DIVERSFY MEETING
2018, November 23-24
BRUSSELS

THE WRECKFISH
Objectives and Progress

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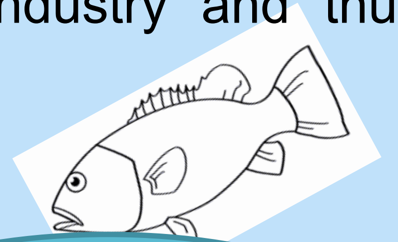
Finding new/emerging finfish species

The project DIVERSIFY has identified new/emerging finfish species in order to support the diversification of the European aquaculture industry and thus contribute to its sustainable expansion.



ECONOMIC CRITERIA

- Economical potential.
- Cover an European geographic area.
- Large finfishes market
- high market price.
- High demanding from consumers.
- New value-added products.

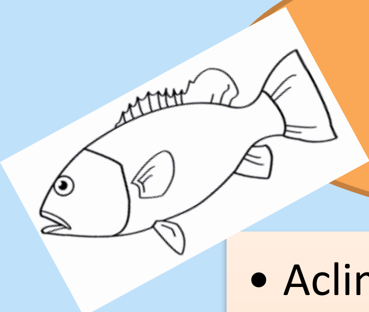
BIOLOGIC CRITERIA

- Biologic potential
- Fast growing.
- Large size.
- Late reproductive maturation.
- Easy manipulation in captivity.
- Extended pelagic juvenile phase.

SUSTAINABLE CRITTERIA

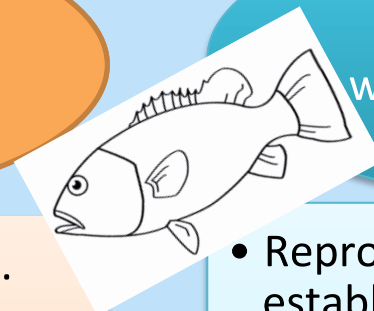
- Nutritional value.
- Limited fisheries landing
- Possibility repoblation.
- Stimulate different aquaculture systems (cage/pond/extensive/ reciculation)
- Aclimatize easily to captivity.

WRECKFISH (*Polyprion americanus*)
New species for the aquaculture



Our first Kowledges: what we did know?

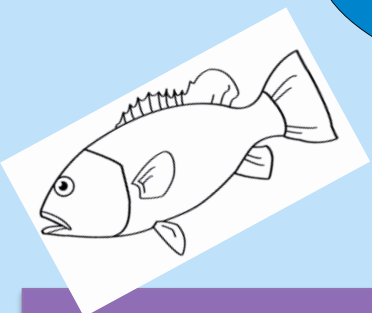
- Aclimatize easily to captivity.
- Low mortalities.
- Accepts inert food easily.
- High growth rate (grew from 1 Kg to 5 Kg in a period of 10 months)
- Late maturation.
- Some of reproduction cycle.
- Existence of wild especimens in captivity



Our unknowledges: what we didn't know?

- Reproduction control. The establishment for the control of spawning.
- Spawning preferences.
- Methods of spawning induction.
- Lack of larval rearing protocols.
- Scarcity of wild broodstocks.
- Nutritional value and preferences.

Our objectives



WP 6 REPRODUCTION

- 6.1 Increase the availability of wreckfish broodstock in captivity
- 6.2 Describe the reproductive cycle in captivity
- 6.3 Develop spawning induction procedures
- 6.4 Develop a CASA for evaluation of wreckfish sperm

WP 12 NUTRITION

- 12.1 Test live prey and influence of enrichment on wreckfish larvae
- 12.1 Broodstock feeds on fecundity and spawning quality

WP 18 LARVAL HUSBANDRY

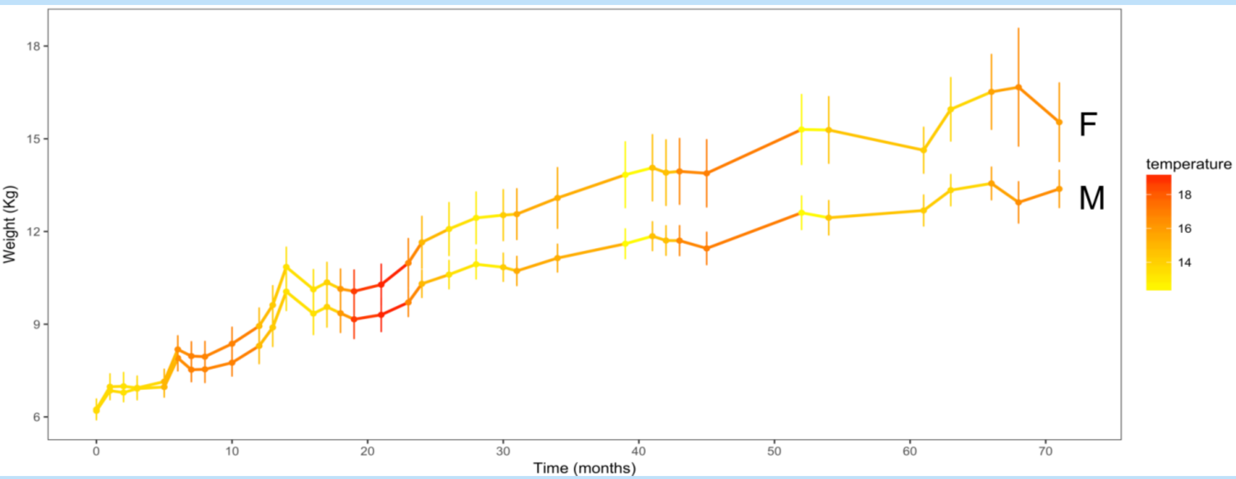
- 18.1 Develop larval rearing protocol
- 18.2 Description the ontogeny of digestive system and vision

WP 6
REPRODUCTION

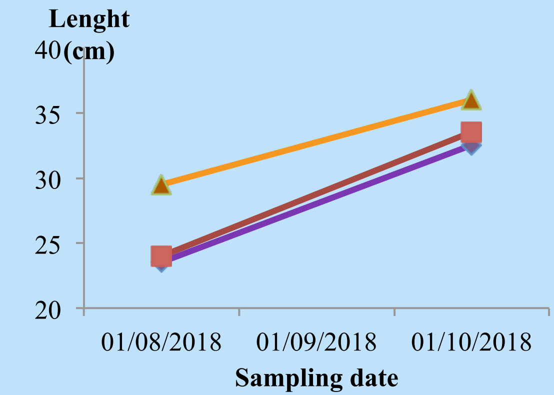
6.1 Increase the availability of wreckfish broodstock in captivity

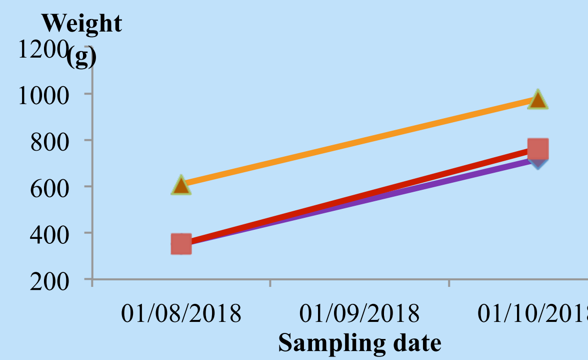
•Sharp drop in catches determined that the fulfillment of one of the objectives of the project that it was to obtain wild fish to increase wreckfish broodstocks was very reduced, despite the efforts to contact with a large number of fishermen in different ports of Galicia.

Evolution of wreckfish weight over 72 month period



The line color represents the temperature measured at each time point.



