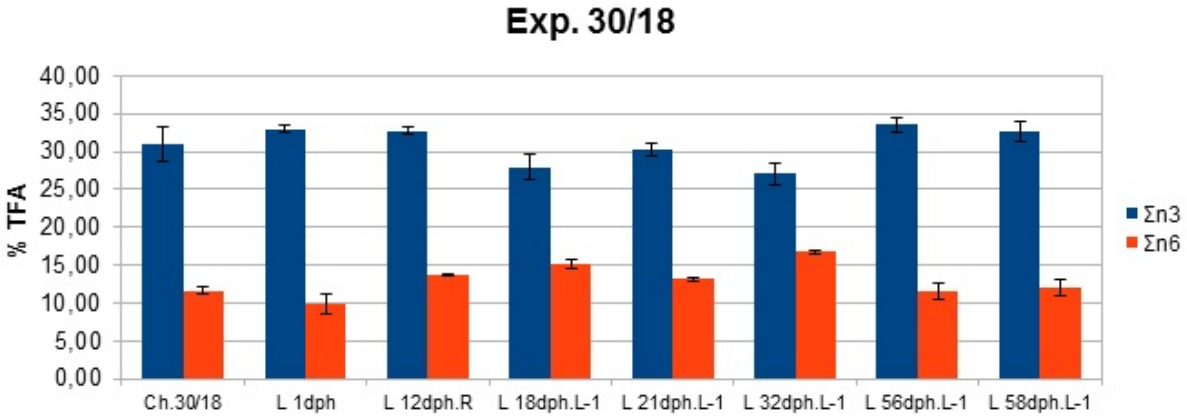

Figure 6. Evolution of n-3 and n-6 PUFA of eggs and through wreckfish larval development (1-58dph).

The evolution of fatty acid profile along the larval development in wreckfish shows that SAFA were maintained in levels of 20-23%TFA up to 32 dph and an increase was found in larvae of 56-58dph (29%) and on the contrary MUFA was 37% in eggs and 1dph larvae, 31-35% in larvae from 12 to 32 dph and the lowest values (24-25%) were found at 56-58 dph. The level of PUFA (**Figs. 5 and 6**) were maintained between 43-47% TFA along the culture (1-58 dph), ARA represents around 6% up to 32 dph and a slight increase (7.5-8) was observed at 56-58 dph. The highest values of EPA were obtained from 32 dph larvae (8-9%TFA) and DHA is higher in larvae from 1 to 12 dph (24-25%). DHA/EPA is higher at 12 and 18 dph larvae (6) than in eggs and 1dph larvae (4) and the lowest values were obtained at 56-58 dph (2-3). EPA/ARA ratio has the lowest values (0.7) at 12-18 dph and 1 in the rest of larvae.

4.- DISCUSSION AND CONCLUSIONS

There are not studies of larvae nutrition of wreckfish so far. Lipids and fatty acids (specifically LC-PUFAs, such as DHA, EPA and ARA) have been shown to the major dietary factors that influence

survival of marine finfish larvae (Cerda et al., 1994; Bell and Sargent, 2003; Nguyen et al., 2010; Carrier et al., 2011; Reza et al., 2013). The intake of LC-PUFAs from the diet is the only way for marine fish to obtain n-3 and n-6 essential fatty acids, such as DHA, EPA and ARA, since most marine fish studied to date have shown a limited ability to synthesize these fatty acids from their precursors, linolenic acid and linoleic acid (Bell et al., 2002; Rasdi and Qin, 2016).

Quantifying larval nutritional requirements is not an easy task and therefore it is usually agreed that the best way is to simulate the lipid content of the eggs or yolk reserves of the species in culture (Mourente and Vásquez, 1996; Narciso, 1999; Pousão Ferreira et al., 1999; Sargent et al.,1999).. Results obtained in DIVERSIFY from tissues composition specially gonads of wild wreckfish and oocytes and eggs from reared wreckfish (results in D12.1 and Linares et al., 2015, 2016, 2018) were very useful to advance in the knowledge of the nutritional requirements of this species. These results and those obtained from eggs and larvae newly hatched were a basis to formulate enrichment products for live prey to feed wreckfish larvae. A high amount of PUFA specially (DHA and ARA) found in gonads from wreckfish wild females and eggs from reared females obtained in the previous studies means that the inclusion of a considerable amount of these fatty acids in prey enrichments to larval feeding will be necessary.

Two enrichment products for rotifer and one for *Artemia* were formulated in Diversify with two levels of ARA with a good results of rotifer enrichment, since rotifers enriched with both enrichments reached 15.5 and 17.4% of total lipids with CER and AER respectively being the last one similar to the one obtained in enriched rotifer with DHA Protein selco (17.3%) used to feed larvae of *Seriola rivoliana* (Roo et al., 2014). In the previous study enriched rotifer had 10.4, 5.9 and 1.4 %TFA in DHA, EPA and ARA respectively. The present work shows that rotifer without enrichment had only 17.5% of PUFA which increased to 49-51% in enriched rotifers being 30.5-34% n-3 and 15-16% n-6. DHA reached 24-27% higher than the one obtained (10%TFA) in the study previously mentioned, EPA 4% and ARA content was 2 and 4% TFA with both enrichment products (Control and ARA enrichments).

