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Effects of Artemia metanauplii substitution by micro-pellet diet on the larval rearing of meagre, *Argyrosomus regius*

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Abstract

The meagre is a species of marine fish that has the most potential for Moroccan and Mediterranean finfish culture production. However, several constraints still limit its production (cannibalism, larval survival low rate and size heterogeneity of hatchery produced juveniles).

This study is an attempt to produce meagre fry without recourse to meta-nauplii of *Artemia sp*, in order to further simplify the protocol for larval rearing of this species. In this study, *Artemia* was substituted by micro-diets in larval feeding. We used two feeding treatments in this study, treatment T1 and treatment T2, were we are comparing their effects on survival and growth of meagre larvae. In the treatment T1 we are using rotifers, *Artemia*, and micro-diets in larval feeding protocol while in treatment T2, *Artemia* was substituted by micro-diets. The results demonstrated that the larval growth in both treatments did not varied significantly from 1st until 12th day post hatching (dph), and thereafter larval growth increased significantly in T1 ($P < 0.007$). Significant difference in survival rates were found at 20 dph between both treatments ($P < 0,009$), with 23% and 15% in T1 and T2 respectively.

the results obtained during this study have shown the technical feasibility of producing meagre fry without using the Artemia metanauplii. However, further research is still needed to improve and optimize the results obtained in this study compared to the common larval rearing protocol.

Keywords

Meagre larvae, feeding treatment, Artemia metanauplii, micro-diet, growth.

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I. INTRODUCTION

The common meagre is a marine fish species of the family of Sciaenidae. It is a species present in the Mediterranean, in the Black Sea, on the Atlantic coasts of Europe and East Africa [1]. It is a coastal species found at a depth between 15 and 200 m both at the bottom of the continental shelf and near the surface [1].

It's a very important species for Moroccan and Mediterranean fish-culture diversification. It is highly appreciated for its nutritional quality, its low rate of fat in its meat, his performance at the thread very important and for the absence of intramuscular spines [2] [3] and also for the rapid juvenile’s growth reared in sea cages [4].

Meagre has a good potential for aquaculture; its culture characteristics include controlled spawning in captivity, relatively easy larval rearing, fast growth, good feed conversion ratios [5]. Meagre withstands perfectly well tank captivity, reaching high growth rates during on-growing, with good feed conversion rates [6] [7].

The meagre is a species with a fast growth [8] and high fecundity [9]. Adults migrate for spawning to estuaries and other coastal areas [10] [11] [12] [13] [14]. It is an emerging species in European aquaculture and in particular in the Mediterranean [15]. Currently, the main producers of large-scale meagre in cages at sea are Spain, Turkey, Italy and France. However, there are other Mediterranean countries, such as Malta or Croatia that produce meagre on a small scale [15] [16]. The meagre has growth rates above 1 kg per year [15], which is much higher than currently grown species such as gilthead seabream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) [17].

For these strengths, meagre has been considered as one the strategic species for the diversification of the Moroccan aquaculture following strategic objectives national marine fisheries strategy (Halieutis Plan). Great efforts have been done in applied research both for hatchery fry production and net-cages growing. In Morocco, larval rearing of meagre larvae only began in 2009 at M'diq's Center Specialized in Zootechnical Engineering and Marine Aquaculture, using techniques similar to those of sea bream [18].

Currently, meagre larvae are reared with facilities and methodologies similar to those of other marine fish, using rotifers, Artemia nauplii, Artemia metanauplii and inert foods. [19] [20] [21] [22] [23] [24] [25] [26]. In commercial hatcheries, meagre larvae still depend on rotifers and Artemia in their larval diet, so no artificial diet can, so far, fully satisfy their nutritional needs [18].

Since the 1980s, tremendous efforts have been made to develop microdiets to replace, in whole or in part, live prey (rotifers and Artemia) in the diet of marine fish larvae [27] [28].

The effectiveness of the use of food particles (live or inert) by marine fish larvae depends on many external and internal factors [29] [30], as well as by physical and chemical such as colour, shape, size, movement, and olfactory stimuli at the molecular level [31].

