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Rearing diet may determine fish restocking success: a case study of hatchery-reared juvenile meagre, *Argyrosomus regius*

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Abstract: The resilience of released hatchery-reared specimens increases with age and size, but production costs are also greater for these individuals. Therefore, for a given budget, the consequences of increasing age and size impose a trade-off between producing a large number of vulnerable (small) fish or a small number of resilient (large) fish. Once the optimal size for releasing fish has been defined, the choice of rearing protocol will determine the number and quality of the fish that can be released. In this study, different rearing protocols were compared using meagre juveniles (*Argyrosomus regius*), which are presently the target of a restocking programme conducted in the Balearic Islands (western Mediterranean). Seven different diets were compared during the grow-out phase to identify the diets that produced good-quality juveniles of a given size at the lowest cost. Most of the diets produced juveniles of suitable biological quality in terms of growth, fish condition (relationships between length and total weight, liver weight and mesenteric fat weight) and tissue biochemical composition. A semi-moist diet (Diet G) provided the best growth rate, closely followed by commercial meagre pellets (Diet A). In contrast, the cost of growing fish with Diet A was lower for any possible size at release. This study demonstrates the need to consider both growth rate and production cost to select the rearing protocol that maximizes the number of juveniles that can be produced for a given budget and desired release size. These considerations will ultimately increase the chance of success of restocking programmes.

Keywords: *Argyrosomus regius*; restocking programme; production cost; fish quality; diet.

La dieta usada en la cría puede determinar el éxito en la repoblación de peces: el caso de juveniles de corvina *Argyrosomus regius* criados en cautividad

Resumen: La resiliencia de los ejemplares criados en cautividad y liberados se incrementa con la edad y el tamaño, pero los costes de producción también aumentan. Por lo tanto, para un presupuesto dado, estas consecuencias del incremento de la edad y el tamaño imponen un compromiso entre la producción de un gran número de peces vulnerables (pequeños) o un pequeño número de peces resistentes (grandes y de más edad). Una vez que el tamaño óptimo para la liberación de peces se ha definido, la elección del protocolo de cría determinará el número y la calidad de los peces que se pueden liberar con un presupuesto dado. En este estudio, se comparó la aplicación de diferentes protocolos de cría a juveniles de corvina (*Argyrosomus regius*), que es la especie objetivo de un programa de repoblación llevado a cabo en las Islas Baleares (Mediterráneo occidental). Se compararon siete dietas diferentes durante la fase de engorde para identificar cuáles de ellas podrían producir juveniles de buena calidad, de un tamaño dado y al menor coste. La mayoría de las dietas produjeron juveniles de calidad biológica adecuada en términos de crecimiento, condición del pez (estimada a partir de la relación de la longitud con el peso total, el peso del hígado y el peso de la grasa mesentérica) y composición bioquímica del tejido. Una dieta semi-húmeda (Dieta G) proporcionó la mejor tasa de crecimiento, seguida de cerca por un pienso comercial de corvina (Dieta A). Por el contrario, el coste del cultivo de peces con la Dieta A fue menor para cualquier posible tamaño de suelta. Este estudio demuestra la necesidad de considerar tanto la tasa de crecimiento como los costes de producción, con el fin de seleccionar el protocolo de cría que maximiza el número de juveniles que pueden ser producidos para un determinado presupuesto y tamaño de liberación deseado. Estas consideraciones aumentarán, en última instancia, las posibilidades de éxito de los programas de repoblación.

Palabras clave: *Argyrosomus regius*; programa de repoblación; coste de producción; calidad de los peces; dieta.

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INTRODUCTION

The use of hatchery-reared fishes to enhance wild stocks (hereafter "restocking") has attracted increasing interest (Cowx 1994, Munro and Bell 1997, Bartley and Bell 2008) due to dramatic decreases in yield and even the collapse of some fisheries (Wilby et al. 2009). A steadily growing number of species and countries have been involved in restocking programmes over recent years (Born et al. 2004). The annual budget invested in rearing hatchery fish and releasing them to the wild is not known precisely, but it is in the range of billions of dollars (Brown and Day 2002). Restocking has been criticized because it may be neither effective nor economically viable (Bell et al. 2006). Therefore, an appropriate restocking-based management strategy should employ a number of pilot studies to assess the actual effects of restocking on stock enhancement and to develop strategies that maximize the profit of stocking while minimizing its environmental impact and economic cost (Blankenship and Leber 1995).

The success of restocking programmes depends on how well the hatchery-reared fishes can adapt to natural habitat conditions and survive once released (Ireland et al. 2002). However, post-release survival may be low due to the inexperience of released animals in wild conditions (Iglesias et al. 2003, Sparrevohn and Støttrup 2007). Thus, releasing a very large number of juveniles is often needed to ensure the success of restocking programmes (Bartley and Bell 2008), which implies high cost. Moreover, post-release survival is mediated by the size of individuals at the time of release (Tsukamoto et al. 1989, Svåsand and Kristiansen 1990, Leber et al. 2005), and releasing undersized fish seems to be largely ineffective (Kristiansen et al. 1997). However, the size at which fishes are released is also strongly governed by economic constraints. Most of the budget of restocking projects is invested in production costs (Ungson et al. 1993). These costs include the maturation, hatchery, nursery and grow-out phases (Leung et al. 1993, Kam et al. 2002). Therefore, the longer the fish remain in captivity, the greater the cost to feed and house them (Tominaga and Watanabe 1998, Brown and Day 2002), and fewer fish may be released within a fixed budget. Consequently, the optimum release size would result from a trade-off between size-dependent survival rates, growth rates and production costs (Leber 1995).

