Deliverable Report

Deliverable No:	D20.1	**Delivery Month:**	24
Deliverable Title	Methodology to avoid size variability in meagre juveniles		
WP No:	20	**WP Lead beneficiary:**	P3. IRTA
WP Title:	Grow out husbandry - meagre		
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Task Title:	Size variability at juveniles		
Other beneficiaries:			
Status:	Delivered	**Expected month:**	24
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INTRODUCTION

The meagre (*Argyrosomus regius*) is a sciaenid fish found in the Mediterranean and Black Sea, and along the eastern Atlantic coast of Europe (Haffray et al., 2012). This fish has attractive attributes for the market that include large size, good processing yield, low fat content, excellent taste and firm texture (Monfort, 2010). The species also has the biological characteristics required for commercial aquaculture using well-established gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) culture technologies (Duncan et al., 2013). These characteristics include a fast growth of ~1 kg per year (Duncan et al., 2013), a low feed conversion ratio of 0.9-1.2 (Monfort, 2010; Duncan et al., 2013), relatively easy larval rearing (Roo et al., 2010; Vallés and Estévez, 2013) and established induced spawning protocols for the production of viable eggs (Duncan et al., 2012, 2013; Mylonas et al., 2013A; 2013b; 2015; Fernandez-Palacios et al., 2014). Meagre was first produced in 1997 in a commercial hatchery in France and since then it has exhibited annual production increases as high as 7 fold (FAO, 2005-2011). In 2010, European meagre aquaculture production was 2,387 t, produced mainly in Spain, with smaller quantities produced in France, Portugal, Italy, Greece, Cyprus and Croatia (FAO, 2005-2011).

DELIVERABLE DESCRIPTION (from the DOW)

The objective if this deliverables was to developed a method to avoid size variability of meagre juveniles. The deliverable will define the influence of genetic origin on the size variability in juveniles and on the bases of this provide recommendations on how to avoid variability (e.g. genetic improvement and / or size grading including the possibility of recovering slow growing fish). The deliverable will include results from growth trails and genetic analysis of fast and slow growers to support the recommendations.

TASK DESCRIPTION (from the DOW)

Size variability in juvenile pre-grow out makes regular grading essential to avoid cannibalism and grades of smaller fish may be related to poor performance when transferred to sea cages. Experiments were carried out with meagre juveniles of a mixture of 4-6 known families (from specific breeding groups), to simulate the commercial hatchery situation and in order to study differences in growth rate. Juvenile fish were stocked in triplicate tanks at the same initial density and fed the same commercial diet (IRTA). At the end of the experiment, fish were genetically characterised for parentage assignment (HCMR, Task 2.4 from WP2 Reproduction and genetics - meagre) to establish if differences in growth rate was a consequence of genetic origin. Fish with low growth rates were used for compensatory growth studies to determine growth potential of small juveniles and estimate the economic cost of using these fish for production, compared to discarding and using only larger juveniles.

The genetic analysis of fast and slow growers will be presented in ***Deliverable 2.5 Genetic characterization of fast and slow growing meagre*** (Mo 36). The genetic analysis for the latter deliverable is in progress (see **Deviations**).

MATERIAL AND METHODS

Two experiments were carried out, one in 2014 keeping the families separated during larval rearing and another in 2015 keeping the families together during larval rearing.

In 2014 six different spawnings obtained from hormonal induction of paired fish, which were used for larval rearing. Two families (V8-1 and C2 spawning on April 24th) hatched on April 28th and the other four (V8-1 (2), C1, V6 and V8-2, spawning on May 1st) hatched on May 5th (**Table 1**). Three spawns were from half-sib families (families 1-3) and three spawns were from unrelated breeders (families 4-6). Two cultured females were used as breeders and all other fish were from wild origin.

Table 1. Parents that contributed to each family or half-sib family and spawning date. The female and male number refers to the breeders unique ID and wild or cultured indicates the origin of the breeder

Family	Related half-sib family	Spawning Date (Tank)	Female	Male
1	2 and 3	24/04/2014 (V8-1)	5-wild	19-wild
2	1	01/05/2014 (V8-1)	5-wild	20-wild
3	1	01/05/2014 (V8-2)	1-wild	19-wild
4	-	24/04/2014 (C2)	16-cultured	21-wild
5	-	01/05/2014 (C1)	2-wild	22-wild
6	-	01/05/2014 (V6)	13-cultured	17-wild

In 2015 the experimental design was changed according to the suggestions given by the other participants in this task. In this trial four different spawnings obtained from hormonal induction of paired fish (**Table 6**) were used for larval rearing. All the spawnings were obtained on the same day (May 13th 2015) and after incubation the newly hatched larvae (May 15th 2015) were **mixed together** and distributed in four 1500-l tanks.

