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Early weaning in meagre *Argyrosomus regius*: Effects on growth, survival, digestion and skeletal deformities

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Abstract

Meagre *Argyrosomus regius* is considered a new species for the diversification of finfish aquaculture in the Mediterranean. Several bottlenecks have been identified by producers, and among them, the necessity to establish early weaning protocols to reduce production costs. In this study, two experiments were carried out with meagre larvae from 2 to 35 days post hatch (dph) using different weaning strategies, including the early introduction of artificial diets and the reduction of *Artemia* metanauplii to half of the normal amounts. A high frequency of cannibalism and high variability in growth rate and survival were obtained in one of the trials and several changes were introduced (reduction of light intensity, higher frequency of food distribution) in the second trial to increase the survival rate. In both trials, weaning started before the complete morphological and functional development of the stomach; thus, pancreatic enzymes, mainly trypsin and lipase tended to be more active in early weaned larvae compared to the control groups. Early weaning delayed the development of the stomach formation and secretion of acid proteases, which may explain the lower growth rates observed in our study. The effect of weaning on skeletal development was also studied and in this sense the results obtained showed no major influence of the early weaning on the incidence of skeletal deformities. Weaning of meagre larvae can be performed as early as 12 dph, but important aspects such as avoiding cannibalism and co-feeding live prey and artificial diets for at least 5 days were recommended.

KEYWORDS
cannibalism, digestive enzymes, growth, meagre, skeletal deformities, weaning

1 | INTRODUCTION

Larval rearing of meagre *Argyrosomus regius* is usually carried out following a protocol based in European sea bass (*Dicentrarchus labrax*) and gilthead sea bream (*Sparus aurata*) larval rearing (Estévez, Treviño & Gisbert, 2007; Roo, Hernández-Cruz, Borrero, Fernández-Palacios & Schuchardt, 2007; Roo, Hernández-Cruz, Borrero, Schuchardt & Fernandez-Palacios, 2010), including the use of enriched live feeds (rotifers, *Brachionus* sp. and *Artemia* sp. metanauplii). However, larviculture practices based on live preys represent a high cost compared to inert diets, both in terms of production and labour costs. Different studies have revealed that these protocols need to be adapted to the biological demands of this species, as meagre larvae are quite sensitive to stress produced by high light intensity (>500 lux at water surface), long photoperiods or high densities of live prey (Roo et al., 2010; Suzer, Kamaci, Coban, Firat & Saka, 2013; Vallés & Estevez, 2013). Although the precise nutritional requirements for meagre have not been completely established, larvae show very good growth performance and survival rates using commercially available products for live prey enrichment and feeds

for weaning (Vallés & Estevez, 2015). Meagre producers do not consider larval rearing to be a major bottleneck for meagre culture (Lazo, Holt, Fauvel, Suquet & Quemener, 2010), and only cannibalism and variable size distribution in juveniles are considered the main concern in the intensive production of meagre fry, as they reduce production yield and increase the production costs. Therefore, advancing the early weaning of larvae from its dependence on *Artemia* onto a dry feed is a priority and the major focus of the current larval research on meagre. In this sense, a better knowledge of larval digestive physiology under a new feeding protocol may contribute to the optimization of diets (Zambonino-Infante et al., 2008) and may help to understand functions and limitations in the processing capacity of the digestive system, and consequently the delivery of nutrients to the rapidly growing larval tissues under an earlier weaning protocol (Rønnestad et al., 2013). Thus, establishing an adequate feeding protocol adapted to the digestive capacities and nutritional needs during early development while also addressing options to reduce cannibalism and size dispersion are of primary importance to improve survival and growth in meagre.

Skeletal deformities in cultured fish are a major factor that reduces production, suppresses growth and increases economic loss, as well as leads to high mortality rates. Most skeletal abnormalities appear during the larval and juvenile stages where several factors can interfere with the normal development of larvae. Existing literature clearly suggests that unfavourable abiotic conditions, inappropriate nutrition and genetic factors are the most possible causative factors of skeletal anomalies in reared fish (Boglione et al., 2013). In this regard, nutritional imbalances are known to play a key role in morphogenesis and skeletogenesis at early stages (Boglione et al., 2013; Person Le Ruyet, Alexandre, Thebaud & Mugnier, 1993). Thus, the efficiency of early weaning practices and its effects on skeletal development in meagre larvae needs also to be considered.

Thus, the objectives of the present study were (1) establish a weaning protocol for meagre to reduce production losses due to cannibalism, (2) study the changes in digestive enzyme activity when live preys are replaced by dry feed and (3) describe possible skeletal deformities derived from early weaning onto artificial diets.

2 | MATERIAL AND METHODS

2.1 | Larval rearing and experimental design

Fertilized eggs of meagre were obtained from a wild broodstock maintained in 4,000 l circular tanks connected to recirculation units (IRTAMar®, IRTA, Spain) at IRTA Centre of San Carles de la Rapita under controlled conditions and after hormonal induction (Duncan et al., 2012). Floating eggs were incubated in 35 L cylindrical PVC containers provided with air-lift systems and high aeration supply. On day 2 post hatching (dph), larvae were stocked into 100 L tanks at a density of 100 larvae/L and cultured from 2 to 35 dph on different dietary treatments. The 100 L tanks were connected to IRTAmar® units with 50% daily water renewal. Temperature (18.2 ± 0.5°C), salinity (35.4 ± 0.3 g/L), dissolved oxygen (7.9 ± 0.3 mg/L) and pH (7.9 ± 0.2) were checked daily, whereas nitrites (<0.25 mg/L) and ammonia (<0.07 mg/L) were measured once per week (Hach Colorimeter DR/890, USA). Light intensity was regulated with a manual potentiometer connected to each fluorescent lamp (Philips LPS100) and measured at the water surface in the middle of the tank with a luxometer (Lutron LX-101 LUX METER) and maintained at 500 lux at water surface, whereas the light regime was 12-hr light: 12-hr dark.

