Parentage assignment, estimates of heritability and genetic correlation for growth-related traits in meagre *Argyrosomus regius*

Orestis Nousias[1,2], Alexandros Tsakogiannis[1], Neil Duncan[3], Javier Villa[4], Kostas Tzokas[5], Alicia Estevez[3], Dimitrios Chatziplis[6], Costas S. Tsigenopoulos[1]

1. Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research (HCMR) Crete, Greece
2. Department of Biology, University of Crete, Greece
3. IRTA Institute of Agrifood Research and Technology, Barcelona, Catalonia, Spain
4. Andromeda S.A., Acuicola Marina, Valencia Spain
5. Andromeda S.A., Agios Vasilios, Rion, Greece
6. Laboratory of Agrobiotechnology and Inspection of Agricultural Products, Technological Educational Institute of Thessaloniki, Greece

* Corresponding author
Costas S. Tsigenopoulos
Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR) Crete, Thalassocosmos, 715 00, Gournes Pediados, Heraklion, Greece
Email: tsigeno@hcmr.gr

Authors e-mails:
Orestis Nousias: nousiaso@hcmr.gr
Alexandros Tsakogiannis: tsakalex@hcmr.gr
Neil Duncan: Neil.Duncan@irta.cat
Javier Villa: Jvilla@andromedagroup.es
Kostas Tzokas: ktzokas@andromedagroup.gr
Alicia Estevez: alicia.estevez@irta.cat
Dimitrios Chatziplis: chatz@ap.teithe.gr
Costas S. Tsigenopoulos: tsigeno@hcmr.gr

Abstract

Meagre is a relatively new aquaculture species with great potential in large scale European aquaculture. The primary objective of the study was to describe, for the first time, parentage allocation and assign offspring to their parents for an industrial scale production system. A total of 800 meagre fish were sampled from two large cages in January and May 2016, both part of a commercial farm site in Valencia, Spain. All fish originated from the same spawning event obtained from a broodstock of 6 females and 13 males. However, due to differential growth during the juvenile stage the fish were graded into two groups, a group of larger juveniles that was transferred to one cage (batch 1) and a group of smaller juveniles that were transferred to the second cage (batch 2). Total length and weight was measured for all fish that were genotyped with a 10 microsatellite loci multiplex to infer parentage based on parental genotypes. Parentage assignment rate was high (87.5% for batch 1 and 95% for batch 2) and provided evidence that offspring belonged to 20 families. Half of the broodstock was identified as probable parents of the offspring (five females and seven males).Between the two sea-cages, a slight differential composition for the same families was encountered. The fifteen shared families that the offspring were assigned to, were analyzed for statistical significant differences concerning body weight and total body length, differences which were observed in 3 families for both batches. We estimated the heritability for body weight and total body length, as well as the genetic and phenotypic correlations for these two traits. Batch 1 showed higher heritability estimates than batch 2 with the genetic and phenotypic correlation estimates being almost the same for both batches. Certain parents contributed more offspring and exhibited dominance in spawning. Similarly, the growth related traits of body weight and total body length of the dominant parents correlate, putatively, with the statistical important differences that are observed in these three families.

Keywords: *Argyrosomus regius* - parentage assignment - microsatellites - growth - - heritability - production

Introduction

Over the last decades, significant improvements have been made for established groups in aquaculture such as tilapias, carps, shrimps and salmonids. Improvements that increase production performance with prominent disease resistance are essential to reduce costs to achieve a competitive product. Meagre (*Argyrosomus regius*) is a species of the Sciaenidae family that is currently being studied as it has high potential for intensive farming. It is distributed in the Mediterranean and Black Sea and along the Atlantic coasts of Europe and Africa (Poli *et al*. 2003). The species attributes that demonstrate the good potential for large scale aquaculture are the good feed conversion ratio (FCR) ratio and the growth rate (Fountoulaki *et al.* 2017) and has emerged as a species for Mediterranean aquaculture (Duncan *et al.* 2013) with an annual production exceeding 23.000 tonnes (FAO 2016). Meagre production started in the 1990's in France and has expanded to most of the Mediterranean. The species is characterized by a tolerance to wide ranges of salinity (5-39‰) as well as temperature (13-28^{o}C). For the market, meagre have an attractive body shape as a whole fish commodity, low fillet fat and good processing yield. Additional benefits of meagre aquaculture are the relatively easy broodstock management for good quality eggs (Duncan *et al.*, 2012; 2013; 2018; Mylonas *et al.*, 2013; 2015; 2016; Fernandez-Palacios *et al.*, 2014) and larvae rearing as well as standard industry live feeds and formulated diets (Duncan *et al.*, 2013; Vallés and Estévez, 2013). Microsatellite makers have been reported to be very useful for pedigree description in aquaculture species (Herbinger *et al.*, 1995; Moore *et al.*, 1999). Results from many studies suggest that the pedigree of mixed populations could be determined through the use of microsatellite markers (O'Reilly *et al.*, 1995; Perez-Enriquez *et al.*, 1999; Waldbieser and Wolters, 1999; Dong *et al.*, 2008). The primary aim of this study was to successfully assign the offspring to their putative parents, and then having the two meristic counts to investigate the genetic makeup of the length and weight attributes in meagre as two important production characteristics. Having followed the standard industrial practices of grading, i.e. the sorting of same-sized fish, the two batches we assigned showed different growth rates. We examine the possible impact of the differential growth between these two batches on body weight and total length. The differences in weight and length as well as the heritability and genetic correlation estimates, were examined in this paper as a secondary focus that can potentially shed

light on our future research concerning QTL identification for the weight and length attributes in meagre.

Materials and methods

Biological material

Fin clips from nineteen broodstock fish (Tank M7) were sampled in December 2012; all fish had more than 6-years old and all (6) females and 7 out of 13 broodstock males were injected with GnRHa to induce spawning in April 2013. Juveniles coming from eggs hatched on April 17th were graded twice, approximately forty and seventy days after hatch following company's protocols. The first batch of bigger juveniles was put in a cage on August 2nd 2013 and the second batch with smaller juveniles on August 14th 2013, both at a commercial farm site off the Spanish coast in the community of Valencia, ie. the two batches entered the cages with 12 days difference. On January 20th 2016, 400 meagre fish (Batch 1) were sampled at the market size (~2kg), fin-clipped and total length and weight was measured for all fish in the company's processing plant. On May 5th 2016, the same procedure was performed for another 400 meagre fish from the second batch sea-cage held on the same commercial farm site (Batch 2).