Table 2. Parents that contributed to each family and spawning date in 2015. The female and male number refers to the breeders unique ID and wild or cultured indicates the origin of the breeder.

Family	Spawning Date (Tank)	Female	Male	Hatched larvae (N)
1	13/05/2015 (V7)	5-wild	19-wild	122617
2	13/05/2015 (V6)	6-wild	23-cultured	141983
3	13/05/2015 (V8-1)	1-wild	20-wild	66500
4	13/05/2015 (V8-2)	8-wild	22-wild	8050

Larvae were reared under intensive conditions following the standard protocol of meagre culture (Vallés & Estévez, 2015). Fifty larvae per litre were used as initial density, a photoperiod of 16L:8D, 500 lux intensity (Vallés & Estévez. 2013), in the water surface, and fed enriched rotifers from 2 to 14 days post hatch (dph), enriched *Artemia* metanauplii from 8 to 30 dph and weaned a commercial diet (Gemma Micro, Skretting, Norway) from 20 dph onwards. For enrichment, the commercial product Red Pepper was used following the enrichment procedure provided by Bernaqua (Belgium).

Every week, 20-30 larvae were sampled and anaesthetized with MS222 to estimate growth in weight and length. Standard length was determined by observation in a stereomicroscope Nikon SMZ800 (Nikon, Japan) equipped with a digital camera Olympus DP25 (Olympus, Germany) and an image analyzer (analysis, SIS GmbH, Germany). The same larvae were used to estimate wet and dry weight, placing the larvae on a pre-weighted coverslip and after drying at 60ºC for 24h in an oven, with a microbalance Mettler Toledo MX5 (Mettler Toledo, Spain).

Juveniles were fed *ad libitum* with a commercial diet for European sea bass (Mar Perla, Skretting, Norway) in 2014 and using a fixed feeding rate in 2015 (7.5% body weight for fish between 10-30 grams, 5% for fish from 30-60 grams). Growth of juveniles was also registered every 2-3 weeks during ongrowing and specific growth rate calculated following the formula: SGR = (lnWf - lnWi x100) / t, where Wf and Wi are the final and initial weights and t the time (days).

For statistics analysis (one-way analysis of variance, ANOVA) and calculation of regression lines and growth curves the Sigma Plot 12.0 program (SyStat, USA) was used.

Problems observed during rearing, weaning and ongrowing

In 2014 larval growth was very different among the six families, thus the larvae from the first week grew faster and bigger than those of the second week. Groups C1 and V6 grew very slowly, larvae were always very small and the weaning became very difficult because the larvae were too small to eat the microdiet.

During the weaning phase a high incidence of cannibalism was detected causing very high mortality rates and very high dispersion of sizes was detected. As a consequence on June 19th when the first grading was carried out the survival was very low and a clear high dispersion of weights was detected, with fish of 8 g and of 0.4 g together in the same tank.

In 2015 we tried to avoid cannibalism reducing the light intensity of the tanks, increasing the number of feed doses (either *Artemia* and weaning diets during weaning but also increasing the number of doses of the microdiet after weaning) and separating the floating small weaned larvae from the big ones usually distributed in the lower part of the water column.

RESULTS

Trial 1 (2014)

The results in growth (dry weight) of the larvae are shown in **Fig. 1**. Growth in dry weight was variable between families obtained in the first (spawning of April 24th) and second week (spawning of May 1st).

