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Sardine-Based Diet Mitigates Growth Depression at Low Temperatures in Juvenile Meagre (*Argyrosomus regius*, Asso 1801)

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Abstract

Low seawater temperatures are expected to depress fish growth in aquaculture. However, recent evidence suggests diet composition may offer mitigation for some species. This study evaluated the impact of different diets on juvenile meagre (*Argyrosomus regius*) in cage farming at low seawater temperatures (average 15.19 °C), conditions known to typically suppress meagre growth. Three replicated groups of fish (initial weight ≈ 107 g) were fed for six months either sardines (group A) or commercial pellets (groups B/C, with group C moisturized). The results demonstrate that the nutritional profile of sardines effectively mitigates cold-induced growth reduction in meagre. While pellet-fed meagre experienced expected growth depression, sardine-fed meagre exhibited a doubled temperature growth coefficient (TGC) and an 80% higher final average weight than the pellet groups (A: 346.13 g, B: 194.44 g, C: 188.93 g).

Keywords: feed; growth; cold water; nutritional habitat component; wet diet; carnivore nutrition; diet composition

Key Contribution: Nutrition can allow continuous growth of farmed meagre during the otherwise unproductive cold season, thus shortening the growth culturing cycle.

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1. Introduction

Meagre (*Argirosomus regius*, Asso 1801) is a carnivorous fish [1] highly suited for aquaculture due to its high salinity and temperature tolerance, good adaptability to captivity, and rapid growth rates up to 2.5 kg in two years [2]. Its promising market potential stems from high flesh quality, good flavor, firm texture, and good processing yield [3,4]. Global production in 2021 exceeded 43,000 metric tons [5].

Temperature fundamentally influences fish growth [6], and suboptimal temperatures typically suppress growth, posing a significant challenge for cage aquaculture where fish cannot avoid environmental fluctuations [7,8]. While meagre exhibits a broad temperature tolerance (10–30 °C) [9] and experiences large temperature fluctuations in nature (13.1–24.9 °C) [10], optimal growth occurs between 17 and 21 °C [11], with feeding considerably reduced below 13 °C [12]. Increased incidence of health issues and potential losses during winter periods with temperatures below 13 °C highlights the critical need for an understanding of meagre's nutritional requirements at these lower temperatures [13,14].

Comprehensive understanding of meagre's nutritional requirements necessary for efficient cultivation is still developing. Building upon early growth studies in captivity [11], recent decades have seen numerous rearing experiments aimed at determining these basic needs. These investigations have explored protein and lipid requirements [15–19] and the potential of vegetable materials as substitutes for fishmeal and fish oil [20–22]. Characteristically, many of these studies were short-term (up to six months) and focused on juvenile fish. Some long-term research involving larger, commercial-sized fish has also been conducted [2,23], including one industrial-scale feeding experiment [19]. These findings collectively contributed to the formulation of commercial meagre feed, primarily an extruded pellet designed to meet the fish's needs.

Commercial meagre pellets, due to biochemical stability, are harder and less moist than natural food, potentially reducing growth and disease resistance [24–26] and hindering further formulation improvements due to data scarcity. While natural diet studies have benefited other Mediterranean aquaculture species [27,28], meagre data are scarce, limited to broodstock management [29] and a single fry report [30]. This data deficiency emphasizes the necessity for further research into meagre nutrition, with the study of natural diets offering a promising avenue for developing improved commercial feeds.

This study compared effects of natural and pellet-based diets on the growth performance of juvenile meagre in cage farming conditions during low seawater temperatures over six months. Surprisingly, we found a large positive effect of natural feed, suggesting substantial potential for improving pellet formulations.

2. Materials and Methods

2.1. Fish Rearing and Experimental Design

Juvenile meagres, previously fed industrial food for juvenile meagre, were randomly sourced from a single production cage located at the producer's fish rearing plant in the Middle Eastern Adriatic Sea (island of Ugljan, Croatia). Meagre were then distributed into six floating cages (9 × 5 × 4 m) at the same facility. A total of 1100 meagres were sequentially allocated to each cage, forming three experimental groups (2200 meagres in two cages per group). The initial characteristics of each group were as follows:

- Group A: 20.35 cm ± 0.207 total length, 106.30 g ± 3.12 wet weight;
- Group B: 20.6 cm ± 0.27 total length, 108.25 g ± 4.38 wet weight;
- Group C: 20.65 cm ± 0.19 total length, 107.14 g ± 3.04 wet weight.

During the experiment, each group received a different diet (without adaptation):

- Group A (diet S—sardines). All sardines (*Sardina pilchardus*, Walbaum, 1792) were from a single haul, individually quick-frozen (IQF) on the same day. Before feeding, sardines were thawed and cut into appropriately sized pieces;
- Group B (diet P—commercial dry pellets). Commercial pellets, which were declared for meagre farming;
- Group C (diet MP—moisturized commercial pellets). Commercial pellets, sourced identically to diet P, were sprayed with 10% water by weight to achieve 15% moisture, and soaked for 30 min before feeding.

The MP diet was included to rule out moisture as the primary determinant of growth variation. The experiment lasted for six months, from November 2015 to April 2016. Seawater temperature and dissolved oxygen levels were measured daily, with oxygen saturation consistently at 95% or above throughout the trial. Fish were fed by hand to apparent satiation twice daily (at 8:00 and 15:00) six days a week during November and December. During the colder months (January to April), feeding frequency was reduced to once daily at 9:00. The total amount of feed distributed to each group was recorded

throughout the experiment. Seawater temperature was measured daily at a depth of 2 m. Mortality rates during the experiment ranged from 7% to 9%, with no statistically significant differences observed between the groups.

2.2. Determination of Somatic and Growth Indices

2.2.1. Morphometric Measurements

At the beginning of the experiment and monthly thereafter, the total length (L, in centimeters) and wet weight (W, in grams) of 100 randomly selected fish per cage were individually measured. Prior to these measurements, the fish were anesthetized using benzocaine at a concentration of 30–40 mg/L (Aquacen benzocaine 200 mg/mL, Cenavisa, Reus, Spain).

