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Deliverable Report

	D2.5	**Delivery Month:**	36
Deliverable Title	Identification of genetic markers related to growth for use in marker assisted breeding programs for meagre through QTL map		
WP No:	2	**WP Lead beneficiary:**	P3. IRTA
WP Title:	Reproduction and Genetics - meagre		
Task No:	2.5	**Task Lead beneficiary:**	P1. HCMR
Task Title:	Development of Single Nucleotide Polymorphisms (SNP) marker tools for the genetic characterization of fast and slow growers		
Other beneficiaries:	P3. IRTA		
Status:	Delivered	**Expected month:**	36
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Lead Scientist preparing the Deliverable: Tsigenopoulos, C.S. (HCMR)

Other Scientists participating: Manousaki, T. (HCMR), Chatziplis, D. (HCMR partner), Duncan, N. (IRTA)

Objective: Identification of genetic markers related to growth for use in marker assisted breeding programs for meagre through quantitative trait loci (QTL) mapping: a report that genetically characterizes more than 250 fish from Tasks in WP20. The report will present the results to establish a genetic basis of growth in meagre and the differences in genetic variation between fast and slow growing meagre.

Description: Growth is an economically important trait in the fish farming industry because it is directly related to fish production. Fast and predictable growth is an important and highly desired trait which affects the profitability of food animal production, since feed costs account for the largest proportion of production costs; improving growth rate increases benefits for aquaculture companies because it decreases the raising time at farm facilities, leading to lower costs and higher harvests (Gjerde, 1986). Growth-related traits such as body weight, total length, standard length, body depth, and body thickness are quantitative traits influenced by both environmental factors and multiple genes with relatively small effects according to the infinitesimal model (Mackay, 2001). Traditional selective breeding techniques are valuable in achieving improved fish growth, but the genetic gain can be increased far faster with marker-assisted selection (MAS) (Liu and Cordes, 2004).

In the last years, there has been an increasing number of studies that identify quantitative trait loci (QTLs) for growth-related traits in food fish species and most have been carried out in salmonids, e.g. Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and coho salmon (*Oncorhynchus kisutch*), tilapia (*Oreochromis niloticus*), Asian sea bass (*Lates calcacifer*), and turbot (*Scophthalmus maximus*) as well as in common carp (*Cyprinus carpio*) (see in Lv et al., 2016). However, in meagre (*Argyrosomus regius*) there are no QTL studies to identify the distribution and variation of QTLs for any trait.

As mentioned in ***Deliverable 2.4 Construction of a genetic linkage map in meagre***, identification and mapping of QTL in both terrestrial and aquatic farmed species are enabled through the construction of

genetic linkage maps and the use of high throughput sequencing (HTS) technologies to discover millions of single nucleotide polymorphism (SNP) markers. SNPs explain the greatest part of the genetic differences between individuals and are suitable for genetic evaluation and strategies that employ molecular genetics for selective breeding. Comparing QTL analyses of multiple pair-mating families can provide a better understanding of important allelic variations and distributions. In order to improve our understanding of heredity and variation of QTLs in different families and identify important QTLs, we performed QTL analysis of growth-related traits in multiple segregating families. We completed a genome scan for QTLs that affect body weight (BW) and total length (TL) of 232 individuals from five full-sib families using the 1,008 markers developed for the linkage map of meagre distributed across 27 linkage groups (LGs). Model mapping from the two larger families (170 fish in total) identified 5 QTLs on only two LGs. All five QTLs exhibited significant evidence of linkage at the genome level ($P < 0.05$). Multiple QTLs on LG20 obtained from the two different families affect BW and TL and are located at close positions. It suggests that the same genetic factors may control variability in these traits. These common QTLs are valuable candidates in MAS.