DNA techniques / PCR

DNA was extracted from all fish using standard protocols (Miller *et al.*,1988) and DNA quality and quantity was evaluated using a NanoDrop ND 1000 spectrophotometer (Thermo Fisher Scientific; wwwthermofisher.com). All fish were genotyped using a 10 microsatellite loci multiplex (Soula *et al.*, 2011) (Casmic14, UBA005, UBA006, UBA050, UBA054, Soc11, Soc405, Soc431, Soc35, Soc428) (see Table 1) using the Qiagen multiplex PCR kit.

The PCR reactions were performed in a 10µl reaction mix with concentration of 10 µmol/l for each primer and 5ng/µl template DNA. The thermal profile included a pre-denaturation step at 95 ^{o}C for 15 mins followed by 30 cycles of denaturation-annealing-extension at 94^{o}C for 30 secs, 57 ^{o}C for 1.3 mins and 72 ^{o}C for 1 min and one final elongation steps of 60 ^{o}C for 30 mins. Amplicons were resolved by

capillary electrophoresis on an ABI 3730 sequencers (Applied Biosystems, Foster City, CA) using LIZ500 size standard marker. The fragment size analysis software STRand (http://www.vgl.ucdavis.edu/informatics/STRand) was used for genotyping.

Parentage assignment

Assignment of the offspring to their parents was done via Vitassign software (Vandeputte *et al.* 2006). Thirteen sires and six dams were crossed and since there was no previous information about pedigree, full-sibs, half-sibs and unrelated pairs were the only possible relationships for any couple of fish. Vitassign uses the exclusion-based computation method based on the Mendelian segregation of alleles as its sole hypothesis. Analyses were run allowing for one allele mismatch in total.

Data analysis

Weight (kg) and total length (cm) were measured, in all 800 fish of the two batches, constituting the phenotypic data set. In the two batches, the mean values for length were 61.92 cm for batch 1 and 62.17 cm in batch 2 and for weight were 2,289 kg for batch 1 and 2,317 kg for batch 2. Levene's test was used to examine the heterogeneity of variance for weight and length traits between the two batches. The differences in weight and length for each family that had assigned progeny in both of the batches (15 families), were tested for statistical significant differences. We conducted two t-tests for each family, one for the weight attribute and one for the length attribute using the two-sample t-test assuming unequal variances using SPSS v24.0 (IBM Corp.). Last, we calculated the effective population size of the population (Falconer and McKay, 1998) using:

a) the formula of the unequal contribution of males and females,

$N_p=(4N_mN_f)/ N_m+ N_f)$ (1)

where N_m and N_f are the number of male and female parents, respectively, and

b) the formula that takes into account the variation of family size,

$N_V=8N/(V_m+V_f+4)$ (2).

where V_m and V_f are the variances in family sizes for males and females, respectively (Hill, 1979). In the calculation of N_v, we used the values of V_m and V_f calculated from the data (N_{v1}), and also the variance values corresponding to a Poisson distribution of family size for N_{v2}.

The estimates of heritabilities and genetic correlations were obtained from the assigned 728 progeny of both batches using an animal model utilizing Maximum Likelihood method with VCE4.0 (Kovač *et al.*, 2002) with the batch being the sole fixed effect, in order to take into account any differences due to placements in the cage and growth period. Two additional runs were performed to obtain heritability, genetic and phenotypic correlation estimates for each batch separately, with no fixed effect included in the analysis.

Results

Parentage assignment

Batch 1 offspring were genotyped successfully and the parentage assignment was successful at 87.5%, allowing for one mismatch. In total, 348 offspring were assigned to their putative parents. The offspring, were attributed to 18 families, out of the 78 theoretically expected. Only 5 out of the 6 females were identified as probable parents of the offspring; females 4F and 3F participated the most in spawning, and to a smaller extend females 1F and 5F. Likewise, 7 out of the 13 males were identified as probable parents of the offspring; Male 6M seems to be responsible for nearly half of the offspring followed by males 2M, 1M and 5M (see Table 3). For batch 2 fish, parentage was based on the same 10 loci multiplex, with the final assignment rate being 95%, or 380 assigned offspring out of 400. The 17 families of the batch 2 offspring followed a similar distribution especially in relation to the males (Table 4). In overall, parentage assignment rate is calculated at 91.1% in both batches.

Chi-square tests show that the distribution of the family sizes between batches is not independent (P=0.044); consequently the variation in family composition between the two batches is not significantly different and the batches are homogenous in terms of family size (see also Figure 1). In both batches, three females, 1F, 3F and 4F, participated the most (26.9, 28.4 and 29.3%, respectively), and to a much smaller extend female 5F (0.7%); female 2F is identified as the parent in only one offspring. Likewise, seven out of the 13 males were identified as probable parents of the offspring; Male No 6M seems to be responsible for nearly half of the offspring (49.9%) followed by males 2M, 1M, 5M and 4M (25.3, 12.4, 8.1 and 3.3%, respectively). From the six females injected with GnRHa, only one of them did not give any offspring (6F). From the seven males that were injected with GnRHa, only 7M and 11M had no putative offspring assigned; on the contrary, male 1M that was not injected, participated highly in the putative families with a percentage of 11.2% and 13.4%, respectively (Tables 3 and 4).

Effective population size

The variation of an idealized population, with unequal number of males and females is given by the equation $N_V=8N/(V_m+V_f+4)$, where $V_m=V_f=\mu$ (mean family size). In

Table 2, we show the calculations of N_{v1}, N_{v2} and N_p. The ratios we obtained N_{v1}/N, N_{v2}/N and N_p/N were 0.02, 0.08 and 0.02, respectively. Our values of N_e were significantly lower than N (the nineteen fish of the broodstock), with $V_{family\ size}$ = 2,351 and the mean average family size μ=42.6.

