New species for EU aquaculture

Deliverable Report

Deliverable No:	D18.4		**Delivery Month:**	60
Deliverable Title	Determine the most effective culture system (RAS vs flow-through) for wreckfish larvae			
WP No:	18		**WP Lead beneficiary:**	P8. IEO
WP Title:	Larval husbandry –wreckfish			
Task No:	18.2. Subtask 18.2.2		**Task Lead beneficiary:**	P8. IEO
Task Title:	Test of two culture systems RAS (CMRM) and flow-through (IEO) in terms of larval culture conditions and feeding protocols.			
Other beneficiaries:	P1.HCMR	P19. CMRM	P32. MC2	
Status:	Delivered/delayed		**Expected month:**	36
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Objective:

Deterrmine the most effective culture system, recirculating aquaculture system (RAS) vs flow-through (FT) for wreckfish larvae: three culture systems were tested; two are representative of intensive culture (RAS and FT) while the other is a semi-intensive (Mesocosm) system.

This deliverable presents and defines the culture protocols for the three systems: 1) management of RAS and FT under intensive and semi-intensive conditions, 2) different larval and prey densities, 3) feeding sequence, according to the system used, 4) control of the physical and chemical parameters, 5) feeding protocols for the three systems during the first 30 dph. In addition, this deliverable includes information on larvae survival, growth parameters on larval quality, deformities and size distribution. Samples were also taken to determine biochemical profiles of the larvae (proteins, lipids and EFA contents).

Background:

The wreckfish artificial culture is one of the more viable options beside the new species to develop in the field of commercial aquaculture. Its fast growth, the specific characteristics relation with the management, high price in market and shortage in our coasts, us well us its genetic homogeneity in the current stocks (Sedberry et al., 1999; Ball et al., 2000), the high growth index during the pelagic period (Kentouri et al., 1995; Papandroulakis et al, 1997) and the long phase on juvenile growth, seems to appear wreckfish like a firm candidate for diversify of the marine aquacultures species in Europe.

The first references about wreckfish natural spawns in captivity were cited by Papandroulakis et al., who maintained a wreckfish stock during 8 years in that advances in knowing sperm and oocytes quality were made. Fertilized eggs in captivity was obtained for stripping an artificial fertilization in quantity enough to be cultured in experimental conditions, allowing realize a detailed description of embryonic and larvae development until mouth opening (Papandroulakis et al., 2004).

Recently, Peleteiro et al. (2011) obtained viable spawns by artificial fertilization with sperm and oocytes coming from Aquarium Finisterrae (MC2). Besides this precedents a private industry, Isidro 1952 (formerly

Isidro de la Cal) with facilities of marine cultures and with a wreckfish broodstock in Valdoviño (A Coruña), achieved to close the cycle in 2013 with one individual that actually survive in the MC2.

During 2018, more advances in achieving natural spawns and larval husbandry have been done in the three Galician wreckfish stocks resulting in very good larval hatching (42-82%) and survival until 34-37 dph. At Instituto Galego de Formación en Acuicultura, IGAFA (CMRM) two batches of larvae, one from Instituto Español de Oceanografía (IEO) natural spawn and one from MC2, produced larvae and juveniles at least with 5 months of live. This was the first time in the project that we succeeded in producing juveniles weaned to inert food, and it signifies a milestone in the efforts to produce wreckfish under aquaculture conditions. This trial acquired important data on growth and increased our knowledge about the feeding protocol and the specific behavior and metamorphosis of wreckfish larvae.

Material and methods:

2014

Only one of all spawns in MC2 facilities had acceptable quality to attempt culture. Once egg incubation was concluded (hatching percentage: 14%), the 11340 larvae (3.8±0.3 mm) with 100% yolk sac were transferred to a 85 L culture tank (**Fig. 1**) in FT at 1l..min^{-1}. The yolk sac was consumed in 11 days post hatching (dph) at 14±0.6ºC (**Fig. 2**); mortality was 100% at 20 dph. A daily dose of 8 rot.ml^{-1} (*Brachionus plicatilis*) were used as diet, but no ingestion of this live prey was observed. Larvae at 20 dph had a functional digestive system but all presented empty stomachs.

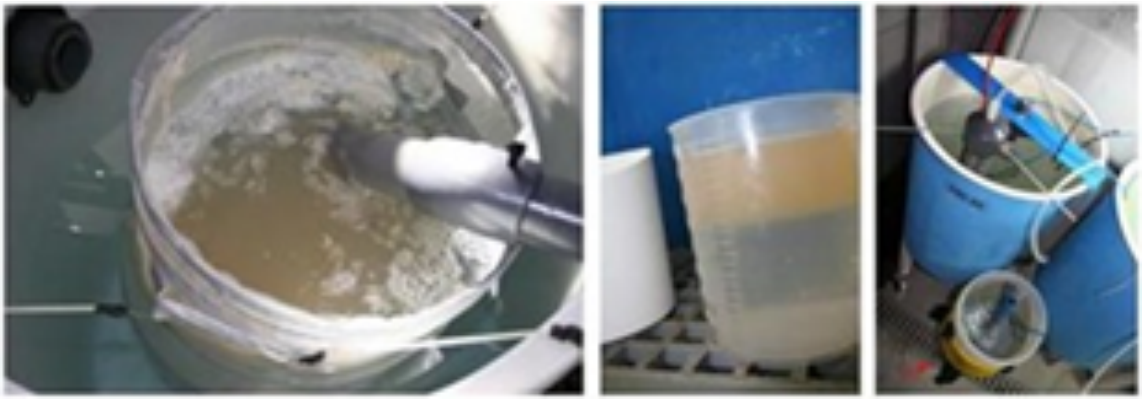

Figure 1. Eggs inside a collector tank, separation of viable eggs and incubation, at the MC2 facilities.