2.2.2. Fulton's Condition Factor (K)

The measured length (L) and weight (W) were used to estimate fish robustness using Fulton's condition factor, K, according to Bagenal and Tesch [31]:

$$K = \frac{W}{L^3} * 100$$

2.2.3. Somatic Indices

At the beginning and end of the six-month trial, a subsample of 20 fish per cage was taken to determine somatic indices. These fish were euthanized in a melting ice bath, individually weighed, and then dissected to remove the liver and viscera. The weights of the liver (in grams) and viscera (in grams) were recorded for the calculation of the hepatosomatic index (HSI) and viscerosomatic index (VSI) using the following formulas:

- Hepatosomatic index (HSI):

$$HSI = \frac{\text{liver wet weight}}{W} * 100;$$

- Viscerosomatic index (VSI):

$$VSI = \frac{\text{visceral wet weight}}{W} * 100.$$

2.2.4. Growth Parameters

Fish biomass within each cage was estimated based on the weight measurements of the 100 sampled fish and the total number of fish present in the cage at the beginning and end of the experiment, assuming a constant mortality rate. These biomass data, along with the individual weight measurements, were used to calculate growth indices and feed consumption parameters:

- Thermal growth coefficient, TGC:

$$TGC = \frac{{W_2}^{1/3} - {W_1}^{1/3}}{T * t},$$

where

- W_1 = initial mean weight (g);
- W_2 = final mean weight (g);
- T = daily seawater temperature (°C);
- t = duration of the experiment (days).

2.2.5. Feed Consumption Parameters:

The following parameters were calculated to assess feed consumption:

- Daily feeding ratio, DFR:

$$\text{DFR} = \frac{\text{AF (g/day)}}{\text{average biomass of fish per group}} * 100,$$

where AF is the dry matter of the administered feed per group:

$$\text{AF (g)} = \text{feed weight (g)} \frac{100 - \%\ \text{moisture}}{100};$$

- Total feed intake per fish (TFI):

$$\text{TFI} = \frac{\text{AF (g)}}{\text{number of fish per group}};$$

- Gross energy intake per fish (GEI):

$$\text{GEI} = \text{TFI (g/fish)} * \text{gross energy of feed (kJ/g)}.$$

2.3. Analytical Methods

2.3.1. Diet Composition Analysis:

The chemical composition of the experimental diets was determined in triplicate following standard Association of Official Analytical Chemists (AOAC) procedures [32]. The specific methods employed were as follows:

- **Lipid content:** lipids were extracted according to the method of Folch et al. [33]. The total lipid content was then gravimetrically determined after solvent evaporation using a Soxhlet apparatus (Ser 158, Velp Scientifica Srl. Usmate, Italy);
- **Protein content:** protein content was determined using the Kjeldahl method; total nitrogen content was measured and multiplied by a conversion factor of 6.25 to estimate protein levels;
- **Water content:** water content of the feed was determined by oven-drying of minced feed at 105 °C until a constant weight was achieved, followed by reweighing;
- **Ash content:** ash content was determined by combusting a 1 g homogenized feed sample in a muffle furnace until a stable weight was obtained;
- **Carbohydrate content:** carbohydrate content was calculated as the following remaining fraction:

$$100 - (\%\ \text{lipid content} + \%\ \text{protein content} + \%\ \text{water content} + \%\ \text{ash content});$$

- **Gross energy, GE:** gross energy was calculated based on the determined macronutrient content using the energy conversion factors provided by Steffens [34]: 23.9 kJ/g for protein, 39.8 kJ/g for lipids, and 17.6 kJ/g for carbohydrates.

2.3.2. Fatty Acid Composition Analysis:

The fatty acid composition of diets S and P, as well as the whole body of fish from groups A, B, and C at the end of the experiment, was analyzed using gas chromatography (GC). Although there is some potential for oxidation and/or leaching of lipids after moisturizing, due to the short soaking time and hydrophobic nature of the lipids, the composition of the moisturized pellet (diet MP) was assumed to be the same as that of the pellet alone (diet S) because the same pellets were used for both diets.

Whole-body fish samples were pooled (n = 10 fish per group). Both diets and the pooled fish samples were minced using a Grindomix GM 200 (Retsch Gmbh, Haan, Germany) to ensure homogeneity. Lipids were extracted from 3 g of the homogenized samples with petroleum ether after acid hydrolysis in 4 mol/dm^3 HCl. The extracted fatty acids were converted into their corresponding methyl esters (FAMEs) through trans-esterification with a methanolic solution of potassium hydroxide [35].

GC analyses [36] were performed on a Scion 436 GC instrument (Bruker, Billerica, MA, USA) equipped with a flame ionization detector and a Famewax capillary column (30 m length, 0.32 mm internal diameter, 0.25 µm film thickness; Restek Famewax, Bellafonte, PA, USA). Individual FAMEs were identified by comparing their retention times to those of known standards (Marine Oil FAME Mix, Restek, USA). The content of each fatty acid was expressed as a percentage of the total fatty acids.

2.4. Statistical Analysis

All statistical calculations were performed using TIBCO Statistica version 14.0.015 software (TIBCO Software Inc., Palo Alto, CA, USA). The results are presented as the mean ± standard error of the mean (SEM).

First, data were checked for normality and homogeneity. Data expressed as percentages were subjected to arcsine square root transformation to ensure assumptions of normality and homogeneity were satisfied. Normality of the data was assessed using the Kolmogorov–Smirnov test, while homogeneity in the variance of the data was assessed using Cochran's test, Hartley's F-max test, and Bartlett's test.

To determine statistically significant differences in growth data, TGC, TFI, and somatic indices among the experimental groups, a one-way analysis of variance (ANOVA) was performed. Where significant differences were identified by ANOVA, a post hoc Tukey's Honestly Significant Difference (HSD) test was applied for pairwise comparisons. The significance level for all statistical tests was set at $p < 0.05$.

The relationship between the fatty acid profiles of the diets and the whole-body fatty acid profiles of the fish at the end of the experiment was examined using Pearson linear regression analysis. Following the regression analysis, the z-score of the residuals for each fatty acid was calculated to identify any significant deviations from the predicted linear relationship.