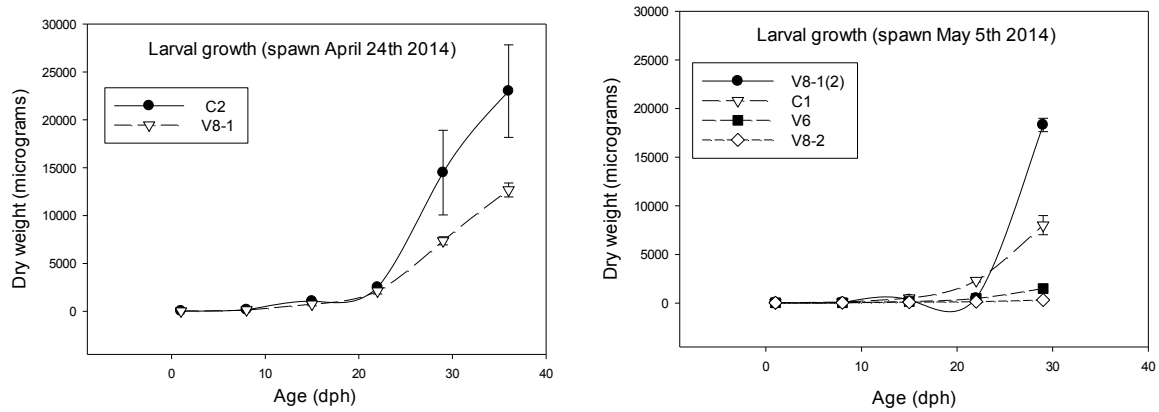

Fig 1. Growth (DW, µg) of the six groups of larvae during the first month.

On June 19th all the fish were graded according to weight, separated in big (8 g for fish of the first spawning on 24th April and 3 g for fish of the second spawning on 5th May) and small size juveniles (0.6 g from 1st spawn and 0.4 g from 2nd spawn, see Table 3). The results of the number of fish graded are shown in **Table 3** and the distribution of sizes of the different tanks in **Fig 2**. After grading the fish were transferred to the nursery. The fish from the different spawning dates (1st date 24th April and 2nd date 1st May) were maintained

separate to give a total of four groups in the nursery: big and small fish from the 1st spawns on April 24th and big and small fish from the 2nd spawns on May 1st.

Table 3. Big and small juveniles transferred to the nursery on June 19th.

Tank	V8-1	C2	V8-1 (2)	C1	V6	V8-2
Big	5	8	5	14		
Small	219	141	176		36	89
Total	224	181	181	14	36	89
% Survival	0.64	0.43	0.52	0.04	0.10	0.25

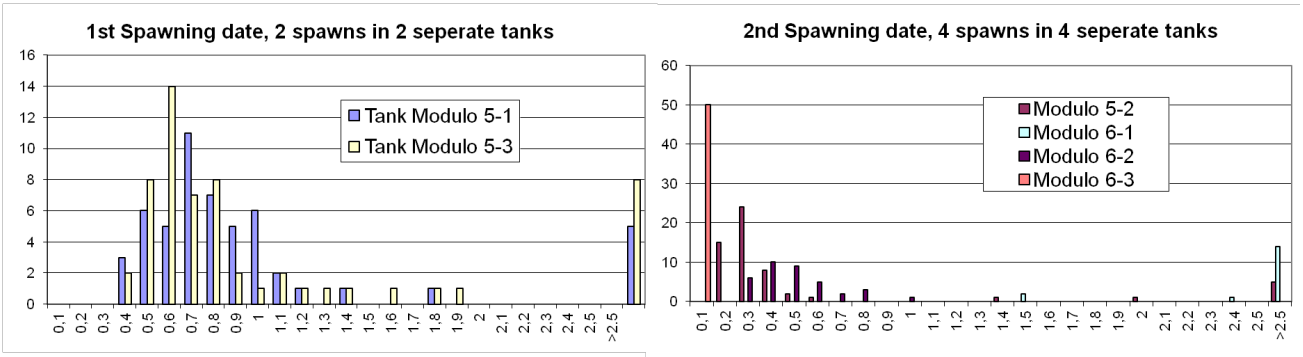

Fig 2. Weight (WW, g) distribution of fish in different recirculation systems (modulos 5 and 6 each with 3 tanks) from first spawning on April 24th and second spawning on May 1st.

The number and weights of the fish transferred to the nursery (1500-l tanks) is shown in **Table 4**. The fish were stocked into tanks of 1500 l with >400% flow through water supply daily. Water temperature and photoperiod was natural. The fish were fed a commercial meagre diet to satiation, by both hand feeding and automatic feeders.

Table 4. Distribution of fish in nursery on June 19th.