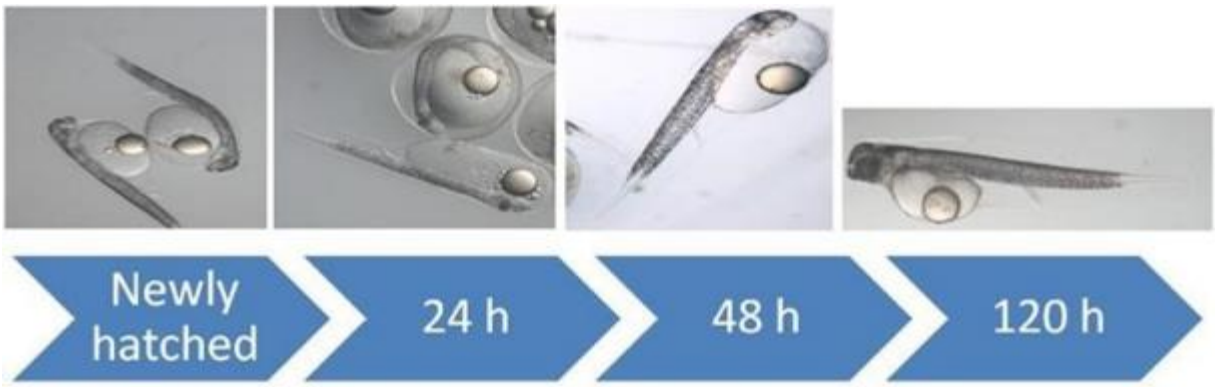

Figure 2. Yolk consumption from 1 to 120 hours after hatching.

2015

In the IEO, three experiments with a batch of larvae from an artificial fecundation of own stock and two from two spontaneous spawns from MC2 broodstock were developed (**Table 1**). The culture system was traditional, in close circuit of water until 10 dph and the rotifer enriched with Isochrysis. Biometric data of the yolk sac and the droplet consumption were taken (**Fig. 1a**). Samples for biochemical analysis (fatty acid profile) were collected and carried out in CIMA (CMRM) (see D12.1).

Trials in HCMR and MC2 with recirculation aquaculture system (RAS) were done (**Table 1**). Densities between 0.2-52.0 larvae l^{-1} (MC2) and 2.0 larvae.l^{-1} (HCMR) in 85 L tanks were tested. Larval food based in live prey of enriched rotifer with microalgae and copepods was administrated in MC2 and HCMR facilities (**Table 1**). Batches of larvae from MC2 for biochemical analysis were collected and for ontogeny of digestive system and eye carried out by HCMR (see D18.1). In both facilities larval survival of 22 dph was achieved (**Table 1**).

Table 1. Different spawns at the MC2 and IEO and larval culture during 2015.

STOCK	TRIAL	DATE	LARVAE (nº)	LARVAE DENSITY (nº larv/l)	MEAN Tª	FEED	SURVIVAL (dph)	WATER SYSTEM	TANK VOL (L)
IEO	1	10-04-15	110	0,2	17,4	Enrich rot	17	FT since 10 dph	400
MC2(IEO)	2	27-05-15	100	0,2	19,1		10		400
MC2(IEO)	3	05-06-15	1000	2,0	18,4		10		400
MC2	4	04-05-15	4000	2	16,3	Enrich rot +copepods+Artemia	22	RAS	83
	5	27-05-15	2000	2	16,3	Enrich rot +copepods	22	RAS	83
	6	18-05-15	20	0,2	16	Enrich rot +copepods	10	RAS	83
	7	22-05-15	2600	31,3	15	Enrich rot +copepods	19	RAS	83
	8	27-05-15	10600	127,7	14,5	Enrich rot +copepods	21	RAS	83
	9	01-06-15	180000	2168,7	14,5	Enrich rot +copepods	22	RAS	83
	10	05-06-15	18500	222,9	15	Enrich rot +copepods	18	RAS	83
	11	08-06-15	3700	44,6	15,3	Enrich rot +copepods	18	RAS	83
HCMR	12	04-05-15	4000	2	16,3	Enrich rot +copépods+Artemia	22	RAS	85
MC2 (HCMR)	13	27-05-15	2000	2	16,3	AF+EG	22	RAS	85

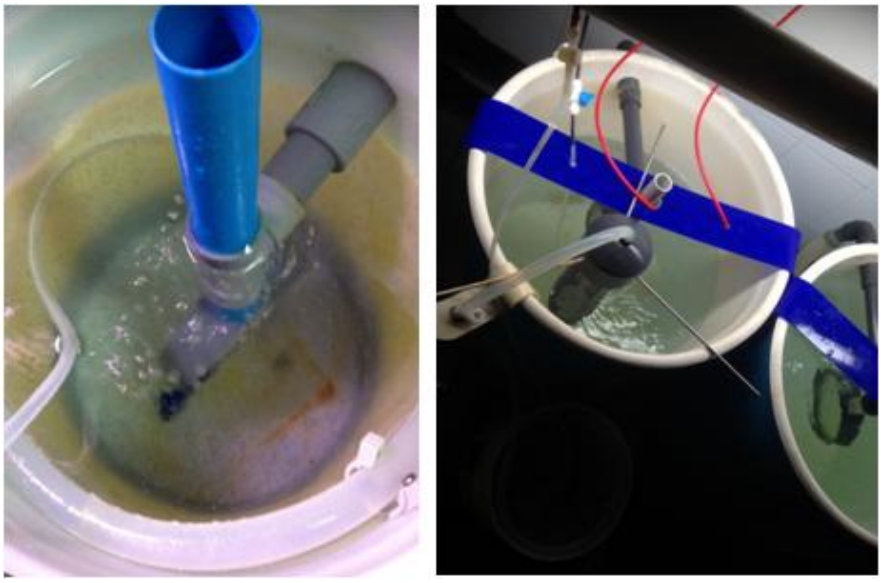

Figure 3. Culture tanks with recirculation (RAS) at MC2 facilities.