3. Results

3.1. Environmental Conditions and Diet Composition

During the six-month experimental period, the seawater temperature ranged between 18.7 °C and 13 °C, with an average temperature of 15.19 °C. Notably, 141 days the temperatures were below 17 °C, and 71 days they were below 14 °C (Figure 1). The proximate compositions of the three experimental diets (S, P, and MP) are presented in Table 1. The most significant difference observed between the diets was the higher water content in diet S, which consisted of sardines. To facilitate a more direct comparison of nutrient content, the proximate compositions were also recalculated and expressed on a dry matter basis in Table 2. This recalculation revealed substantial differences in the proportions of the main constituents between the sardine-based diet S and the artificial pelleted diets (P and MP).

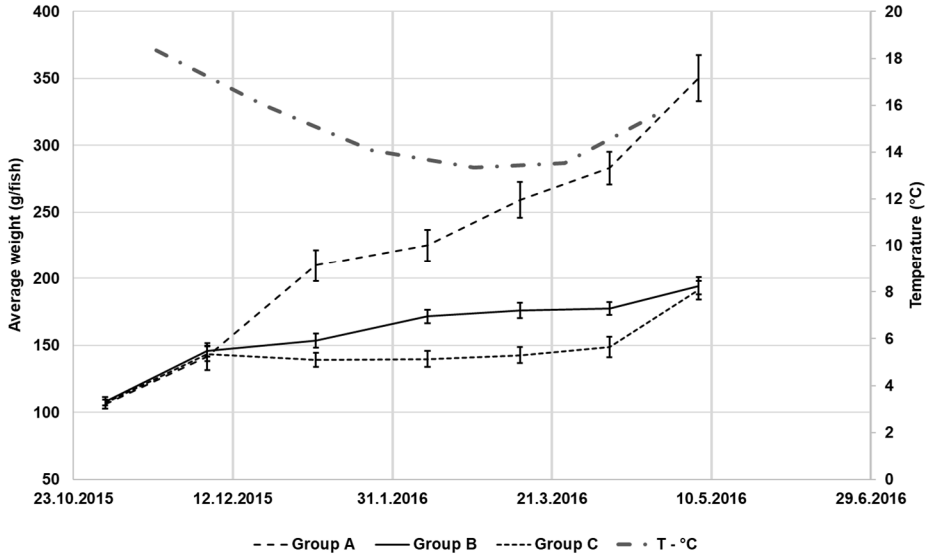

Figure 1. Average body weight (g/fish) of meagre in experimental groups A (sardine diet), B (commercial pellets), and C (moisturized pellets) from October 2015 to June 2016. The dashed–dotted line indicates the average seawater temperature (°C). Note the divergence in growth between group A and groups B/C as temperatures dropped below 18 °C. Data are presented as means ± SE.

Table 1. Proximate composition of diets (%). Diet S consisted of sardines, whereas diet P was commercial pelleted feed and diet MP was obtained by adding water to diet P. Values are means ± SEM.

	Diet S	Diet P	Diet MP
Moisture	72.6 ± 0.15	6.367 ± 0.10	15.01 ± 025
Crude protein	17.27 ± 0.14	46.97 ± 0.27	42.66 ± 0.39
Crude lipid	6.65 ± 0.21	16.05 ± 0.05	14.58 ± 0.18
Carbohydrates	0.21 ± 0.01	21.24 ± 1.02	18.74 ± 1.13
Ash	3.36 ± 0.06	9.27 ± 0.30	8.42 ± 0.13

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

	Diet S	Diet P	Diet MP	F	*p*
Crude protein	62.8 ± 0.90 ^{a}	50.23 ± 0.29 ^{b}	50.49 ± 0.25 ^{b}	157.56	0.000006
Crude lipid	24.17 ± 1.11 ^{a}	17.17 ± 0.16 ^{b}	17.23 ± 0.21 ^{b}	42.67	0.000284
Carbohydrate	0.76 ± 0.04 ^{a}	22.68 ± 0.05 ^{b}	22.36 ± 0.21 ^{b}	4051.42	0.000000
Ash	12.23 ± 0.48 ^{a}	9.90 ± 0.54 ^{b}	9.90 ± 0.0.76 ^{b}	10.05	0.012137
GE (kJ/g feed)	24.51 ± 0.26 ^{a}	22.56 ± 0.11 ^{b}	22.59 ± 0.03 ^{b}	47.243	0.000212
GP/GE (g/MJ)	25.6 ± 0.6 ^{a}	22.3 ± 0.2 ^{b}	22.4 ± 0.1 ^{b}	29.202	0.000809

Data in the same row with different superscripts differ at $p < 0.05$ (ANOVA, Tukey's test).

3.2. Growth and Somatic Indices

Growth and somatic indices are summarized in Table 3. Even though initial weights were not significantly different, the final weight of fish in group A, receiving sardines (diet S), exhibited a significantly higher final weight compared to groups B and C, which were receiving the dry (diet P) and moisturized (diet MP) pelleted diets, respectively (Table 3, see also Figure 1). The same pattern emerged for growth in length (Table 3, see also Figure 2), and the condition factor (K) indicated better overall fitness at the end of the experiment (Table 3).

The temperature growth coefficient (TGC) also revealed significantly superior results for the sardine-fed group A ($p < 0.05$). HSI and VSI experienced significant drops during the cold period, but the final hepatosomatic (HSI) and viscerosomatic (VSI) indices are

practically the same with only group B showing a statistically detectable but very small difference of 0.7%. In contrast, the different diets did not have a statistically significant effect on the hepatosomatic index (HSI) or the viscerosomatic index (VSI).

Table 3. Effects of diets on growth and somatic indices of meagre. Fish of group A were fed with sardines while groups B and C received pellets and moisturized pellets, respectively. Values are means ± SEM.