Materials and Methods

Biological material

On January 20th 2016, 400 meagre fish were sampled from a large fish-cage that formed part of a commercial farm site on the Spanish coast in the community of Valencia. The cage contained approximately 80,000 fish, which were stocked into the cage as juveniles. The juveniles were from the largest grade of fish that came from the same group of spawns collected from a broodstock that contained 19 breeders (8 females and 11 males) that were injected with gonadotropin releasing hormone agonist (GnRHa) to induce spawning. Total length and weight was measured for all 400 sampled fish (**Figure 1**).

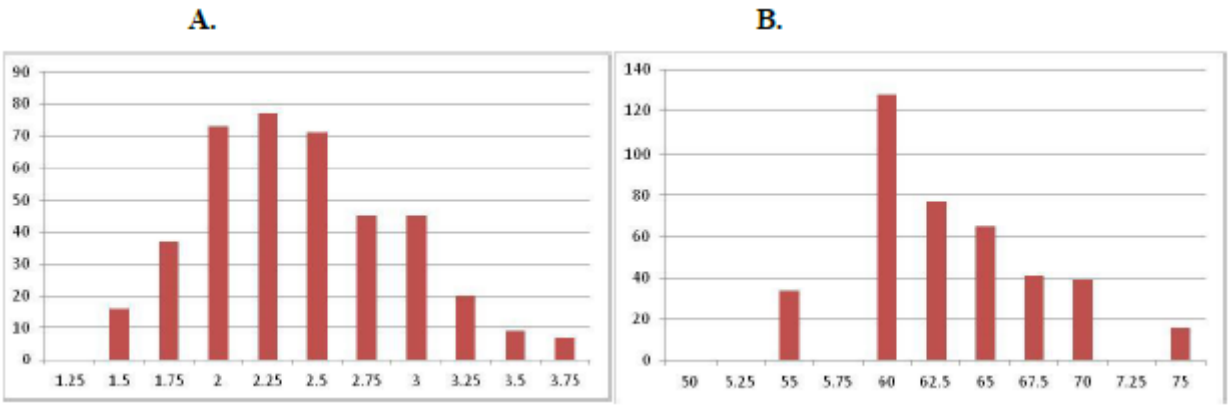

Figure 1. A. Weight (in kg) and B. total length (in cm) of the 400 meagre fish sampled.

Average weight for all 400 fish was 2.35 kg (ranging from 1.33 to 3.71 kg) and average total length 62.3 cm (ranging from 53.0 to 74.0 cm). The 15 families were ranked according to their median weight and body length (**Table 1**); the two families with only one offspring were excluded.

Table 1. Ranking of the 15 meagre families for body weight (kg) and total length (cm).

Code	Dam	Sire	bwt	len	bwt_rank	len_rank	Nb of Fish
A	BR391F	BR405M	0.236017	22.88583	2	1	25
B	BR404F	BR388M	0.179912	20.58313	5	2	29
C	BR404F	BR405M	0.226776	19.63287	3	3	95
D	BR403F	BR405M	0.176233	19.55027	7	4	24
E	BR404F	BR402M	0.26567	18	1	5	6
F	BR403F	BR397M	0.21635	15.92225	4	6	77
G	BR406F	BR405M	0.136498	12.95112	10	7	40
H	BR391F	BR398M	0.1519	12.33333	9	8	3
I	BR406F	BR397M	0.177244	11.875	6	9	8
J	BR406F	BR402M	0.1008	9.083333	11	10	3
K	BR391F	BR401M	0.075707	7.602679	12	11	7
L	BR391F	BR388M	0.075143	6.1	13	12	10
M	BR391F	BR402M	0.155067	5.525	8	13	10
N	BR391F	BR397M	0.021233	1.75	15	14	3
O	BR403F	BR402M	0.033233	1.583333	14	15	6

From the above fish, 232 fish from the five families A, B, C, F and M that exhibited the greatest phenotypic variation together with their seven breeders (three females: 391, 403 and 404 and four males: 388, 397, 402 and 405) were selected for the construction of two ddRAD libraries according to the protocol described and successfully applied in Manousaki et al (2016).

