DIVERSIFICATION

- European aquaculture production scheme (seabream - sea bass)
  - 12-18 months to reach 350-600 g

- Sector's demand: open new markets
  introduce new species/products

- A profitable activity is targeting products (whole fish or processed) with high added value and high export potential
- Species satisfying these criteria should have
  - fast growth
  - wide distribution and
  - solved basic biological problems

A good candidate !

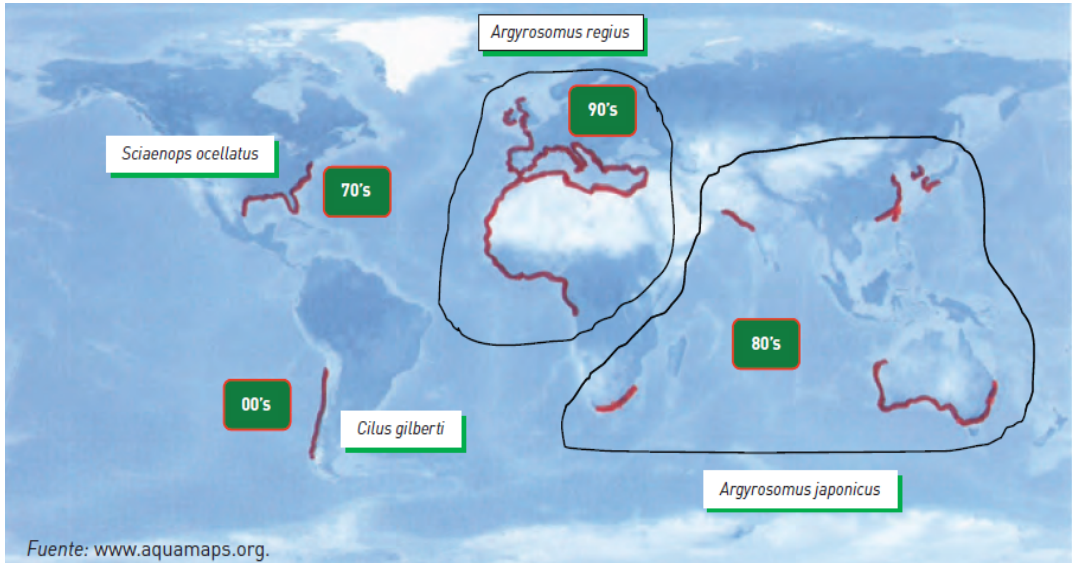
- Widespread all over the Mediterranean Sea. Senegal, bay of Dakar, seems to be the southern limit of the species.

Main production countries www.fao.org

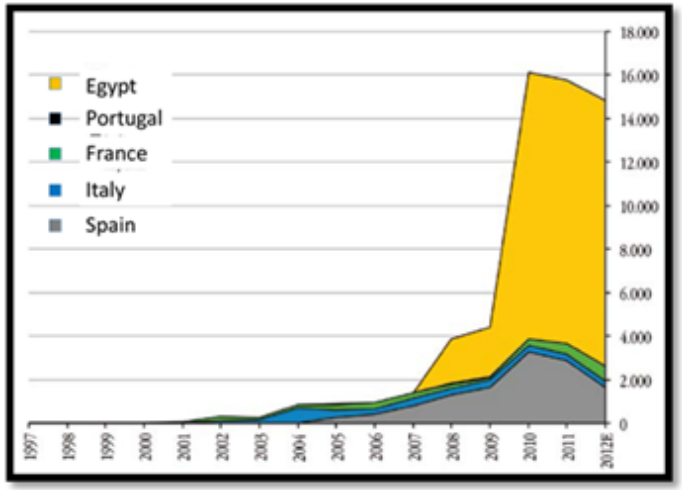
- Reaches up to 2 m in length and 50 kg in weight
- Important commercially
  - Global distribution
  - Fast growth
  - Excellent flesh quality and global market
- Innovative products with added value
  - Large size attained
    - marketed as whole or as processed food
    - **suitable for development of value added products**
- Efforts to develop/improve aquaculture methods
  - Economic potential in the EU market
  - Significant potential for exports

Other species are produced in other countries

Especie	Nombre en Español	Nombre en Inglés	Otros nombres
Argyrosomus japonicus	Verrugato del sur	Japanese meagre	Dusky kob (Sudáfrica) Mulloway (Australia)
Argyrosomus japonicus	Corvina	Whitemouth Croacker	
Argyrosomus regius	Corvina	Meagre	Maigre (Francia)
Cilus gilberti	Corvina	Corvina drum	
Sciaena umbra	Corvallo	Brown meagre	Corvina negra
Sciaenops ocellatus	Corvinón ocelado	Red drum	Corvina roja (México) Loup des carïbes
Umbrina cirrosa	Verrugato	Shi drum	



Fuente: www.aquamaps.org.