	Group A	Group B	Group C	F	*p*
Initial weight (g)	106.30 ± 3.12 [a]	108.25 ± 4.38 [a]	107.14 ± 3.04 [a]	0.07	0.929
Final weight (g)	346.13 ± 17.39 [a]	194.44 ± 7.45 [b]	188.93 ± 6.22 [b]	67.8	$<1 \times 10^{-5}$
Init. lengths (cm)	20.35 ± 0.207 [a]	20.6 ± 0.27 [a]	20.65 ± 0.19 [a]	0.56	0.573
Final length (cm)	30.01 ± 0.49 [a]	25.31 ± 0.33 [b]	25.07 ± 0.28 [b]	42.17	$<1 \times 10^{-5}$
Initial K	1.18 ± 0.01 [a]	1.17 ± 0.01 [a]	1.17 ± 0.01 [a]	1.2	0.309
Final K	1.22 ± 0.01 [a]	1.14 ± 0.11 [b]	1.16 ± 0.01 [b]	9.41	0.0001
Initial HSI	2.83 ± 0.14 [a]	2.81 ± 0.17 [a]	2.93 ± 0.14 [a]	0.16	0.849
Final HSI	1.85 ± 0.17 [a]	2.03 ± 0.09 [a]	2.17 ± 0.12 [a]	1.55	0.230
Initial VSI	8.34 ± 0.51 [a]	8.38 ± 0.33 [a]	8.73 ± 0.31 [a]	0.272	0.764
Final VSI	6.70 ± 0.31 [a]	6.05 ± 0.20 [b]	6.92 ± 0.14 [a]	3.996	0.030
TGC	0.808 ± 0.003 [a]	0.359 ± 0.003 [b]	0.358 ± 0.021 [b]	386.5	0.0002
DFR (ratio)	0.583 ± 0.11 [a]	0.465 ± 0.07 [b]	0.478 ± 0.08 [b]	3.654	0.029
TFI (g/fish)	227.8	139.6	117.0		
GEI (kJ/fish)	5584.3	3150.0	2642.1		

Data in the same row with different superscripts differ at $p < 0.05$ (ANOVA, Tukey's test). Acronyms: K (Fulton's condition factor), HSI (hepatosomatic index), VSI (viscerosomatic index), TGC (temperature growth coefficient), DFR (daily feeding ratio), TFI (total food intake per fish), GEI (gross energy intake per fish).

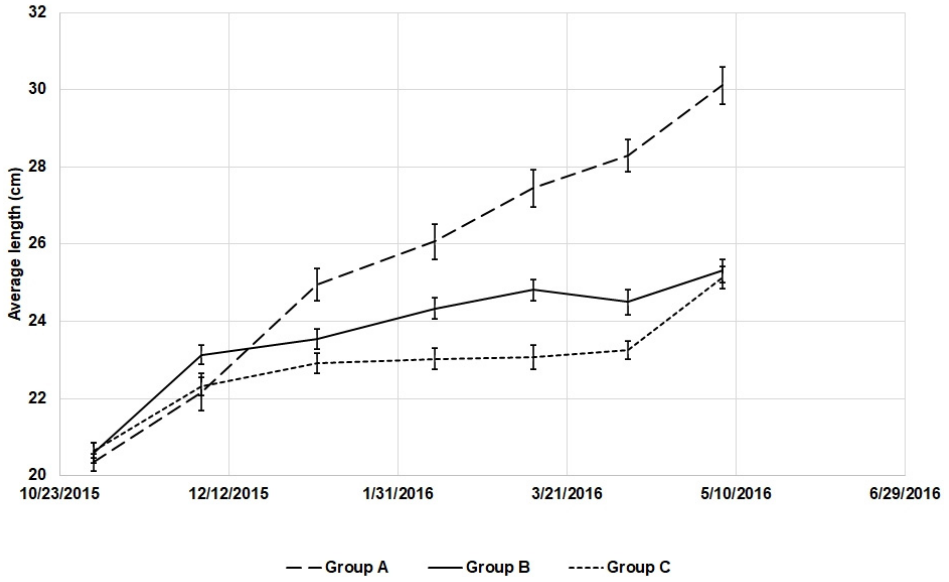

Figure 2. Average length (cm) of meagre in experimental groups A (sardine diet), B (commercial pellets), and C (moisturized pellets) from October 2015 to June 2016. Similar to weight gain (Figure 1), group A shows a continued increase in length, while growth in groups B and C appears to slow down during colder periods. Data are presented as means ± SE.

3.3. Feed Consumption

The daily feed ratio (DFR) was slightly higher for group A compared to groups B and C. However, the differences in feed consumption were most pronounced when considering the total feed intake (TFI) and gross energy intake (GEI), with group A exhibiting considerably higher values for both parameters.

3.4. Fatty Acid Profiles

Table 4 contains the fatty acid profiles of diets S and P, as well as the whole-body fatty acid compositions of fish from all groups at the end of the experiment. As diet MP was simply diet P with added moisture, the fatty acid profile of MP is identical to that of P.

Table 4. Fatty acid composition (g/100 g total fatty acids) of diet S and P, and whole-body fatty acid compositions of fish from groups A, B, and C at the end of the experiment.

	Diet S	Diet P	Group A	Group B	Group C
C12:0	0.2	0.1	0.0	0.0	0.1
C14:0	12.3	3.3	7.3	2.8	3.2
C15:0	1.5	0.3	0.94	0.45	0.5
C16:0	29.2	13.5	23.2	18.7	19.8
C16:1n7t	0.5	0.2	0.0	0.0	0.41
C16:1n7c	7.4	3.5	7.3	4.28	4.38
C17:0	1.6	0.4	0.94	0.6	0.69
C17:1	0.3	0.2	0.49	0.3	0.29
C18:0	7.8	3.6	6.1	4.9	4.81
C18:1n9c	9.8	30.4	17.69	32.1	33.78
C18:1n7	3.4	2.8	3.77	3.24	3.23
C18:2n6t	0.2	0.1	0.2	0.0	0.1
C18:2n6c	0.2	13.9	5.5	13.6	11.3
C18:3n6	0.1	0.1	0.01	0.34	0.0
C18:3n3	1.3	3.9	1.55	2.21	1.51
C18:4n3	1.6	1.1	1.2	0.4	0.22
C20:0	1.4	0.5	0.71	0.5	0.48
C20:1n9	1.4	2.4	1.09	2.12	2.98
C20:2n6	0.4	0.3	0.37	0.33	0.31
C21:0	0.2	0.0	0.0	0.0	0.0
C20:3n6	0	0.1	0.21	0.1	0.0
C20:4n6 (ARA)	0	0.6	0.8	0.99	0.28
C20:3n3	0.1	0.1	0.18	0.31	0.0
C20:4n3	0.6	0.5	0.64	0.49	0.23
C20:5n3 (EPA)	4.9	5.8	5.23	2.31	2.24
C22:0	0.3	0.2	0.0	0.0	0.3
C22:1n11	0.3	2	0.74	1.9	2.12
C22:1n9	0.3	0.4	0.12	0.69	0.72
C22:5n3	0.3	0.3	1.65	0.19	0.79
C24:0	0.7	1.1	0.0	0.0	0.4
C22:6n3 (DHA)	8.9	7.8	10.6	4.42	3.99
C24:1n9	1.3	0.4	1.2	0.82	0.89

While most fatty acids were present in similar quantities in both diets S and P, there were some notable exceptions. Diet S contained a higher percentage of saturated fatty acids C14:0 and C16:0 compared to diet P. Conversely, the monounsaturated fatty acid C18:1n9c and the polyunsaturated fatty acid C18:2n6c were more abundant in diet P.