In addition to fish size, maximizing survival of released fish depends on biological quality (Leber 1995, Tominaga and Watanabe 1998, Tsukamoto et al. 1999, Le Vay et al. 2007). Quality not only refers to the release of disease- and deformity-free fish but also implies particular physiological and nutritional characteristics. Physiological welfare can be evaluated by body condition, and nutritional quality is directly assessed by tissue composition (Pepper et al. 1992, Le Vay et al. 2007). Biological quality has been directly related to the quality of the diet used to feed the larvae or juveniles (Le Vay et al. 2007). Therefore, an optimal diet allows the production of quality fish and also provides good growth rates.

Here we examine the meagre, *Argyrosomus regius* (Asso, 1801). This fish, belonging to the family Sciaenidae, is distributed along the eastern Atlantic coast (from Norway to Congo) and throughout the entire Mediterranean (Chao 1986). In Mediterranean waters, the abundance of meagre has decreased alarmingly (Quéro and Vayne 1987, Sadovy and Cheung 2003). Indeed, this species is considered extinct in the Balearic Islands, where it was frequently captured by the artisanal fleet only a few decades ago (Mayol et al. 2000). Consequently, a consortium composed of policy makers from the local government (Balearic Government), researchers from LIMIA (Laboratori d'Investigacions Marines i Aqüicultura, Balearic Government) and IMEDEA (Instituto Mediterráneo de Estudios Avanzados, CSIC-UIB), and local fishermen (associations of artisanal fishermen and recreational fishing clubs) have launched a restocking programme.

As meagre is considered an emerging species in European aquaculture (Monfort 2010), numerous studies related to rearing conditions and growth have been conducted in recent years with a view to improving production (Piccolo et al. 2008, Roo et al. 2010, Estévez et al. 2011, Vargas-Chacoff et al. 2014, Velazco-Vargas et al. 2014). In contrast, the main objective of this study was to determine the optimal rearing protocol for obtaining juveniles suitable for restocking, considering both biological and economic aspects. To this end, growth, physiological quality (body condition indices), nutritional quality (tissue composition) and cost of seven different diets were compared to identify the optimal diet for producing juveniles of a desired release size at the lowest cost, thus facilitating the release of the greatest number of specimens.

MATERIALS AND METHODS

Meagre were spawned in May at LIMIA facilities using protocols developed by LIMIA (Pastor and Grau 2013). Eggs were obtained by hormonal induction of meagre breeders captured in the Bay of Cádiz. During the hatchery phase, the larvae were reared under controlled conditions and fed a diet composed of rotifers (day 2-15) and *Artemia* sp. (day 8-30). The fish fry were fed commercial feed (Skretting$^{®}$, Burgos, Spain; day >30) and were maintained in tanks and later in small sea cages during the nursery and pre-grow-out phases.

Table 1. – Tested diets.

Experiment	Diet tested	Origin
1	A	Meagre commercial pellet 1
	B	Seabass commercial pellet
2	C	45P/17F experimental composition
	D	47P/20F experimental composition
	E	49P/22F experimental composition
3	A	Meagre commercial pellet 1
	F	Meagre commercial pellet 2
	G	Semi-moist diet (OMP, Oregon Moist Pellet)

Diet experiments

Juvenile meagre obtained from the pre-grow-out phase were transferred to experimental sea cages (8 m^3) and fed seven different diets (Table 1). Due to logistical constraints (i.e. only eight experimental cages were available at the LIMIA facilities), it was not possible to compare the seven diets within a single year, and three experiments were performed with juveniles born in each of 2006, 2007 and 2008. Some cages received the same treatment (Diet A) in two different years (Table 1) to estimate the between-year variability. Assuming that between-year variability in growth was similar for all diets, meagre length-at-age could be compared between diets even with these logistical constraints (see details of the statistical model below).

The macronutrient composition of the tested diets is shown in Table 2. Gross energy was calculated using energy coefficients (Miglavs and Jobling 1989).

The food ration (FR, i.e. amount of food provided daily per cage) was continuously adjusted as a function of water temperature, fish size, total biomass and pellet size, following the instructions given by Skretting®. Every month, approximately 20-30 fish per cage were measured (total length, L) and weighed (W) to monitor growth and to adjust the food ration for the increasing biomass.

Experiment 1

During the first year, we compared meagre and seabass (*Dicentrarchus labrax*) commercial pellets, Diets A and B, respectively (Table 1). This experiment was performed over a period of 11 months (November 2006-October 2007) with juveniles born in 2006. A total of 635 fish (mean W±sd: 154.7±48.5 g; mean L±sd: 23.7±2.6 cm) were distributed in 4 sea cages (2 replicate cages per treatment).

Experiment 2

Juvenile meagre born in 2007 were subjected to three experimental diets for eight months (December 2007-August 2008). These experimental diets were formulated using fish meal, fish oil, and wheat and soya oil. Feed was produced by extrusion with a Clextral BC-45 (France). The diets had different protein/fat ratios (45P/17F, 47P/20F and 49P/22F). Approximately 250 fish per cage (mean W±sd: 121.3±15.5 g; mean L±sd: 21.5±0.9 cm) were transferred to eight experimental cages. Three of the cages were supplied with experimental Diet C, two with Diet D and three with Diet E (see Table 1 for details on the diets).