Evolution of meagre aquaculture production in Europe and Egypt from 1997 to 2012

The product Argyrosomus regius

Meagre has a number of attractive features:

- It is a particularly lean fish that produce high quality marketable products.
- It has a high dressing percentage, low adiposity, healthy muscular lipid content, and long shelf life.
- It reaches relatively large commercial sizes quite rapidly (1 Kg/ year) with a low FCR 0.9-1.2

Argyrosomus regius rearing in the Mediterranean región

- Started in 1997 when it was reproduced for the first time in captivity in France
  - fish of ~25 g reached ~1 kg in a year
  - standard culture conditions in cages
  - feeding on live feed passing quickly to artificial feeds

- The Mediterranean production in 2017 was 7934 tons (Apromar, 2018). Egypt has 16000 tons production although it is not clear that it is the same species
  - Hatcheries exits in Greece, Spain and France
  - Successfully produced by various aquaculture companies in Spain, France, Greece, Italy, Croatia

- The market price value in Spain 13-14 € kg^{-1}
  - Since 2002, producers differentiate between meagre products: smaller fish (600 g to 1 kg) are sold whole or filleted, while bigger fish (1 kg to 3-5 kg) are sliced or filleted and smoked.

Major bottlenecks for the EU aquaculture industry

- **Reliable reproduction**
  - In captivity reproduction is not considered a bottleneck, although there is an unknown genetic variability of captive broodstocks that needs to be characterised
  - Wild and captive-reared breeders reproduced after hormonal treatments, and in some cases also spontaneously.
  - There is a need to provide tools for genetic breeding programs

- **Production of adequate numbers of juveniles**.
  - Larval rearing is not considered a bottleneck for the expansion of meagre culture.
  - Cannibalism and variable size distribution in larvae and juveniles is an increasing concern
  - Feeds must be improved to consistently obtain high growth rates

- **Fish health**: an area of concern for commercial production
  - Several diseases and pathogens as potential threats
    - Systemic Granulomatosis (SG)
    - Chronic Ulcerative Dermatopathy (CUD)
    - Monogenean *Sciaenocotyle panceri*
  - Study meagre immune system and responses for the development of future vaccines

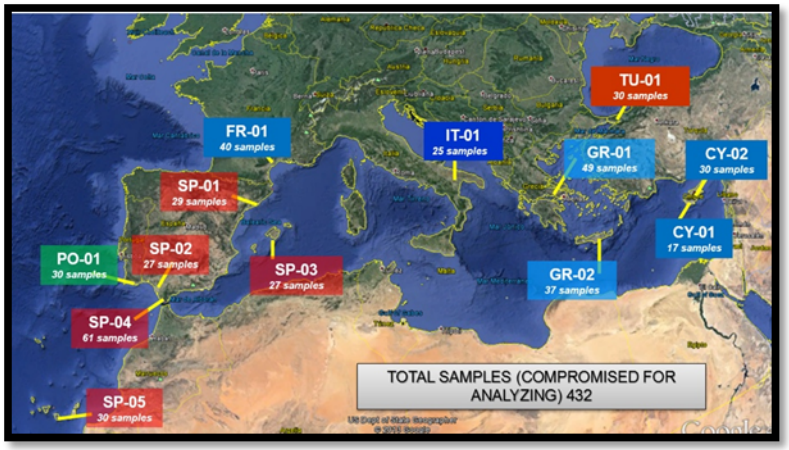
What DIVERSIFY promised at the beginning?

- **Reproduction**
  - Develop spawning induction methods (GnRHa-based spawning protocols) to produce the families required for genetic breeding programs
  - Characterise genetically available broodstock and fast/slow growers
  - Improve and develop new genetic tools

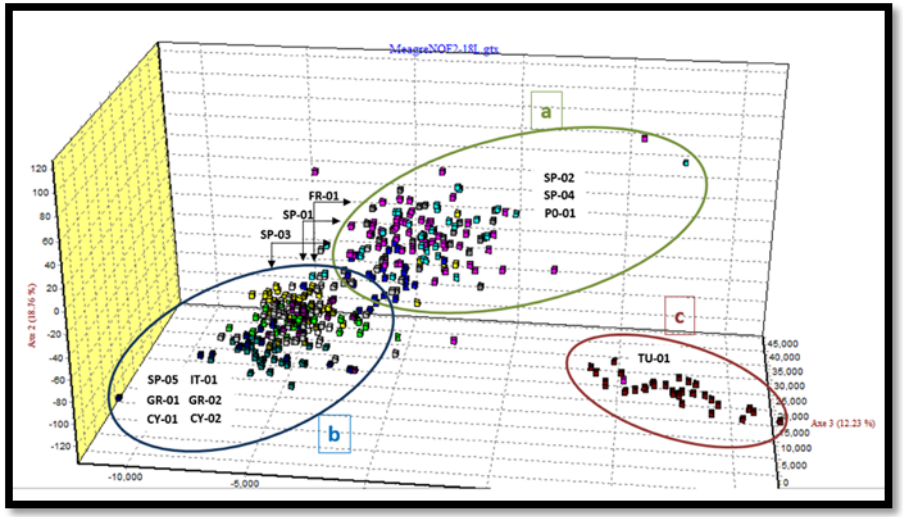
- **Larval husbandry**
  - Develop appropriate weaning protocols adapted to the development of the digestive system of the larvae