Pearson linear regression analysis revealed a strong positive correlation ($r = 0.91$) between the fatty acid profile of diet S and the fatty acid profile of the related fish (group A) at the end of the experiment. Z-score analysis of the residuals identified significant differences ($p < 0.05$) only for the fatty acids C18:1n9c and C18:2n6c, where the observed values in the fish deviated significantly from those predicted by the regression. Similarly, strong positive correlations were found between the fatty acid profile of diet P and the fatty

acid profiles of fish in groups B (r = 0.98) and C (r = 0.97). However, z-score analysis of the residuals for diet P versus group B showed significant departures for C16:0, EPA, and DHA. Comparable results were obtained for diet P versus group C, with significant differences ($p < 0.05$) in the z-scores of residuals for C16:0, C18:2n6c, EPA, and DHA.

4. Discussion

Reduced yields are a common challenge in fish aquaculture during low water temperatures, primarily due to decreased feed uptake and growth, and a heightened risk of winter syndrome. This is also true for meagre, which exhibit poor feeding and increased disease susceptibility during winter–spring cultivation (December to May) [13], and even show negative growth below 17 °C with various feed formulations [37]. Consistently, the expected growth slowdown occurred in our experiment with industrial pellet-based diets, even when moisturized. Such suppressed growth at low seawater temperatures poses a serious threat to successful meagre rearing in floating cages in the northwestern Mediterranean, where sub-17 °C temperatures are common and prolonged during colder months.

However, contrary to the expected reduction, sardine-based feed resulted in significantly superior yields across key metrics like growth in length and weight, feed and energy intake (TFI and GEI), and growth efficiency (TGC). While the significant impact of feeding and feed composition on farmed fish growth is well documented [16,38–41], our study is the first to demonstrate that feed composition can effectively mitigate the typical growth reduction in meagre during cold conditions.

Feed composition analysis suggests water content, carbohydrates, and lipids are driving the difference in feed intake and growth. The sardine-based diet (S) had an almost five-times-higher water content than even the moisturized pellet-based diet (MP), negligible carbohydrates of about only 3% relative to both pellet-based diets, and a 40% higher lipid fraction (Tables 1 and 2). Given that moisturizing the commercial pellets (MP) did not improve growth (groups B and C, Table 3), the substantial difference in carbohydrate and lipid content, rather than water content alone, likely drove the variations in feed intake and growth rates during cold months. The slight reduction in growth on moisturized compared to dry pellets is consistent with the possibility that a small fraction of fatty acids may have been oxidized and/or leached from the pellets after adding water.

The high protein and lipid content of the sardine diet appears to be particularly favorable for growing meagre at low temperatures, aligning with prior research on meagre protein [17,18,42,43] and lipid requirements [18,42]. The significantly higher condition factor (K) in group A at the end of the experiment suggests better energy reserves, potentially contributing to their resilience against stressors during this unfavorable period. This finding challenges the hypothesis that K decreases with meagre growth [19] when a natural diet is provided.

The doubled values of growth indicators (TGC) for the sardine diet (Table 3) further underscore the superior growth rate and efficiency of the sardine-based diet at low temperatures, supported by the 70% higher feed intake (TFI) and 94% greater energy intake (GEI) compared to the pellet-fed groups. Notably, the sardine diet's positive impact on growth was evident throughout the 141 days where seawater temperatures were below 17 °C (Figures 1 and 2). This highlights the diet's capacity to counteract the typical growth limitations experienced below the 17 °C threshold reported in previous studies [29,44].

Our findings should be extrapolated to adult meagre with caution. Since meagre reach adult sizes up to 103 kg [45], the fish in our study of around 0.3 kg should be considered juvenile. The higher growth potential of juveniles necessitates protein-rich diets, potentially amplifying the benefits of the sardine's higher protein content.

HSI, reflecting the relative size of the liver, and VSI, indicating the proportion of visceral fat, are useful indicators of fish energy status, nutrient metabolism, and overall physiological health. During the experimental period, a general trend of decreasing HSI and VSI values was observed across all experimental groups. This common result of physiological response to cold stress in poikilothermic animals reflects increased mobilization of energy reserves (including liver glycogen and visceral lipids) to cope with the unfavorable thermal conditions and maintain basic physiological functions.

The continuous and robust growth of group A throughout the experiment is particularly noteworthy given the general decline observed in HSI and VSI indices. This further suggests that the higher protein and lipid content of the sardine diet supported efficient growth. While diet composition did not induce statistically significant changes in HSI and VSI overall, the slightly lower final HSI in group A, despite its higher lipid intake, could be attributed to the low carbohydrate content in the sardine diet. This aligns with observations in other species [46].

Consistent with existing literature [18,19,47], we found a correlation between the fatty acid profiles of fish and the diet. The fatty acid composition of group A reflected the sardine diet (higher C14:0 and C16:0), while groups B and C mirrored the pellet diet (higher C18:1n9c and C18:2n6c).

Notably, our findings diverge from the typical observation of lower EPA levels in fish fed commercial diets [3,4,15], a trend we also observed for EPA and DHA in the pellet-fed groups. Instead, the sardine-based diet resulted in higher EPA and DHA levels in the fish than were present in the diet, indicating potential synergistic effects that promote the uptake and/or retention of these valuable long-chain omega-3 fatty acids.