QTL mapping

For the QTL mapping purposes, the two bigger families (C and F, **Table 1**) were used; 731 SNP markers spread on 27 LGs that were common to both families were used for a genome wide QTL scan in meagre. The three other smaller families (A, B and M) were used to crosscheck and validate the existence of certain SNP combinations that could explain hereditary variation in BW and TL.

The software QxPAk (http://nce.ads.uga.edu/~ignacy/numpub/blupf90/docs/qxpak.pdf) was selected. The genotypic data were transformed appropriately with custom perl scripts. The genotypic data, the linkage map and phenotypic data were imported in QxPAk and the analysis did not include any fixed effects since fish were grown under the same environment. The QTL linkage analysis between the SNP markers and the phenotypes in question was performed. In all analyses, a polygenic effect component was included in the model.

Results and Discussion

Searching for QTL for growth in meagre

The genotypes of individuals and their parents, the genetic linkage map and phenotypic data were analyzed and used in a search for QTL. The genome-wide scan revealed two areas containing QTL on LG 11 and 20

for both traits under investigation. For both traits the likely position of the QTL was the same. The highest test-statistic (Log Likelihood Ratio) was observed at the beginning of LG 11 followed by the LG 20 (18.745 to 22.042cM). In **Figure 2**, the likelihood ratios over all the LGs are presented.

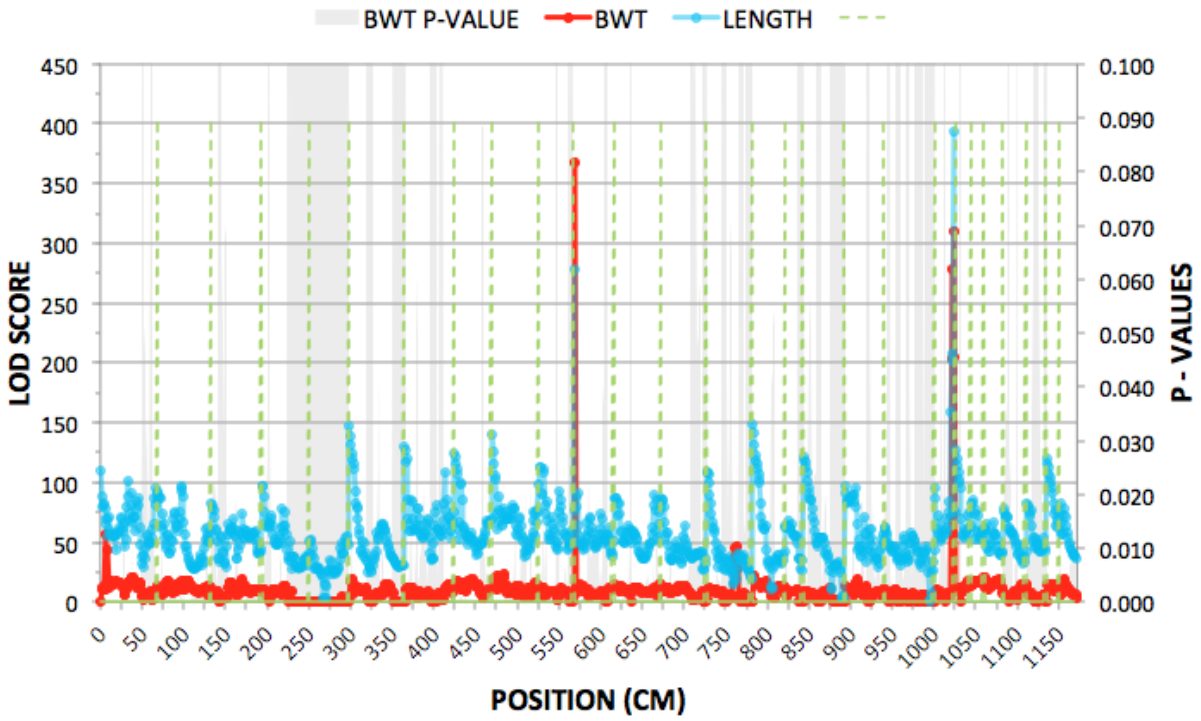

Figure 2. Likelihood Ratio scores, every cM, from a QTL genome scan. The green dashed lines are for separating the different linkage groups. The gray shaded areas indicate those areas where the likelihood ratio is not significant.