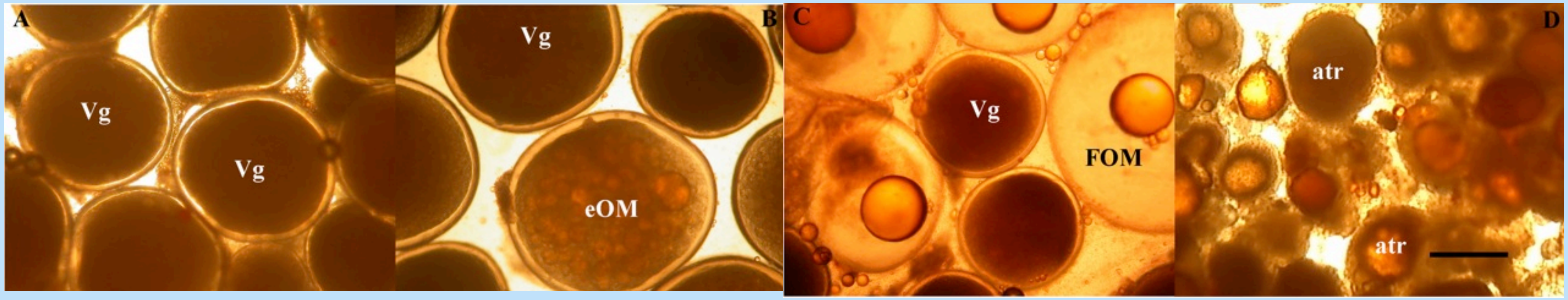


During two months in captivity two juveniles have doubled their weight, while the another one achieved a weight of 1 Kg.
Ingestion rate 1%.
FCR 0.8

Wreckfish reproductive period

✓Oocytes reaching vitellogenesis.
✓Early oocyte maturation and lipid droplets coalescence.
✓During final oocyte maturation, yolk coalescence was completed and the germinal vesicle was located in the periphery.
✓after ovulation, lipid vesicle and atretic oocytes could be observed in female fish gonads, together with post-ovulatory follicles.

2 months (Ø1mm)   1 month   3-4 months (Ø2 mm)   5-6 months



Vitellogenesis

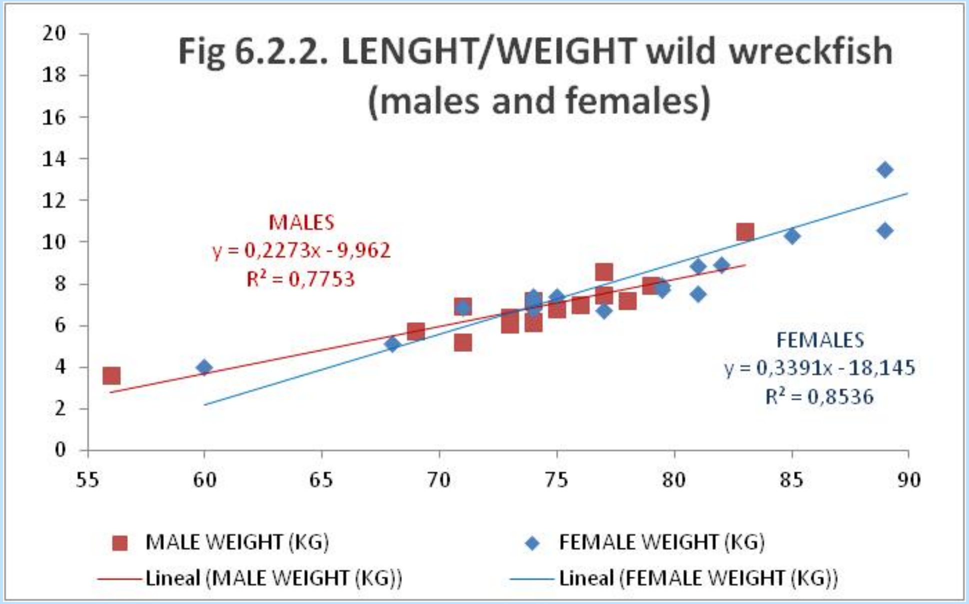
(lipid droplet coalescence) Early Oocyte maturation

Final oocyte maturation (yolk coalescence completed). Germinal vesicle located in the preriphery of the oocyte

Atretic oocytes

✓Biometric parameters of 60 wild wreckfish from market, captured in 2014.
✓A relation between weight and length was established, both for males and females

Relation between weight and length



Biometric parameters

BIOMETRIC PARAMETER (60 WILD WRECKFISH)	MEAN	STD
TOTAL LENGHT	76,09	6,788
ST LENGHT	66,38	7,629
PERÍMETER	55,68	5,986
WEIGHT (Kg)	7,52	2,169
EVIS. WEIGHT (Kg)	6,99	1,967
GONAD WEIGHT (g)	17,10	20,831
LIVER WEIGHT (g)	95,70	71,671
FAT PERIVIS. WEIGHT (g)	76,25	72,233
STOMACH WEIGHT (g)	125,90	56,183
INTESTINE LENGHT (cm)	94,53	15,555
INTESTINE WEIGHT (g)	99,27	62,688
GSI FEMALES	0,30	0,184
GSI MALES	0,13	0,126
SHI	1,21	0,497
VSI	10,31	17,233

Our wreckfish broodstocks

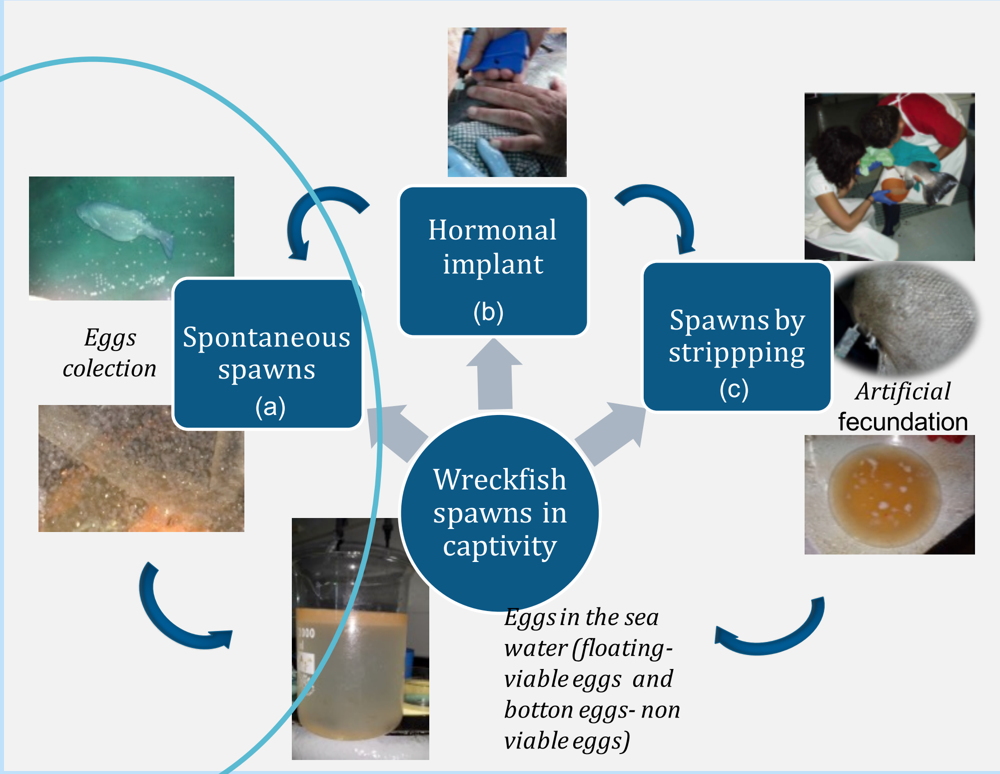
- **MC2.** Exhibition tank (3500m^3) and auxiliary tank for breeders (33m^3). Natural Tª and simulated natural photoperiod
- **CMRM.** Two tanks (40m^3). Natural Tª and photoperiod
- **IEO.** Two tanks (110m^3). Natural Tª and photoperiod
- **HCMR.** One tank (15m^3). Constant Tª and simulated natural photoperiod

Procedures to obtain wreckfish spawns in captivity

b) Spawning induction with exogenous hormones (GnRHa)

•In large tanks (>40 m3) under controlled photothermal conditions, allowing the fish to spawn spontaneously, and fecund by the male.

•Or in smaller tanks (< 15 m3) by stripping will be conducted and fish will monitored for ovulation.