Larval yolk sac and lipid droplet length was tested in the IEO in flow-through (FT) larval culture (**Fig. 4**) and photos of all sequence were taken **(Fig. 5).** In FT conditions larvae alive until maximum of 17 dph.

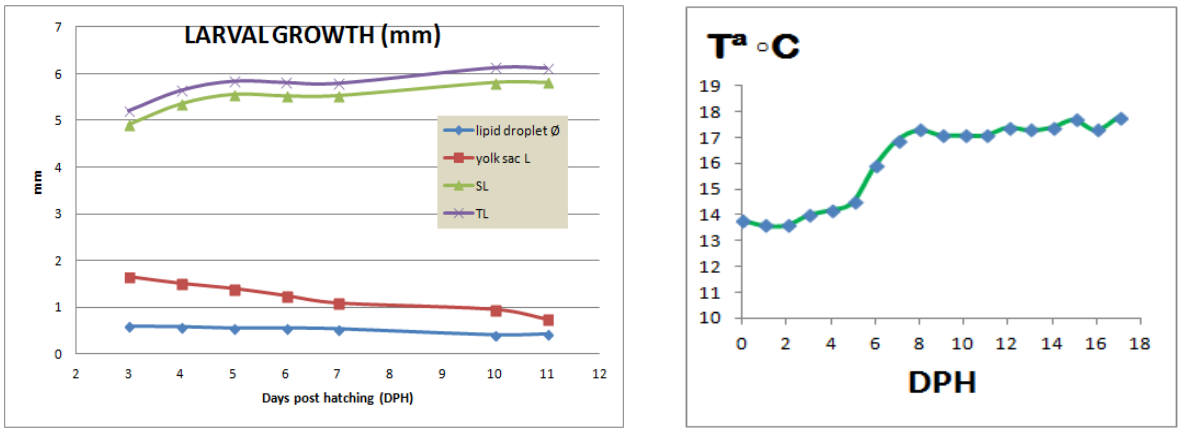

Figure 4. Water temperature (13.8-17.9ºC) and larval growth until 11 dph. Yolk sac (mm) and changes in the fat drop (mm).

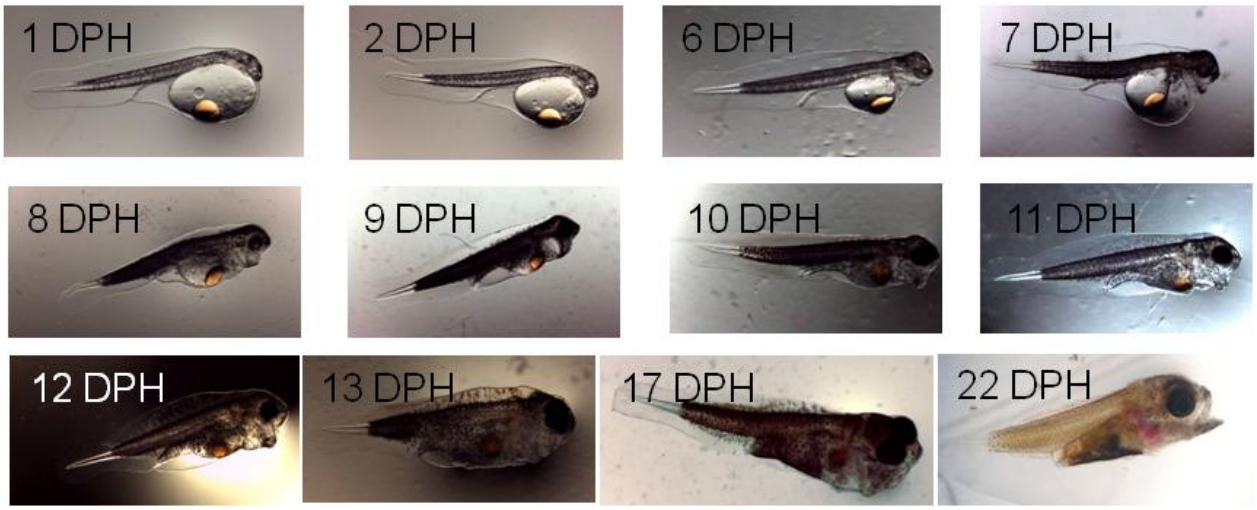

Figure 5. Larval growth until 22 days post-hatching

2016

During 2016, spawn quality at MC2 improved considerably and the IEO stock started to spawn as well (**Table 2)** and trials with different culture methods were tested. Larvae at the IEO were reared in 500 l tanks in closed circuit until 9 and 10 dph, using a "green water" system with a rotifer and enriched artemia with Isochrysis diet and natural photoperiod during endogenous feeding. After opening mouth and consumption of all yolk sac, artificial light (410 Lux) was used for 12 h per day until the end of the culture period. Different ranges of sea water temperature were used (see D18.2). Values between 7.3 and 8.8 mg.l^{-1} water oxygen dissolver were measure during the experiments.