The nearest SNP in LG 11 was the M_16364, though in LG 20 four SNPs (M_2513, M_7577, M_7581, M_15320) seem to produce high likelihood ratio with M_15320 expressing the highest test statistic. In **Figure 3**, the likelihood ratios for LG 10, 11 and 20 are presented as well as the most likely positions of the QTL within each LG (**Figure 3** is actually a zoomed caption of **Figure 2**).

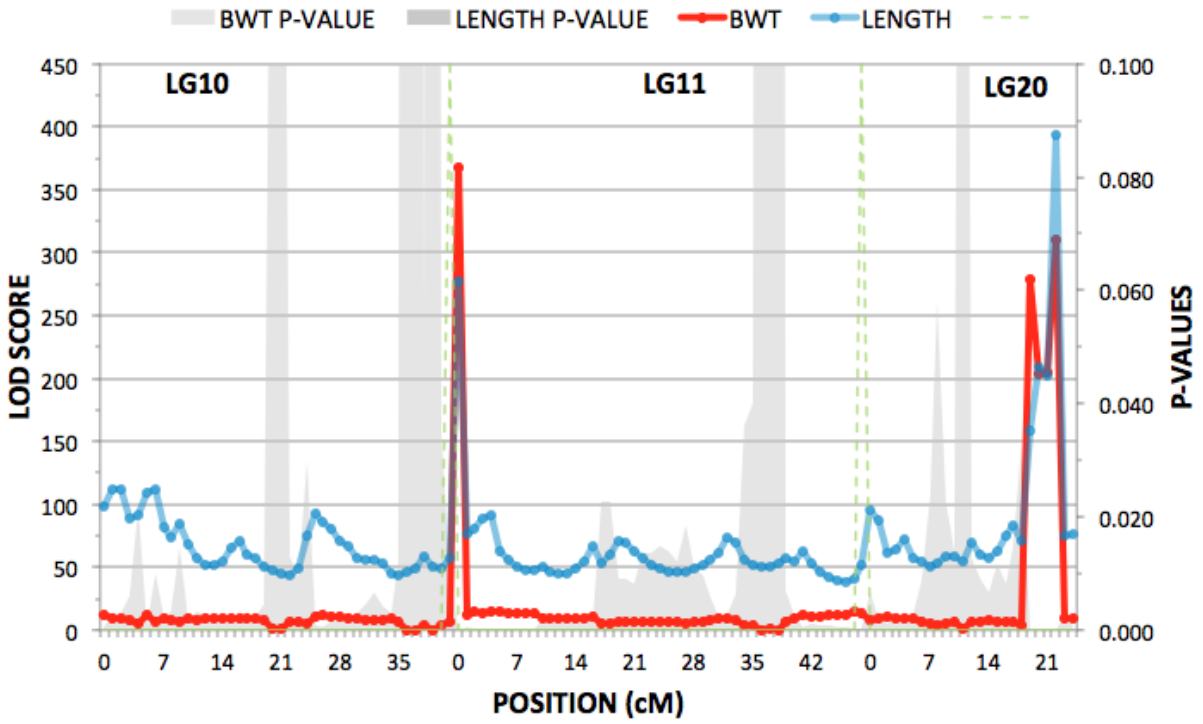

Figure 3. Likelihood Ratio scores, every cM, for linkage groups 10, 11 and 20. The peak of the test statistic is indicating the most likely position of a QTL. The green dashed lines are for separating the different LGs. The gray shaded areas indicate those areas where the likelihood ratio is not significant.