Heterogeneity of variances for weight and length

Due to the fact that we are conducting a bivariate analysis on a combined dataset, we assess the assumption that the variances of weight and length which are drawn from two different samples (batches) are not equal. Results are shown in Table 3 for the weight trait and for the length trait. Length values do not have equal variances between the two batches; this is expected due to the application of grading. On the contrary, weight values show equal variances and homogeneity criteria are met. A significant result with $p<0.05$ indicates heterogeneity of variances and the null hypothesis is confirmed (data not shown). Consequently, the origin of the fish (Batch1 or 2) was fitted as a fixed effect in the genetic analysis, in order to take into account differences in the variability of length.

Heritability and genetic correlation estimates

Heritability estimates for body weight ranged from 0.64 to 0.76, with batch 1 progeny having 12% higher heritability estimates than batch 2 (Table 4). Heritability estimates for the length trait ranged from 0.69 to 0.72 with batch 1 progeny showing 3% higher estimates that batch 2. The estimates for both of the batches were 0.62 for the weight trait and 0.64 for the length trait. Genetic correlation estimates between body weight and length were 0.97 for batch 1, 0.94 for batch 2 and 0.96 for both of the batches. Phenotypic correlation estimates between body weight and length were 0.78 for batch 1, 0.80 for batch 2 and 0.82 for both of the batches.

Statistical significant differences for growth related traits

Having two batches of progeny with differential growth rate originating from the same broodstock, gives rise to the question of statistically significant differences in body weight and length among the batches. For each shared family in the two batches,

the progeny forms two distinct populations of unequal variance and sample sizes. We performed two t-tests for each of the 15 shared families, one for the weight and one for the length attribute. *P*-value threshold for statistical significance was set at $P \leq 0.05$. *P* value, was estimated for a two-tail test, i.e. the mean is considered significantly different if it is in the top 2.5% or bottom 2.5% of its probability distribution, resulting in a *p*-value less than 0.05. Three families showed statistical significant differences in weight and two families in length (Tables 6 and 7).

Furthermore, we estimated heritabilities and genetic and phenotypic correlations of the growth traits in a tetravariate context, i.e. body weight and length measurements were considered as different traits in different batches. Results have shown that heritability estimates had decreased drastically (data not shown) in the first batch (0.27 and 0.26 for bodyweight and length, respectively) and at a lesser extend in the second batch (0.46 and 0.52 for body weight and length, respectively). However, the genetic correlations between the body weight and length traits were high between the measurements of the two batches (0.90 and 0.88, respectively). Moreover, the ranking correlations (i.e. Spearman's) between the estimated breeding values of body weight and of length in the two batches were very high (0.95 for both traits).

Discussion

In this study, we assess for the first time in meagre (*Argyrosomus regius*) the relative contribution of each broodstock used in the offspring composition. The assignment rate differs slightly between two batches of 400 fish each examined, ranging from 87.5% to 95.0%, and calculated at 91.1% in both batches (728 fish successfully assigned). The use of multilocus genotypes to ascertain parentage for genetic studies is a widely used method (Milner *et al.*, 2000; Garant and Kruuk, 2005). A major disadvantage of the exclusion method is that a single mismatch is enough to exclude a putative parent (Jones and Ardren, 2003). One crucial factor for obtaining high levels of unique assignments is the assignment power of the marker set used. Microsatellite markers due to their high number and variability allow efficient parentage assignment (Perez-Enriquez *et al.*, 1999; Waldbieser and Wolters, 1999; Boudry *et al.*, 2002; Brown *et al.*, 2005; Fessehaye *et al.*, 2006; Herlin *et al.*, 2007; Wang *et al.*, 2008). The development of a marker set for parentage assignment, is of importance to estimate the efficiency of the assignment from the initial data. Allele frequencies are used to calculate the exclusion probabilities and the quantification of individual markers. Vitassign uses simulation to determine the assignment power of the whole marker set, with the parents genotyped, using the mating scheme declared. The output given is the expected percentage of unique assignments in the absence of genotyping errors. Current parentage assignment rate (91.1% for both batches) is comparable to those already reported for members of the Sciaenidae family. In yellow croaker offspring, *Larimichthys crocea*, the assignment using 6-7 microsatellite loci was estimated at 99.6% (Liu *et al.*, 2012), whereas in *Argyrosomus japonicus* offspring, the correct assignment was at 84.7% using 14 microsatellite loci (Mirimin *et al.*, 2015).

The theoretical number of families is 78 with the families of the assigned offspring being 20. In the two batches, the contribution of putative parents shows slight and non significant variation. The distribution of families in batch 1 population of offspring shows that certain parents have possibly an increased contribution in comparison with the rest. From Figure 1, we see that 1F-1M crossing, 1F-5M, 1F-6M, 4F-1M and 4F-6M show some variation. Among these parents, 1F and 3F from the female breeders and 4M, 5M and 6M from the male breeders playing a possible dominant role in the

mass spawning reproduction practice, usually followed by commercial companies in a breeding program.

Overall the most dominant females were 4F with 37% in batch 1 and 1F with 35.53% participation in batch 2, and male 6M is equally dominant in both batches with males 5M and 4M showing variation in participation in the spawning process. Only three of the broodstock fish injected with GnRHa did not give any offspring, one female and two males; this might be due to sampling bias since other fish, like the 13M and 2F, appear to have a single offspring in the two batches analyzed. Surprisingly, a single non-injected male was responsible for 11 and 13% of the offspring in the two batches. The t-tests we performed among the shared families of the two batches showed that there is some substantial variation within families, which was statistically important. Three families, show variation for the weight attribute (Tables 5 and 6).These families include females 1F, 3F, 4F and males 2M, 4M, 6M.

Nevertheless, size variability and growth in general, is very common among same-age farmed fish in many species. This variability in growth can be a major hurdle in the long-term viability of a commercial aquaculture farm. Fish grading optimizes production by limiting cannibalism, decreasing size variability among harvested fish, and improving feed conversion efficiency (Loughnan *et al.,* 2013). Stocking non-graded fish can result in under- or overstocking and may lead to poor feeding practices (Ghozlan *et al.,* 2018). Selection practices (i.e. breeding programs) could be used to reduce variability in growth and improve in the long term growth, product uniformity and decrease management costs.