Larvae were fed enriched rotifers (*Brachionus sp*) from 2 dph until 14 dph at a density of 10 rotifers/ml and *Artemia* metanauplii (Sep Art Artemia, Inve, Belgium) from 9 dph starting with 0.5 metanauplii/ml, increasing the density up to 6 metanauplii/ml at 20 dph and decreasing the density down to 1.5 nauplii/ml at 25 dph, and keeping that density until the end of the trial. Both live preys were enriched using Red Pepper™ (Bernaqua, Belgium) for 12 hr at 28°C in the case of rotifers and 6 h at 25°C in the case of *Artemia*. Larvae were fed two doses of live prey (morning and evening) every day, whereas dry feed (Gemma Micro, Skretting, Norway) was administered by hand every morning at 9:00 hours and using automatic feeders every hour, from 9:00 to 20:00 hours. The amount of feed was adjusted to reach the level of apparent feeding satiation. Every day, the bottom of the tank was siphoned to remove dead fish, uneaten food and faeces.

Two experiments were carried out with meagre larvae. In Trial 1, the following experimental protocols were tested in triplicate:

Group A: weaning on dry feed started from 20 dph and it was completed at 30 dph (control group) following the standard protocol described above; Group B: weaning started from 20 dph and it was completed at 30 dph (the same as in Group A but using half the amount of *Artemia* metanauplii); Group C: weaning started from 15 dph and it ended at 25 dph using also half the amount of *Artemia* metanauplii; and Group D: weaning started at 12 dph and it was completed at 23 dph using half the amount of *Artemia* metanauplii.

Due to the high incidence of cannibalism (so-called coeval cannibalism; Folkvord, 1997) among similar-aged individuals observed in Trial 1, several changes in the rearing protocol were introduced in Trial 2. Thus, light intensity was reduced from 500 lux to 150–200 lux from 13 dph onwards, as well as the number of doses/meals of *Artemia* (given at 10:00, 13:00, 16:00 and 18:00 hours) and the artificial diet that were increased in order to provide enough food to larvae. This strategy was chosen according to Smith and Reay (1991) who reported that cannibalism is enhanced by low food availability, high fish densities, size disparity and lack of refuges. Thus, in Trial 2, only two treatments were tested (five replicates each): Group E: weaning started from 20 dph and it was completed at 30 dph (control group); and Group F: weaning started at 12 dph and it ended at 23 dph.

In both trials, 10 larvae from each tank were randomly collected to measure growth (standard length, SL and dry weight, DW) every week. Fish were previously anaesthetized with tricaine methanosulphonate (MS-222, Sigma-Aldrich, Spain) and SL measured under a stereomicroscope Nikon SMZ800 (Nikon, Tokyo, Japan) equipped with a digital camera Olympus DP70 (Olympus, Hamburg, Germany)

and an image analyser (AnalySIS, SIS Gmbh, Hamburg, Germany). The same larvae were used to estimate wet and dry weight, placing them, previously washed with distilled water, on preweighted coverslips, dried in an oven at 60°C for 24 hr, and weighted in a microbalance Mettler MX5 (Mettler Toledo, Barcelona, Spain).

Specific growth rate (SGR) was calculated using the following formula: SGR (%day^{-1}) = Ln DW_f − Ln $DW_i/t_f - t_i$; where DW_f and DW_i were the final and initial larval dry weight ant $t_f - t_i$ the days from the initial until the end of the sampling periods.

The coefficient of variation (CV, %) of weight was calculated according to the formula CV = standard deviation/mean × 100.

Larvae were randomly collected when the weaning started and the feed was changed from live to inert diets that was at 12 dph (Group D), 15 dph (Group C), 20 dph (Groups A and B) and at 24 dph in Trial 1 and at the end of Trial 2 (35 dph), to analyse the activity of digestive enzymes. In this case, sampled larvae were sacrificed with overdose of MS-222, rinsed in distilled water and conserved at −80°C until analysis. At the end of the trial, survival was evaluated by counting the survivors at the end of the experiment and calculated according to Buckley et al. (1984) that consider the number of sampled individuals during the experiment.

2.2 | Determination of digestive enzyme activities

Larvae in both Trials 1 and 2 (5–150 individuals depending on age and size) were collected for enzyme analyses at 12, 15, 20 and 24 dph in Trial 1 and at 35 dph in Trial 2. For quantifying the activity of pancreatic and gastric enzymes (total alkaline proteases, α-amylase, lipase and pepsin), samples were homogenized (Ultra-Turrax T25 basic, IKA©-Werke, Germany) in 5 volumes (v/w) of ice-cold Milli-Q water, centrifuged at 3,300 g for 3 min at 4°C, the supernatant removed for enzyme quantification and kept at −80°C until further analysis. For the quantification of the intestinal brush border (BB) enzyme, alkaline phosphatase samples were homogenized in cold 50 mM mannitol, 2 mM Tris-HCl buffer (pH 7.0) and intestinal BB membranes purified according to Crane et al. (1979).