Table 2. Parameters of the different larvae culture at MC2 and IEO in 2016

STOCK	TRIAL	DATE	LARVAE (nº)	LARVAL DENSITY (nº Larv/l)	MEAN Tª	FEED	SURVIVAL (dph)	WATER SYSTEM	TANK VOL. L
IEO	1	13-05-16	3921	7,8	19,8	Enrich rot + Art. AF	20	CC UNTIL 12 DPH	500
IEO	2	18-05-16	7793	8,7	20,7	Enrich rot + Art. AF	20	CC UNTIL 9 DPH	500
			2560	2,8	16,2	Enrich rot + Art. AF + EG	27		
IEO	3	20-05-16	2200	9,2	17,1	NONE	4	FT	1000
MC2(IEO)	4	13-06-16	2500	2,8	18,3	Enrich rot + Art. AF	20	FT	1000
MC2(IEO)	5	12-07-16	1495	3,3	16,7	Enrich rot + Art. AF	23	FT	1000
			3937	8,8	16,8	Enrich rot + Art. AF	25	FT	1000
MC2	6	26-05-16	1600	0,2	16,5	Copepod Tisbe + Acartia	22	MESOCOSM	10000
			1000	1,0	17,5		11	FT	10000
MC2	10	13-06-16	6000	10,0	17,5	Copepod Tisbe + Acartia	10	CC	1000
MC2	11	08-07-16	600	0,1	15,5	Copepod Tisbe + Acartia	3	RAS	3 x30 L
	12	12-07-16	6000	0,8	15,5	Copepod Tisbe + Acartia	23		
MC2	13	16-07-16	100	2,5	17,5	NONE	4	CC	1000
MC2	14	20-07-16	2500	62,5	17,5	Copepod Tisbe + Acartia	15	FT	10000

Samples of larvae were taken once a week and morphological parameters were measured, showing decrease in both values due to deformities along the period of the culture and digestibility problems with live food (**Fig. 6**).

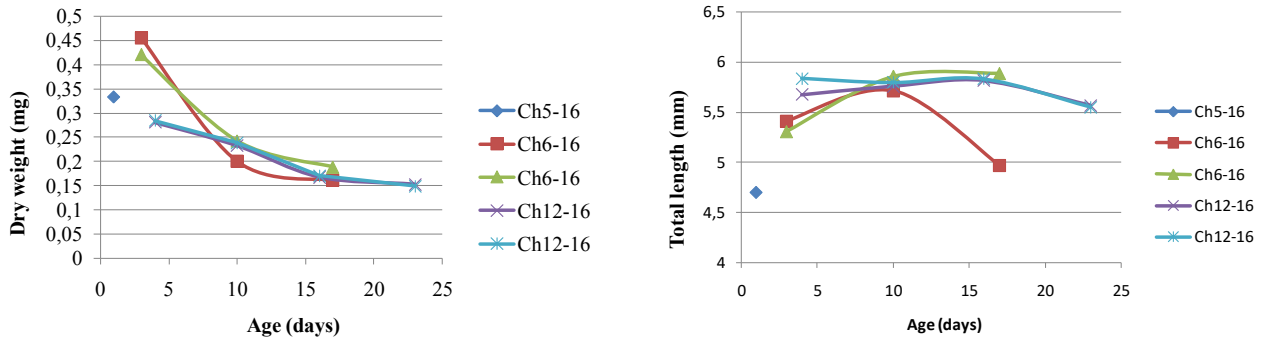

Figure 6. Larvae growth (mg dry weight) and length (mm) during different larvae cultures.

Trials with close circuit (MESOCOSM) and another with water renovation (FT) and recirculation (RAS) adding copepods (Tisbe and Acartia) and Artemia as diet were done in MC2, with poor results in terms of growth and survival until 22 dph (Álvarez-Blázquez, 2016) (**Table 2**).
Maximum larval survival was 27 dph in FT, eating enriched rotifer with microalgae and AF/EG Artemia nauplii since 9 dph. Mean sea water temperature was 16.2ºC and larvae density 2.8 larvae.l^{-1}.
Respect to the biochemical profiles, larvae batches were analyzed at different days of life, which were useful for designing enrichments for larval feeding (Task 12.1).

2017
During this year, only larvae from 40% of the spawns were reared (**Table 3**), due to zootechnical problems during the eggs incubation phase. In the case of MC2 stock a problem with the casual death of part of the adult female stock occurred. In IEO and IGAFA (CMRM) a total of five trials with larval culture in FT were done (**Table 3**). The larvae survived a maximum of 29 days at the IEO facilities fed enriched rotifers with Isochrysis, with a mean sea water temperature of 16ºC and larvae density of 6.8 larvae.ml^{-1}.

Table 3. Parameters of the different spawns at the MC2 and IEO and larval culture.

STOCK	TRIAL	DATE	LARVAE (nº)	LARVAL DENSITY (nº Larv/l)	MEAN Tª	FEED	SURVIVAL (dph)	WATER SYSTEM	TANK VOL.
IEO	1	13-04-17	2049	6,8	15-17	Enrich rot	29	FT	400
			2049	6,8	19-21	Enrich rot	24		
IEO	2	22-04-17	15400	4,4	16-19	Enrich rot	26	FT	
CMRM	3	05-04-17	18400		15,1	Enrich rot	13	FT	400
CMRM	4	18-04-17	1050	14	14-17	Enrich rot	11	FT (MESS BOOTON)	3X25 L
CMRM	5	26-04-17	164	3,3	14-17,4	Enrich rot	11	FT	400

Trials were focused in egg incubation and larvae culture at different sea water temperatures (D.18.2) and also in trials with different enrichments for live prey (see D12.1). Larvae samples at different days of life were taken. Length, yolk sac and droplet were measured, obtaining curves of growth in length and yolk sac and droplet consumption (**Fig. 7**).