Our study is the first effort to quantify in the species how much of the size and growth variability is of genetic origin (i.e. genetic variation) which in turn can provide the first indications for the feasibility of a breeding program. However, since the two batches of meagre stock contain individuals, originating from one spawning event, that were harvested from two cages on two different time points, the first on January 2016 and the second on May 2016, we introduced two time points in the combined analysis of both batches as a fixed effect. As it was pinpointed, due to the grading process, the two batches were not measured at the same time but when the individuals reached their commercial weight. Taking this fact into consideration and the number of progeny in our dataset, as far as heritability and genetic correlation estimates of weight and length are concerned, it is likely that an overestimation of these values

might occur. Nevertheless, the estimation and evaluation of these values cannot be dramatically affected, thus providing useful indications. The heritability values for the two traits of the two batches combined for weight and length (0.62 for weight and 0.64for length) indicate that there is high probability that the specific traits are tightly correlated and that the environment plays, potentially, a less important role, especially when the organisms were grown in essentially the same or very similar environments. Moreover, the anticipated high genetic correlation (96%) between these two traits implies that there is a link between weight and length attributes in meagre which is also shown for other species (Chatziplis *et al.* 2007, Fernandes *et al.* 2017, Rutten *et al.* 2005). Moreover, the separate estimation of the heritability values for weight and length for the two batches independently, outlines differences in weight and length heritability values between them. The batch 1 stock with the higher growth rate, shows higher values for weight and length heritability (0.76 and 0.72) compared to 0.64 and 0.69, respectively, for batch 2. The population that showed highest growth rate shows higher heritability values than the population of lower growth rate. The circumstances under which selection is done may affect the response to that selection. Commercial and non commercial environments are different; hence the range of GxE interactions may differ among different environments(Lynch and Walch, 1998). In tilapia, no significant differences were observed in heritability estimates between commercial and test environment (Khaw *et al.*, 2009) whereas, differences, albeit low, were observed in a European sea bass study (Dupont-Nivet *et al.*, 2009). When grading is applied in bivariate or multivariate analyses, an option is to create classes for the trait that the grading is based upon. The division in separate smaller groups correlates with an underestimation in heritability values (Blonk *et al.*, 2010). In our study, the division in classes (i.e. batches) lead to a drastic decrease of the heritability estimates (data not shown). However, the genetic correlations between the same traits in different batches as well as the high ranking correlations (0.95) between the estimated breeding values of the same traits indicate that we analyze the same trait. On the other hand, the cage confounding effect, the heterogeneity of the length trait in progeny, has potentially lead to the aforementioned could also lead to overestimation of genetic parameter values which could be tackled by increasing the size of the dataset. The relatively high standard errors that are observed (see Table 6) in relation to other studies (Gjedrem, 2000) underline these facts.

One of the problems in the fish breeding programs utilizing mass spawning in their matings' designs is the unequal gamete contribution and the non-random distribution of family sizes. Unfortunately, the unequal distribution in family sizes coming from a given broodstock has negative effects on effective population size (Ne). In an idealized population, each breeding individual has the same probability with the others to contribute genes, or progeny, and this contribution is randomly distributed among the parents with the subsequent variation in family sizes (Falconer and McKay, 1998). However, in reality, parents (broodstock) do not have an equal chance of contribution due to differences in fertility, survival of the progeny etc. The variation among parents leads to variation of family size and this leads to a next generation originating from a smaller number of parents. The ratios we obtained (Table 2) show that the values of N_e were significantly lower than the actual number of fis hin the broodstock (19) which might be attributed to the increased variation in family size ($V_{family\ size}$ = 2,351 and μ=42.6), as well as to the reduced contribution of males relative to their female counterparts.

Our study is a preliminary analysis which concentrated to quantify the magnitude of the genetic origin of differences in growth traits in meagre and also to provide the first indications concerning the feasibility of a selection program. To our best understanding, these estimates of heritabilities would be the lower and upper bound of heritabilities for growth traits in meagre. However, much bigger data sets will be needed in order to estimate with sufficient accuracy genetic parameters for growth traits in meagre in order to be used for selection purposes in a future well-structured breeding program. Finally, having examined, several aspects of both batches, the results of this research will provide useful indications of our future approach concerning QTL research for growth related traits in meagre.

References

Archangi, B., Chand, V., Mather, P.B., 2009. Isolation and characterization of 15 polymorphic microsatellite DNA loci from *Argyrosomus japonicus* (mulloway), a new aquaculture species in Australia. Molecular Ecology Resources 9, 412–414. https://doi.org/10.1111/j.1755-0998.2008.02464.x

Boudry, P., Collet, B., Cornette, F., Hervouet, V., Bonhomme, F., 2002. High variance in reproductive success of the Pacific oyster *Crassostrea gigas*, Thunberg/ revealed by microsatellite-based parentage analysis of multifactorial crosses. https://doi.org/10.1016/S0044-8486(01)00841-9

Brown, C. R., Woolliams, J.A., McAndrew, B.J., 2005. Factors influencing effective population size in commercial populations of gilthead seabream, *Sparus aurata*. Aquaculture 247, 219–225. https://doi.org/10.1016/j.aquaculture.2005.02.002

Chatziplis, D., Batargias, C., Tsigenopoulos, C.S., Magoulas, A., Kollias, S., Kotoulas, G., Volckaert, F.A.M., Haley, C.S., 2007. Mapping quantitative trait loci in European sea bass (*Dicentrarchus labrax*): The BASSMAP pilot study. Aquaculture 272, S172–S182. https://doi.org/10.1016/j.aquaculture.2007.08.022

Dong, S., Kong, J., Meng, X., Zhang, Q., Zhang, T., & Wang, R. (2008). Microsatellite DNA markers associated with resistance to WSSV in *Penaeus* (Fenneropenaeus) *chinensis*. Aquaculture, 282(1-4), 138–141. https://doi.org/10.1016/j.aquaculture.2008.05.037

Duncan, N.J., Estévez, A., Porta, J., Carazo, I., Norambuena, F., Aguilera, C., Gairin, I., Bucci, F., Vallés, R., Mylonas, C.C., 2012. Reproductive development, GnRHa- induced spawning and egg quality of wild meagre (*Argyrosomus regius*) acclimatised to captivity. Fish Physiology and Biochemistry 38, 1273-1286. https://doi.org/10.1007/s10695-012-9615-3