What DIVERSIFY has produced at the end

1.- Characterize genetically the available broodstocks in the Mediterranean



432 meagre breeders were sampled from broodstocks in 13 centers from 7 countries and studied using 18 microsatellite markers

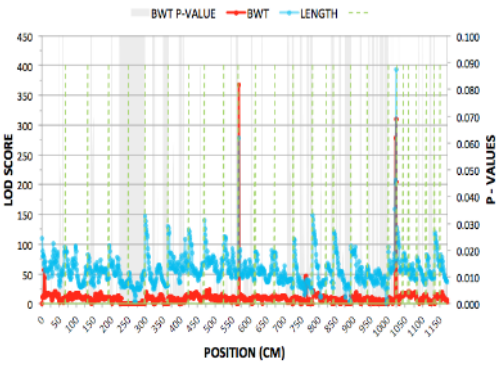


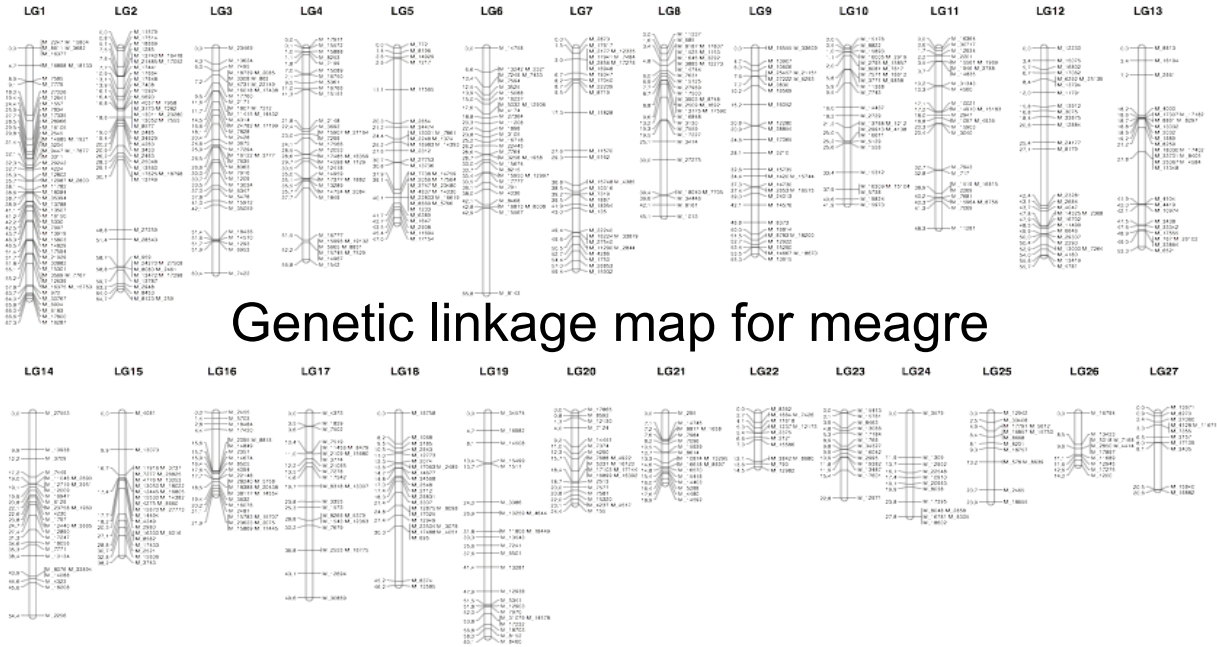
A Factorial Correspondence Analysis showed two clusters correlated with the geographical distribution of populations (Atlantic and Mediterranean), and a third cluster for a population from Turkey

What DIVERSIFY has produced at the end

2.- Genetic tools, markers and description of the transcriptome

- A total of 400 meagre from commercial farm characterized
- Description of transcriptome, 1000s of DNA sequences registered
- Genetic linkage map constructed for meagre
- A total of 731 genetic markers (SNPs) - Marker-assisted selection
- Two genetic markers for growth (QTLs) - Marker-assisted selection