Natural and spontaneous spawns:

Eggs were fertilized by the male in the sea water and were collected by floating in the tank

In large tanks (>40 m3)

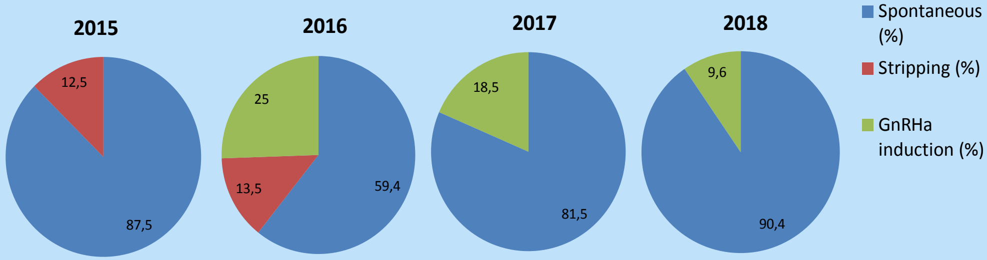
c) By stripping of the mature males and females followed by in vitro fertilization

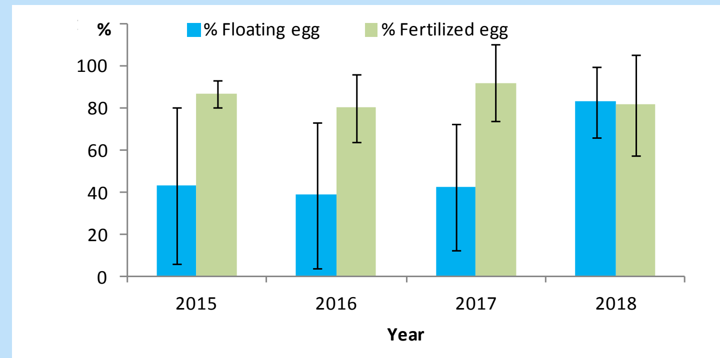
•Maintained in smaller tanks (<15 m3)

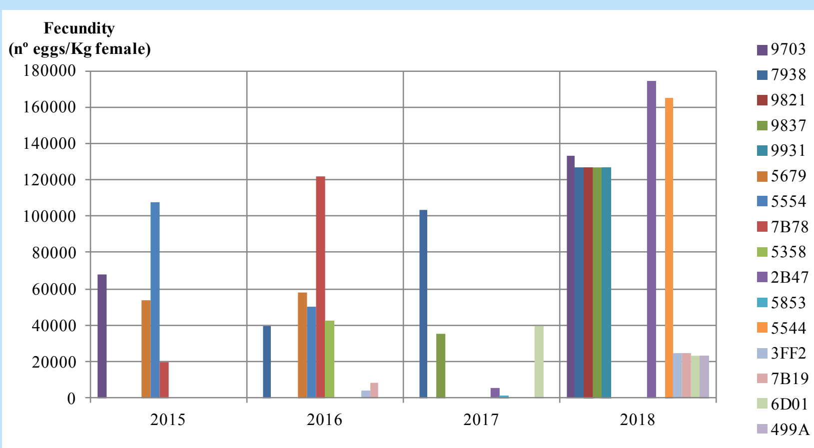
WP 6
REPRODUCTION

6.3 Develop spawning induction procedures

The number of spontaneous spawns was increased in all the broodstocks along the years. Females and males produced a large number of fertilized eggs and achieved satisfactory fertilization success.





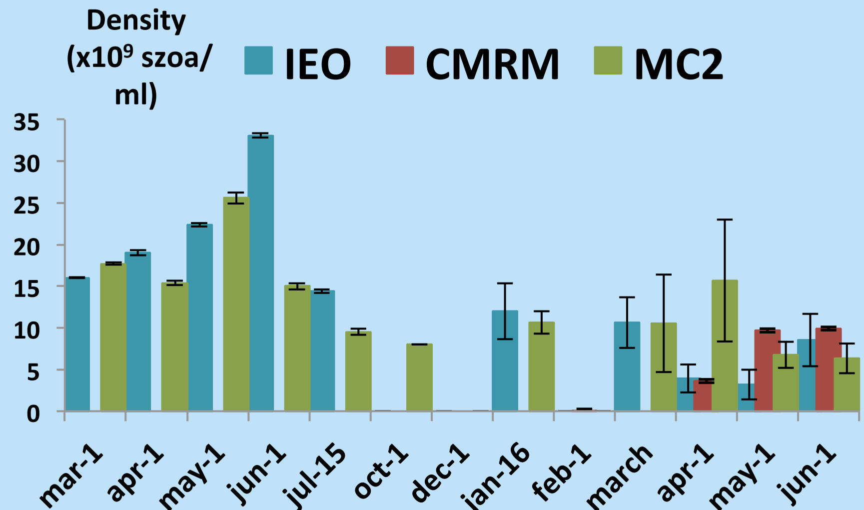


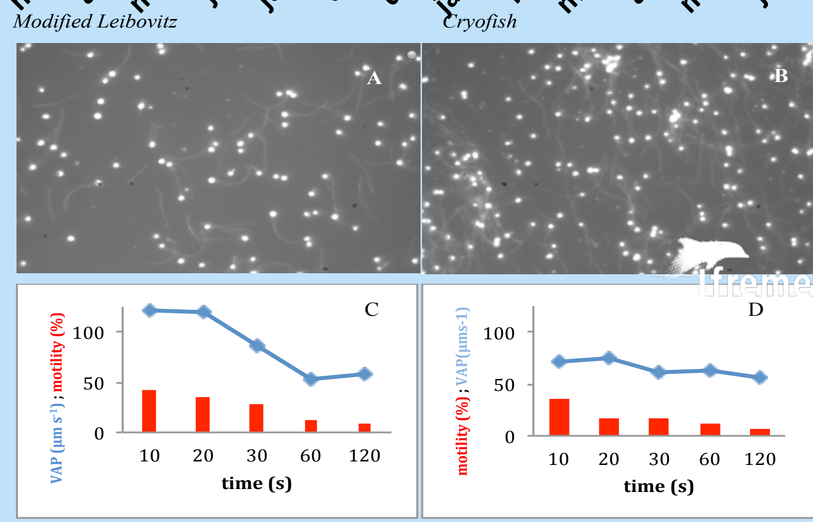
Increase individual relative fecundity (nº eggs/kg female) along this five years, as well as the quantity of fecund females, with a maximum values of relative fecundity of 174466 eggs/kg female in 2018 spawning season (MC2 stock).

WP 6
REPRODUCTION

6.4 Develop a CASA for evaluation of wreckfish sperm

Sperm samples from mature male were obtained by stripping.





- Sexual maturation of males covers the same period of females, reaching its peak in the months of April and June, with concentrations of 25-35 x 10^9 szoa/ml of sperm.
- The mobility ratio is high, with mean values between 2- 3 min. 30sec.
- The mean survival time of the sperm, conserved refrigerated at 4ºC, is 4 days. However, in some cases it reaches 18 days of survival after its recollection.

SUMMARY

✓The spawning season covers the months of January to June, and occurs sequentially in batches. Spawning takes place during the night or early in the morning, mainly between 05:00 and 08:00 h, with some exceptions that took place at mid day.

✓All wreckfish broodstocks produce a large number of fertilized eggs and achieve satisfactory fertilization success.

✓Spawning periodicity is 3-5 days and fertilization success is between 50 and 100% with better quality eggs towards the mid or end of the spawning season for each female.

✓It has been found that one female is able to spawn an average of 10 times per breeding season.

✓One male has the capability to fertilize at least 30 spawns in a period of 150 days (during 5 months in 2018 at IEO broodstock).

WP 6
REPRODUCTION

SUMMARY

✓Sperm exhibits a high percentage of motile cells at activation and one of the highest initial speeds recorded for fish sperm. This high speed was associated with a long swimming duration compared to other marine fish. The mean survival time of sperm maintained at 4ºC is 4 days. However, in some cases (male from IEO) sperm may reach 18 days of survival after collection.

✓The spermatozoa concentration in wreckfish stripped sperm is of the same order of magnitude as that of pelagic fish such as European seabass (*Dicentrarchus labrax*), gilthead seabream (*Sparus aurata*) or meagre (*Argyrosomus regius*) and it is higher than that of sole (*Solea solea*) and Turbot (*Scophthalmus maximus*).

✓Males chasing the females followed by the release of the gametes and produces large volume of good quality sperm for a very long period of time, covering the same period of females, reaching maximum values in the months of April and June. The mean concentration of wreckfish sperm is 2.41 $x10^{10}$ (sd: 0.4 $x10^{10}$, n=9) spermatozoa ml^{-1} in Galicia in January, while it remains around $1x10^{10}$ from April to September with no significant variation between sampling dates in Crete, Greece.