Experiment 3

The third experiment was performed with fish born in 2008 and lasted for 8 months (February 2009-October 2009). Approximately 86-87 juveniles per cage (mean W±sd: 95.8±20.9 g; mean L±sd: 20.4±1.4 cm) were stocked in 8 experimental cages. In this experiment the fish density was lower than in the other experiments, due to the reduced availability of meagre that year. However, in all experiments fish density was low (<4 kg m^{-3}) compared with the 50 kg m^{-3} maximum (Lazo et al. 2010) and was not expected to affect fish growth. Three cages were fed with experimental Diet A (the same meagre commercial pellet used in Experiment 1) and three with Diet F (another type of meagre commercial pellet). Two cages were fed with Diet G, a semi-moist diet (OMP, Oregon Moist Pellet) prepared at the LIMIA facilities by mixing raw fish, fish flour and fish oil in a proportion of 10:10:1, respectively. The rations of Diet G were adjusted for moisture content such that each treatment received the same ration on a dry weight basis (Millamena 2002).

Growth

The length-at-age dataset obtained from the diet experiments was fitted to the growth model proposed by Somers (1988), a version of the conventional von Bertalanffy growth model that incorporates seasonal growth oscillations (García-Berthou et al. 2012). These seasonal oscillations are mainly dependent on temperature but also on photoperiod (Pauly 1990).

The model was also modified to ensure that growth was initiated at the beginning of the grow-out phase because age and length were known at that time. The model was as follows (note that subindices referencing

Table 2. – Proximate composition of the tested diets expressed as a percentage of dry matter.

Diet	A	B	C	D	E	F	G
Dry matter	94.34	93.16	91.52	90.79	89.92	92.73	64.87
Crude protein	48.77	49.20	46.12	48.63	49.38	49.17	53.39
Crude fat	18.70	16.62	14.14	17.12	19.51	16.47	20.43
Ash	7.77	6.80	8.98	9.43	9.76	10.44	9.36
Fibre	1.57	1.84	2.10	1.00	0.89	2.10	1.89
NFE (Nitrogen free extract)	24.13	27.38	28.66	23.82	20.45	21.82	14.93
Gross energy (MJ kg^{-1} feed)	22.81	22.65	21.17	22.11	22.66	21.66	23.04
Protein/energy ratio (g protein/MJ)	21.37	21.72	21.78	21.98	21.79	22.70	23.17

fish have been omitted):

$$L_t = L_{0\,year}+(L_\infty-L_{0\,year})(1-\exp(-K_{cage}(t-t_{0\,year})-\\ -S_{t,\,year}+S_{t0,\,year}))+\varepsilon_t\ ,$$

where

$$S_{t,\,year}=(CK_{cage}/2\pi)\ \sin(2\pi(t-t_{S\,year}))$$

$$S_{t0,\,year}= (CK_{cage}/2\pi)\ \sin(2\pi(t_{0\,year}-t_{S\,year}))$$

$$K_{cage}\sim \text{Normal}(K_{year}+K_{diet},sd_{cage})$$

$$K_{year}\sim \text{Normal}(0,\ sd_{year})$$

$$\varepsilon_t\sim \text{Normal}(0,\ sd_\varepsilon)$$

and where L_0 is the mean fish length at the beginning of the grow-out phase (L_0 was measured and varied between years); L_∞ is the length at asymptotic infinite age; and K is the rate of approach to the asymptotic length (Schnute and Fournier 1980) (hereafter, growth rate). Note that the growth rate estimated for a specific cage (K_{cage}) was considered a random realization from a normal distribution. The mean of this distribution results from combining a fixed component (K_{diet}) and a random component (K_{year}) that accounts for between-year variability. Therefore, data from different cages and years can be combined with a single hierarchical mixed model. In our study, t_0 is the age at the beginning of the grow-out phase rather than the typical theoretical age at which the length would be zero; t_0 was known and varied between years. C modulates the amplitude of the seasonal growth oscillations; t_S is the time between t_0 and the inflection point of the first sinusoidal growth oscillation; and sd_ε, sd_{year} and sd_{cage} are the standard deviations of the corresponding levels of the hierarchical mixed model.

In our study, L_0 was known (depending on the year, approximately 21 cm or 100 g). L_∞ was assumed to be 171.9 cm, as estimated by González-Quirós et al. (2011); although the value of this parameter did not affect the estimates of size-at-age, as previously determined by sensitivity analysis. Sensitivity analyses compared the estimates of size-at-age at L_∞=150, 171.9 and 200 cm. Depending on the year, t_0 oscillated between the ages of 6 and 9 months. The remaining parameters (C, K_{diet} and t_S) were estimated. Note that the seasonal oscillation included in the growth model allowed for comparison of the experiments with different starting dates.

The model parameters were estimated using a Bayesian approach. A non-informative normal distribution (zero mean and tolerance=10^{-6}) for K_{diet} and a uniform distribution for C and t_S were assumed as priors. C was constrained to be within the interval (0, 1) (García-Berthou et al. 2012), and t_S was constrained to be between 1 and 365 days. Three chains were run using randomly selected initial values for each parameter within a reasonable interval, and conventional convergence criteria were checked. The number of iterations was selected for each run to obtain at least 1000 valid values after convergence and thinning. The models were implemented in R (at http://www.r-project.org/) using the library R2jags (http://cran.r-project.org/web/packages/R2jags/R2jags.pdf) with the samplers implemented in JAGS (http://mcmc-jags.sourceforge.net/).

Fish quality

At the beginning, middle and end of each experimental period, 10 animals per cage were sacrificed following the officially authorized animal care protocol, and data on fish length and total weight, as well as weights of liver and mesenteric fat, were recorded. These data were used to evaluate the biological condition of the fish based on the relationships between the length and total weight, liver weight and mesenteric fat weight. The biological condition represents the fatness of individuals at a given length (Marshall et al. 2004); therefore, higher values indicate better quality, or vigour, and greater energy reserves, which accumulate primarily in the liver and mesenteric fat.