	1st spawn April 19th	1st spawn April 19th	2nd spawn May 5th	2nd spawn May 5th
	Big	Small	Big	Small
Nº	13	360	19	212
Avg Weight (g)	8.83	0.64	2.82	0.35
SD	2.24	0.30	0.8	0.16
Biomass (g)	114.73	230.4	53.66	73.78

A new classification of all the fish (big and small, see table 4) was carried out on July 24th to separate the fish in 3 sizes: big, medium and small fish (**Table 5**). In the new classification the small grades from June 19th were separated into medium and small grades for each spawning date to give a total 6 groups: big medium and small for each spawning date: 1st spawns on April 24th and 2nd spawns on May 1st (**Table 5, Fig. 3**).

Table 5. Results of the second grading on July 24th.

	Week 1			Week 2		
	Big	**Medium**	**Small**	**Big**	**Medium**	**Small**
Nº	12	46	168	19	49	122
Av Weight	62.04	14.14	9.22	31.36	16.60	11.01
SD	8.71	3.73	1.48	5.84	3.58	3.80
Min	50.60	11.00	5.20	23.26	12.97	5.65
Max	78.50	30.3	11.80	41.23	23.70	22.70

On the 21st August all the fish were weighed, measured (length), photographed and fin clips were taken for genetic analysis, and in addition to fin clips 16 fish were sacrificed and samples of liver and muscle stored in RNA-Later for transcriptome analysis (see WP2, Task 2.5). During the first two months (June 19th to August 21st) in the nursery the fish exhibited good growth, the largest group from the 1st spawning (April 24th) grew from 8.8±2.2 g to 101.8±22.3 g and the largest fish from 2nd spawning (May 1st) grew from 2.8±0.8 g to 56.7±13.6 g (**Fig 3**). The small and medium grades of fish grew from 0.6±0.3 g to 21.8±5.0 g (small grade) and to 35.2±4.3 g (medium grade) for the first spawning and from 0.3±0.2 g to 26.0±4.1 g (small grade) and to 50.7±12.7 g (medium grade) for the second spawning. The large fish exhibited a SGR of 5.6 and 6.9% day^{-1} for the first period (June to July) and 1.8 and 2.1% day^{-1} for the second period (July to August). The medium and small grades exhibited a SGR from 8.0 to 10.1% day^{-1} for the first period (June to July) and a range from 3.1 to 4.0% day^{-1} for the second period (July to August). The proportion or percentage of the population attaining larger weights was low indicating a few fish grow faster than the majority of the population. The large grades of fish represented 3 and 8% of the population, the medium grades 21 and 26% and the small grades 76 and 65% of the population (**Fig. 3**).

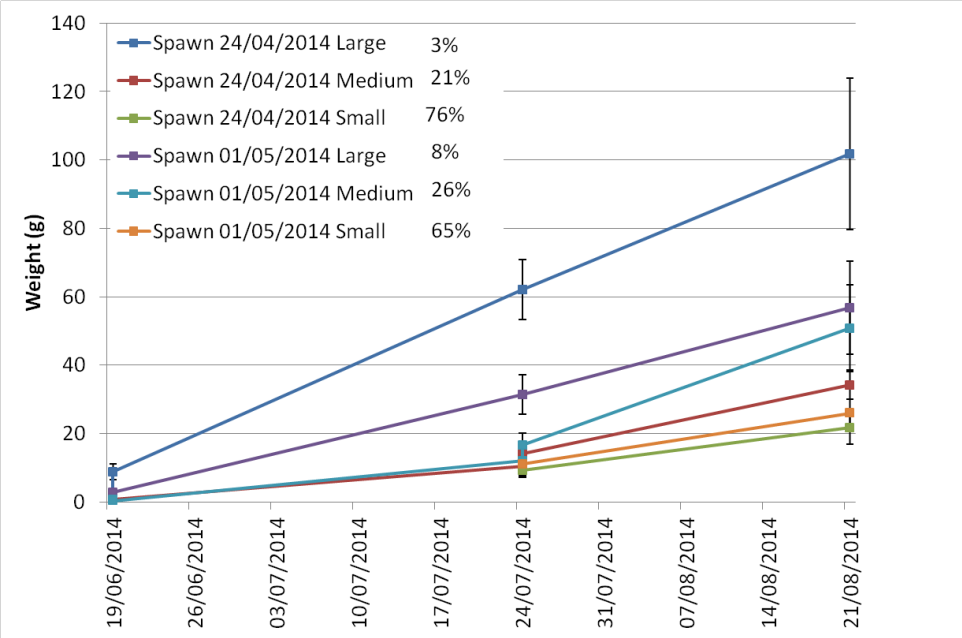

Fig 3. Growth, mean wet weight in g (error bars are the standard deviation of the mean) of the juveniles from different spawning dates and graded by weight. Initially the population was divided into large and medium fish and then the medium fish were divided into medium and small. The percentages in the legend refer to the percentage of the population in each grade.