Test the effectiveness of live prey and influence of enrichment on wreckfish larvae

- ✓ 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.

	Enrichment Product		Preys				
	CE	AE	NoERot	CERot	AERot	NoEArt	AEArt
Proximate analysis (% dry matter)							
Proteins	46,73±0,18	43,00±0,16	62,33±1,11	60,45±0,58	59,41±2,79	61,64±0,41	69,48±1,96
Lipids	29,94±1,74	31,79±6,51	12,43±0,18	15,53±0,72	17,36±3,66	18,89±3,05	19,72±2,74
Fatty acids content (% TFA)							
Saturated (SAFAs)	29,73±2,94	26,01±1,95	17,88±0,26	16,83±0,05	16,80±0,31	19,93±0,00	21,06±0,07
Monoenoics (MUFAs)	7,37±0,54	10,41±2,29	64,91±0,25	32,94±0,28	34,77±0,33	45,58±0,09	42,31±0,03
Polyunsaturated (PUFAs)	62,48±4,69	62,59±2,27	**17,49±0,14**	**50,60±0,24**	**48,81±0,35**	**34,56±0,09**	**36,78±0,06**
Σn-3	41,26±6,47	41,37±1,21	5,40±0,09	33,78±0,20	30,52±0,25	22,48±0,10	22,11±0,02
Σn-6	19,14±0,32	21,95±1,23	8,87±0,04	14,83±0,05	16,23±0,11	11,22±0,01	13,99±0,01
n-3/n-6	2,24±0,26	1,89±0,16	0,61±0,01	2,28±0,01	1,88±0,00	2,00±0,01	1,58±0,00
DHA/EPA	8,30±0,06	7,20±0,37	0,66±0,02	6,57±0,04	6,42±0,05	0,01±0,00	0,31±0,00
EPA/ARA	2,03±0,16	0,87±0,04	1,00±0,03	2,27±0,03	0,92±0,01	3,99±0,02	2,27±0,01
20:4 n-6 (ARA)	**2,13±0,10**	**5,44±0,21**	**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)	4,33±0,53	4,75±0,02	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)	35,84±4,04	34,19±1,58	**0,71±0,01**	**26,71±0,16**	**23,97±0,23**	**0,06±0,00**	**3,38±0,04**

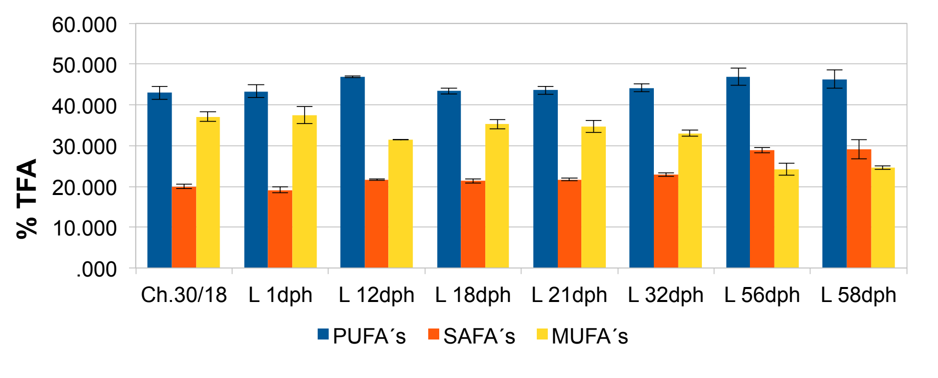
HUFA, highly unsaturated fatty acid; ARA arachidonic acid; DHA docohexaenoic acid; EPA eicosapentaenoic acid. CE, control enrichment product; AE, ARA enrichment product; NoERot, No enriched rotifer, AERot, ARA rotifer, NoEArt, No enriched Artemia, AEArt, ARA enriched Artemia

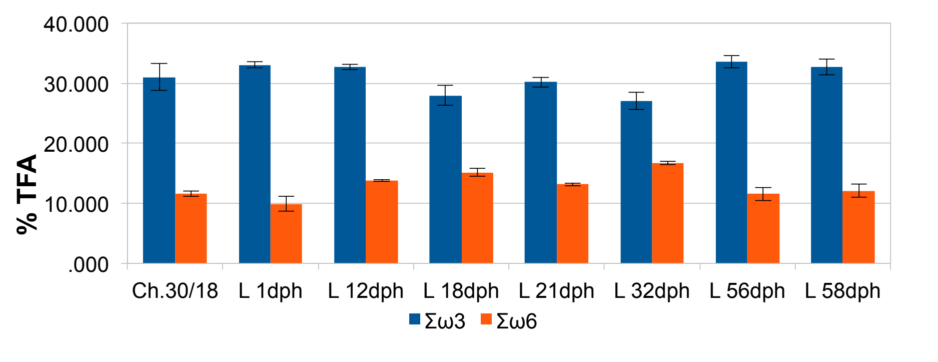
WP 12
NUTRITION

12.1 Test live prey and influence of enrichment on wreckfish larvae

Experiments of wrechfish larvae Nutrition

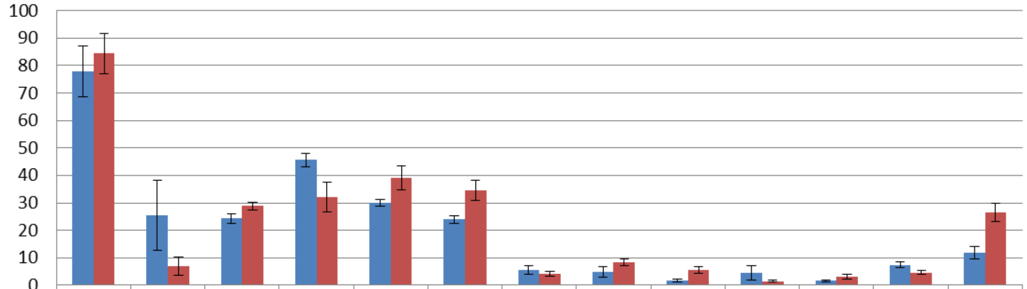
- ✓ Larvae of wreckfish present in general a good acceptance of enriched live prey tested
- ✓ No differences in fatty acid composition of wreckfish larvae fed with the prey enriched with the two enrichment products were found
- ✓ Fatty acid profile of wreckfish larvae along the larval development was obtained showing a big amount of PUFA specially DHA, EPA and ARA.



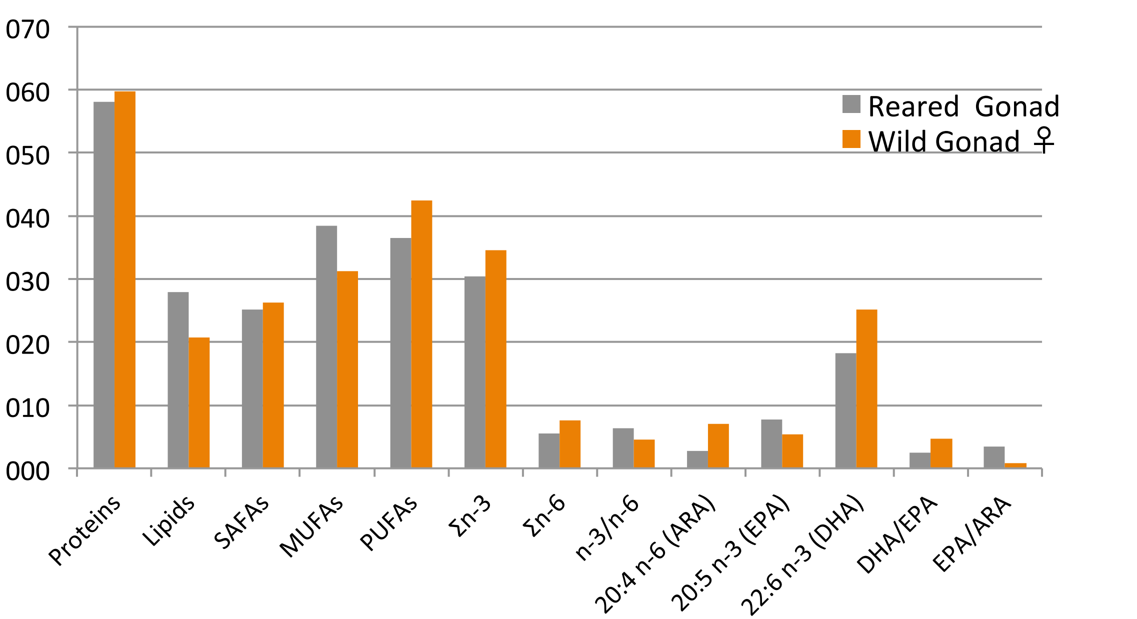


Biochemical profile in wreckfish muscle

Polyprion americanus



	Proteins	Lipids	SAFAs	MUFAs	PUFAs	Σn-3	Σn-6	n-3/n-6	DHA/EPA	EPA/ARA	20:4 n-6 (ARA)	20:5 n-3 (EPA)	22:6 n-3 (DHA)
Reared muscle	77,78	25,41	24,34	45,62	30,04	23,95	5,45	4,91	1,67	4,61	1,58	7,45	11,94
Wild muscle	84,41	6,92	28,83	32,09	39,08	34,51	4,08	8,5	5,69	1,54	3,11	4,55	26,38