The sample size for estimating the total weight-length relationship included the monthly samples obtained to adjust the rations (see above). The sample sizes are detailed below.

The weight-length relationship was defined by the equation $W=\alpha L^\beta$ and was converted into a linear regression by logarithmic transformation. The mesenteric fat weight was zero in some fish; therefore, logarithmic transformation was possible only by adding a factor of 0.01 to all observations. In all cases, the regression line was forced to pass through the known intercept (W_0 and L_0; note that fish measured at t_0 were only used for estimating mean W_0 and L_0 but were not included in other analyses). Therefore, the only unknown parameter of the model was β (the slope):

$$\log(W_i - W_{0\,year}) = \beta_{cage}\ (\log(L_i - L_{0\,year})) + \varepsilon_i$$

$$\beta_{cage} \sim \text{Normal}\ (\beta_{year} + \beta_{diet},sd_{cage})$$

$$\beta_{year} \sim \text{Normal}\ (0,sd_{year})$$

$$\varepsilon_i \sim \text{Normal}\ (0,sd_W)$$

β_{diet} was determined using a Bayesian approach and the same random-effects hierarchical structure used for analysing growth outlined in the previous section. Normal non-informative priors were assumed. Prior to these analyses, the raw data were submitted to an outlier removal procedure using the function *influence.measures* of the R package. To simplify the interpretation of the results and to assess the differences between diets, weights (total, liver and mesenteric fat) were estimated for a desired size at release of 30 cm. This reference point was selected as an example of a reasonable release size because no or very few fish smaller than 30 cm were recaptured in the course of the meagre restocking programme (Gil et al. 2015).

The proximate composition of the diets and of the entire fish were determined following the procedures of the AOAC (1997). After the measurements had been made, all fish samples were frozen at −20°C until further analysis. After homogenization of the samples, the crude protein (Kjeldahl method, with a 6.25 nitrogen-to-protein conversion factor), crude fat (ethyl-ether extraction using a SOXTEC System HT6 extractor), moisture (drying at 105±1°C to constant weight), and total ash (incineration at 450±2°C to constant weight) contents were determined. The diets were also tested for crude fibre (Weende method). All analyses were performed in triplicate. The means and standard deviations of proximate composition were computed for each diet at the end of the three experiments. Statistically significant differences between means were evaluated with a one-way analysis of variance with $\alpha=0.05$. Post hoc pairwise comparisons were conducted with a Tukey test. These statistical analyses were performed with the R package.

Production costs

To determine the length-dependent economic costs of the meagre grow-out phase for the different diets, the costs related to feeding and personnel were analysed. Costs (CO) were calculated per fish and expressed in euros (€) using the following expression:

$$CO_{diet} = FCO_{diet} + PCO_{diet} .$$

The feeding cost (FCO) depends on the accumulated daily food ration (i.e. the amount of feed supplied per day, FR_{day}, kg, which depends on the temperature, fish size and pellet size) and the price (P, € kg^{-1}) of each tested diet. Additionally, because we assumed that the fish were fed only 74% of all the days of the experiments, this cost was multiplied by a factor of 0.74.

$$FCO_t = \sum_{day=1}^{t} FR_{day,diet} P_{diet} 0.74$$

The prices were very similar (approximately 1 € kg^{-1}) for all the feeds with the exception of Diet G. The raw materials for Diet G, especially fresh fish, were more expensive. Accordingly, the cost of the resulting diet was approximately twice that of the other diets. Moreover, due to its higher moisture content, the food ration was greater.

The daily food ration (FR_{day}) depends on the fish weight (W), which was calculated from the length (L) with the model

$$W = \alpha L^{\beta},$$

where α and β were determined empirically by pooling several data sets collected at LIMIA.

The personnel cost per fish (PCO) depends on the salary per day (S), the number of fish finally produced in a given year (estimated to be approximately 3000 juveniles per year for the LIMIA facilities), the days (D) required on each diet to achieve the desired length and the factor 0.74 because the fish were not fed every day.

$$PCO = S\ D\ (0.74/3000)$$

The costs estimated for each diet allowed us to evaluate the most suitable diet for growing the maximum number of juvenile meagre to any desired release size. For example, for comparative purposes, the number of 30-cm fish that can be produced with a given budget of €1000 could simply be estimated by the ratio of budget/cost-per-fish. However, some fish will die before release, thus implying an additional cost that is not accounted for by analysing only released fish. Therefore, the number of fish finally released was readjusted after taking into account fish losses produced during the grow-out phase (i.e. within the cages). The daily mortality rate (0.012% day^{-1}; unpublished data) was assumed on the basis of empirical data from LIMIA, after pooling data from different meagre experiments.

RESULTS

Effect of diet on growth

A total of 5174 length-at-age observations on 3331 fish fed one of seven different diets were successfully fitted to the proposed growth model. Due to the model complexity, preliminary fit of the data to the Somers' growth model at the cage level (i.e. independent analysis for each cage) was verified through visual exploration (Fig. 1). Next, all the data were combined in a single analysis using the hierarchical mixed model described above to compare between-diet performance. The fitted growth curves for each diet are shown in Figure 2. The best growth was obtained with diet G, although diet A also showed good growth performance. The growth rates of the fish fed the experimental diets (C, D and E) were clearly slower in comparison with the fish fed the other diets (Table 3).

Quality of juveniles

The slopes of the weight-length relationships (log-transformed), i.e. total weight, liver weight and mesenteric fat weight, for the different diets were estimated using the model detailed above based on 5360 fish for total weight and 186 fish for liver and mesenteric fat weight.