While this study demonstrates the superior growth of juvenile meagre on a sardine diet at low temperatures, using whole sardines as a feed source in commercial aquaculture raises economic and sustainability concerns. Sourcing and consistently providing wild-caught sardines can be expensive and logistically challenging due to fluctuating availability, complex storage, and their use for direct human consumption. Furthermore, relying heavily on wild fish for feed raises significant long-term environmental sustainability questions. Therefore, we suggest using sardines as a guiding principle for future pelleted feed formulation research with the goal to reverse-engineer the nutritional advantages of the sardine diet into cost-effective and sustainable pelleted formulations. The research priorities should include the following:

- Optimizing macronutrient ratios: determining the ideal high protein and lipid levels, coupled with minimized carbohydrates, that mimic the sardine diet's success in cold-water meagre such as increasing the proportion of n-3 PUFA to above 35% [48] and/or increasing dietary tryptophan to mitigate stress [49];
- Enhancing essential fatty acid bioavailability: investigating how to achieve the superior EPA and DHA deposition seen with sardines, potentially through specific lipid sources, processing methods, or functional additives in pellets such as *Fucus vesiculosus*, shown to promote feed efficiency [50];
- Improving pellet functionality: formulating pellets that retain high palatability and are easily digestible even when fish appetite and metabolism are reduced in cold temperatures;
- Improving sustainability: investigate alternative protein and lipid sources such as plant-based proteins and animal by-products suggested by Matias et al. [51], which replicate the sardine's beneficial profile without relying on wild fisheries.

5. Conclusions

In conclusion, this study demonstrated that a sardine-based diet significantly enhances the growth performance of juvenile meagre in cage farming conditions at low seawater temperatures that typically suppress growth. This finding contrasts with the limited success of commercial pellet-based diets, even with moisturization, and highlights the potential of natural feed sources to overcome the challenges of cold-water aquaculture for this species. The superior growth, feed utilization, and condition factor observed in meagre fed sardines suggest that the nutritional profile of this natural diet, particularly its high protein and lipid content, is better suited to their requirements at lower temperatures. While our results are confirmed only for juvenile meagre, further research into the specific components of sardines responsible for these benefits could inform the development of more effective and sustainable commercial feeds for meagre aquaculture, especially in regions experiencing prolonged cold periods such as the northwestern Mediterranean.

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References

1. Jiménez, M.T.; Pastor, E.; Grau, A.; Alconchel, J.I.; Cárdenas, S. Revisión sobre el cultivo de esciénidos en el mundo, con especial atención a la corvina (*Argyrosomus regius*). *Bol. Inst. Esp. Oceanogr.* **2005**, *21*, 169–176.
2. Pastor, G.E.; Grau, J.A.; Massutí, P.E.; Sánchez, L.R.Y.A. Preliminary results on growth of meagre, *Argyrosomus regius* (Asso, 1801) in sea cages and indoor tanks. Aquaculture Europe Seafarming, today and tommorow. *EAS/Spec. Publ.* **2002**, *32*, 422–423.
3. Poli, B.M.; Parisi, G.; Zampacavallo, G.; Iurzan, F.; Mecatti, M.; Lupi, P.; Bonelli, A. Preliminary results on quality and quality changes in reared meagre (*Argyrosomus regius*): Body and fillet traits and freshness changes in refrigerated commercial-size fish. *Aquac. Int.* **2003**, *11*, 301–311. [CrossRef]
4. Grigorakis, K.; Fountoulaki, E.; Vasilaki, A.; Mittakos, I.; Nathanailides, C. Lipid quality and filleting yield of reared meagre (*Argyrosomus regius*). *Int. J. Food Sci. Technol.* **2011**, *46*, 711–716. [CrossRef]
5. FAO. Global Aquaculture Production Quantity (1950–2021). 2023. Available online: https://www.fao.org/fishery/statistics-query/en/aquaculture/aquaculture_quantity (accessed on 20 November 2023).
6. Brett, J.R. Environmental factors and growth. In *Fish Physiology*; Hoar, D.J., Randall, J.R., Brett, J.R., Eds.; Academic Press: New York, NY, USA, 1979; pp. 599–667.
7. Ibarz, A.; Padrós, F.; Galardo, M.Á.; Fernández-Borràs, J.; Blasco, J.; Tort, L. Low-temperature challenges to gilthead sea bream culture: Review of cold-induced alterations and "Winter Syndrome". *Rev. Fish Biol. Fish.* **2010**, *20*, 539–556. [CrossRef]