- ✓ Results obtained from tissues composition of wild wreckfish were very useful to advance the knowledge of the nutritional requirements of this species.
- ✓ These results and those obtained from eggs and larvae newly hatched were very useful for the formulation of a **specific dry food for wreckfish broodstock**.

WP 12
NUTRITION

12.1 Broodstock feeds on fecundity and spawning quality

Ingredients	*Dry food %*
Fishmeal 70 LT FF Skagen	**25.000**
CPSP 90	**10.000**
Squid meal	**34.200**
Krill meal (Aker Biomarine)	**7.500**
Wheat Gluten	**7.000**
Wheat Meal	**7.250**
Tuna oil	**1.000**
Algatrium 70% DHA	**0.200**
Incromega DHA 500TG	**1.000**
VEVODAR	**1.300**
Vit & Min Premix PV01	**2.000**
Lutavit E50	**0.050**
Soy lecithin - Powder	**1.500**
Macroalgae mix	**1.000**
Antioxidant powder (Paramega)	**0.200**
Antioxidant liquid (Naturax)	**0.200**
SelPlex - Se yeast	**0.020**
Carophyll Pink 10% - astaxanthin	**0.050**
Nucleotides (Nucleoforce)	**0.030**
L – Taurine	**0.500**
Total	**100.000**

Diets used for wreckfish broodstocks feeding

	2016	*2017*
Stock IEO Tank S1 n = 5	Semi-moist diet	Semi-moist diet
Stock IEO Tank S2 n = 6	Dry food	Dry food
Stock IGAFA n = 10	Squid	Hake/Squid

Semimoist diet:

- **14.8% blue fish**
- **14.8% white fish**
- **18% mussel**
- **17.6% squid**
- **34.8% fishmeal**

WP 12
NUTRITION

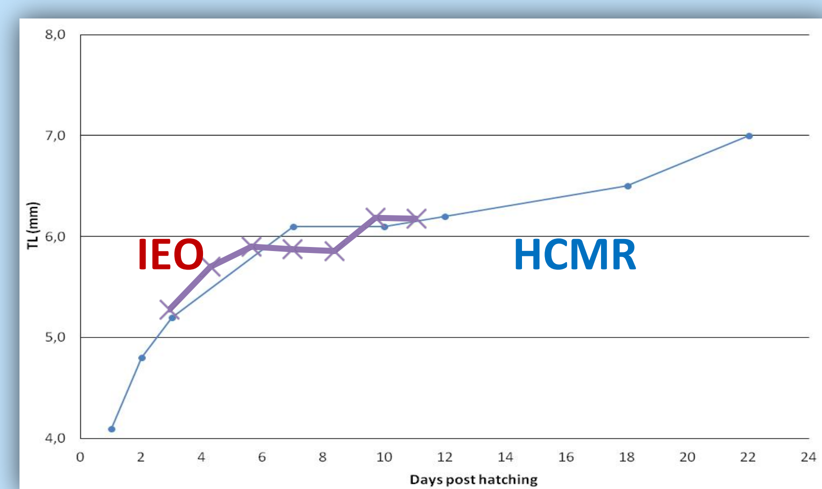
SUMMARY

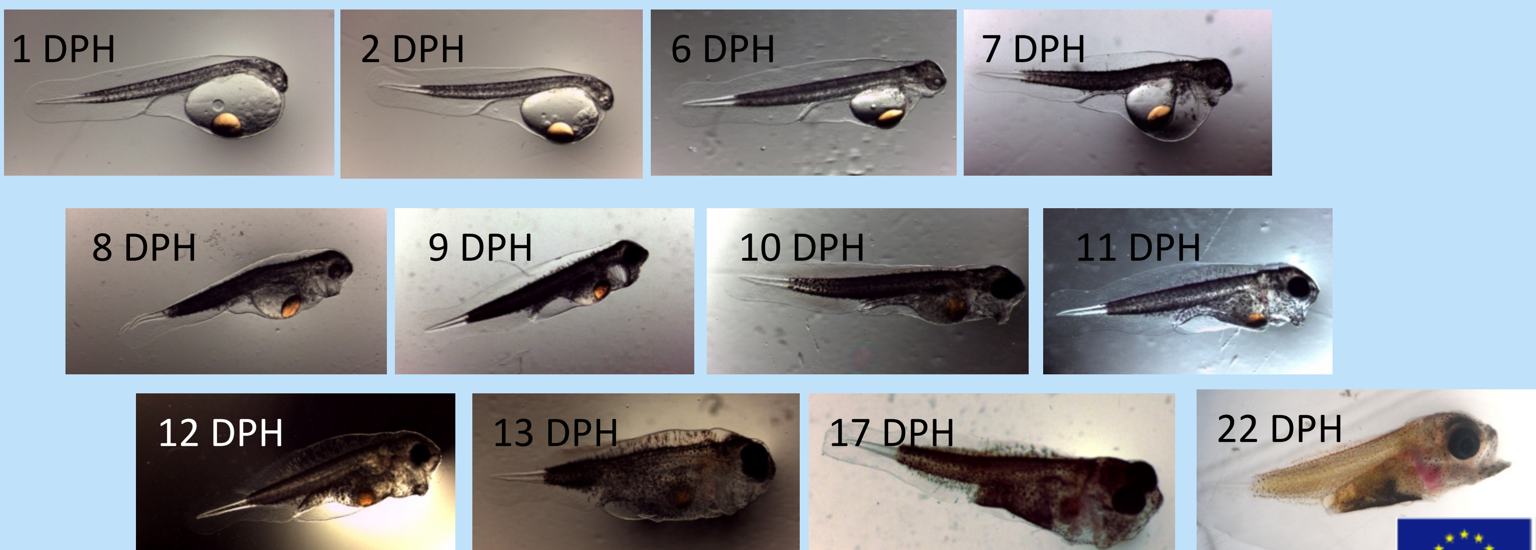
- Enrichment products for live prey (rotifers and *Artemia*) were developed with two levels of ARA being the enrichment less effective in *Artemia* than in rotifers.
- Wreckfish larvae present in general a good acceptance of enriched live prey tested.
- Fatty acid profile of wreckfish larvae along the larval development shows a big amount of PUFA specially DHA, EPA and ARA.
- Results obtained from tissues composition of wild wreckfish and those obtained from eggs and larvae newly hatched were very useful to advance the knowledge of the nutritional requirements of this species and for the formulation of a specific dry food for wreckfish broodstock.
- 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.
- Results obtained with dry food demonstrated that the wreckfish diet must contain a big amount of proteins, low level of lipids, a high amount of n-3 PUFA and the EPA/ARA ratio must be similar to that obtained in wild females gonads (about 1-1.5). New diet was specially formulated.