Enzyme activities of pancreatic, gastric and intestinal enzymes were conducted as described in Gisbert, Giménez, Fernández, Kotzamanis and Estévez (2009) and Solovyev et al. (2016). In addition, spectrophotometric analyses were performed as recommended by Solovyev and Gisbert (2016) in order to prevent sample deterioration due to their short- and long-term storage. In brief, trypsin (E.C. 3.4.21.4) activity was assayed at 25°C using BAPNA (N-α-benzoyl-DL-arginine p-nitroanilide) as substrate. One unit of trypsin per ml (U) was defined as 1 μmol BAPNA hydrolysed per min per ml of enzyme extract at 407 nm (Holm, Hanssen, Krogdahl & Florholmen, 1988). Alpha-amylase (E.C. 3.2.1.1) activity was determined according to Métais and Bieth (1968), using 0.3% soluble starch as substrate, and its activity (U) was defined as the mg of starch hydrolysed during 30 min and ml of tissue homogenate at 37°C at 580 nm. Bile salt-activated lipase (BALT, E.C. 3.1.1) activity was assayed for 30 min at 30°C using p-nitrophenyl myristate as substrate. The reaction was stopped with a mixture of acetone: n-heptane (5:2), the extract centrifuged for 2 min at 6,080 *g* and 4°C and the absorbance of the supernatant read at 405 nm. Bile salt-activated lipase activity (U/ml) was defined as the nmol of substrate hydrolysed per min per ml of enzyme extract (Iijima, Tanaka & Ota, 1998). Regarding intestinal enzymes, alkaline phosphatase (E.C. 3.1.3.1) was quantified at 37°C using 4-nitrophenyl phosphate (PNPP) as substrate. One unit (U) was defined as 1 μg BTEE released per min per ml of brush border homogenate at 407 nm (Bessey, Lowry & Brock, 1946). Finally, pepsin activity (U) was defined as the μmol of tyrosine liberated per min at 37°C per ml of tissue homogenate at 280 nm (Worthington Biochemical Corporation, 1991). All enzymatic activities were expressed as specific activity defined as milliunits per milligram of protein (mU/mg protein). Soluble protein of crude enzyme extracts was quantified by means of the Bradford's method (Bradford, 1976) using bovine serum albumin as standard. All the assays were made in triplicate from each pool of larvae and absorbance read using a spectrophotometer (Tecan™ Infinite M200, Mannedorf, Switzerland).

2.3 | Analysis of skeletal deformities

To evaluate the impact of different weaning strategies on the incidence of skeletal deformities in meagre, 20 early juveniles aged 35 dph per tank were randomly sampled at the end of Trial 2. Fish were preserved in 10% buffered formalin and stored until double staining. Animals were stained with Alcian blue 8X and alizarin red (Sigma-Aldrich, Barcelona, Spain) to detect cartilaginous and bony tissues as described in Darias, Lan Chow Wing, Cahu, Zambonino-Infante and Mazurais (2010). Once stained, fish were individually examined under a dissection microscope by two independent observers. The incidence of skeletal abnormalities was determined in the cranium, vertebral column and caudal fin complex. Special attention was given to vertebral deformities, which were divided in two categories: "severe," which included the fusion and compression of adjacent vertebral bodies, deformation of vertebral bodies and changes in the anterior–posterior alignment of vertebrae (kyphosis and lordosis), and "light," including deformed haemal spines and neural spines, and changes in the osteological organization of the caudal fin complex. The nomenclature of skeletal elements was conducted according to the description of meagre skeletogenesis (Cardeira et al., 2012).

2.4 | Statistical analyses

Data were expressed as mean ± standard deviation (SD) except for skeletal anomalies that were expressed in mean ± standard error of the mean (SEM) and tested by Student's t test (Trial 2, with only two treatments) or one-way ANOVA (Trial 1, with four treatments). When a significant difference was found between treatments, a Tukey's test was performed for multiple range comparisons with the level of significant difference set at $p < 0.05$. All the data were tested for normality, homogeneity and independence to satisfy the

assumptions of ANOVA, whereas data expressed as percentage were previously arcsine-transformed.

3 | RESULTS

3.1 | Growth and survival

The results in terms of larval growth in SL and DW from Trial 1 are shown in Figure 1. Growth in SL and DW of meagre aged 35 dph from Groups C, weaned at 15 dph with half the amount of *Artemia* metanauplii, and A, control, was higher than in the rest of the other groups ($p < 0.005$), whereas fish from Group B showed the lowest values in SL and DW among groups (data not shown). Specific growth rates of the different groups were 25.74% day^{-1} for Group A and 21.86%, 24.79% and 23.39% for Groups B, C and D respectively. Survival rates were significantly affected by the weaning strategy ($p < 0.05$), being higher in fish from the Group B (2.8 ± 0.6%), whereas meagre from Groups A, C and D showed similar and lower values (1.7 ± 0.1%, 1.2 ± 0.3% and 1.8 ± 0.3% respectively).

Large differences in size were detected among experimental groups at the end of the Trial 1, as indicated by the values of the coefficient of variation (CV) for SL (Figure 2). In particular, fish showing the largest size dispersion values were those from Group D (49.2 ± 20.2%), whereas fish from Groups A, B and C showed similar CV values (23.2 ± 3.4%, 24.1 ± 5.3% and 23.8 ± 8.1% respectively). Differences in DW among small, medium and large larvae are also shown in Table 1, with larvae weighing from 1.28 to 38.28 mg of dry weight depending on the group, such wide range of sizes might have enhanced larval cannibalism.