8. Bowyer, J.N.; Qin, J.G.; Stone, D.A.J. Protein, lipid and energy requirements of cultured marine fish in cold, temperate and warm water. *Rev. Aquac.* **2013**, *5*, 10–32. [CrossRef]
9. Lavié, A.; Rodríguez-Rúa, A.; Ruiz-Jarabo, I.; Vargas-Chacoff, L.; Mancera, J.M.; Cárdenas, S. Influencia de la densidad de cultivo y la temperatura sobre el crecimiento y el metabolismo en alevines de corvina, (*Argyrosomus regius*, Asso, 1801). In Proceedings of the IV Jornadas de Acuicultura del Litoral Suratlántico, Cartaya, Spain, 16–17 April 2008.
10. Gandra, M.; Winkler, A.C.; Afonso, P.; Abecasis, D. Long-distance migrations and seasonal movements of meagre (*Argyrosomus regius*), a large coastal predator, along the Iberian Peninsula coast. *Mov. Ecol.* **2024**, *12*, 35. [CrossRef]
11. Calderón, J.A.; Esteban, J.C.; Carrascosa, M.A.; Ruiz, P.L.; Valera, F. Rearing and growth in captivity of a lot of meagre reproducers (*Argyrosomus regius*). In Proceedings of the Actas VI Congreso Nacional de Acuicultura, Cartagena, Murcia, Spain, 9–11 July 1997; Ministerio de Agricultura, Pesca y Alimentación: Madrid, Spain, 1997; pp. 365–370.
12. FAO. *Argyrosomus regius*. Cultured Aquatic Species Information Programme. Text by Stipa, P.; Angelini, M. Fisheries and Aquaculture Division [online]. Rome. 2022. Available online: https://www.fao.org/fishery/en/culturedspecies/argyrosomus_regius/en (accessed on 29 November 2022).
13. Kružić, N.; Mustać, B.; Župan, I.; Čolak, S. Meagre (*Argyrosomus regius*, Asso, 1801) Aquaculture in Croatia. *Croat. J. Fish.* **2016**, *74*, 14–19. [CrossRef]
14. Gustinelli, A.; Čolak, S.; Quaglio, F.; Sirri, R.; Kolega, M.; Mejdandžić, D.; Caffara, M.; Barić, R.; Fioravanti, M.L. Histological assessment of systemic granulomatosis progression in meagre *Argyrosomus regius* during cage ongrowing phase. *Dis. Aquat. Org.* **2021**, *145*, 165–172. [CrossRef]
15. Piccolo, G.; Bovera, F.; De Riu, N.; Marono, S.; Salati, F.; Cappuccinelli, R.; Moniello, G. Effect of two different protein/fat ratios of the diet on meagre (*Argyrosomus regius*) traits. *Ital. J. Anim. Sci.* **2008**, *7*, 363–371. [CrossRef]
16. Chatzifotis, S.; Panagiotidou, M.; Papaioannou, N.; Pavlidis, M.; Nengas, I.; Mylonas, C.C. Effect of dietary lipid levels on growth, feed utilization, body composition and serum metabolites of meagre (*Argyrosomus regius*) juveniles. *Aquaculture* **2010**, *301*, 65–70. [CrossRef]
17. Chatzifotis, S.; Panagiotidou, M.; Divanach, P. Effect of protein and lipid dietary levels on the growth of juvenile meagre (*Argyrosomus regius*). *Aquac. Int.* **2012**, *20*, 91–98. [CrossRef]
18. Martínez-Llorens, S.; Espert, J.; Moya, J.; Cerdá, M.J.; Tomás-Vidal, A. Growth and nutrient efficiency of meagre (*Argyrosomus regius*, Asso, 1801) fed extruded diets with different protein and lipid levels. *Int. J. Aquac. Fish. Sci.* **2011**, *3*, 195–203.
19. Fountoulaki, E.; Grigorakis, K.; Kounna, C.; Rigos, G.; Papandroulakis, N.; Diakogeorgakis, J.; Kokou, F. Growth performance and product quality of meagre (*Argyrosomus regius*) fed diets of different protein/lipid levels at industrial scale. *Ital. J. Anim. Sci.* **2017**, *16*, 685–694. [CrossRef]
20. Velazco-Vargas, J.; Martínez-Llorens, S.; Cerda, M.J.; Tomás-Vidal, A. Evaluation of soybean meal as protein source for *Argyrosomus regius* (Asso, 1801) (Sciaenidae). *Int. J. Fish. Aquac.* **2013**, *5*, 35–44.
21. Emre, Y.; Kurtoğlu, A.; Emre, N.; Güroy, B.; Güroy, D. Effect of replacing dietary fish oil with soybean oil on growth performance, fatty acid composition and haematological parameters of juvenile meagre, *Argyrosomus regius*. *Aquac. Res.* **2015**, *47*, 2256–2265. [CrossRef]
22. Ribeiro, L.; Moura, J.; Santos, M.; Colen, R.; Rodrigues, V.; Bandarra, N.; Soares, F.; Ramalho, P.; Barata, M.; Moura, P.; et al. Effect of vegetable-based diets on growth, intestinal morphology, activity of intestinal enzymes and haematological stress indicators in meagre (*Argyrosomus regius*). *Aquaculture* **2015**, *447*, 116–128. [CrossRef]
23. Vargas-Chacoff, L.; Ruiz-Jarabo, I.; Páscoa, I.; Gonçalves, O.; Mancera, J.M. Yearly growth and metabolic changes in earthen pond-cultured meagre *Argyrosomus regius*. *Sci. Mar.* **2014**, *78*, 193–202. [CrossRef]
24. Chatzifotis, S.; Papadakis, A.E.; Divanach, P. Effects of dietary water on growth of dentex *Dentex dentex*. *Fish. Sci.* **2005**, *71*, 1243–1248. [CrossRef]
25. Papadakis, I.E.; Chatzifotis, S.; Divanach, P.; Kentouri, M. Weaning of greater amberjack (*Seriola dumerilli* Risso, 1810) juveniles from moist to dry pellet. *Aquac. Int.* **2008**, *16*, 13–25. [CrossRef]
26. Pryzybla, C.; Fievet, J.; Callier, M.; Blancheton, J.P. Effect of dietary water content on European sea bass (*Dicentrarchus labrax*) growth and disease resisitance. *Aquat. Living Resour.* **2014**, *27*, 73–81. [CrossRef]
27. Cardenete, G.; Skalli, A.; Hidalgo, M.C.; Abellán, E.; Massuti, S. Feeding *Dentex dentex* with dry diets: Growth response and diet utilization. In *Feeding Tomorrow's Fish*; Tacon, A.G.J., Basurco, B., Eds.; CIHEAM Cahiers Options Méditerranéennes: Zaragoza, Spain, 1997; Volume 22, pp. 141–151.
28. Mazzola, A.; Favaloro, E.; Sarà, G. Cultivation of the Mediterranean amberjack, *Seriola dumerili* (Risso, 1810), in submerged cages in the Western Mediterranean Sea. *Aquaculture* **2000**, *181*, 257–268. [CrossRef]
29. Duncan, N.J.; Estévez, A.; Fernández-Palacios, H.; Gairin, I.; Hernández-Cruz, C.M.; Roo, J.; Schuchardt, D.; Vallés, R. Aquaculture production of meagre (*Argyrosomus regius*): Hatchery techniques, ongrowing and market. In *Advances in Aquaculture Hatchery Technology*; Alan, G., Burnell, G., Eds.; Woodhead Publishing: Cambridge, UK, 2013; pp. 519–541.