The distribution of all the size grades across the different tanks / grades was compared and 70% of the population was observed in the size range from 15 to 30 g (**Fig 4**). Therefore, on August 21st this 70% of the population that formed a normal distribution was graded into three grades: 73 large (25-30 g) fish, 89 medium (20-25 g) fish and 86 small (15-20 g) fish. Each grade of fish was stocked into a separate tank and to monitor the growth.

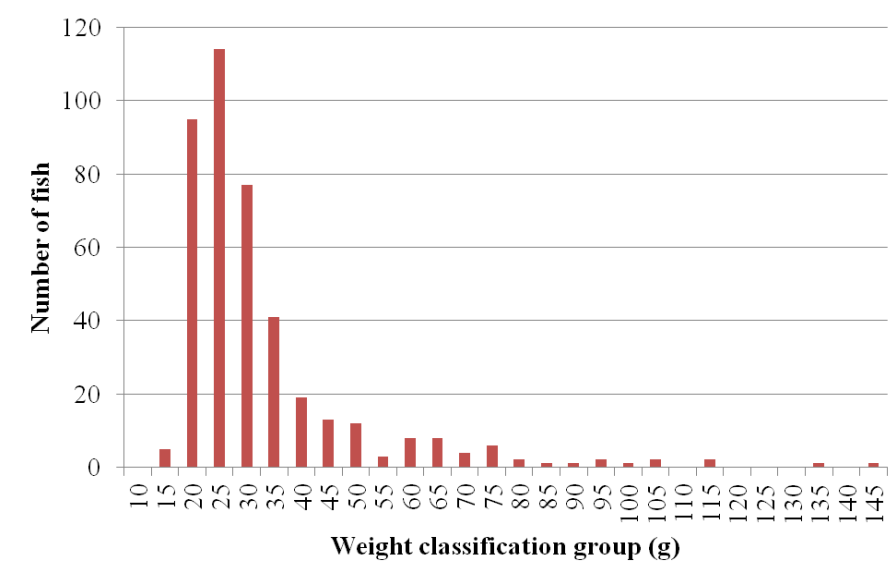

Fig 4. Frequency distribution of number of fish in each 10g size classification. The weight shown is the upper value of the classification, for example classification 15g contains fish from 10.1g to 15g.

The fish were stocked into tanks of 1500-l with >400% flow through water supply daily. Water temperature and photoperiod was natural. The fish were fed a commercial sea bass diet (Perla Skretting, Norway) to satiation, by both hand feeding and automatic feeders. A random sample of 50 fish from each group was weighed and measured (length) on the 18th Sept., 8th Oct., 29th Oct., and 19th Nov. The growth performance of these three groups was similar and the SGR in the first period (August to Sept.) was 2 % day^{-1} in all groups, 1.6-1.8 % day^{-1} in all groups in the second period (Sept. to Oct.), 1.4-1.7 % day^{-1} in all groups in the third period (Oct.) and 0.8-0.9 % day^{-1} in all groups in the fourth period (Oct. To Nov.). The large fish have grown from 27.2±1.5g to 113.9±21.0g, medium fish have grown from 22.7±12.2g to 94.2±19.8g and small fish have grown from 17.9±1.8g to 71.6±21.3g (Fig 5). On all sample dates there have been significant differences (P<0.05) between the grades and the fish in each group have grown significantly (P<0.05). The different size grades appear to have very similar growth potential, but the large and medium grades always presented the same of slightly higher growth than the small grade. This trail finished in November 2014.