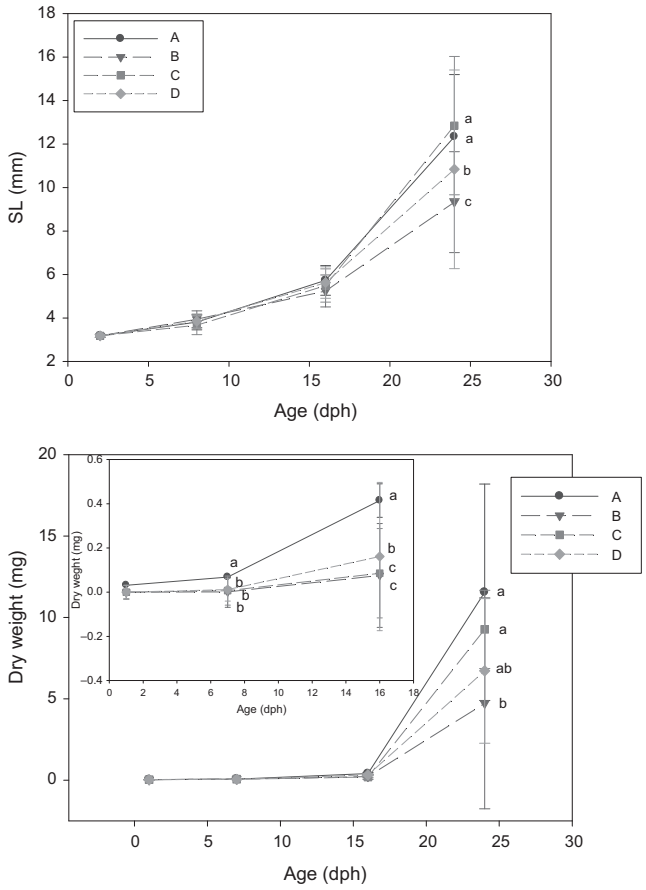

FIGURE 1 Growth in standard length (SL) (mm, mean ± SD) and dry weight (DW) (mg, mean ± SD) of meagre (*Argyrosomus regius*) larvae from the different groups at different sampling times in Trial 1. Different letters show significant differences (ANOVA, $p < 0.001$)

The results in larval growth in SL and DW from Trial 2 are shown in Figure 3a. Growth performance and survival rates were higher in meagre from the Group E, weaned at 20 dph and fed using the standard protocol, than those obtained in Group F (larvae weaned at 12 dph) ($p < 0.05$). Specific growth rates of larvae between 1 and 30 dph were 18.95%/day for the control group and 17.06%/day for early weaned larvae, values that were almost 1.5 times lower than those observed in Trial 1. In this case, the size between the groups was similar, with a CV value of 0.12 and 0.13 for groups E and F respectively (Figure 3b). Survival rate from Group E (4.9 ± 0.7%) was also higher than in the early weaned Group F (3.9 ± 0.5%), similarly to the results obtained in Trial 1. Measures adopted in Trial 2 to reduce cannibalistic behaviour (reduction of light intensity and higher feeding frequency) had positive results lowering the size differences among the larvae and improving the survival rate at the end of the trial.

3.2 | Activity of digestive enzymes

In both trials, digestive enzyme activities analysed in larvae at the end of live prey feeding period were the same for all the treatments (data not shown). The activity of the pancreatic and intestinal digestive enzymes was assessed in 24 dph larvae in the case of Trial 1 and 35 dph larvae for Trial 2; the results are shown in Figure 4. In Trial 1, trypsin activity was higher in meagre from Group C in comparison with larvae from Groups A and B, whereas fish from Group D showed intermediate values in trypsin activity ($p < 0.05$). Similarly, lipase activity was higher in larvae from Group B in comparison with the rest of the treatments ($p < 0.05$), whereas no statistically significant differences were found with regard to α-amylase activity between groups ($p > 0.05$). The activity of the brush border enzyme, alkaline phosphatase, was highest in fish from Group A, whereas the lowest values were observed in meagre from Group D ($p < 0.05$), and Groups B and C showed intermediate values. Similarly to Trial 1, there were no differences neither in α-amylase activity nor in trypsin regardless of the weaning strategy used in Trial 2 ($p > 0.05$), whereas lipase activity was higher in early weaned larvae (Group F, $p < 0.05$). The activity of alkaline phosphatase was higher in larvae from the control Group E than in Group F ($p < 0.05$). Regarding the activity of acid proteases, pepsin was only analysed in larvae from Trial 2; in particular, early weaned larvae (12 dph) showed a significantly lower activity of this acid protease (66.97 ± 20.03 U mg protein^{-1}) compared with larvae weaned at 20 dph (91.83 ± 10.76 U mg protein^{-1}) ($p < 0.05$).