WP 18
LARVAL HUSBANDRY

18.1 Develop larval rearing protocol

First results of larval growth and developmet



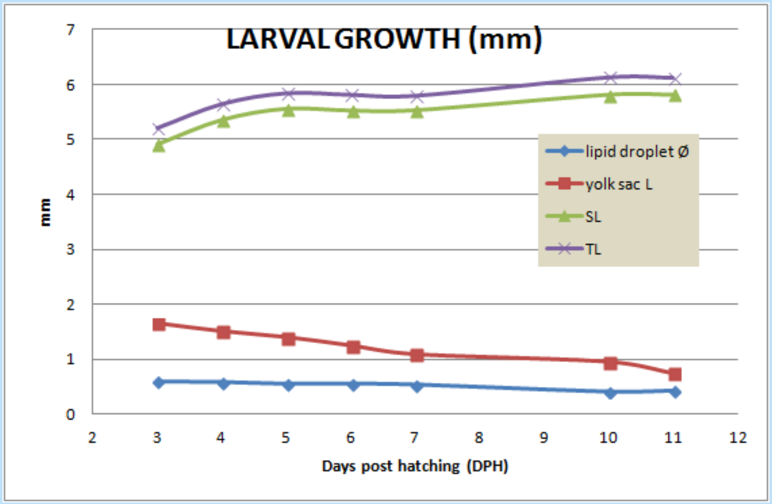


WP 18
LARVAL HUSBANDRY

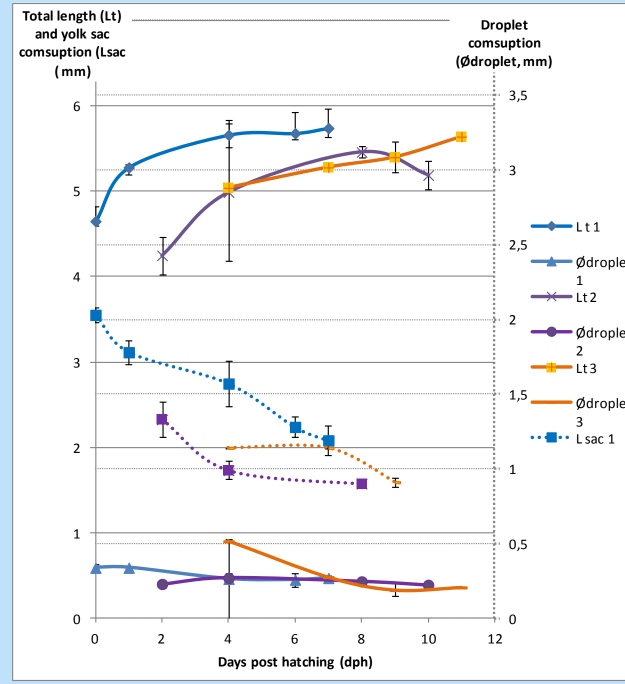
18.1 Develop larval rearing protocol

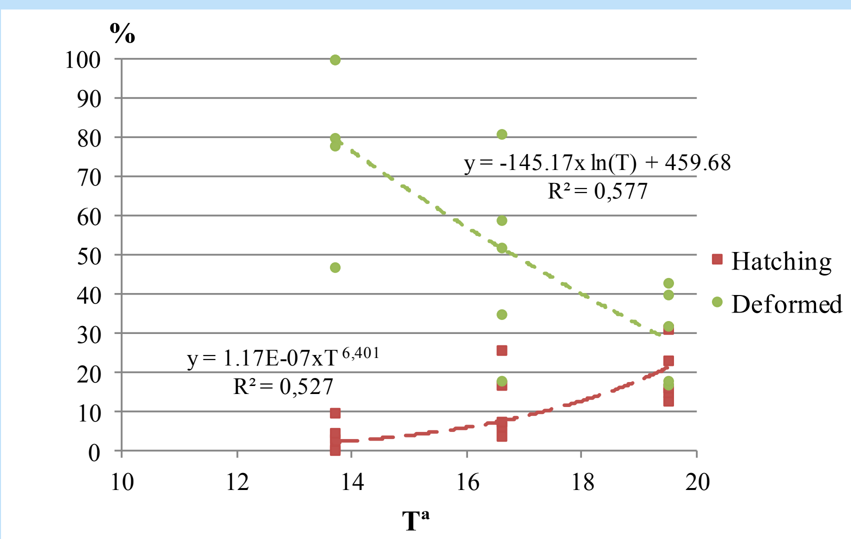
Curves of growth in length and yolk sac and droplet consumption in FT and RAS

FT



RAS





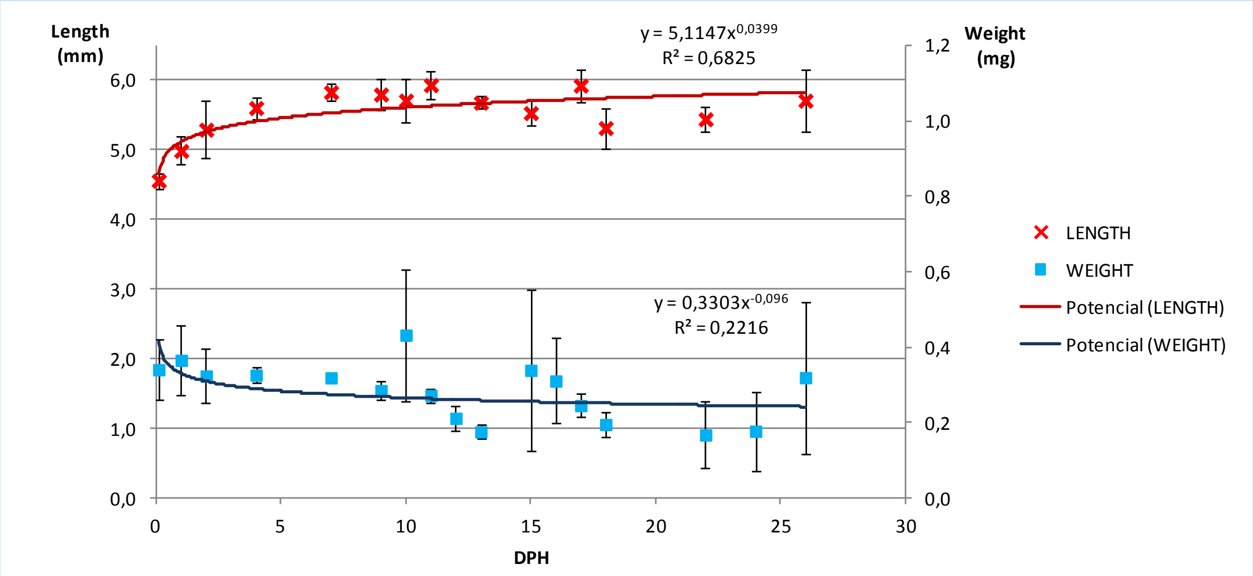
Another trials were focused in incubation eggs and larvae culture at different sea water temperatures

Regression adjustment for percent of hatched and deformed larvae as a function of incubation temperature.