The Bayesian credibility intervals of the predicted population averages (and their variances) for total weight, liver weight and mesenteric fat weight for each of the diets and for a size at release of 30 cm are shown in Table 4. These results demonstrate that the total weight was greatest for Diet G, closely followed by Diets A, B and F. The median total weight was 4.9 g less for Diet A than for Diet G. Furthermore, the experimental diets (C, D and E) yielded substantially lower weights than the other diets, with differences greater than 50 g. Diet A had the highest liver weight (Table 4), although the values for diets A, B and G were all very similar. The results for liver weight were similar to those for total weight, with diets C, D and E yielding substantially lower liver weights than the other diets. The mesenteric fat weight was only weakly related to length for any diet ($R^2<0.78$), and the pattern of the relationship contrasted with the pattern found for total weight and for liver weight. For a size at release of 30 cm, experimental Diets D and E yielded the highest

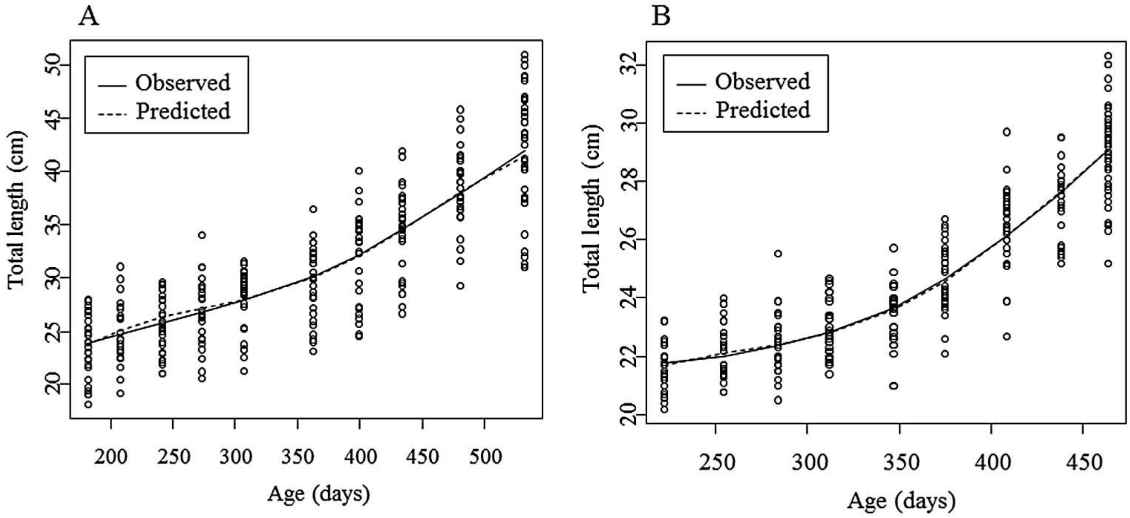

Fig. 1. – An example of the fit of the Somers' growth model to data from two randomly selected cages. A) Total length of the fish for the first replicate of Diet B in experiment 1. B) Total length of the fish for the third replicate of Diet E in experiment 2.

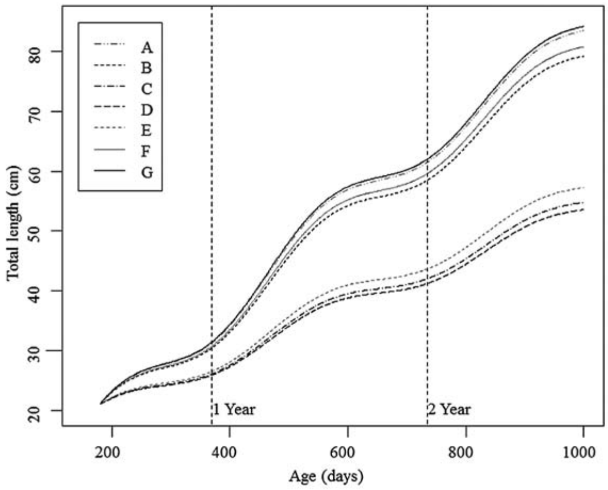

Fig. 2. – Estimated growth of *A. regius* juveniles for the seven tested diets during the grow-out phase.

Table 3. – Posterior descriptors (median and 95% credibility interval) of the relevant model parameters, where t_S is given in days and K in days^{-1}. Variability at fish, cage and year levels is indicated by tolerance (1/variance). Note that variability of K_{cage} and K_{year} is small.

	2.5%	Median	97.5%
t_S	104.3	107.5	110.9
C	0.68	0.71	0.74
K_{diet} Diet A	3.39 10^{-4}	4.77 10^{-4}	6.12 10^{-4}
K_{diet} Diet B	3.09 10^{-4}	4.41 10^{-4}	5.95 10^{-4}
K_{diet} Diet C	0.62 10^{-4}	2.26 10^{-4}	4.15 10^{-4}
K_{diet} Diet D	0.56 10^{-4}	2.20 10^{-4}	4.08 10^{-4}
K_{diet} Diet E	0.84 10^{-4}	2.48 10^{-4}	4.34 10^{-4}
K_{diet} Diet F	3.11 10^{-4}	4.51 10^{-4}	5.84 10^{-4}
K_{diet} Diet G	3.36 10^{-4}	4.86 10^{-4}	6.19 10^{-4}
Tolerance fish	0.20	0.21	0.22
Tolerance cage (K_{cage})	9.9 10^{8}	9.9 10^{8}	1.0 10^{9}
Tolerance year (K_{year})	1.4 10^{7}	2.8 10^{8}	6.0 10^{9}

median mesenteric fat weight (Table 4). Diet B also provided a considerable quantity of mesenteric fat. However, the other diets yielded median mesenteric fat weights of less than 1 g.