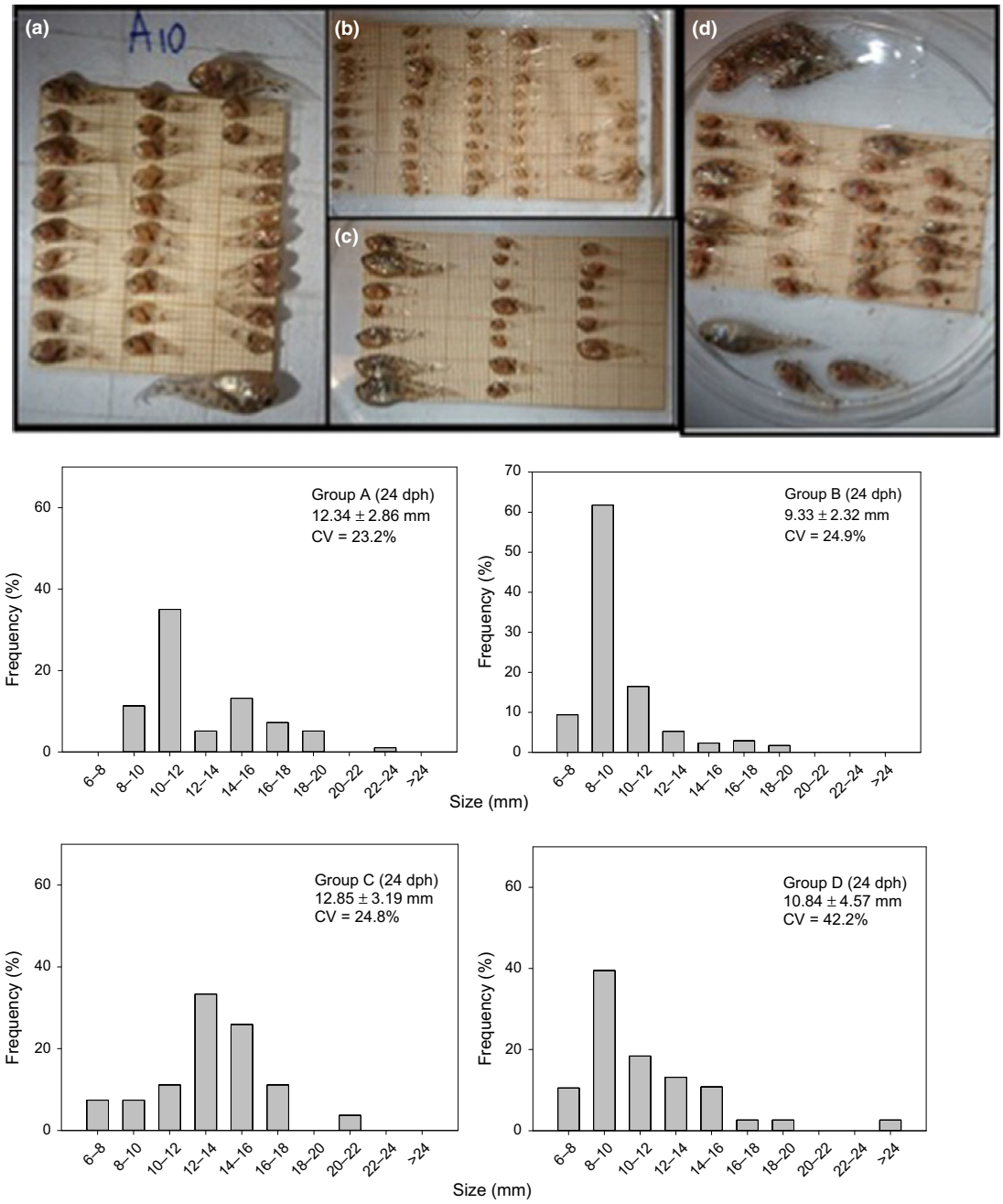

FIGURE 2 Photographs and frequency diagrams showing the differences in the size (SL) of the larvae (24 dph) of the experiment 1 in the groups a (left), b (centre, up), c (centre, down) and d (right), due to the high incidence of cannibalism. CV, coefficient of variation

TABLE 1 Differences in dry weight (mg, average ± SD) among small, medium and large larvae in the four feeding groups (A, B, C and D)

Group	Small	Medium	Large
A	4.52 ± 0.97	16.06 ± 9.58	38.28 ± 8.41
B	1.28 ± 0.16	8.00 ± 7.69	18.67 ± 9.75
C	3.73 ± 1.55	12.27 ± 3.79	23.17 ± 5.11
D	3.01 ± 1.29	8.91 ± 3.89	26.41 ± 2.77

3.3 | Skeletal deformities

In Trial 2, early weaning of the larvae did not have any effect on the incidence of total skeletal deformities in early juveniles of meagre ($p > 0.05$) with an average frequency of deformed fish ranging from 17.6% to 21.0% nor in the number of vertebral bodies (98.7% specimens with 25 vertebrae and 1.3% with 24). In addition, no differences were found in the incidence of light and severe skeletal deformities among groups ($p < 0.05$), being the light one the most

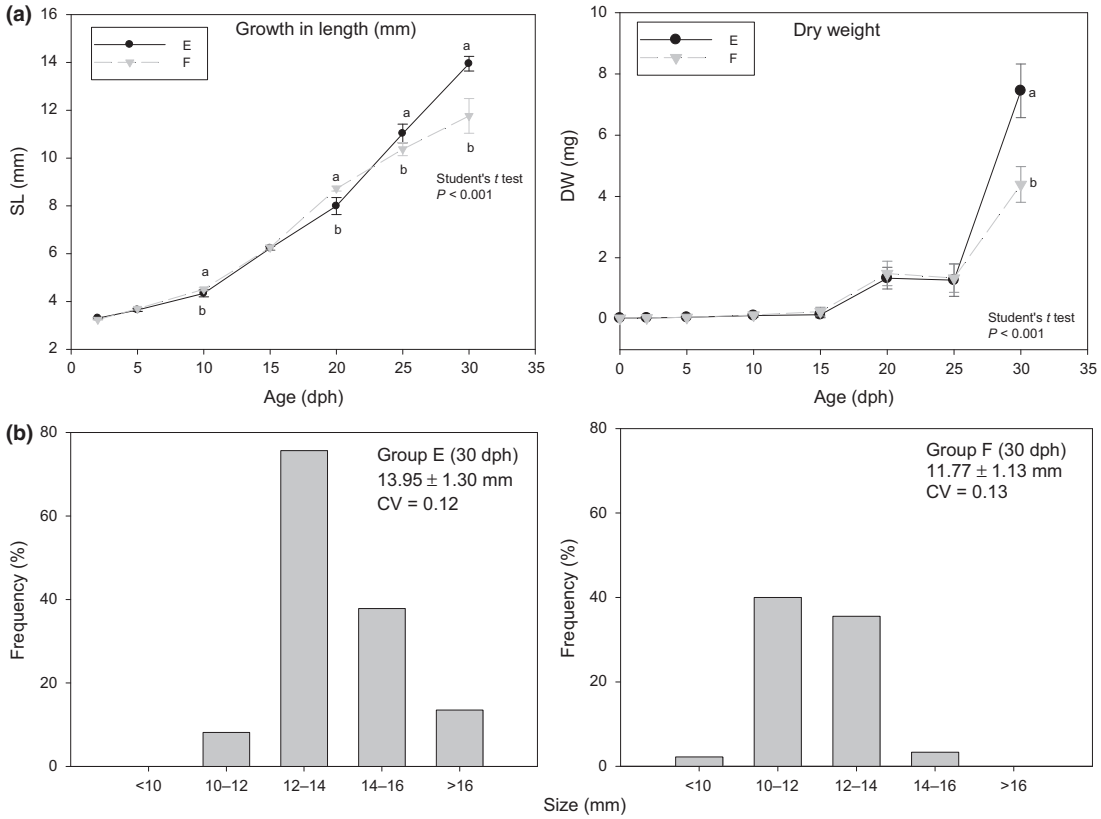

FIGURE 3 (a) Growth in standard length (SL) (mm, mean±SD) and dry weight (DW) (mg, mean± SD) of Trial 2 larvae, weaned at 20 dph (group E) and 12 dph (group F). Letters indicate significant differences (Student's *t* test, $p < 0.001$). (b) Frequency diagrams showing the differences in the size (SL) of the larvae (30 dph) in the groups E (left) and F (right) of Trial 2. CV, coefficient of variation