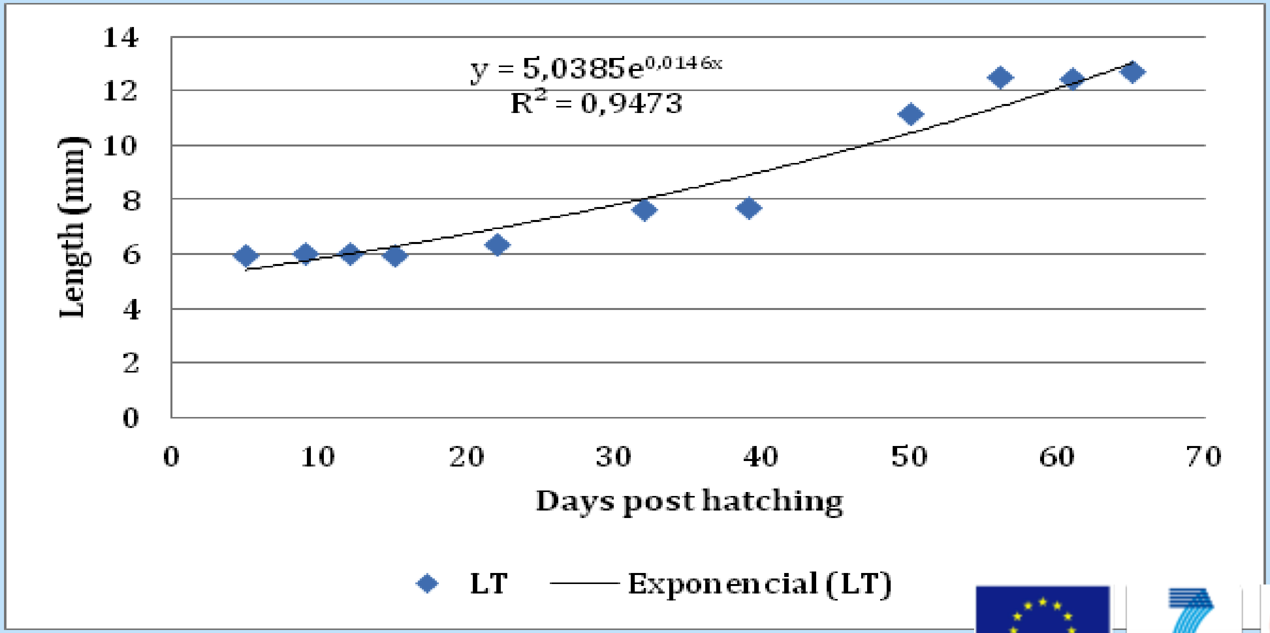
WP 18
LARVAL HUSBANDRY

18.1 Develop larval rearing protocol

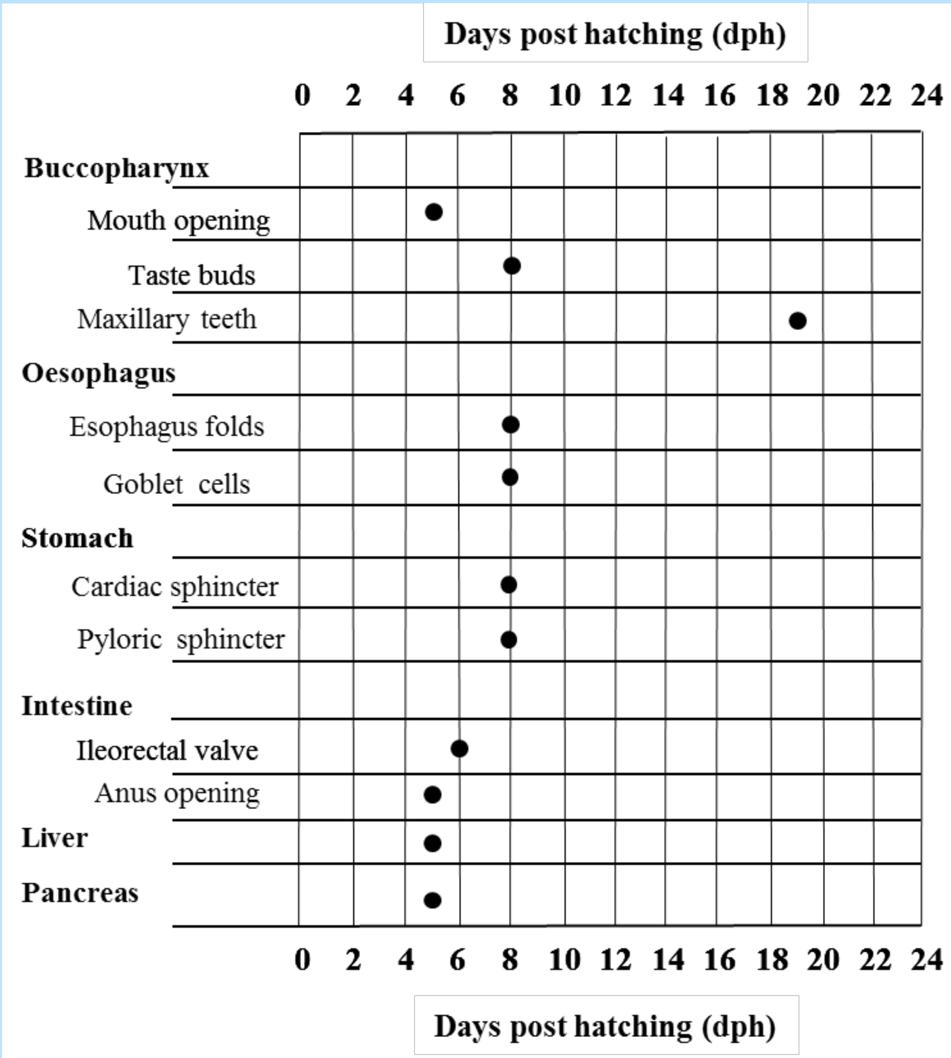
Growth in weight and length of wreckfish larvae from 0 to 26 dph (FT)



Growth in length until weaning, increasing from 5.6 mm to 12.8 mm at 5 and 65 dph, respectively (RAS).

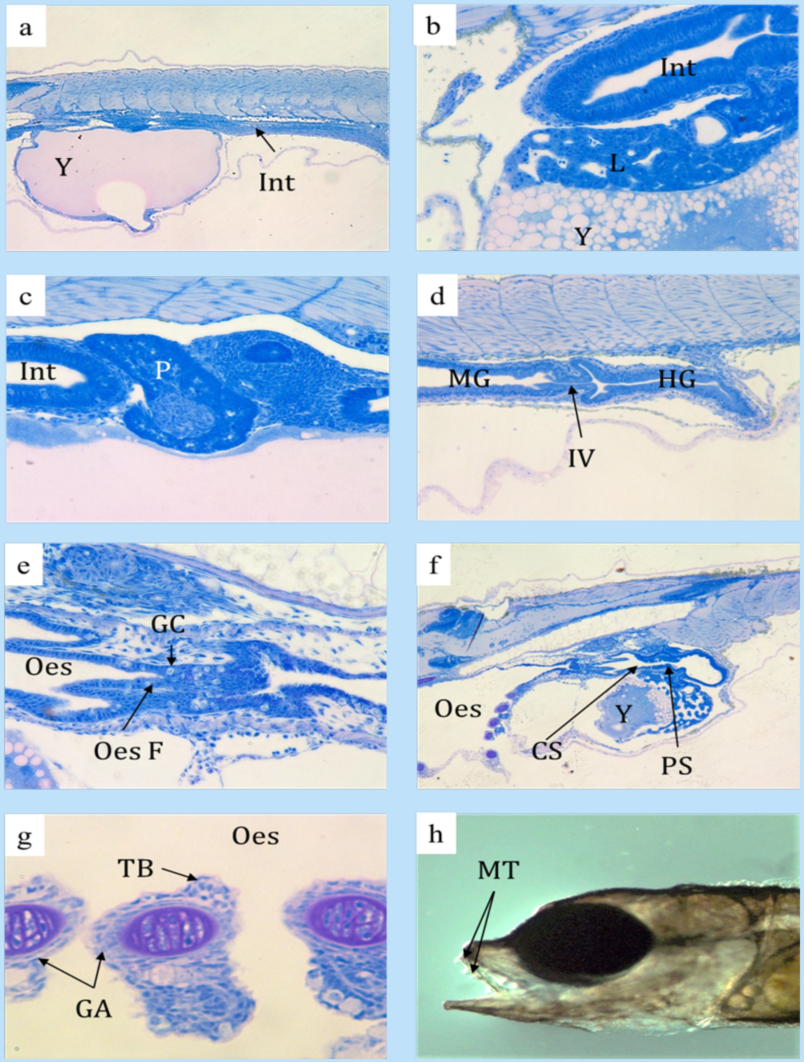


Time of appearance of each structure



Big period that the endotrophic phase occurred.
According to the results from the histological analysis, material from the lecithotrophic sack was visible until 23 dph

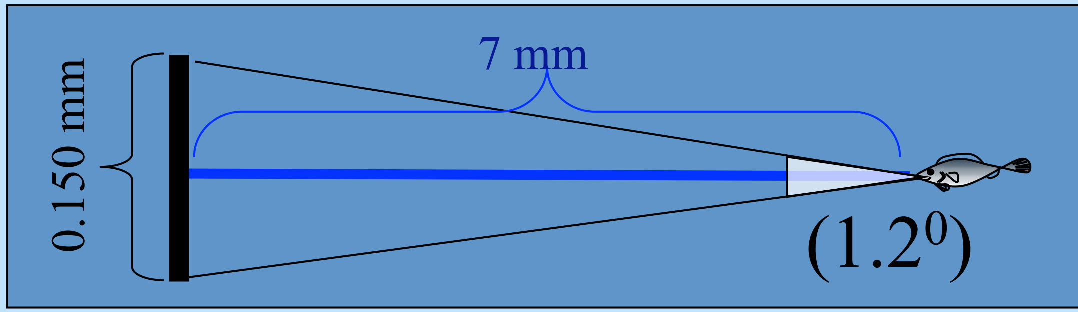
Microphotographs of histological sections from wreckfish larvae at different developmental stages.