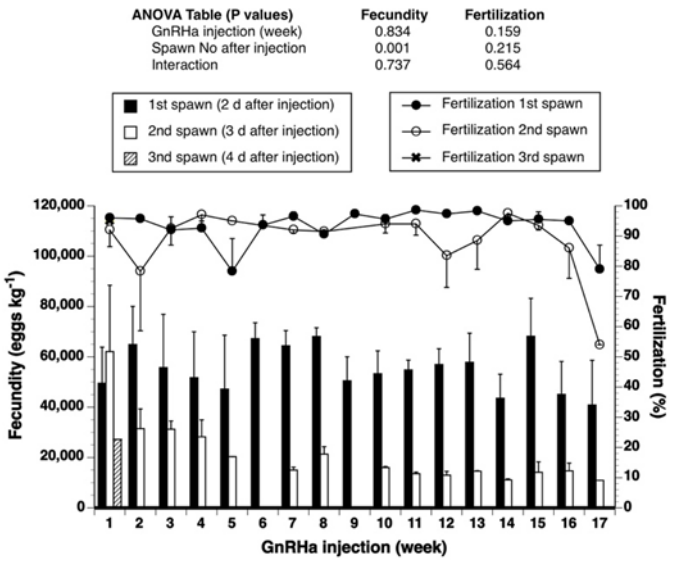




Genetic linkage map for meagre

What DIVERSIFY has produced at the end

3.- Paired crossings using hormonal induction. Important to establish genetic improvement programs



- GnRHa injected pairs (male + female) spawned for up to 17 consecutive weeks
- Total of 2 – 3 spawns after each weekly injection to give high fecundities
- Fertilization, hatching and larval survival success was high
- Paired tank spawning of meagre with male rotation to produce many families is possible
- Production of large (many siblings) multiple families from parents with known phenotypes.

What DIVERSIFY has produced at the end

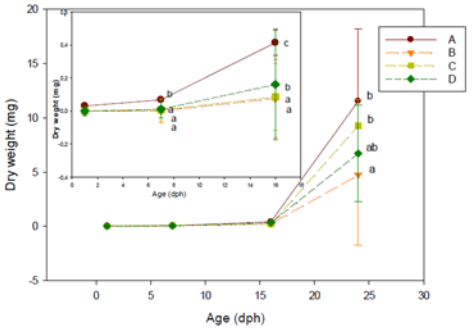
4.- A protocol for industrial level artificial fertilization

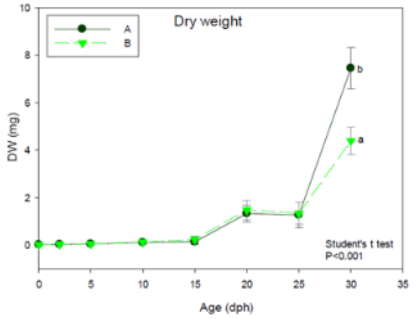
- A protocol for the optimum artificial (*in vitro*) fertilisation of meagre has been designed.
- The broodstock should be examined at 38 h post-injection at 18ºC to obtain optimum egg quality.
- For conventional production, a minimum of 200,000 spermatozoa per egg is recommended to ensure high fertilisation rates.
- It is recommended that eggs, sperm and water are mixed to coordinate activation with the sperm coming into contact with the eggs
- Meagre eggs should be fertilised within the first 50 min post-stripping
- The protocol was scaled up to a large factorial cross of 120 artificial fertilisations using fresh or cryopreserved sperm to confirmed the feasibility

What DIVERSIFY has produced at the end

4.- A protocol for early weaning of larvae onto dry feeds

Early weaning can be carried out with meagre larvae if several measures to reduce cannibalism are in place: (1) increasing feeding frequencies, (2) removing dominant individuals, (3) grading the larvae regularly and (4) keeping the larvae in the dark when food was unavailable or in short supply. The use of low light intensity before feeding in the morning increased survival of the larvae in the trial carried out in 2015 by reducing cannibalism.





What DIVERSIFY promised at the beginning ?