Enriched *Artemia* has 20% (DW) of lipids. With repect to FA, *Artemia* had 37% of PUFA being 22% n-3 and 14% n-6. DHA was 3.4, EPA 11% and ARA 5%. The enrichment was less effective than in rotifer specially in DHA. The low efficiency of DHA enrichment in *Artemia* naupliu has been aknowledged as a major obstacle for their use as live prey for first-feeding larvae of marine fish (Bell et al., 2003; Haché & Plante (2011). Viciano et al.(2015) found higher values 7-10% of DHA and lower values of ARA (1-1.5%) with an experimental emulsion (70%DHA, 2.4%ARA) and the same authors reported an amount of DHA of 4.9% of total fatty acids when de *Artemia* was enriched with DC Superselco enrichment (with 20.6%DHA and 1.6%ARA) and Roo et al. (2014) reported 5.4, 5.8 and 1.3% TFA in DHA, EPA and ARA respectively. In general, rotifers assimilated PUFA in a much higher ratio than *Artemia* (Saidi et al., 2018).

No differences were found in the fatty acid profile of larvae at 11, 18 and 21 dph fed with enriched live prey with the two enrichment products (two levels of ARA) having both a good larvae acceptance. A high amount of PUFA was found in all the cases with values of DHA between 21-24%TFA, EPA 5% and ARA 6% in the three groups of larvae with both enrichments. The large amount of PUFA namely DHA, EPA and ARA found in wreckfish eggs from different spawnings reflect, as it was reported before (D12.2 and Linares et al., 2016, 2018), the composition of broodstock feed supplied (dry food specifically formulated for wreckfish). ARA content is higher in eggs and larvae of wreckfish (from different spawnings and larvae of different days of life until 25 dph) with values around 6% of total fatty acids (except in larvae AF that is slightly smaller) than in larvae of other marine fish as meagre, *Argyrosomus regius* (Saidi et al., 2018) and Senegalese sole, *Solea senegalensis* (Morais et al., 2004) and the same tendency was observed for EPA and DHA.

The evolution of fatty acids along the larval development until 58 dph shows again the maintenance of the high level of PUFA. Furthermore an increase in SAFA and a decrease in MUFA from 32 to 56 dph was found. High levels of ARA, EPA and DHA was maintained along the larval culture and EPA and ARA were even increased in larvae with 56-58 dph. The EPA/ARA ratio was about 1 in eggs and 1 dph larvae and larvae from 21 dph to 56-58 dph similar to those obtained in wild female gonads. Migaud et

al., (2013) pointed out that due to the influence of both ARA and EPA on tissue eicosanoid production it is likely that maintaining both n-6 and n-3 LC-PUFA at values close to wild values will be beneficial to subsequent egg and larvae success.

Although these are the first results in wreckfish larvae composition and it is necessary to continue the research in nutritional requirements of this species, the results suggests that the enriched live prey used has beneficial effects on the larvae composition.

Even though so far wreckfish larvae have very low survival rate, the results obtained in 2018 with the obtention of the first alevines of this species at the IGAFA facilities from two spawnings (IEO and AF) demonstrated that they have a good rotifer and *Artemia* acceptance and are very promising for the future. Finding a specific dry food for weaning and wreckfish fry would have a great importance too to have success in the culture.

Concluding remarks:

- Enrichment products for live prey (rotifers and *Artemia*) were developed with two levels of ARA being the enrichment less effective in *Artemia* than in rotifers.
- Larvae of wreckfish exhibit, in general, a good acceptance of the enriched live prey tested.
- The large amount of DHA, EPA and ARA found in wreckfish eggs from different spawnings reflect the composition of broodstock feed supplied.
- No differences in fatty acid composition of wreckfish larvae fed with the prey enriched with the two enrichment products were found at 11, 18 and 21 dph.
- Fatty acid profile of wreckfish larvae along the larval development was obtained, showing big amounts of PUFA specially DHA, EPA and ARA.
- The first results of larval culture are promising for wreckfish, but it is necessary to continue with the research about nutritional requirements and their impact on the growth, survival and larval quality.

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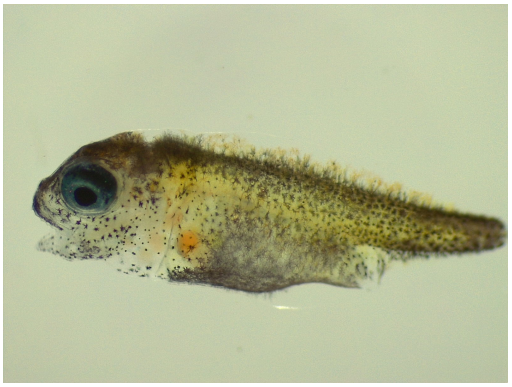

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