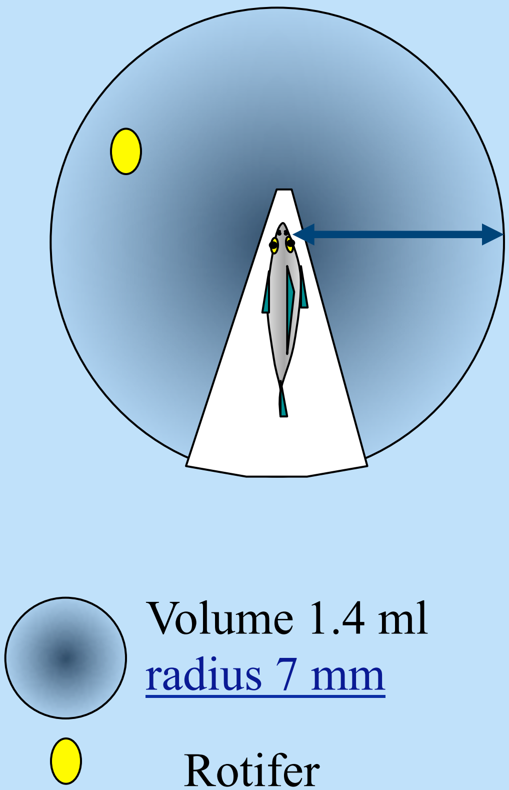


(a) At 3 dph showing the digestive tract as a closed straight tube located dorsal to the yolk sac.
(b) At 5 dph when the liver appeared.
(c) At 5 dph when the pancreas appeared.
(d) At 6 dph when the ileo-rectal valve appeared.
(e) At 8 dph showing the formation of folds and goblet cells at the oesopagus.
(f) At 8 dph showing the formation of the stomach area from the cardiac and pyloric sphincter.
(g) At 8 dph when the taste buds appeared.
(h) At 19 dph showing the formation of the maxillary teeth at the upper jaw. CS = cardiac sphincter, GA = gill arches, GC = goblet cells, HG = hindgut, Int = intestine, IV = ileo-rectal valve, L = liver, MG = midgut, MT = maxillary teeth, Oes = oesophagus, Oes F = oesophageal folds, P= pancreas, PS= pyloric sphincter, TB = taste buds, Y = yolk.

Visual hability

Estimation of the Visual Distance





At mouth opening, the histological visual acuity for wreckfish was estimated around 1.2^0 (degrees).

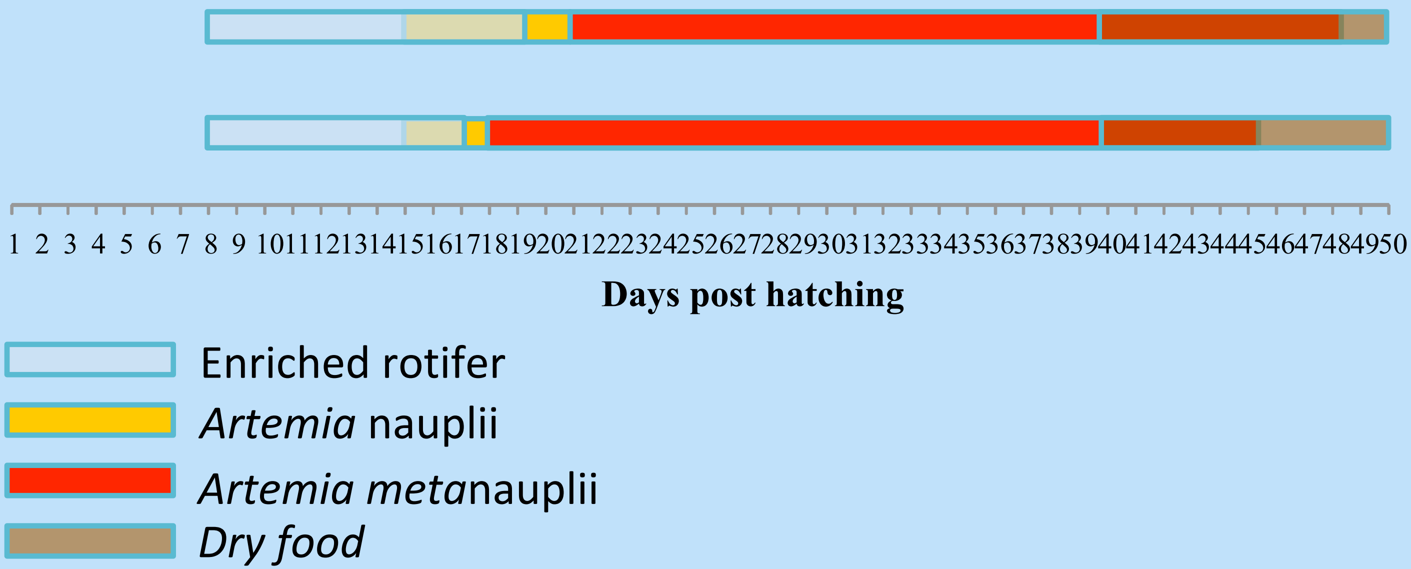
The distance or the radius of the visual field was calculated around 7 mm for the identification of an object size of 0.15 mm (rotifer).

This corresponds to a volume of sphere of around 1.4 ml.

So the minimum concentration of rotifers has to be at least 1 rotifer per 1.4 ml.

Feeding sequence until weaning in RAS sytem in IGAFA (CMRM) facilities with two larvae batches from IEO and MC2

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



SUMMARY

✓The optimal water temperature for artificial incubation of wreckfish eggs is in a range of 16.5-19.5ºC. Lower temperatures (14±0.5ºC) promote more deformed larvae and lower hatching rates, with more egg mortalities during the first three days of incubation.

✓Temperature range between 16-18 ºC may be adequate to improve survival and growth in wreckfish.

✓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.

✓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.

✓The larvae are able to consume food from the time that the mouth opens, although no exogenous feeding items were identified in the stomach contents.

✓The coexistence of autotrophic and heterotrophic stage for a long period during the first larval rearing period has to be taken in consideration for the development of new enrichment protocols for zooplanktonic preys that are used during the first

WP 18
LARVAL HUSBANDRY

SUMMARY

- 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±1.7 ‰ 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.

- 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. It is worth mentioning that, in the IGAFA facilities, there are 25 juveniles that reached 150 dph that were cultured at approximately 18ºC and represents a significant step forward in wreckfish larval culture and provides a basis for further studies.

- 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.

Our challenge has been achieved almost entirely and we hope to continue working to consolidate the culture of the wreckfish

Reasons to continue with wreckfish

- 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.

- Very important knowledge about biology, nutrion, reproduction physiology, behaviour in captivity, incubated and larvae zoothecnic knowledges adapted to the species, larval ontogeny.

- More study of ontogenesis and the formation of the basic systems of the rearing organism, seems to be the basis on which the optimization of the protocol of wreckfish larval rearing should be carried out.

- Trials with ongrowing in captivity and cages and organoleptic conditions of farming wreckfish.

- The scarcity in nature.

- In the coming years with the results obtained from the larval culture in 2018 with the obtaining of 25 juveniles of wreckfish, since in the future they can help to increase the number of individuals in the different wreckfish broodstock.

PRIVATE INDUSTRIES WITH WRECKFISH STOCK IN GALICIA (NW Spanish coast)

Isidro 1952

Acuario O Grove

Nueva PESCANOVA


FARMING?

Thanks!

Partners involved

- ✓ CMRM: CIMA and IGAFA (Xunta de Galicia, Spain)
- ✓ MC2 (A Coruña, Spain)
- ✓ IEO (Vigo. Spain)
- ✓ FCPCT (Las Palmas of Gran Canarias, Spain)
- ✓ HCMR (Greece)
- ✓ IFREMER (France)
- ✓ ULL (Tenerife, Spain)
- ✓ IOLR (Israel)