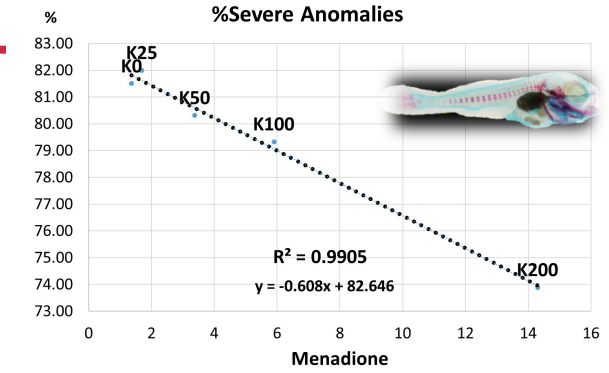
- **Nutrition**
  - Study the most relevant nutritional aspects (Digestible protein and Energy, Essential fatty acids and aminoacids)
  - Study the requirements of antioxidants (vitamin E and C) that affect fish welfare

- **Health**
  - Gene markers for immunity
  - Systemic granulomatosis and nutritional imbalance (P, vitamin C, etc)
  - Chronic ulcerative dermatopathy affecting lateral line organ and development of the disease using different water sources
  - Parasite infections by *Sciaenocotyle panceri*, chemical treatments

What DIVERSIFY has produced at the end

5.- Highlights to improve larval weaning diets

- A 0.4% dietary HUFA is not enough to cover the essential fatty acid requirements of larval meagre and, since their elevation up to 3% markedly improved lipid absorption, essential fatty acids levels and growth.
- High vitamin E and vitamin C requirements in meagre larvae (higher than 1500 and 1800 mg kg-1 for vitamin E and vitamin C, respectively).
- Meagre weaning diets must be supplemented with 2.4 mg/kg vit K, since the absence of this vitamin markedly reduced larval survival.
- Meagre larvae seem to be very sensitive to hypervitaminosis D and, only mildly to hypervitaminosis A, since supplementation with these vitamins leaded to a growth reduction.
- A 5.90 mg/kg of menadione in diet shows a trend to increase growth and survival of meagre larvae, however, large amount seems to decrease growth and survival, possibly due to its toxic potential.

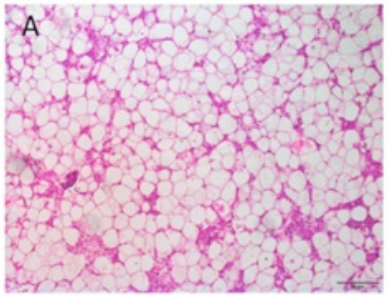


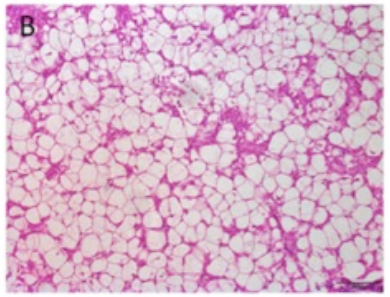
What DIVERSIFY has produced at the end

6.- Highlights on the requirements of essential fatty acids by meagre juveniles.

- Meagre showed the ability to selectively conserve key FA, particularly DHA and ARA over other FA, in response to EFA-deficiency.
- Meagre fingerlings have n-3 HUFA requirement of 2.1% DM in diets containing 16.5% DM lipids, 0.9 EPA/DHA and 0.4% ARA of total FA contents.
- EFA deficient meagre showed higher incidence of granulomas than fish fed adequate levels of n-3 HUFA in diet
- Hepatic steatotic alterations decreased linearly with the increase of the dietary n-3 LCPUFA levels.

	Dietary n-3 LC-PUFA level (% DM*)				
	0.8	1.4	2.0	2.6	3.6
Steatosis[2]	$2.6^{a}\pm0.2$	$2.4^{ab}\pm0.2$	$1.7^{ab}\pm0.2$	$1.2^{b}\pm0.0$	$1.2^{b}\pm0.2$
Granulomas[3]	$5.3^{a}\pm1.6$	$2.1^{ab}\pm1.1$	$1.4^{b}\pm1.0$	$0.1^{b}\pm0.1$	$0.7^{b}\pm0.4$

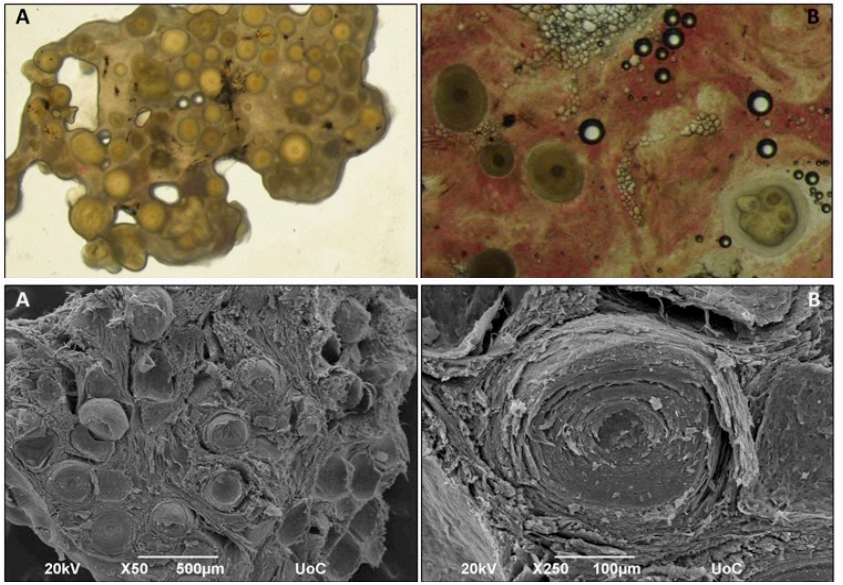




What DIVERSIFY has produced at the end

7.-Symptoms and causes of Systemic Granulomatosis (SG)