common among examined fish. In any of the groups examined, cranial deformities were observed, whereas most of skeletal abnormalities were detected in the vertebral column and caudal fin complex, particularly fusions between 20 to 24 haemal vertebrae, deformation of epurals 1 to 2 and in the last two haemal vertebrae before the urostile (Figure 5).

No significant differences in the frequency of skeletal abnormalities affecting the vertebral column (prehaemal and haemal regions) were observed, although when examining each type of vertebral deformity, the incidence of vertebral fusion in the haemal region was different between the two groups of Trial 2. Figure 5 shows some examples of the skeletal anomalies detected in both groups. Thus, most of the anomalies observed can be considered mild anomalies such as lordosis and scoliosis (0.4% for both groups). Fusion of haemal vertebrates was observed in 0.4 to 1.6% of the examined larvae in Group E and only in 0.4 to 0.8% in the larvae from Group F. Skeletal structures of the caudal fin complex were almost not affected showing some defects in ossification associated with the underdevelopment or absence of epurals (Group E = 3.6 ± 2.5% and Group F = 6.8 ± 2.2%).

The incidence of severe deformities (lordosis, kyphosis, scoliosis, deformed vertebral centra) was similar in both dietary treatments (4.25 ± 1.78% for group E and 4.65 ± 2.22% for group F), as well as in the case of light deformities (haemal spines and neural spines and modified epural) 12.38 ± 7.26% for Group E larvae and 16.80 ± 2.48% for Group F (Figure 6).

4 | DISCUSSION

Weaning, the transfer from live food to an artificial diet, is successful with most marine fish with a completely developed digestive tract (Person Le Ruyet et al., 1993). In the current study, weaning was carried out with a commercial diet (Gemma Micro, Skretting, Norway) using a gradual transfer of live prey to this artificial diet over a minimum of five days, although in some other marine species like European sea bass, there is an abrupt replacement (Person-Le Ruyet, 1990). Durán et al. (2009) using a weaning protocol for meagre, similar to the one used in Trial 2, obtained similar results in growth performance and survival rates than those obtained in the present study. Thus, early weaning can be carried out with meagre larvae if several measures to reduce cannibalism are in place.

In natural environments, cannibalism is regarded as an alternative feeding strategy, more likely to be adopted by larvae and early juveniles which are carnivorous, when resources become limiting (Hecht & Pienaar, 1993) or when the population is too crowded (Babbitt &