Significant differences were observed in the ash, moisture, crude fat and crude protein composition of the fish on the seven diets (ash: F=18.8, $P<0.05$; moisture: F=20.5, $P<0.05$; crude fat: F=19.4, $P<0.05$; crude protein: F=11.9, $P<0.05$). The crude fat content of the fish on Diets A, B, F and G was significantly higher than that of the fish on Diets C, D and E (Fig. 3A). Similar results were observed for the crude protein content (Fig. 3B), with average values in a much narrower range (17-18.5%). The crude protein content of Diet F was intermediate in value between the diets with the highest content (A, B and G) and the diets with the lowest content (C, D and E).

Production costs

The total costs and the percentage of feeding and personnel costs were obtained for each diet (Table 5). As the desired size at release increased, the relative proportion of feeding costs also increased. However, the proportion of the total associated with personnel costs was higher than that of feeding costs for all the

Table 4. – Summary of the weight-length analysis results for the total, liver and mesenteric fat weights of the different diets for a desired size at release of 30 cm, with the median value and lower and upper 2.5% percentiles of the Bayesian credibility intervals for the estimated weights (g).

Diets	Total weight			Liver weight			Mesenteric fat weight		
	2.5%	Median	97.5%	2.5%	Median	97.5%	2.5%	Median	97.5%
A	289.54	326.64	368.80	5.39	7.65	10.93	0.19	0.80	3.39
B	285.78	323.44	365.61	5.30	7.46	10.54	0.29	1.19	5.08
C	237.03	267.78	302.21	2.61	3.69	5.28	0.17	0.73	3.07
D	241.07	272.33	307.30	3.14	4.45	6.30	0.41	1.69	7.06
E	237.42	267.83	302.69	2.90	4.10	5.82	0.36	1.56	6.43
F	284.81	321.69	363.83	4.77	6.72	9.50	0.08	0.34	1.49
G	293.11	331.58	375.49	5.21	7.44	10.57	0.16	0.67	2.78

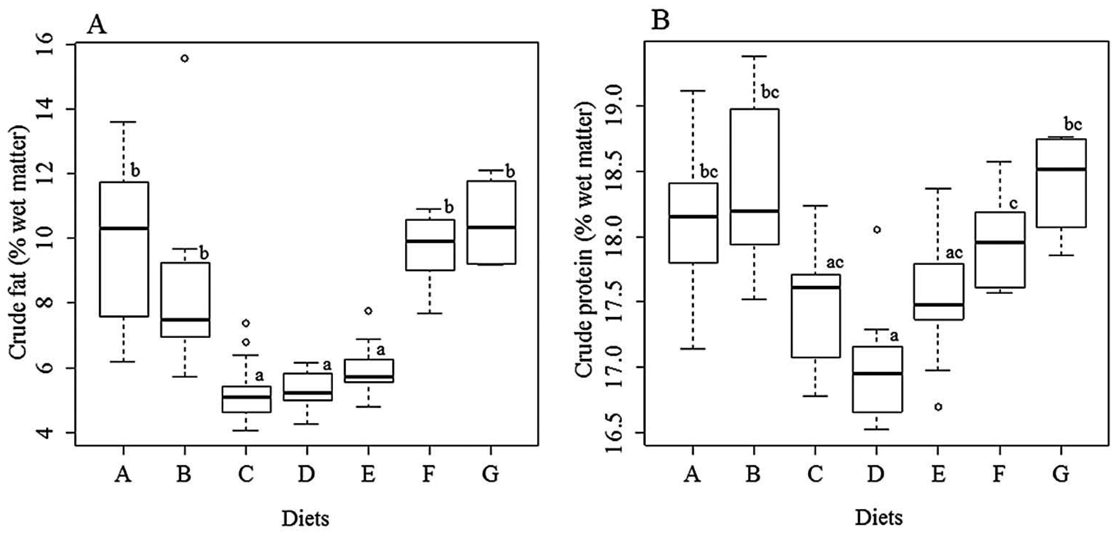

Fig. 3. – Boxplots of the A) crude fat content and B) crude protein content for fish reared on the tested diets. A line within the box marks the median values, and the boundary of the box indicates the 25% and 75% percentiles. The results of the pairwise comparisons, using a Tukey test, are represented by different letters.

Table 5. – The total (CO, in € fish^{-1}), feeding (FCO, in %) and personnel costs (PCO, in %) for each diet relative to some possible size at release (L, in cm).

Diets		L=25			L=30			L=35			L=40	
	FCO	PCO	CO	FCO	PCO	CO	FCO	PCO	CO	FCO	PCO	CO
A	9.9	90.1	1.3	14.0	86.0	4.2	16.2	83.8	5.5	19.0	81.0	6.7
B	10.1	89.9	1.5	14.3	85.7	4.4	16.4	83.6	5.7	19.5	80.5	7.0
C	9.0	91.0	3.8	10.6	89.4	5.9	12.8	87.2	8.9	15.4	84.6	13.2
D	9.5	90.5	3.9	11.2	88.8	6.0	13.7	86.3	9.9	16.3	83.7	13.6
E	9.9	90.1	3.6	11.5	88.5	5.7	14.2	85.8	7.9	16.9	83.1	12.8
F	9.5	90.5	1.5	13.4	86.6	4.4	15.4	84.6	5.6	18.3	81.7	6.8
G	26.6	73.4	1.6	35.0	65.0	5.6	39.1	60.9	7.5	43.9	56.1	9.6