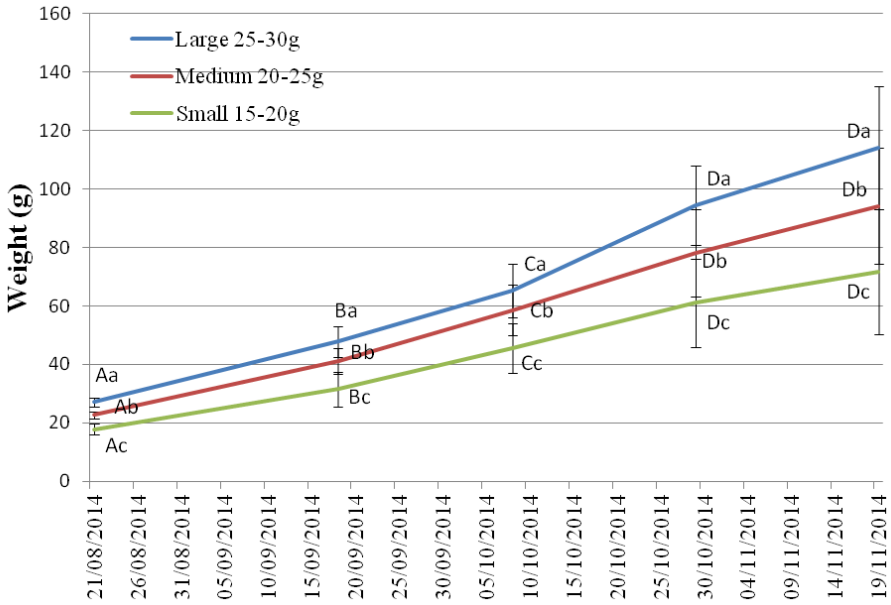

Fig. 5. Growth, mean wet weight (g) with standard deviation of the juveniles classified to three grades large (initially 25-30g), medium (initially 20-25g) and small (initially 15-20g). These fish represented 70% of the population from five spawns on two different dates. Capital letters represent significant differences (P<0.05) between sample dates for the same size grade. Lower case letters represent significant differences (P<0.05) between size grades on the same sample date.

The results obtained in 2014 are also summarized in **Table 6** and **Fig 6**, including the dates of grading, age of the fish (days post hatch), weight and growth rate (SGR).

Table 6. Summary of results obtained in the growth in weight of 2014 juveniles.

	2014																				
	53 dph		88 dph			116 dph			144 dph			164 dph			185 dph			205 dph			227 dph		
	19/06/2014		24/07/2014		SGR	21/08/2014		SGR	18/09/2014		SGR	08/10/2014		SGR	29/10/2014		SGR	19/11/2014		SGR	11/12/2014		SGR
S	0,43	0,37	9,42	1,82	2,27	17,855	1,786	2,80	31,488	6,052	3,35	45,55	8,57	3,65	61,01	15,14	4,04	71,58	21,31	4,07	79,47	24,40	4,18
M			15,47	3,91		22,682	1,288	3,02	41,214	4,488	3,61	58,59	8,55	3,88	78,13	14,81	4,28	94,18	19,79	4,33	101,33	23,48	4,41
L	5,26	3,36	43,23	16,7	3,72	27,184	1,544	3,17	47,768	5,311	3,75	65,26	9,06	3,99	94,39	13,46	4,47	113,93	21,00	4,51	125,55	26,49	4,62