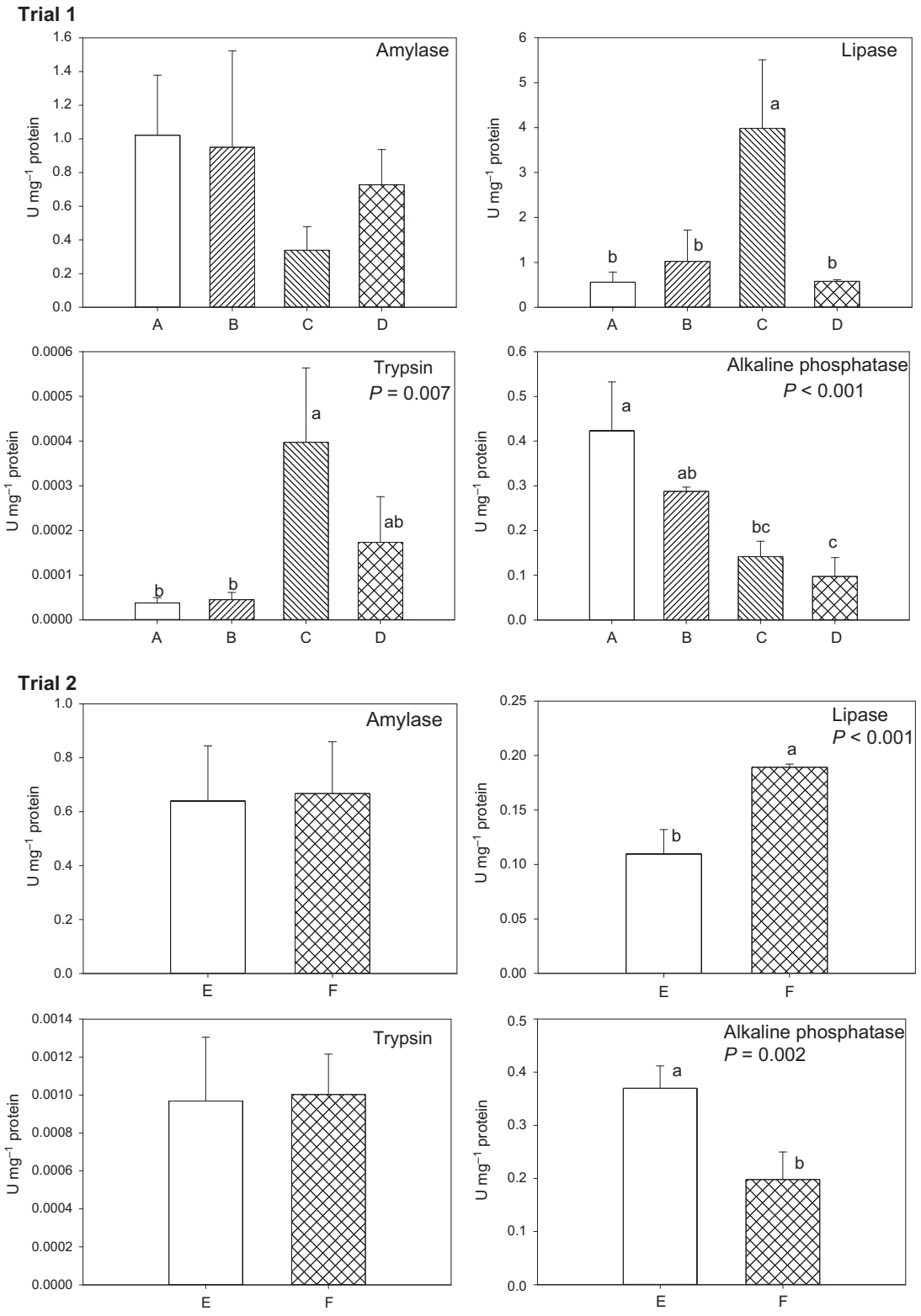

FIGURE 4 Results of digestive enzyme activity (mean± SD) measured in the larvae from Trials 1 and 2 at the end of the experiments. Letters indicate significant differences (ANOVA, data from Trial 1 and Student's *t* test, data from Trial 2, $p < 0.05$)

Meshaka, 2000). It is a major problem in the culture of many marine fish larvae because, being a size-selective form of predation, which has consequences on both the abundance and size structure of the population. Size heterogeneity is the primary cause of cannibalism in larval fish (Katavic, Jug-Dujakovic & Glamuzina, 1989), although other factors such as food availability, larval density, feeding frequency, light intensity, water clarity and shelter have been also identified (see review by Hecht & Pienaar, 1993). In particular,

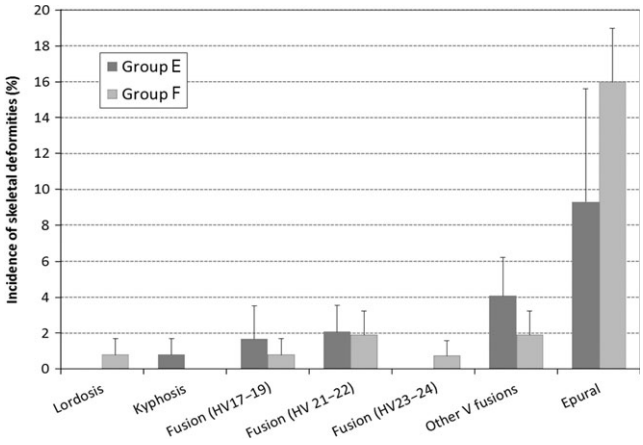

FIGURE 5 Different typologies of skeletal deformities (%) found in 37 dph meagre larvae in Trial 2 (E, control group; F, early weaned larvae), considering the number of abnormal skeletal elements per fish (mean and SEM in brackets). HV, haemal vertebrae; EP, epural

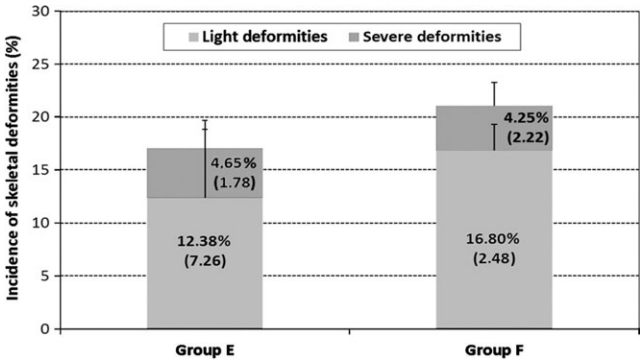

FIGURE 6 Skeletal deformities (in %, mean and SEM in brackets) in meagre from the control group (E) and early weaned (F), considering the degree of the abnormalities among treatments

Kestemont et al. (2003) considered size heterogeneity as the main cause for this behaviour, as the smallest fish are consumed by the largest ones, and considered the period of weaning in carnivorous species as one of the most important during larval rearing because the transition from live to dry diet is generally size dependent and fish that are slightly larger than others may gain a definite advantage while having access to more energetic diets. Depending on species behaviour and morphology, the resulting size advantage may result in more intense and frequent agonistic interactions in the context of dominance hierarchies, or in cannibalism. Cannibalism occurs in other cultured marine fish such as cod *Gadus morhua* (Puvanendran, Laurel & Brown, 2008), yellowtail *Seriola quinqueradiata* (Sakakura & Tsukamoto, 1996) or dusky kob *A. japonicus* (Timmer & Magellan, 2011), and authors attributed the aggressions to the high size variation within the cohorts with frequencies of cannibalistic behaviour increasing as the size differences between prey and predator increased. Density-dependent cannibalism has also been observed (Otterlei, Folkvord & Moller, 1994), being very rare when fish are cultured in extensive pond systems or mesocosm (N. Papandroulakis, pers. com.). In the present study, high cannibalistic behaviour in Trial 1 resulted in a high reduction in survival rates and high dispersion in fish size especially from 16 dph onwards when aggressions, fin napping and attacks from cannibal larvae were more frequent, which was completely unsuitable for commercial rearing purposes. Subsequently, several measures were adopted in Trial 2 to avoid this behaviour, such as increasing the feeding frequency and keeping the larvae in low light (150–200 lux) when food was unavailable or in short supply. The modification of feeding practices and the use of low light intensity before feeding in the morning increased the survival and reduced the size differences among the larvae of Trial 2 by reducing larval cannibalism.