- Nocardiosis is present in Greece, most probably in a confined geographical region; however it is not the cause of SG.
- Vitamin D3 supplementation did not affect the development of the SG,
- High P content in the diet seems to improve the condition
- Plant protein replacement affects negatively the progression of the SG.
- High dietary content of the antioxidants vitamin E and C increased the incidence and number of fish with lower severity of SG
- The addition of Se, Mn and Se did not ameliorate the granuloma incidence or severity.



What DIVERSIFY has produced at the end

8.- Symptoms and causes of Chronic Ulcerative Dermatopathy (CUD)

Although the disease is directly associated with the use of borehole water, the causative agent is still unknown for meagre, it is recommended to avoid borehole seawater for the rearing of meagre if natural sea water sources are available and to pay careful attention to the source of the water used. Alternatively, the residence time of meagre in borehole water should be reduced to the minimum necessary, and fish should be moved to natural seawater (e.g. in sea cages) as soon as possible once the nursery phase is completed, in order to allow the tissue regeneration process to complete before marketing the fish.

What DIVERSIFY promised at the beginning (3)

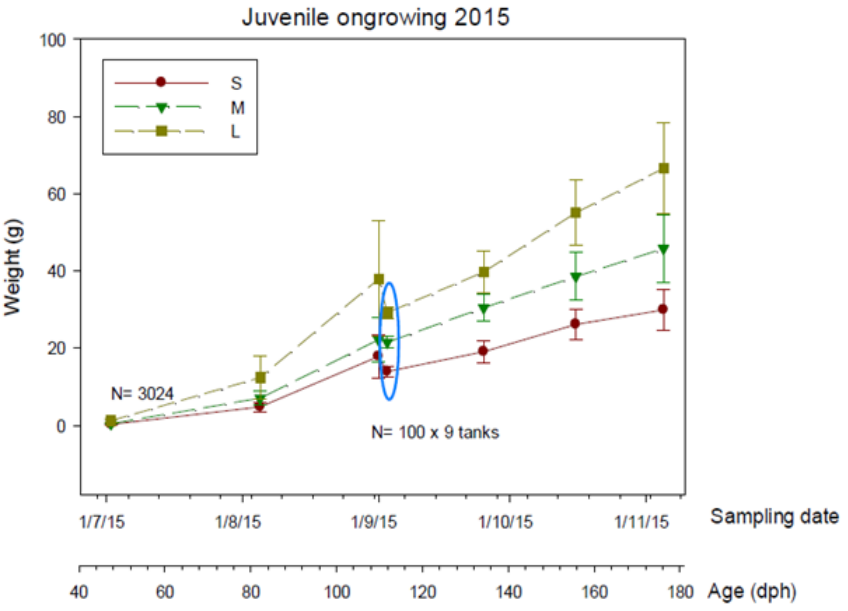
- **Growout husbandry**
  - Define an appropriate feeding method that respects the behaviour of meagre in the cages
  - Modify existing methods for cage culture related to volume and ligh conditions to maximize performance
  - Study the behavior of fish in sea cages

- **Consumer market analysis**
  - Develop new products with physical prototypes
    - incorporating consumer, market and buying criteria
    - monitoring the quality for organoleptic characteristics
    - marketing and communication strategies, and market and business models development

What DIVERSIFY has produced at the end

9.- Avoid size variability in meagre juveniles

We have observed that in meagre there is no compensatory growth when the fish are graded in sizes during ongrowing. Slow growing fish (S) always show a lower growth rate than medium or fast (L) growing fish that have as a consequence a delay of around 6 months to reach commercial size with clear economical consequences for producers.