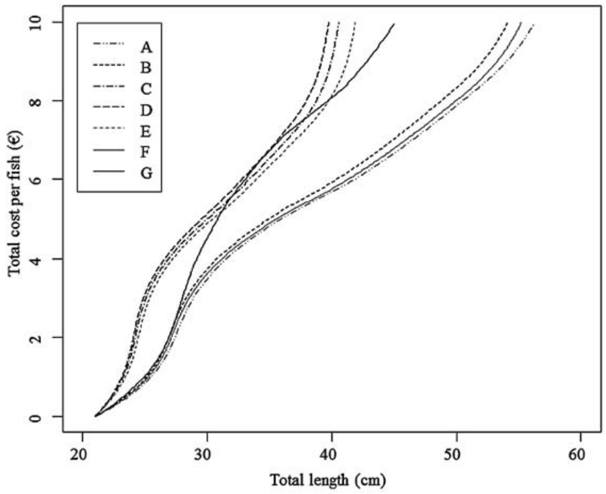

Fig. 4. – Size-dependent total cost (€) for a fish fed with each of the seven tested diets during the grow-out phase.

analysed lengths. Even in the case of diet G, for which the cost of raw materials was highest, the proportion of personnel costs was greater than the feeding cost, although the feeding and personnel costs were almost equal for a length of 40 cm.

Diet A yielded the lowest production cost at any release length (Fig. 4). The experimental diets (C, D and E) had the highest total cost because of the poor growth observed. This implies that the fish had to be fed for a longer time to reach the desired length than the fish fed with the other diets, thus leading to greater personnel costs. Although the costs of Diet G and Diet A were similar at the beginning of the grow-out phase, the total cost for producing fish larger than 25 cm with Diet G increased substantially over time. Therefore, despite the better growth furnished by Diet G, this diet was not advantageous in terms of the total production costs.

Table 6. – Number of fish that can be produced for a target size of 30 cm with a given budget of €1000. Daily mortality was assumed to be 0.012%.

Diets	Number of fish
A	2328
B	2234
C	1665
D	1626
E	1724
F	2267
G	1768

The estimated number of fish for a given size (30 cm) and with a given budget (1000 €) is compared at Table 6. Diet A produced the largest number of fish.

DISCUSSION

The success of a restocking programme depends on complex tradeoffs, but in practice it could be considered a two-fold process. First, the post-release survival rate of hatchery-reared specimens is primarily dependent on the size/age and the quality of specimens at the time of release (Tsukamoto et al. 1999, Leber et al. 2005); larger fish tend to be more resilient. Second, success may depend on the number of fish released. However, both processes are limited by economic factors. The economic factors of restocking programmes have rarely been studied (Kellison and Eggleston 2004, Leber et al. 2005, Patrick et al. 2006), but play important roles in the recovery of several species.

While the size-dependent mortality rate of restocked meagre in the wild has been analysed elsewhere (Gil et al. 2015), here we focused on the methods for maximizing the number of fish to be released.

Growth

Growth of meagre is high during the grow-out phase and feed conversion rates (i.e. the proportion of food that is converted into biomass) are good (Duncan et al. 2013). Recently, meagre growth has been widely studied in different culture systems (Pastor et al. 2002, El-Shebly et al. 2007, Chatzifotis et al. 2010, Vargas-Chacoff et al. 2014) and with different diet compositions (Piccolo et al. 2008, Estévez et al. 2011, Chatzifotis et al. 2012, Velazco-Vargas et al. 2014) due to the increasing interest in meagre for diversification of aquaculture.

To compare the growth rate of juveniles that were fed with different diets was challenging in this study because fish showed a clear shift in growth rate following the winter (Fig. 1). As in most marine organisms (Pauly 1990, Alcoverro et al. 1995, Coma et al. 2000), the growth of *A. regius* appears to be influenced by seasonal changes. The growth rate clearly decreases at temperatures less than 16ºC (Quéméner 2002, El-Shebly et al. 2007). For this reason, the conventional von Bertalanffy growth model did not adequately fit the growth pattern of the juvenile meagre on the diets tested in this study. The observed growth pattern was better described by introducing a seasonal oscillation rather than using the conventional formulation (Somers 1988, García-Berthou et al. 2012, Gil et al. 2014b). This method yielded season-independent growth rates that were fully comparable among diets.

The diet experiments conducted with juvenile *A. regius* revealed that Diet G provided the best growth, because semi-moist diets appear to have better palatability than dry pellets. The second-best growth rate was obtained with Diet A (dry commercial pellets), but this diet minimized the cost of growing fish for any desired size at release (see below). The experimental diets (C, D and E) clearly underperformed when compared with the others. This result supports the need for completing pilot studies for comparing different rearing protocols.

Fish quality

The expected fitness of fish released into a natural environment is a result of both fish quality and size (Tsukamoto et al. 1999). There is a long history of research on hatchery technologies aiming to produce high-quality juveniles not only for aquaculture but also for stock enhancement (Fushimi 2001, Shields 2001). Studies on red sea bream, *Pagrus major* have shown that the quality of hatchery-reared juveniles depends primarily on the nutritional quality of the diet (Nakano 1996, Le Vay et al. 2007). Similarly, the current study showed that the biological condition of the fish (estimated from the relationships between length and total weight, liver weight and mesenteric fat weight) and the biochemical composition of the entire fish body were influenced by the diets used to feed juvenile meagre.

The biochemical composition of the fish body (specifically, crude fat and protein) was similar and optimal for diets A, B, F and G but deficient for diets C, D and E. The proximate composition of cultured fish is affected by endogenous and exogenous factors. The levels of protein and ash are primarily related to fish size (endogenous factors), whereas fat content depends on exogenous factors, such as diet (Shearer 1994). Although exceptions occur, muscle with relatively low lipid content is common in many demersal species. These species are more sedentary than pelagic carnivores, which typically show burst swimming behaviour and thus sustain higher lipid levels in muscle tissue to fuel this response (Sheridan 1988).