Duncan, N.J., Estévez, A., Fernández-Palacios, H., Gairin, I., Hernández-Cruz, C.M., Roo, F.J., Schuchardt, D., Vallés, R., 2013. Aquaculture production of meagre (*Argyrosomus regius*): hatchery techniques, ongrowing and market, in: Allan, G., Burnell, G. (Eds.), Advances in Aquaculture Hatchery Technology. Woodhead Publishing Limited, Cambridge, UK, pp. 519-541. https://doi.org/10.1533/9780857097460.3.519

Duncan, N.J., Mylonas, C.C., Milton Sullon, E., Karamanlidis, D., França Nogueira, M.C., Ibarra-Zatarain, Z., Chiumento, M., Aviles Carrillo, R.O., 2018. Paired spawning with male rotation of meagre *Argyrosomus regius* using GnRHa injections, as a method for producing multiple families for breeding selection programs. Aquaculture 495, 506-512. https://doi.org/10.1016/j.aquaculture.2018.06.017

411 Dupont-Nivet, M., Vandeputte, M., Vergnet, A., Merdy, O., Haffray, P., Chavanne, H.,
412 Chatain, B., 2008. Heritabilities and GxE interactions for growth in the European sea bass
413 (*Dicentrarchus labrax* L.) using a marker-based pedigree. Aquaculture 275, 81–
414 87.doi:10.1016/j.aquaculture.2007.12.032
415 Estoup, A., Gharbi, K., SanCristobal, M., Chevalet, C., Haffray, P., Guyomard, R., 1998.
416 Parentage assignment using microsatellites in turbot (*Scophthalmus maximus*) and rainbow
417 trout (*Oncorhynchus mykiss*) hatchery populations. https://doi.org/10.1139/f97-268
418 Falconer, D.S., Mackay, T.F.C., 1996. Introduction to Quantitative Genetics, Fourth edition.
419 Longman Group Limited, Harlow, Essex, U.K. 464 pp.
420 Farias, I. P., Muniz, L. B., Astolfi-Filho, S., & Sampaio, I. 2006. Isolation and
421 characterization of DNA microsatellite primers for *Cynoscion acoupa*, the most exploited
422 sciaenid fish along the coast of Brazil. Molecular Ecology Notes, 6(3), 660–663.
423 https://doi.org/10.1111/j.1471-8286.2006.01289.x
424 Fernandes, T., Herlin, M., Belluga, M.D.L., Ballón, G., Martinez, P., Toro, M.A., Fernández,
425 J., 2017. Estimation of genetic parameters for growth traits in a hatchery population of
426 gilthead sea bream (*Sparus aurata* L.). Aquac. Int. 25, 499–514.
427 https://doi.org/10.1007/s10499-016-0046-5
428 Fessehaye, Y., El-bialy, Z., Rezk, M.A., Crooijmans, R., Bovenhuis, H., Komen, H., 2006.
429 Mating systems and male reproductive success in Nile tilapia (*Oreochromis niloticus*) in
430 breeding hapas: A microsatellite analysis. Aquaculture 256, 148–158.
431 https://doi.org/10.1016/j.aquaculture.2006.02.024
432 Fernandez-Palacios, H, Schuchardt, D, Roo, J, Izquierdo, M, Hernandez-Cruz, C, Duncan, N,
433 2014. Dose-dependent effect of a single GnRHa injection on the spawning of meagre
434 (*Argyrosomus regius*) broodstock reared in captivity. Spanish Journal of Agricultural
435 Research 12(4): 1038-1048. https://doi.org/10.5424/sjar/2014124-6276
436 Fountoulaki, E., Grigorakis, K., Kounna, C., Rigos, G., Papandroulakis, N., Diakogeorgakis,
437 J., Kokou, F., 2017. Growth performance and product quality of meagre (*Argyrosomus*
438 *regius*) fed diets of different protein/lipid levels at industrial scale. Italian Journal of
439 Animal Science 16, 685–694. https://doi.org/10.1080/1828051X.2017.1305259
440 Garant, D., Kruuk, L.E.B., 2005. How to use molecular marker data to measure evolutionary
441 parameters in wild populations: Quantitative Genetics and selection estimates. Molecular
442 Ecology 14, 1843–1859. https://doi.org/10.1111/j.1365-294X.2005.02561.x
443 Ghozlan, A., Gaber,M.M., Zaki, M.A. and Nour, A. 2018. Effect of stocking density on
444 growth performance, production trait, food utilization and body composition, of meagre
445 (*Argyrosomus regius*). World Journal of Engineering and Technology, 6, 37-47.
446 https://doi.org/10.4236/wjet.2018.63B005
447 Gjedrem, T., 2000.Genetic improvement of cold-water fish species. Aquaculture Research 31,

448 25–33. https://doi.org/10.1046/j.1365-2109.2000.00389.x
449 Herbinger, C.M., Doyle, R.W., Pitman, E.R., Paquet, D., Mesa, K.A., Morris, D.B., Wright,
450 J.M., Cook, D., 1995. DNA fingerprint based analysis of paternal and maternal effects on
451 offspring growth and survival in communally reared rainbow trout. Aquaculture 137, 245–
452 256. https://doi.org/10.1016/0044-8486(95)01109-9
453 Herlin, M., Taggart, J.B., McAndrew, B.J., Penman, D.J., 2007. Parentage allocation in a
454 complex situation: A large commercial Atlantic cod (*Gadus morhua*) mass spawning tank.
455 Aquaculture 272, S195–S203. https://doi.org/10.1016/j.aquaculture.2007.08.018
456 Hill, W.G. 1979. A note on effective population size with overlapping generations Genetics
457 92, 317–322.PMID:17248921 PMCID:PMC1213952
458 IBM Corp. Released 2016. IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY:
459 IBM Corp.
460 Jones, A.G., Ardren, W.R., 2003. Methods of parentage analysis in natural populations.
461 Molecular Ecology 12, 2511–2523. https://doi.org/10.1046/j.1365-294X.2003.01928.x
462 Khaw, H.L., Bovenhuis, H., Ponzoni, R.W., Rezk, M.A., Charo-Karisa, H., Komen, H., 2009.
463 Genetic analysis of Nile tilapia (*Oreochromis niloticus*) selection line reared in two input
464 environments. Aquaculture 294, 37-42. https://doi.org/10.1016/j.aquaculture.2009.05.025
465 Kovač, M., Groeneveld, E., García-Cortés, L.A., 2002. VCE-5: a package for the optimization
466 of dispersion parameters. Proceedings of the 7th World Congress on Genetics Applied to
467 Livestock Production, 20–23 August 2002, Montpellier, France.
468 Liu X., Zhao, G., Wang, Z., Cai, M., Ye, H., Wang, Q., 2012. Parentage assignment and
469 parental contribution analysis in large yellow croaker *Larimichthys crocea* using
470 microsatellite markers. Current Zoology 58, 244–249.
471 https://doi.org/10.1093/czoolo/58.2.244
472 Loughnan, S.R., Domingos, J.A., Smith-Keune, C., Forrester, J.P., Jerry, D.R., Beheregaray,
473 L.B., Robinson, N.A., 2013. Broodstock contribution after mass spawning and size grading
474 in barramundi (*Lates calcarifer*, Bloch). Aquaculture 404–405, 139–149.
475 https://doi.org/10.1016/j.aquaculture.2013.04.014
476 Lynch, M., Walsh, B. 1998. Genetic and Analysis of Quantitative traits. Sinauer Associates
477 Inc., Sunderland, USA.
478 Miller, S.A., Dykes, D.D., and H F Polesky 1988. A simple salting out procedure for
479 extracting DNA from human nucleated cells. Nucleic Acids Research Volume 16 Number
480 3, I R L Press Limited, Oxford, England. https://doi.org/10.1093/nar/16.3.1215
481 Milner, Pemberton, Brotherstone, Albon, 2000. Estimating variance components and
482 heritabilities in the wild: a case study using the “animal model” approach. Journal of
483 Evolutionary Biology 13, 804–813. https://doi.org/10.1046/j.1420-9101.2000.00222.x
484 Mirimin, L., Roodt-Wilding, R., 2015. Testing and validating a modified CTAB DNA