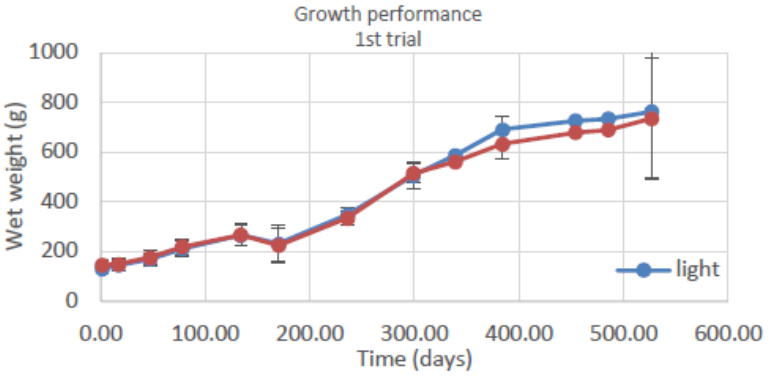
PRODUCTION COST OF L- AND S- GROWING FISH (1000 juveniles)		
	L- fish	S- fish
Juveniles (0.6€/unit)	600	600
Food 10-30 gr (2.4€/Kg)	90	136,8
30-250 gr (2.04€/Kg)	1526	2557,7
250-500 gr	1943,1	3243,6
Total	4159,1	6538,1
Market price (9,3€/Kg)	4650	4650

Growth of fish in 2014			
	S	M	L
100 d	7,82	10,84	15,67
200 d	66,67	85,94	104,18
360 d	160,83	206,10	261,79
540 d	266,76	341,28	439,11

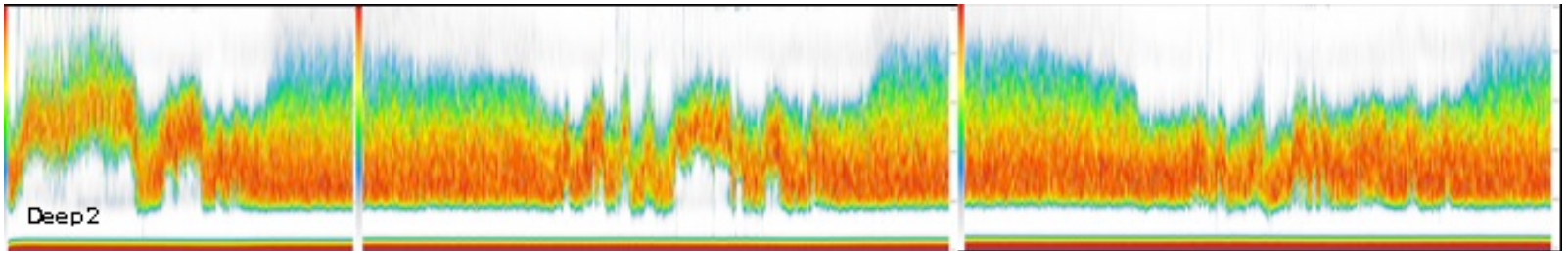
Growth of fish in 2015			
	S	M	L
100 d	12,27	19,26	24,24
200 d	33,17	50,33	73,33
360 d	66,61	100,04	151,87
540 d	104,23	155,97	240,24

What DIVERSIFY has produced at the end

10.- Study the effect of cage depth and light intensity on growth



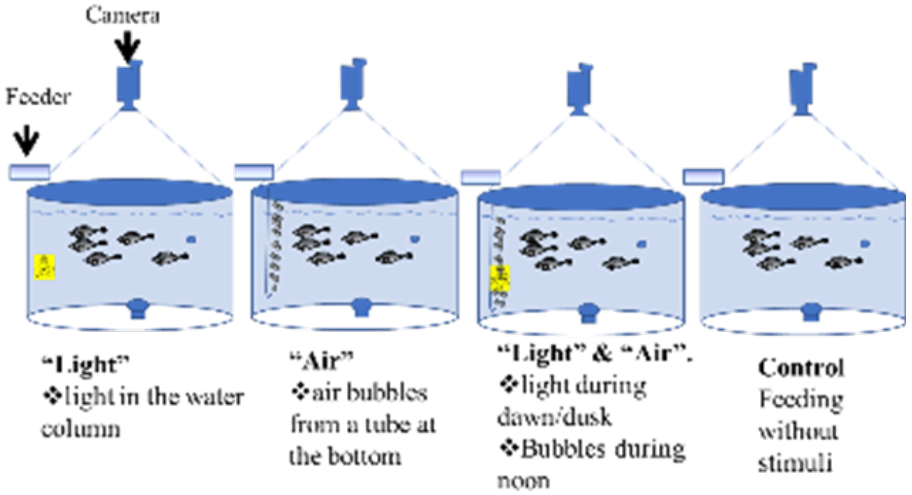
Several studies carried out to check the effect of shading or changing the depth of the sea cages gave no significant differences in growth or food conversion, however the fish tended to distribute in the bottom of the cage during the day and in all the volume of the cage in the night.