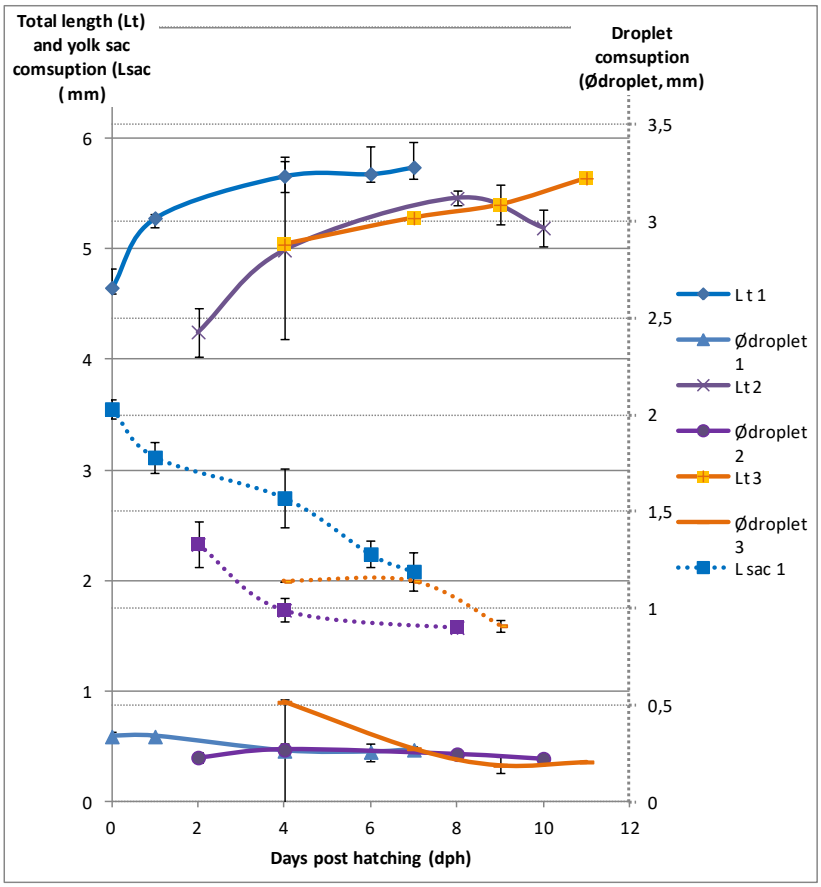

Figure 7. Total length, yolk sac and droplet consumption (mm) of larvae since 0 to 11 dph.

2018

During this year a lot of experimental trials in larval husbandry were done in IEO, MC2 and IGAFA (CMRM) facilities focused in adjust different culture system (FT, MESOCOSM and RAS) and environmental parameters such as water temperature, volume, density, food sequence, air, light, tanks shape and colour (**Table 4**).

Table 4. Larvae cultures at IEO facilities during the spawning season of 2018.

STOCK	SPAWN	DATE	LARVAE (nº)	LARVAL DENSITY (nº Larv/l)	MEAN Tª	FOOD	SURVIVAL (dph)	WATER SYSTEM	TANK VOLUME (L)
IEO	1	09-03-18	171921	21	15,2	ROT 10-26 dph. A0 11-28 dph	29	FT. NATURAL Tª	8000
	2	02-04-18	110740	7,1	17,2	ROT ORIGREEN 7-14 dph. A0 11-13. A-1 ORIGREEN 14-17 dp	17	CC. HOT WATER	8500
	3	07-04-18	134802	5,5	17,5	ROT 7-10 DPH. A0 8-11 dph.	23	CC. HOT WATER	8000

STOCK	SPAWN	DATE	LARVAE (nº)	LARVAE DENSITY (nº /l)	MEAN Tª	FOOD	SURVIVAL (dph)	WATER SYSTEM	TANK VOL (L)	COLOUR BOTTON TANK
IEO	1	16/02/2018	3429	7	18,3	ROT ARAq 12-18 dph	18 DPH	CC until 14 dph	500	WHITE
	2	21/02/2018	7500	13	17,9	ROT ARAq 11- 24dph. A0 15-24 dph	24 DPH	CC until 11 DPH	1000	WHITE
	3	27/02/2018	73260	36	16,8	ROT ARAq 10-19 dph. A0 13-23 dph	23 dph	CC until 12 dph	2000	WHITE
	4	04/03/2018	7282	1,8	15,4	ROT ARAq 12-27 dph. A0 20-27 dph	27 DPH	CC until 11 dph. FT 120 cc/15 seg	500	BLACK
	5	10/03/2018	37844	18,9	15,6	ROT enrich. T-Iso 11-25 dph. A0 19-27 dph	27 DPH	FT 500 cc/15 seg	2000	WHITE
	6	19/03/2018	102570	39	16	ROT ARAq 10-20 dph. A0-A1: 12-24 dph	24 DPH	FT 500 cc/15 seg	800	WHITE
				26	15,9	ROT enrich. T-Iso 10-18 dph. A0 11-18 dph	19 DPH	CC until 9 dph	800	WHITE
	7	24/03/2018	23320	10	16,5	ROT CONTROL 9-17 dph. A0 12-20 dph	21 DPH	FT	400	
	8	24/03/2018	21888	33	18,9	ROT enrich T-Iso10-15 dph. A 0 12-17 dph	19 DPH	FT	1750	WHITE
				4,5	17,6	ROT CONTROL 9-14 dph. A0 11-13. A-1 T-ISO 13-22 dph	22 DPH		400	WHITE
	9	28/03/2018	11170	46	17,1	ROT enrich. T-Iso 8-15 dph. A0 11-14. A1-15-22 dph	22 DPH	FT	2000	BLACK
	10	29/03/2018	71524							
	11	29/03/2018	45182 47456	24	16,8	ROT ORIGREEN 7-15 dph. A0 10-14. A1 ORIGREEN 14-19 dph	19	CA	2000	WHITE
	12	07/04/2018	134802	5,5	15,1	ROT ORI-GREEN 7-10 dph. A0 8-11 dph.	11 DPH	FT	8000	WHITE
	13			20,4	18,4	ROT ORI-GREEN 7-10 dph. A0 8-10. A1 10-18 dph	18 DPH	C C + AIR	2000	WHITE
	14			14,9	18,2	ROT ORI-GREEN8-10 dph. A0 9-10. A1 11-16 dph	18 DPH	C C + AIR	900	WHITE
	15	12/04/2018	54116	12,8	17,6	ROT ORI-GREEN 7-14 dph. A0 7-9, A1 10-20 dph.	20 DPH	FT	600	WHITE
	16	16/04/2018	67428	14	18,7	ROT ORI-GREEN 6-11 dph. A0 7-9. A1 10-20 dph	23 DPH	FT	2000	WHITE
				18,6	19,1	ROT ORI-GREEN 6-11 dph. A0 6-9. A1 10-20 dph	21 DPH		400	WHITE
				16,4	18,8	ROT ORI-GREEN 6-13 dph. A0 6-9. A1 10-17 dph	17 DPH		400	WHITE
				20,4	18,4	ROT ORI-GREEN 6-13 dph. A0 6-9. A1 10-17 dph	17 DPH		800	WHITE
	18	25/04/2018	39798	5,7	19,2	ROT ARA 6-7 dph	8 DPH	FT	3X100 L	WHITE
				5,7	19,2	ROT CONTROL 6-7 dph	8 DPH		3X100 L	WHITE
				19,2	19,1	ROT T-ISO 5-8dph. A0 9-10. A1 11-22dph	22 DPH		400 L	WHITE
				18,3	18,9	ROT T-ISO 5-8 dph. A0 9-10. A1 18 dph	18 DPH		400 L	WHITE