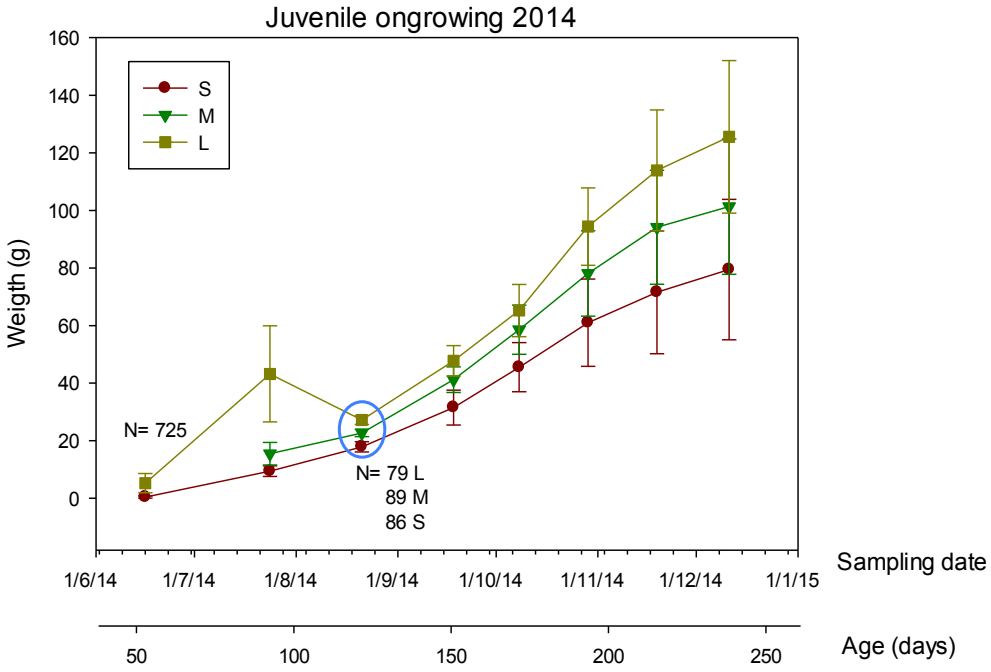

Fig 6. Growth in weight of juveniles obtained in 2014 after grading in small (S), medium (M) and large (L).

Trial 2 (2015)

All the larvae obtained from the 4 spawns were reared mixed together in 4 tanks. On July 2nd 150 fish were individually weighed to check the size distribution before separating the fish in small, medium and large individuals (**Fig 7**).

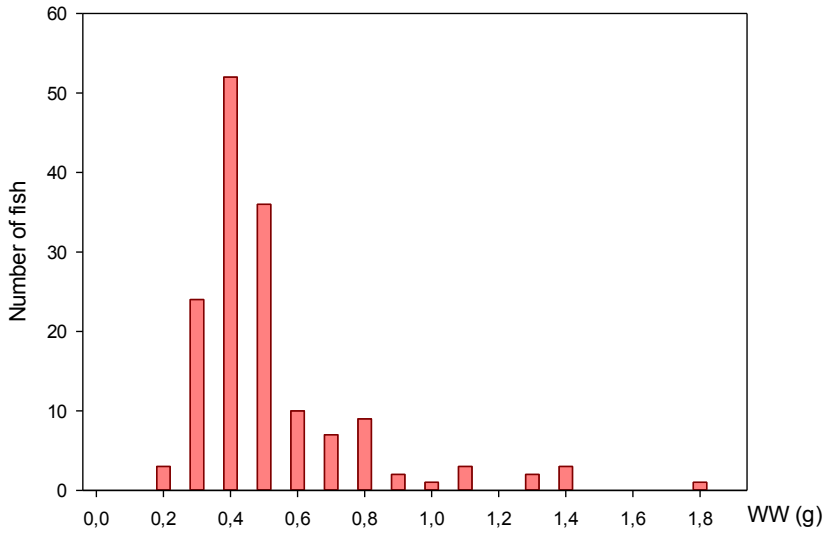

Fig 7. Size distribution of juveniles on July 2015

On July 17th all the fish were counted and graded into large, medium and small fish and distributed in two RAS modules according to **Table 7**.

Table 7. Distribution of fish in ongrowing tanks on july 17th 2015

14/07/2015									
Mod 5						Mod 3			
Tank 1		Tank 2		Tank 3		Tank 1		Tank 2	
N	Average WW (g)	N	Average WW (g)	N	Average WW (g)	N	Average WW (g)	N	Average WW (g)
551	0,26	802	0,43	361	1,20	660	0,44	650	0,43
Biomass (g)	145,00		343,72		433,94		291,90		278,57
7,5% feeding rate (g)	10,88		25,78		32,55		21,89		20,89

The fish were kept in these tanks (an additional movement was carried out in August to redistribute M size fish and reduce the biomass in tank 2 Mod 5 and tanks 1 and 2 Mod 3) for 2 months and fed using automatic feeders at feeding rate of 7.5% from July 17th until September 3rd when the fish were graded again in large (L 28-32 g), medium (M, 19-24 g) and small (S, 12-16 g) fish and fin clips taken for parental assignment. Fish were graded in L, M and S, and distributed in triplicate tanks each with 100 fish that were fed also using automatic feeders at a feeding rate of 7.5% for fish between 12 and 30 grams and 5% for fish weighing more than 30 g. Every 2-3 weeks until November 5th 2015, the fish were weighed to build the growth curve and calculate the standard growth rate as in 2014. Results are summarized in **Table 8** and **Fig. 8.**