30. Abdelhamid, A.M.; Fedekar, F.; Madkour, F.F.; Abou Elregal, M.A.; Radwan, A.M. Effect of feeding regimen on growth performance, feed utilization, body composition and economic efficiency by *Argyrosomus regius* (Asso, 1801). *J. Arab. Aquac. Soc.* **2013**, *8*, 53–56.
31. Bagenal, T.B.; Tesch, F.W. *Age and Growth in Methods for Assessment of Fish Production in Fresh Waters*; Blackwell Scientific Publications: Oxford, UK, 1978; pp. 101–136.
32. Association of Official Analytical Chemists. *Association of Official Analytical Chemists (AOAC) Official Methods of Analysis*, 16th ed.; Association of Official Analytical Chemists: Arlington, VA, USA, 1995.
33. Folch, J.; Lees, M.; Stanley, G.H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem.* **1957**, *226*, 497–509. [CrossRef] [PubMed]
34. Steffens, W. *Principles of Fish Nutrition*; Ellis Horwood: Chichester, UK, 1989; 397p.
35. *ISO 12966-2*; Animal and Vegetable Fats and Oils—Gas Chromatography of Fatty Acid Methyl Esters-Part 2: Preparation of Methyl Esters of Fatty Acids. International Standards Organization (ISO): Geneva, Switzerland, 2017.
36. *ISO 12966-4*; Animal and Vegetable Fats and Oils—Gas Chromatography of Fatty Acid Methyl Esters-Part 4: Determination by Capillary Gas Chromatography. International Standards Organization (ISO): Geneva, Switzerland, 2015.
37. Estévez, A.; Treviño, L.; Kotzamanis, Y.; Karacosta, I.; Tort, L.; Gisbert, E. Effects of different levels of plant proteins on the ongrowing of meagre (*Argyrosomus regius*) juveniles at low temperatures. *Aquac. Nutr.* **2011**, *17*, 572–582. [CrossRef]
38. Hardy, R.W.; Barrows, F.T. Diet Formulation and Manufacture. In *Fish Nutrition*; Halver, J.E., Hardy, R.W., Eds.; Academic Press: London, UK, 2002; pp. 505–600.
39. Kokou, F.; Henry, M.; Alexis, M. Growth performance, feed utilization and non-specific immune response of gilthead sea bream (*Sparus aurata* L.) fed graded levels of a bioprocessed soybean meal. *Aquaculture* **2012**, *364*, 74–81. [CrossRef]
40. Mongile, F.; Mandrioli, L.; Mazzoni, M.; Pirini, M.; Zaccaroni, A.; Sirri, R.; Parma, L.; Gatta, P.P.; Sarli, G.; Bonaldo, A. Dietary Inclusion of Mussel Meal Enhances Performance and Improves Feed and Protein Utilization in Common Sole (*Solea solea*, Linnaeus, 1758) Juveniles. *J. Appl. Ichtyol.* **2015**, *31*, 1077–1085. [CrossRef]
41. Boujard, T.; Gélineau, A.; Covès, D.; Corraze, G.; Dutto, G.; Gasset, E.; Kaushik, S. Regulation of feed intake, growth, nutrient and energy utilisation in European sea bass (*Dicentrarchus labrax*) fed high fat diets. *Aquaculture* **2004**, *231*, 529–545. [CrossRef]
42. Velazco-Vargas, J.; Tomás-Vidal, A.; Hamdan, M.; Moyano López, F.J.; Jover Cerda, M.; Martínez-Llorens, S. Influence of digestible protein levels on growth and feed utilization of juvenile meagre *Argyrosomus regius*. *Aquac. Nutr.* **2014**, *20*, 520–531. [CrossRef]
43. Gil, M.M.; Palmer, M.; Hernández, M.D.; Grau, A.; Durán, J.; García, B.G.; Jover, M.; Pastor, E. Rearing diet may determine fish restocking success: A case study of hatchery-reared juvenile meagre, *Argyrosomus regius*. *Sci. Mar.* **2015**, *79*, 431–441. [CrossRef]
44. Kounna, C.; Fountoulaki, E.; Miliou, H.; Chatzifotis, S. Water temperature effects on growth performance, proximate body and tissue composition, morphometric characteristics and gastrointestinal evacuation processes of juvenile meagre, *Argyrosomus regius* (Asso, 1801). *Aquaculture* **2021**, *540*, 736683. [CrossRef]
45. Quéro, J.C.; Vayne, J.J. Le maigre, *Argyrosomus regius* (Asso 1801) (Pisces, Perciformes, Sciaenidae) du Golfe de Gascogne et des eaux plus septentrionales. *Rev. Trav. Péches Marit.* **1987**, *49*, 35–66.
46. Mongile, F.; Bonaldo, A.; Fontanillas, R.; Mariani, L.; Badiani, A.; Bonvini, E.; Parma, L. Effects of Dietary Lipid Level on Growth and Feed Utilisation of Gilthead Seabream (*Sparus aurata* L.) Reared at Mediterranean Summer Temperature. *Ital. J. Anim. Sci.* **2014**, *13*, 2999. [CrossRef]
47. Tocher, D.R. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fish. Sci.* **2003**, *11*, 107–184. [CrossRef]
48. Matulić, D.; Blažina, M.; Pritišanac, E.; Čolak, S.; Bavčević, L.; Barić, R.; Križanac, S.; Vitlov, B.; Šuran, J.; Strunjak Perović, I.; et al. Growth, Fatty Acid Profile and Malondialdehyde Concentration of Meagre *Argyrosomus regius* Fed Diets with Different Lipid Content. *Appl. Sci.* **2024**, *14*, 4842. [CrossRef]
49. Vasconcelos, A.; Soares, M.C.; Barata, M.; Couto, A.; Teixeira, B.; Ribeiro, L.; Pousão-Ferreira, P.; Mendes, R.; Saavedra, M. Effect of Tryptophan Dietary Content on Meagre, *Argyrosomus regius*, Juveniles Stress and Behavioral Response. *Animals* **2023**, *13*, 3762. [CrossRef]
50. Monteiro, M.; Sousa, C.; Coutinho, F.; Castro, C.; Fontinha, F.; Guerreiro, I.; Pousão, P.; Matos, E.; Díaz-Rosales, P.; Oliva-Teles, A.; et al. Functional Feeds to TackleMeagre (*Argyrosomus regius*) Stress: Physiological Responses under Acute Stressful Handling Conditions. *Mar. Drugs* **2021**, *19*, 598. [CrossRef]
51. Matias, A.C.; Quental-Ferreira, H.; Dias, J.; Saavedra, M.; Bandarra, N.M.; Araújo, R.L.; Gamboa, M.; Soares, F.; Pousão-Ferreira, P. Evaluation of Alternative Dietary Ingredients as a Sustainable and Ecological Solution for Meagre (*Argyrosomus regius*) Production in Earthen Ponds. *Fishes* **2024**, *9*, 517. [CrossRef]