More than 50 experiments of larval culture have been carried out in the IEO facilities. Several rearing conditions such as photoperiod, water circuit, use of aeration or sequence of feeding used were modified as the season progressed.

The first experiments were carried out in closed circuit up to 6-12 dph, photoperiod of 12h light:12h dark, with moderate aeration in the center of the tank, green water from day zero and a temperature between 15-18ºC. Larvae density ranged from 2 to 30 larvae l^{-1} and survival varied between 4 and 30 dph. A rotifer feeding sequence (10-25 dph) and Artemia nauplii (11-30 dph) was supplied. Oxigen dissolve was between 7.2-8.4 $mg.l^{-1}$.

In a second stage, new tests were carried out and some of the cultivation parameters were readjusted. Larvae of 1 dph were introduced at densities between 6-20 larvae l^{-1} in the larval culture tanks and kept in darkness until 7 dph. Temperature between 17-20ºC, open circuit (2.5 renewals d^{-1}) and no aeration was maintained. A photoperiod of 10L: 12D and green water culture conditions were applied from 7dph. A feeding sequence for rotifer (7-13 dph), *Artemia* nauplii (8-11 dph) and enriched metanauplii (10-29 dph) was supplied. Survivals between 8 and 30 dph were reported.

In the last tests, some variables were modified. The culture density was maintained between 5-15 larvae l^{-1}, temperature was controlled at 17.9 ± 1ºC, the oxygen dissolve between 7.0 and 8.4 $mg.l^{-1}$, the intensity during the light period was adjusted to 400 lux on the surface of the tank and a new feeding sequence was supplied: rotifer (7-18 dph), *Artemia* nauplii (12-19 dph) and enriched metanauplii (17-30 dph). The rest of the culture conditions were the same as those described in the previous paragraph. Survivals between 18 and 34 dph were observed.

For all the experiments carried out during 2018, live prey concentrations were adjusted between 3-10 ml^{-1}, 0.5-1 ml^{-1} and 0.5-2 ml^{-1} for rotifer, *Artemia* nauplii and metanauplii, respectively.

In MC2 facilities larvae culture was focused in conditions of FT (**Table 5**), with water temperature between 17.5 and 18.8ºC and live food based on rotifer and Acartia since 6 to 22 dph. Larval density was between 0.2 and 11.1 larvae l^{-1} and survival until 22 dph.

Table 5. Larval culture conditions in MC2 facilities during trials in 2018.

STOCK	TRIAL	SPAWN	LARVAE nº	LARVAE DENSITY	MEAN Tº	FOOD	SURVIVAL dph	Water System	Tank Vol	TANK COLOUR	TANK SHAPE	AIR	Light
MC 2	1	28/03/2018	30000	3	17,5	Enrich rot and Acartia 6-20 dph	20	FT 30 Lh^{-1}	1000	Black	CYLINDER gently cone-shaped botom	Botom buble	Natural light
	2	02/04/2018	0		18,5	Enrich rot and Acartia 6-20 dph	12	FT 30 Lh^{-1}	180	White	Cone-bottom, cylindrical tank	Botom buble	Natural light
	3	07/04/2018	200	0,2	18	Enrich rot and Acartia 6-20 dph	17	FT 30 Lh^{-1}	1000	Black	CYLINDER gently cone-shaped botom	Botom buble	Natural light
	4	24/04/2018	10000	10	18,5	Rot <6 dph	7	FT 30 Lh^{-1}	1000	Black	CYLINDER gently cone-shaped botom	Botom buble	Natural light
	5	30/04/2018	2000	11,1	18,8	Enrich rot and Acartia 6-20 dph	19	RAS	6 x 3 1 x 2	White	Jars	Botom buble	Natural light

Different culture systems were used in IGAFA (CMRM) facilities (**Table 6**). The best results were achieved with RAS conditions with two batches, one from IEO and another one from MC2 facilities. Water temperature was 17.6 and 17.9ºC, in tanks volume of 400 L, without air and natural photoperiod since 7 and 9 dph. Life food was based on rotifer, *Artemia* nauplii and enriched metanauplii with dry microalgae supplemented with arachidonic acid (see D12.1). Both larval batches achieved the weaning period and dry food was provided since 40 dph at the same time that A1. At 48 dph larvae were weaning completely.

Table 6. Larval cultures at CMRM facilities during 2018.