Weight-length relationships were used for comparing body condition based on the assumption that heavier fish of a given length are in better condition than thinner fish (Froese 2006). Poor conditions may lower the chances of survival because fish are more susceptible to predation and to a variety of environmental stressors (Lloret et al. 2002). According to these criteria, fish on Diet G exhibited the best condition, followed by those on Diet A. Note, however, that diets A, B, F and G all yielded a very similar total weight for a size at release of 30 cm. These total weights differed by only a few grams (Table 4). Additionally, note that the within-diet differences were large in comparison with the among-diet differences (Table 4).

In fish, liver is considered a major fat and glycogen storage organ, and can provide an additional way to estimate nutritional state (Adams and Greeley 2000). Accordingly, correlations involving liver weight and fish length are calculated to estimate condition in fish (Benejam et al. 2010). In the current study, the measurements of fish condition based on liver weight were highest and almost equal for diets A, B, F and G, indicating a higher accumulation of reserves. Chatzifotis et al. (2006) have suggested that the liver of the sciaenid brown meagre, *Sciaena umbra*, may serve as a storage organ for energy because of its relatively high lipid content (39-43%). Furthermore, the low lipid content of muscle (1-2.5%) observed in certain species of sciaenids (Poli et al. 2003, Hernández et al. 2009, Grigorakis et al. 2011) indicates the minor role of muscle as an energy storage tissue (Chatzifotis et al. 2006).

The experimental diets (C, D and E) clearly resulted in poorer growth, total weight and liver weight-length relationships. However, the mesenteric fat weight-length relationship yielded greater values than the other diets, indicating a substantial accumulation of mesenteric fat during the experiment. Piccolo et al.

(2008) evaluated the effect of two diets with different protein/fat ratios (P/F) and found that, although the biometric traits analysed were not affected by the diet, the amount of mesenteric fat was significantly higher for diets with a lower protein/fat ratio. Too much dietary lipid may result in excessive fat deposition in the visceral cavity and tissues (Lanari et al. 1999). In fish, an appropriate energy-to-protein ratio in the diet contributes to the effective utilization of dietary proteins through the protein sparing effect (Watanabe 1982). Therefore, fish are able to utilize dietary lipids up to a certain level beyond which growth may be retarded because high energy intake depresses appetite before sufficient protein has been consumed to support growth (Ellis and Reigh 1991). However, meagre is a carnivorous species, feeding on Mysidacea, Decapoda and Teleostei in the wild (Cabral and Ohmert 2001, Gil et al. 2014a) (Chatzifotis et al. 2012), and does not appear to require high dietary lipid levels. At lipid levels greater than 17%, Chatzifotis et al. (2010) did not observe a sparing effect on protein. Therefore, the mesenteric fat weight-length relationship should not be viewed as an indicator of good condition.

In summary, fish condition and biochemical composition suggest that all the diets except the experimental ones (i.e. C, D and E) produce fish of similar quality and are fully suited for use in a restocking programme.

Cost

Economic effectiveness is one of the most frequently criticized aspects of restocking programmes, although most restocking programmes have yet to be fully assessed because this aspect has received little or no attention (Hilborn 1998). However, economic viability has been demonstrated in certain cases: e.g. release programmes for Pacific salmon *Oncorhynchus* sp. (Isaksson 1988), Japanese flounder *Paralichthys olivaceus* and red sea bream *P. major* in Japan (Kitada 1999, Okouchi et al. 2004, Kitada and Kishino 2006).

In our case, the costs of the grow-out phase showed marked among-diet differences. The high cost of the semi-moist diet and its required cold storage limit the use of Diet G (Kubitza and Lovshin 1997, Kim and Shin 2006). Diet A was the best option for minimizing production costs for any size at release because this diet has both a reasonable price (unlike Diet G) and good growth performance, allowing fish to spend less time in the grow-out phase before attaining the desired size. This last point is decisive because time savings imply reduced personnel costs, which represent the largest component of the total cost (see Table 5).

Despite the favourable results obtained with Diet A, the absolute cost of the grow-out phase was higher than the costs obtained in other studies of the entire production process (Svåsand et al. 2000, Leber et al. 2005, Patrick et al. 2006). However, it is important to note that LIMIA facilities are designed for research purposes. Continuous improvement in hatchery technology minimizes production costs (Sproul and Tominaga 1992, Ungson et al. 1993). so it is expected that productivity will be largely improved at facilities specialized in producing fish for restocking. Nevertheless, production capability is somewhat limited and producing large numbers of large specimens is unreliable given, for example, the spatial limitations of hatcheries (Kellison and Eggleston 2004). Ultimately, the annual budget imposes a limit to the number and size of the fish that can be produced.

In conclusion, Diet A was the best option for the production of juvenile meagre for a restocking programme. This diet was capable of producing quality juveniles while minimizing production costs for any possible size at release and maximizing the number of juveniles that could be produced on a fixed budget. However, other factors that need further study include the magnitude of the release (i.e. the critical number of released fish), the survival of released fish (Gil et al. 2015) and the economic value of the fish (fisher profits). All of these factors must be considered for a suitable assessment of the cost-effectiveness of a restocking programme. Adapting and improving the programme in light of the results of pilot studies and incorporating the opinions of different stakeholders into decision making may successfully facilitate the design of restocking programmes that are socially acceptable, economically viable and environmentally sustainable (Bartley and Bell 2008).

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