485 extraction method to enable molecular parentage analysis of fertilized eggs and larvae of
486 an emerging South African aquaculture species, the dusky kob *Argyrosomus japonicus*:
487 dna extraction from *Argyrosomus japonicus* eggs and larvae. Journal of Fish Biology 86,
488 1218–1223. https://doi.org/10.1111/jfb.12639
489 Moore, S.S., Whan, V., Davis, G.P., Byrne, K., Hetzel, D.J.., Preston, N., 1999. The
490 development and application of genetic markers for the Kuruma prawn *Penaeus japonicus*.
491 Aquaculture 173, 19–32. https://doi.org/10.1016/S0044-8486(98)00461-X
492 Mylonas, C. C., Mitrizakis, N., Papadaki, M., & Sigelaki, I. (2013). Reproduction of
493 hatchery-produced meagre *Argyrosomus regius* in captivity I. Description of the annual
494 reproductive cycle. Aquaculture, 414-415, 309–317.
495 https://doi.org/10.1016/j.aquaculture.2013.09.009
496 Mylonas, C.C., Fatira, E., Karkut, P., Sigelaki, I., Papadaki, M., Duncan, N., 2015.
497 Reproduction of hatchery-produced meagre *Argyrosomus regius* in captivity III.
498 Comparison between GnRHa implants and injections on spawning kinetics and egg/larval
499 performance parameters. Aquaculture 448, 44-53.doi:10.1016/j.aquaculture.2015.05.036
500 Mylonas, C.C., Salone, S., Biglino, T., de Mello, P.H., Fakriadis, I.,Sigelaki, I., Duncan, N.,
501 2016. Enhancement of oogenesis/spermatogenesis in meagre *Argyrosomus regius* using a
502 combination of temperature control and GnRHa treatments. Aquaculture 464, 323-330.
503 https://doi.org/10.1016/j.aquaculture.2016.07.006
504 O'Malley, K. G., Abbey, C. A., Ross, K., & Gold, J. R. (2003). Microsatellite DNA markers
505 for kinship analysis and genetic mapping in red drum, *Sciaenops ocellatus* (Sciaenidae,
506 Teleostei). Molecular Ecology Notes, 3(1), 155–158. https://doi.org/10.1046/j.1471-
507 8286.2003.00379.x
508 O'Reilly, P., Wright, J.M., 1995. The evolving technology of DNA fingerprinting and its
509 application to fisheries and aquaculture. J. Fish Biol. 47, 29–55. https://doi:10.1046/j.1471-
510 8286.2003.00379.x
511 Perez-Enriquez, R., Takagi, M., Taniguchi, N., 1999. Genetic variability and pedigree tracing
512 of a hatchery-reared stock of red sea bream *Pagrus major* / used for stock enhancement,
513 based on microsatellite DNA markers. https://doi.org/10.1016/S0044-8486(98)00469-4
514 Poli, B.M., Parisi, G., Zampacavallo, G., Iurzan, F., Mecatti, M., n.d. Preliminary results on
515 quality and quality changes in reared meagre (*Argyrosomus regius*): body and fillet traits
516 and freshness changes in refrigerated commercial-size fish.
517 https://doi.org/10.1023/A:1024840804303
518 Renshaw, M.A., Saillant, E., Bradfield, S.C., Gold, J.R., 2006a. Microsatellite multiplex
519 panels for genetic studies of three species of marine fishes: red drum (*Sciaenopso*
520 *cellatus*), red snapper (*Lutjanus campechanus*), and cobia (*Rachycentron canadum*).
521 Aquaculture 253, 731–735. https://doi.org/10.1016/j.aquaculture.2005.09.012

Renshaw, M.A., Saillant, E., Bradfield, S.C., Gold, J.R., 2006b. Microsatellite multiplex panels for genetic studies of three species of marine fishes: red drum (*Sciaenops ocellatus*), red snapper (*Lutjanus campechanus*), and cobia (*Rachycentron canadum*). Aquaculture 253, 731–735. https://doi.org/10.1016/j.aquaculture.2005.09.012

Renshaw, M.A., Saillant, E., Lem, S., Berry, P., Gold, J.R., 2007. Microsatellite multiplex panels for genetic studies of gray snapper (*Lutjanus griseus*) and lane snapper (*Lutjanus synagris*). Fish. Bull. 105:436-439 (2007)

Rutten, M.J.M., Bovenhuis, H., Komen, H., 2005. Genetic parameters for fillet traits and body measurements in Nile tilapia (*Oreochromis niloticus* L.). Aquaculture 246, 125–132. https://doi.org/10.1016/j.aquaculture.2005.01.006

Saillant E., K. Cizdziel, K. G. O'Malley, T. F. Turner, c. L. Pruett, and. R. Gold, 2004, Microsatellite Markers for Red Drum, *Sciaenops ocellatus* Gulf of Mexico Science, Vol. 22, No. 1, Art. 10, https://doi.org/10.18785/goms.2201.10

Soula, M., Zamorano, M. J., Navarro, A., Sánchez, J.J., Neil, D., Alejandro, G., Afonso, J. M. 2011. Diseño de dos nuevas PCRs múltiplex para corvina (*Argyrosomus regius*), Congreso National de Acuicultura, Barcelona España 2011.