STOCK	TRIAL	SPAWN	LARVAL DENSITY (nº Larv/l)	MEAN Tº	FOOD	SURVIVAL (dph)	WATER SYSTEM	TANK VOL.	TANK COLOUR	TANK SHAPE	AIR	LIGHT
IGAFA (CMRM)	1	27-02-18	10,0	16,3	Enrich. Copepod Isoch 11-23 dph	24 DPH	CLOSED	200	WHITE		NO	since 11 dph
	2	02-04-18	13,0	16,9	Enrich. Rot. Araq 13-28 dph. A0 15-28 dph	28	CLOSED	200	WHITE	ROTATION (SMILAR KREISLER)	NO	since 9 dph
	3	22-03-18	10,0	15,9	Enrich Rot control and araq. 9-15 dph. A0 12-17 dph. Enrich A1 control and araq. 17-21	21 DPH	CLOSED	200	WHITE		NO	NO
	4	27-03-18	11,0	15,1	NON	3 DPH	RAS	400	BROWN	FLAT	YES	NO
	5	02-04-18	12,5	17,6	Enrich rot araq 9-17 dph. A0 15-18 dph. Enrich A1 araq. 18-48 dph. Dry food 40DPH until now	ALIVE	RAS	400	BROWN	FLAT	NO	since 9 dph
	6	08-04-18	10,0	15,6	Enrich Rot araq 10-25 dph	25 DPH	RAS	400	BROWN	FLAT	NO	since 10 dph
	7	08-05-18	9,0	19,0	Enrich Rot araq 10-25 dph	4 DPH	CLOSED	200	WHITE	ROTATION	NO	NO
	8	10-05-18	12,5	17,9	Enrich Rot araq. 8-19 dph. A0 18-23 dph. Enrich araq. A1 23-48 dph. Dry food 40 dph until now.	ALIVE	RAS	400	BROWN	FLAT	NO	since 7 dph

In IGAFA facilities juveniles alive until now were reared at ±18ºC of water temperature, achieving the first results in larval growth (see D18.4).

Conclusions and perspectives:

The mean weight in growth and length during trials at IEO were reflected in the **Fig. 8**. Growth in weight and length were adjusted to a potential regression curves. More data of weight will be needed to achieve a clear conclusion about growth in weight of wreckfish larvae.

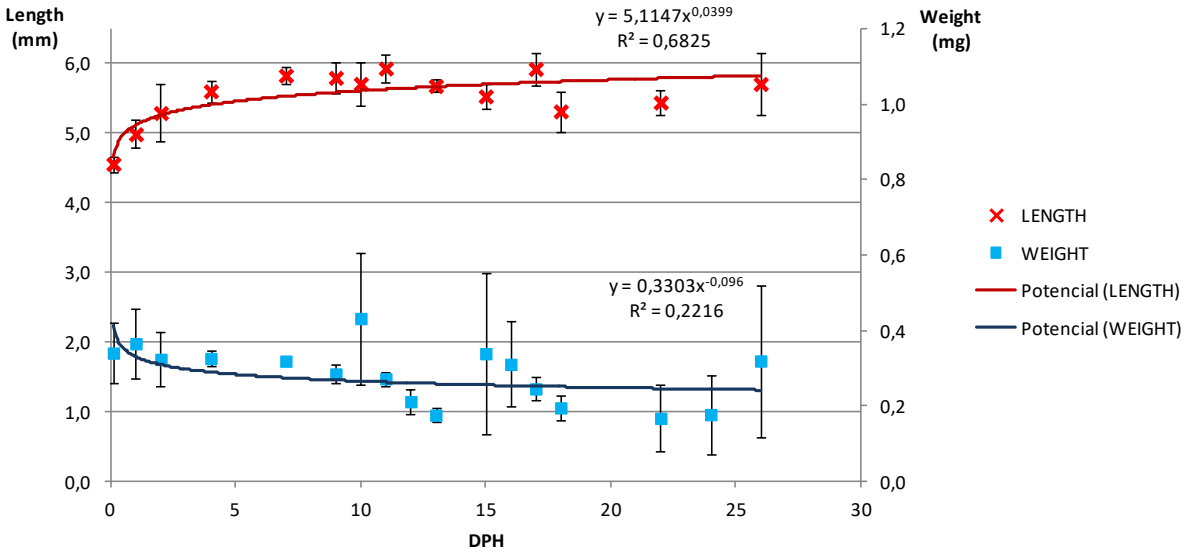

Figure 8. Growth in weight and length of wreckfish larvae from 0 to 26 dph

With data of length obtained in IGAFA facilities until 65 dph, the growth in length of the wreckfish larvae until weaning was knew, increasing from 5.6 mm to 12.8 mm at 5 and 65 dph, respectively **(Fig. 9)**.

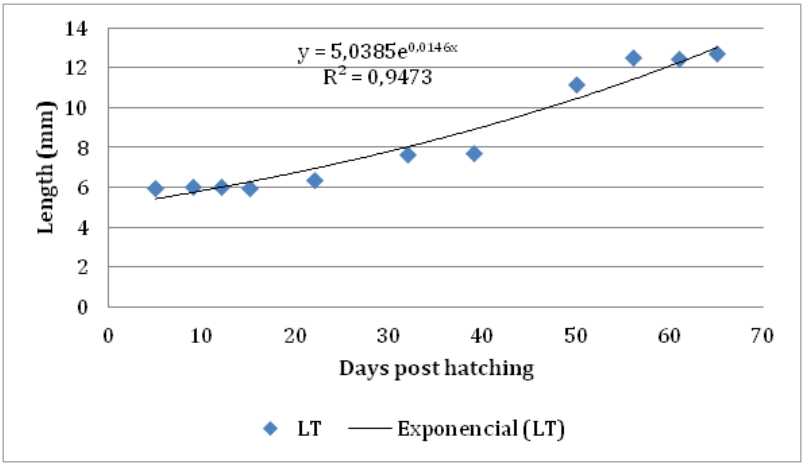

Figure 9. Wreckfish larvae length increasing (mm) from 5 to 65 dph.

Most important advances were made in the feeding sequence with trials in IGAFA (CMRM) in RAS, where the best survival results were obtained so far **(Fig. 10)**.