A genome wide QTL scan using ddRAD SNP markers revealed two areas of interest for possible QTL in meagre. Despite the power limitations of the experiment (170 fish from two families) the results seem promising for a more focused analysis on these LGs. When we tried to analyze jointly the other three families, there was no specific 'haplotype' or SNP combination that is characteristic of 'big' sized and 'small' sized fish (See **Supplementary File 1**).

A recent approach in mammals to decipher QTL is to construct concordant QTL maps since orthologous genes are expected to have conserved function in biological and biochemical traits. The use of comparative genomics could reveal possible candidate genes that could be sequenced to reveal polymorphic sites within them that are associated with growth. Thus, those genes with a quantitative effect in one species may also be important in another species (see in Louro et al., 2016). The construction of concordant maps for teleosts may prove to be a useful approach in aquaculture where many different species of fish are cultivated but relatively few have extensive molecular resources. However, such an approach is likely to be more challenging than in terrestrial animals because of their richer evolutionary diversity (Canario et al., 2008).

The five ddRAD QTL loci identified when blasted against the European seabass (*Dicentrarchus labrax*) genome showed unfortunately no similarity with known genes. Moreover, these regions are not syntenically related to those reported to be implicated for growth in European sea bass (LG1a, LG4, LG6 and LG15, Louro et al., 2016). Additionally, Palaiokostas et al. (2015) have reported indications for putative sex-determining QTL in European sea bass that were significant at the genome-wide threshold and were detected

on LG 6, 11 and 18 to 21, although a genome-wide association study (GWAS) did not identify individual significant SNPs at a genome-wide threshold; sea bass LG11 is syntenic to meagre's LG11 in which we also identified a genome-wide SNP for growth (see ***Deliverable 2.4 Construction of a genetic linkage map in meagre***). Shen et al (2016) report that in the Asian sea bass genome, growth-associated QTLs are found in the species LG2 which is syntenically linked to Tilapia's LG1, stickleback's (*Gasterosteus aculeatus*) Group II and according to Manousaki et al (2016) to European sea bass LG5; the last LG, is syntenic to meagre's LG1 which does not show any QTL identified.

Moreover, a future more extensive genomic association study, utilizing the same genomic areas (LG11 and 20) but more 'saturated' with SNP markers, could reveal useful haplotypes for broodstock pre-selection purposes. A small number of QTLs were detected in the meagre genome and associated with growth-related traits. Four of the QTLs of different growth-related traits were identified at similar chromosomal regions, suggesting a role for pleiotropy and/or tight linkage and demonstrating a common genetic basis of growth trait variations. Discovery of these common QTLs between families and growth-related traits represents an important step towards understanding of quantitative genetic variation in meagre.

Therefore, in conclusion, two regions (in LG11 and LG20) with SNPs in the genetic linkage map for meagre (***Deliverable 2.4 Construction of a genetic linkage map in meagre***) were associated with BW and TL. Although this association was not conclusive as regarding the application of the SNPs for immediate use in MAS based breeding programs the association gives a clear indication for future work to determine QTLs or SNPs for two growth related traits. Future work could take a number of recommended directions, for example further analysis could be made of other families to build a more robust association between growth and the identified regions or existing phenotype based breeding programs could incorporate these SNPs into the breeding programs and make a transition to a MAS based breeding program in response to the confirmation of the identified SNP related traits.

Deviations: Due to recent technological and research advances concerning molecular methods to genotype and genetically characterize fish (or organisms in general) and some constraints that are related to the biology of the meagre (*Argyrosomus regius*), we proposed (Amendment 3, Nov 2016) to modify the DOW for Task 2.5, in terms of (a) the method to be used and (a) the time-schedule. Because it is the first time that these methods were going to be applied in meagre we believed that we needed a 6-month extension of the associated deliverables, from Mo 30 to 36). No change in budget allocation, staff effort or the number of deliverables has resulted from this modification.

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