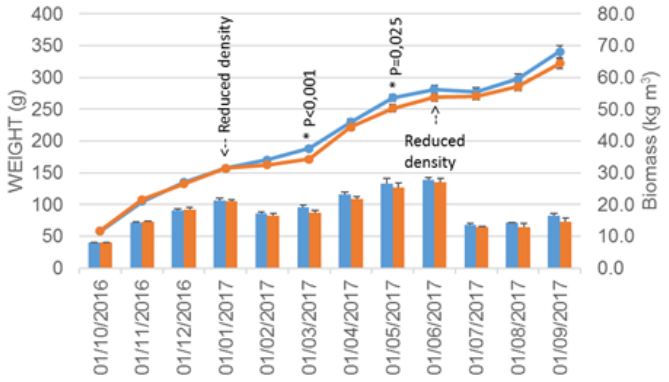
What DIVERSIFY has produced at the end

11.- Study the effect of different stimuli or the use of different feeding systems on growth

A trial using 2 different feeding systems programmed or auto-demand was designed to determine feeding patterns that may improve growth, FCR and size variation, however no significant effects on growth, size variation or feed conversion ratio (FCR) were obtained although in other species fed with auto-demand systems exhibited improved growth, FCR, size variation and showed less aggressive behaviours compared to systems with specific feeding times.



Air bubbles and light were quickly learnt and responded to as feeding stimuli. Both air bubbles and light or a combination of the two can be used in sea cages.

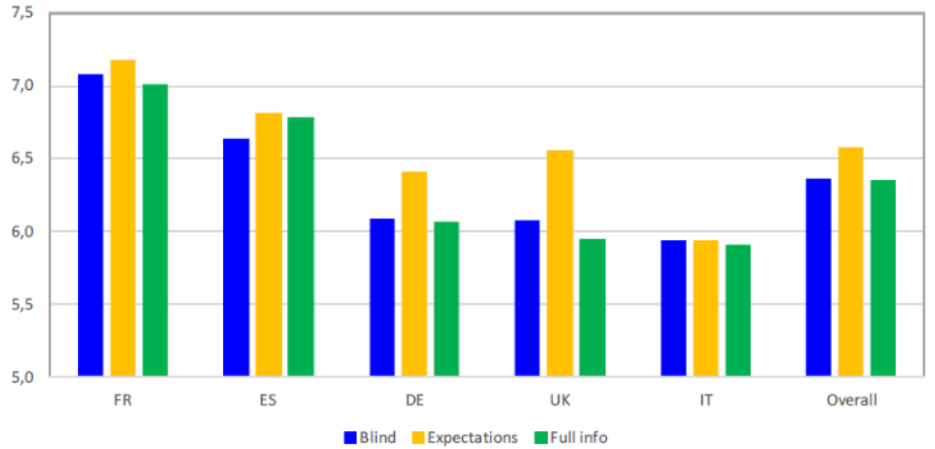


And an S-form submerged feeding system can also be used

What DIVERSIFY has produced at the end

12.- Market, consumer perception, new products and business model

Three product ideas were developed: ready to eat salad with fish, frozen meagre fillets divided in double portions and fish hamburger with the shape of a fish



In a consumer acceptance test, it was demonstrated the influence of providing the product information on the consumer acceptance degree. In the case of meagre, it was presented as a ready to eat fish salad and fish burger with the shape of a fish. In general, products with a lower degree of processing were those who generated higher expected and actual acceptance.

What DIVERSIFY has produced at the end

13.- A Technical Manual for Meagre

DIVERSIFY

New species for EU aquaculture

Technical Manual – Meagre (*Argyrosomus regius*)

Species Leader: Alicia Estevez, (IRTA, Spain),

Other Scientists participating: Mylonas, Constantinos C., Papandroulakis, Nikos, Papadakis, Ioannis (Hellenic Center for Marine Research), Fauvel, Christian (IFREMER, France), Duncan, Neil, Gisbert, Enric, Campoverde, Cindy, Andree, Karl B., Roque, AAna (IRTA, Spain), Rodríguez, Covadonga, Pérez, Jose (Univ. de La Laguna, Spain), Robles, Rocio (CT-AQUA, Spain),

IRTA hcmr ctaqua

ULL Universidad de La Laguna Parque Científico Tecnológico

Ifremer SKRETTING Argosaronikos Fishfarms SA

DTU Technical University of Denmark DTU Aqua National Institute of Aquatic Resources

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What is still needed for meagre

More Research is needed to:

1.- Reduce the problems of cannibalism during weaning and first periods of ongrowing. In other species like cod, domestication of broodstock has contributed to reduce the incidence of this behaviour

2.- Continue working on sea cage ongrowing and use of feeding stimuli. After the workshop carried out in Barcelona with fish farmers we found that they still need help/guidance to ameliorate their ways of working with this particular species and learn how to feed and manage it.

3.- The causes of granulomatosis are still unknown, although it is thought to have a nutritional origin we need to continue doing research in this disease