Vallés, R., Estévez, A., 2013. Light conditions for larval rearing of meagre (*Argyrosomus regius*). Aquaculture, 376-379: 15-19. https://doi.org/10.1016/j.aquaculture.2012.11.011

Vandeputte, M., Mauger, S., Dupont-Nivet, M., 2006. An evaluation of allowing for mismatches as a way to manage genotyping errors in parentage assignment by exclusion. Molecular Ecology Notes 6, 265–267. https://doi.org/10.1111/j.1471-8286.2005.01167.x

Waldbieser, G.C., Wolters, W.R., 1999. Application of Polymorphic Microsatellite Loci in a Channel Catfish *Ictalurus punctatus* Breeding Program. Journal of the World Aquaculture Society 30, 256–262. https://doi.org/10.1111/j.1749-7345.1999.tb00873.x

Wang, C.M., Lo, L.C., Zhu, Z.Y., Lin, G., Feng, F., Li, J., Yang, W.T., Tan, J., Chou, R., Lim, H.S., Orban, L., Yue, G.H., 2008. Estimating reproductive success of brooders and heritability of growth traits in Asian sea bass (*Lates calcarifer*) using microsatellites. Aquaculture Research, https://doi.org/10.1111/j.1365-2109.2008.02034.x

Figure Captions

Figure 1: Differential parental assignment in two meagre batches; all fish were taken out of the sea at an average weight of 2 kg and some 100 days of difference. Families are depicted on X axis, and their percentage on the Y axis.

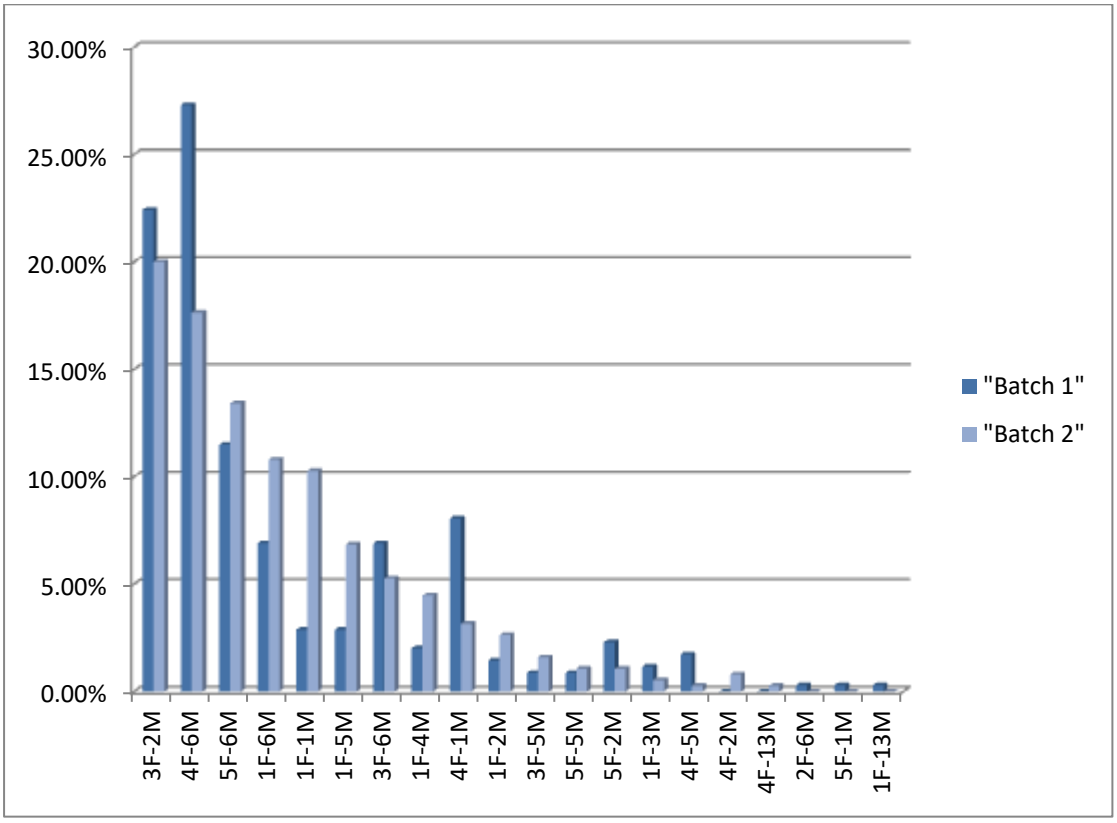

Table Legends

Table 1. Name of locus, (M) motif of repetition, (F) fluorescent tag, forward and reverse sequences of the primers.