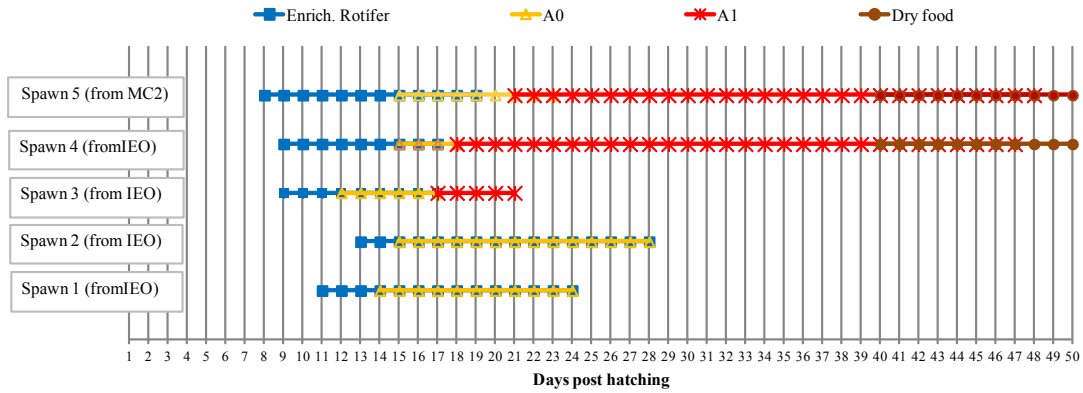

Figure 10. Larval rearing protocol for wreckfish used in the CMRM facilities.

As a result, we can conclude that the optimal feeding sequence for the larval culture of wreckfish in RAS at 12.5 larvae l^{-1}, 17.5-18ºC of water temperature, 36.4±1.7th 5º% of salinity and oxygen dissolve bewteen 7.2-8.4 mg.l^{-1}, without air and natural photoperiod since 7-9 dph, was the following:

- Enriched rotifer (arachidonic supplemented): 8-19 dph (4-6 rot.ml^{-2})
- *Artemia* nauplii: 15-23 dph (0.2-0.7 A0.ml^{-1})
- *Artemia* metanauplii (arachidonic supplemented): 18-48 dph (0.2-0.7 ml^{-1})
- Dry food: since 40 dph.

Some data about behavior were known during the project:

- Peak of mortality: between 7-11 dph.
- Air vesicles and deformities: since 10 dph (with bubble pump).
- They eat voraciously since 6 dph at 18-19ºC (open mouth)
- Remain at the bottom of the tank and through the walls up to 10 dph
- They go up to surface between 7-15 dph where they remain the rest of the time.
- Wreckfish larvae were also characterized by the large size of the yolk sac and the total absorption endogenous reserves last until 20 dph at 17 ± 0.5°C. The presence of the large yolk sac and the large oil droplet, indicates the presence of a long autotrophic larval stage (see D18.1)

Regarding biochemical profiles of the larvae, first data of fatty acid profile were obtained from 1 dph until 26 dph of larvae to complete the data obtained previously until 10dph. In addition, enrichment products for living prey (rotifers and Artemia) were designed. Two levels of ARA content were used for enrichment product for rotifer and one level of ARA for Artemia and the effect of the new enrichment products on the biochemical composition of rotifers and Artemia was evaluated (see D12.1).

The study of the technical conditions and the adequate parameters regarding the aeration, the flow and form of creating an adequate circulation of water, as well as continue investigating the larval malformations that occur in a high percentage are needs for the immediate future.

Very important results were achieved in larvae feeding sequence in RAS system culture. These data could be the starting point for future experiments and a reality to propose the cultivation of wreckfish as a possibility as diversification of aquaculture

Concluding, the perspectives regarding wreckfish larval rearing are very encouraging, thinking about in new useful species for the aquaculture with very good perspectives of future. The increase of our knowledge of this species in the larval and juvenile period could be very interesting for subsequent studies.

References

Álvarez-Blázquez, B., Vilar, Papandroulakis, C.A, Linares, F., Cal, R., Rodríguez, J.L., Lluch, N., Gómez, C., Pérez, E., and and Peleteiro J.B. (2016). First experiences of wreckfish (Polyprion americanus) larval husbandry in NW Atlantic and East Mediterranea. Aquaculture Europe 2016 (EAS), September 2016. Edimburg. United Kingdom.

Ball, A.O., Sedberry, G.R., Zatcoff, M.S., Chapman, R.W., Carlin, J.L., 2000. Population structure of wreckfish *Polyprion americanus* determined with microsatellite genetic markers. Mar. Biol. 137: 1077-1090.

Kentouri M., Papandroulakis N & Divanach P. 1995. Species diversification in Greek finfish mariculture. Cah. Options Mediterr 14: 129-136.

Papandroulakis N, Divanach P and Kentouri M. 1997. Specific diversification in finfish mariculture: the Mediterranean case. Proceeding of the International Conference Martinique Special Publication EAS pp 223-224.

Papandroulakis N, M. Suquet, M.T. Spedicato, A.Machias, C. Fauvel and P Divanach. 2004. Feeding rates, growth performance and gametogénesis of wreckfish (Polyprion americanus) kept in captivity. Aquaculture International 12: 395-407

Peleteiro, J.B., Saavedra Penas, C., Pérez Rial, E., Álvarez-Blázquez, B., Soares, E.C., Vilar Perón, A., 2011. Diversificación de especies en acuicultura. Estudio preliminar de la reproducción de la cherna (*Polyprion americanus*) en cautividad, Congreso Nacional de Acuicultura, Castelldefels, Spain

Sedberry, G.R., Andrade, C.A.P., Carlin, J.L., Chapman, R.W., Luckhurst, B.E., Manooch, C.S. III, Menezes, G., Thomsen, B., Ulrich, G.F., 1999. Wreckfish *Polyprion americanus* in the North Atlantic: fisheries, biology and management of a widely distributed and long-lived fish. American Fisheries Society Symposium. Life in slow lane: ecology and conservation of long-lived marine animals, nº23, pp. 27-50

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