Table 8. Summary of results obtained in the growth in weight of 2015 juveniles

	2015																	
	49 dph		83 dph			110 dph			112 dph			134 dph			155 dph			190 dph		
	02/07/2015		05/08/2015		SGR	01/09/2015		SGR	03/09/2015		SGR	25/09/2015		SGR	16/10/2015		SGR	05/11/2015		SGR
S	0,263	0,030	4,806	1,20	1,61	17,841	5,646	2,82	13,96	1,39	1,20	19,07	2,79	2,83	26,13	4,04	3,12	29,89	5,31	3,30
M	0,434	0,093	7,030	1,83	1,97	22,171	5,776	3,03	21,50	1,47	1,52	30,43	3,53	3,28	38,61	6,13	3,49	45,83	8,79	3,72
L	1,202	0,494	12,359	5,69	2,51	37,950	14,961	3,54	29,18	1,56	1,56	39,76	5,40	3,53	55,12	8,40	3,83	66,62	11,88	4,08

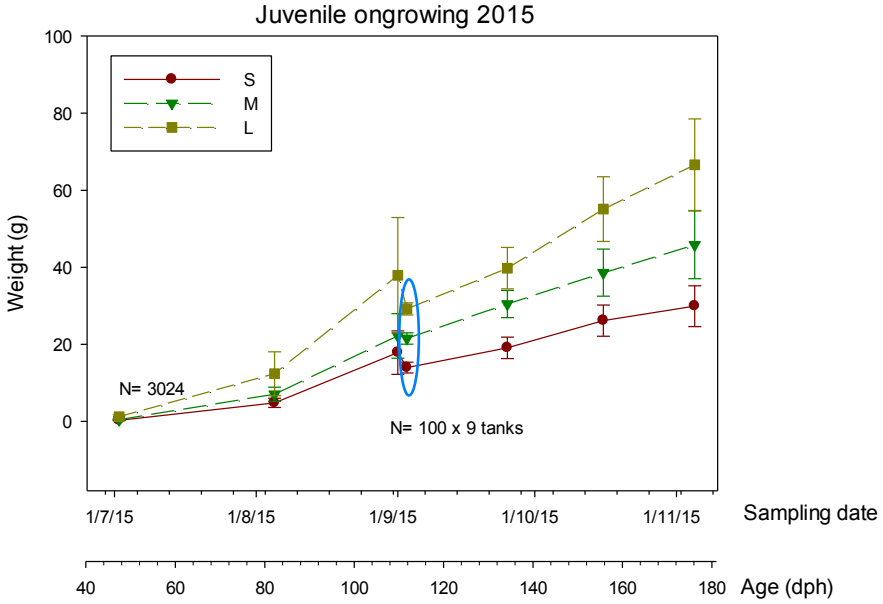

Fig 8. Growth in weight of juveniles obtained in 2014 after grading in small (S), medium (M) and large (L).

With the results obtained we have calculated the growth curves for 2014 and 2015 for S, M and L fish and calculated the differences in growth for the different groups of fish. The results are presented in table 9 and clearly show that S fish always grow more slowly than M and L fish and there is no compensatory growth when the fish are graded in different sizes. Thus, if S fish are kept in the fish farm they will have a delay of around 6 months to attain the same size of L fish. Although the growth of the fish was different between the two years (2014 and 2015) the differences in growth of S versus L fish were almost the same.

Table 9. Theoretical weight (g) of S, M and L fish in 2014 and 2015.

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

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 get commercial size with clear economical consequences for producers (**Table 10).** The prices used were obtained from a feed producer (Skretting) and the central market of Madrid, Spain.

Table 10. Production cost of L- and S- growing fish.

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

CONCLUSIONS

1.- Size variability and different growth rate in meagre juveniles exist and seems to have a genetic origin (parental assignment still in progress).

2.- There is no compensatory growth of the small size juveniles when graded and transferred to new tanks and offered enough food.

3.- After grading, large fish always show a higher growth rate that it is maintained along the ongrowing period. On the contrary, slow growing fish always show a lower growth rate that is maintained along the whole ongrowing period causing a delay of approx. 6 months in attaining commercial size.

4.- Our recommendation for hatchery producers is to cull and eliminate slow growing fish from the production chain before the fish are transferred to ongrowing facilities.

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DEVIATIONS

The deliverable deviates from the deliverable description in the DOW, in that the genetic analyses are not included. Erroneously, the deliverable description and task description in the DOW are not consistent. The task description refers to the genetic analysis, but indicates this is part of Work Package 2, and that will be delivered with ***Deliverable 2.5 Genetic characterization of fast and slow growing meagre*** (Mo 36). If requested, the present deliverable can be updated to include the genetic analysis when this information becomes available in the near future. However, we believe this deliverable is complete according to the work described for the task in the DOW.

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