Previous larval rearing studies carried out with meagre (Papadakis, Kentouri, Divanach & Mylonas, 2013; Roo et al., 2010; Suzer et al., 2013) reported higher survival rates (around 50% at 40 dph) and lower growth rates (SGR = 10.55%/day, Suzer et al., 2013) than those obtained in the present study. Present results in terms of growth performance and survival were similar to those recently reported by Solovyev et al. (2016), although the former authors had an initial rearing density of 200 larvae/L. Cannibalism was observed by these authors from 20 dph, but it was not considered as an important issue and other factors related to larval stress and hyperinflation of the swim bladder were considered as the main causes of mortality. Pastor et al. (2013) obtained similar results in growth rate and high incidence of cannibalism from 15 dph using a larval rearing density of 50 larvae/L; although the authors did not provide survival results, they considered size differences and larval density the main cause for this behaviour. Similarly, in the larval rearing protocols (Ballagh, Fielder & Pankhurst, 2010; Fielder & Heasman, 2011) developed for the mulloway (*A. japonicus*), weaning starts when the larvae are 10.5 mm length (approx. 20–22 dph), achieving higher survival rates (40%) although using low larval densities (20 larvae/L).

The weaning success of any finfish larvae from live feeds onto a formulated diet is partly dependant on the composition of the diet and the ability of larvae to digest it. Thus, stomach development and the production of gastric digestive enzymes (acid digestion) are generally regarded as the key indicators for the transition from live feeds to microdiets (Cahu & Zambonino Infante, 2001; Rønnestad et al., 2013; Watanabe & Kiron, 1994). In the present study, weaning was started before the complete morphological and functional development of the stomach (Solovyev et al., 2016); thus, protein digestion at early weaning stages was mainly based on alkaline proteolytic enzymes produced by the pancreas as it has been reported for European sea bass (Cahu & Zambonino Infante, 2001). Under current experimental conditions, pancreatic enzymes tended to be more active in the early weaned larvae, with a significantly higher lipase activity compared to the control groups (Groups A and E). Among different pancreatic digestive enzymes, proteolytic enzymes (alkaline proteases) are

regarded as being particularly significant in the early life stages of fish because of the absence of a functional stomach with its acid protease, pepsin (Rønnestad et al., 2013), as it has been recently demonstrated in meagre by means of histological and biochemical procedures (Solovyev et al., 2016). Lipase plays an active role in lipid digestion, especially in the breakdown of triacylglycerol to diacylglycerol and then to monoacylglycerol (Zambonino Infante & Cahu, 2001). In many fish species, including meagre, lipase is active during resorption of the oil globule and the complete transition to exogenous feeding, being relevant for the digestion of high levels of triacylglycerols present in the enriched live prey, as it was shown by Solovyev et al. (2016). On the contrary, the capacity to digest proteins by means of acid digestion (pepsin activity) was significantly lower in the early weaned larvae in Trial 2, also coinciding with the significantly lower growth rate achieved by this group (see results section and Figures 4 and 5). On the other hand, pepsin activity becomes apparent concurrently with formation of functional stomach. According to different authors, the stomach and gastric activity becomes functional in meagre between 15 dph (5.1–5.4 mm in SL; Suzer et al., 2013), 20 dph (6.6 mm in SL; Papadakis et al., 2013) and even at 31 dph (6.0–6.8 mm SL; Solovyev et al., 2016), which confirmed that the functional development of the digestive system in this species is a well-conserved process that generally occurs within a range of body size (notochord flexion) regardless of larval age and rearing conditions (Solovyev et al., 2016). The results of the present study indicated that 12 dph (weaning ages used in both trials) was probably a bit too early for weaning, especially having into account the reported differences in larval growth at the end of the weaning period. The weak ability of early weaned larvae for acid proteolytic cleavage of proteins from microdiets could be one of the reasons for the lower larval growth achieved. In a similar study with shi drum (*Umbrina cirrosa*), Papadakis et al. (2009) also observed lower growth in early weaned larvae and detected a short period of starvation during the adaptation to the artificial feed, without any influence on the timing of the appearance of the various components of the digestive system, although the differentiation or maturation of some organs might be delayed when inappropriate feeding protocols or diets are evaluated at early life stages of development (Gisbert, Ortiz-Delgado & Sarasquete, 2008).

Several studies have shown that nutrients are responsible for the appearance of skeletal deformities when their levels in the diet are inappropriate or unbalanced (Afonso et al., 2000; Boglione et al., 2013; Cahu, Zambonino Infante & Takeuchi, 2003; Lall & Lewis-McCrea, 2007). During early larval development as well as during weaning, a change to an inappropriate diet, or in the hydrodynamic conditions of the rearing tank, might cause problems in the skeleton. In the present study, malformation rate of the larvae was not affected by the feeding regime, which may indicate that the weaning protocol used, co-feeding for several days enriched Artemia metanauplii with commercial microdiets, can supply adequate nutrients for larvae without compromising the harmonious development of the skeleton, without affecting larval quality. As similar typology of deformities was observed in both treatments (larvae weaned at 20 dph or at 12 dph), we cannot discard other factors such as the rearing conditions (Sfakianakis, Koumoundouros, Divanach & Kentouri, 2004), genetic background (Afonso et al., 2000) or broodstock nutrition (Boglione et al., 2013; Cahu et al., 2003) as the main causative agents for such skeletal disorders.

5 | CONCLUSIONS

Based on these results, meagre larvae can be weaned from live feed to artificial diets at as early as 12 dph, but other important aspects for production success including larval performance and survival should be considered. Special care should be taken to avoid cannibalistic behaviour in the rearing tanks, either by reducing the light intensity at the water surface and increasing larval feeding rate and daily doses. Early weaning did not affect the incidence of skeletal deformities in meagre, which is of special relevance in terms of assuring fry quality for further on-growing purposes.

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