Meagre STR loci	M	F	Forward sequences (5′-3′)	Reverse sequences (5′ -3′)	Reference
Casmic 14	$(CT)_{12}$	5′ 6-FAM	TGTCCTCACTCCTCTTTTTCTTTC	GTTTAAGGCGCATCTCCAGTCTC	Farias *et al.* 2006
UBA005	$(CT)_{16}$	5′ NED	CATCAGGATTGGCAACTAGC	GTTTCCTCCAGGTTTATTCTTCATTGAC	Archangi *et al.* 2009
UBA006	$(CA)_{22}$	5′ PET	AGCACACGTAATCACACACAGAT	GTTTCCACTAGTGCAAAACGGTGGT	Archangi *et al.* 2009
UBA050	$(GT)_{26}/(GT)_{9}$	5′ 6-FAM	GCACAACTGCATCCCTTAGAT	GTTTAGAAGTGAAGACTGCGGACTG	Archangi *et al.* 2009
UBA054	$(CA)_{17}$	5′ 6-FAM	CCTTGTGAGAACATTAATTTGGATG	GTTTCCAAACCCTGATAGATGGATAGTT	Archangi *et al.* 2009
SOC11	$(GA)_{11}$	5′ 6-FAM	GCCGAGTCACGAAGGAACAGAGAA	TGTCGTCTCATCTATCTCCATCTC	Saillant *et al.* 2004
SOC405	$(CA)_{12}$	5′ PET	AGGCTTTTGTTTAGTTTCCCTCAT	GGGGTGTAGCAGAACCACAC	O'Malley *et al.* 2003
SOC431	$(TG)_{30}$	5′ HEX	GTGGTAGATGAAAACGTATAAAAGGAG	GTTTCATATATATAGTGTACAGCTCCAGCT	O'Malley *et al.* 2003
SOC35	$(CT)_{5}/(GA)_{9}$	5′ PET	GAGGGTGACGCTAACAGTTGA	CTCTACCTCACACTCCTCAAAGT	Saillant *et al.* 2004
SOC428	$(TG)_{38}$	5′ NED	GACATCGCATTTGTCTACAGAGTC	AACTCCCAGTCATAATATCCCTT	Saillant *et al.* 2004

Table 2. Effective population sizes accounting for variance in family sizes. C_f,C_m represent average contribution of females and males, V_f, V_m stand for variance of contribution of females and males. N is the number of assigned progeny, N_p is the effective population number according to $N_p=(4N_mN_f)/ N_m+ N_f)$, N_{v1} and N_{v2} are the values for the two batches according to $N_V=8N/(V_m+V_f+4)$ with $V_m=V_f=\mu$ (mean family size) as a Poissonian distribution.

	N	N_p	N_{v1}	N_{v2}	C_f	C_m	V_f	V_m
Batch1&Batch2 assigned progeny	728	16.42	14.92	64.95	120.83	55	8364	10555
N_e/N	-	0.02	0.02	0.08	-	-	-	-

Table 3. The distribution of 18 families in batch 1 of meagre stock. Families 5F-1M 2F-6M and 1F-13M were present only in batch 1 (bold).

Batch 1		**Males**							
		1M	2M	3M	4M	5M	6M	13M	Sum
	1F	2.87%	1.44%	1.15%	2.01%	2.87%	6.90%	**0.29%**	17.53%
	2F						**0.29%**		0.29%
Females	3F		22.41%			0.86%	6.90%		30.17%
	4F	8.05%				1.72%	27.30%		37.07%
	5F	**0.29%**	2.30%			0.86%	11.49%		14.94%
	Sum	11.21%	26.15%	1.15%	2.01%	6.32%	52.87%	0.29%	

Table 4. The distribution of 17 families in batch 2 of meagre stock. Families 4F-2M , 3F-6M and 4F-13M are present only in batch 2 (bold).

Batch 2		**Males**							
		1M	2M	3M	4M	5M	6M	13M	Sum
	1F	10.26%	2.63%	0.53%	4.47%	6.84%	10.79%		35.53%
	2F								0
Females	3F		20.00%			1.58%	**5.26%**		26.84%
	4F	3.16%	**0.79%**			0.26%	17.63%	**0.26%**	22.11%
	5F		1.05%			1.05%	13.42%		15.53%
	Sum	13.42%	24.47%	0.53%	4.47%	9.74%	47.11%	0.26%	

Table 5. Heritability and genetic correlation estimates for both batches of meagre as well as for each batch separately. Heritability estimates are shown in bold and standard errors are in parentheses.

Meagre stock	**Weight**	**Length**	**Genetic correlation**	**Phenotypic correlation**
Batch 1	**0.769** (0.14)	**0.725** (0.14)	0.974 (0.01)	0.788 (0.09)
Batch 2	**0.643** (0.13)	**0.699** (0.14)	0.945 (0.02)	0.809 (0.06)
Batch 1&Batch 2	**0.627** (0.12)	**0.643** (0.12)	0.962 (0.01)	0.826 (0.04)

Table 6. t-test for three shared families of meagre stock among batches 1 and 2 that showed statistical significant difference for the weight attribute. Observations represent the number of progeny in each batch inside a family.

Weight						
Family	3F/2M		4F/6M		1F/4M	
Meagre stock	Batch 1	Batch 2	Batch 1	Batch 2	Batch 1	Batch 2
Mean	2.366	2.497	2.379	2.143	3.178	2.806
Observations	77	76	95	67	8	17
P	0.04998		0.00045		0.01386	

Table 7. t-test for two shared families of meagre stock that showed statistical significant differences for the length attribute.

Length				
Family	4F-6M		1F-4M	
Meagre stock	Batch 1	Batch 2	Batch 1	Batch 2
Mean	63.01	61.44	70.06	65.97
Observations	95	67	8	17
P	0.01552		0.00448	

Declarations

1 Ethics approval and consent to participate

Sample providers complied with institutional, national, and international guidelines and regulations as well as Nagoya protocol to obtain our fish clip samples. No ethic committee approval was necessary for the collection of fish clips. All fish treatments used for sampling were in accordance with the guidelines of the European Directive (2010/63/EU) on the protection of animals used for scientific purposes. In addition, *A. regius* is neither an endangered species nor a species at risk of Extinction according to the IUCN (Red List category: Least Concern).

2 Competing interests

Declarations of interest: none

3 Funding

This work has received funding from the European Union's Seventh Framework Programme for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY), and partial funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No. 652831 (AQUAEXCEL^{2020}). This output reflects only the author's views and the European Union cannot be held responsible for any use that may be made of the information contained therein.

4 Author Contributions

CT, KT, ND, DC and ON designed the study. ND and JV contributed to the collection of fin samples. ON and AT contributed to laboratory analyses. ON, AT, ND, KT, and DC analysed and interpreted the results. All authors participated to manuscript writing. All authors read and approved the final manuscript.

5 Availability of data and materials

Genotyping data are available upon request

Acknowledgements

Authors are grateful to Katerina Ekonomaki for her help with laboratory analyses, and C.C. Mylonas and P. Kolios for their help during the project. This work has received funding from the European Union's FP7 for research, technological development and demonstration (KBBE-2013-07 single stage, GA 603121, DIVERSIFY) and H2020 (H2020-INFRAIA-2014-2015, GA 652831, AQUAEXCEL2020).

640