Weaning to a dry diet can be performed at metamorphosis [32] [33] [34]. The use of early weaning is highly desirable to reduce production costs, especially in industrialized countries where the cost of labour is high, which would have a beneficial impact on the development of marine fish aquaculture.

Many studies have tested the effectiveness of different diets for meagre larval rearing [35] [16], but much work remains to be done to develop microdiets who can replace live foods (rotifers and Artemia) partially or completely in the diet of the meagre larvae.

The replacement of metanauplii of *Artemia* by inert micro-diets aims to accelerate the process of weaning meagre larvae, reduce the production cost and simplify the rearing protocol of meagre larval.

In this study, we tested a new larval rearing protocol using microdiets in replacement to Artemia metanauplii. The main objective was to minimize the use of live prey and to determine if substitution of Artemia metanauplii for larval feeding is possible while maintaining satisfactory growth and survival.

II. MATERIALS AND METHODS

II.1. Animal ethics statement

In our study, we referred to the Directive 2010/63 / EU of The European Parliament and The Council of 22 September 2010 on the protection of laboratory animals, where we used optimal condition for the preservation and the good care of fish larvae inside the present experience.

II.2. Experiment

During this experiment, we used two treatments (T1 and T2) with two repetitions each. The four tanks used are fibers-reinforced plastic with a unit volume of 2 m^3, with blue color and central evacuation. The larvae were counted prior to transfer to the experimental tanks with an initial density of 25 larvae / L and an ambient temperature of 21 ± 1.0 ° C with an open circulating system. Moderate aeration was applied to ensure a homogeneous distribution of live prey and micro-diets.

In the first treatment (T1), the rearing protocol followed is similar to the rearing protocol used for sea bream where the larvae were first fed with enriched rotifers (Sparkle, INVE, aquaculture) from 3 to 6 dph with a rotifers density of 5 to 7 rotifers / ml. then larvae were fed with Artemia nauplii (AF Artemia> 280,000 npl / g INVE Aquaculture in Salt Lake City Utah / USA) from 5 to 14 dph, then with Artemia metanauplii (Artemia EG> 260,000 npl / g INVE Aquaculture in Salt Lake City Utah / USA) from 12 to 20 dph.

Light intensity was measured at the water surface in the middle of the tank with a light meter and maintained at 500 lux at water surface, whereas the light regime was 12-hr light / 12-hr dark, under artificial photoperiod [36]. This treatment was using green water technique from 2 to 6 dph. Indeed, microalgae (Nannochloropsis gaditana) have been added to maintain a concentration of between 140,000 and 180,000 cells / ml.

Micro-diets of 100 µm have been distributed from 15 to 18 dph while micro-diets of 200 µm were distributed to meagre larvae from 17 to 20 dph.

In treatment 2 (T2), meagre larvae were first fed with enriched rotifers (Sparkle, INVE, Aquaculture) from 3 to 6 dph, with a rotifers density of 5 to 7 rotifers/ml. Then, larvae were fed with Artemia nauplii (Artemia AF >280.000 npl/g INVE Aquaculture Salt Lake City Utah/USA) from 5 to 14 dph with 3 to 5 Artemia nauplii / ml. Micro-diets of 100 µm were introduced precociously from 12 to 18 dph while micro-diets of 200 µm were distributed to meagre larvae from 17 to 20 dph. The light regime was 12-hr light / 12-hr dark, under artificial photoperiod [36]. This treatment was also conducted under artificial photoperiod 12-hr light / 12-hr dark using the green water technique from 2 to 6 dph.

II.3. Date collection

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Daily sampling of 15 larvae was carried out from 1 to 20 dph. The standard length (SL) was magnified using a Nikon Profile Projector (V-12B). The survival rate was evaluated at 5 and 20 dph. Sampling was done by core sampling, at three time, in each thank.

II.4. Statistical analysis

Differences in standard length and survival rates between treatments (T1 and T2) were performed using the one-way ANOVA test after arcsin transformation. A value of $p < 0.05$ was considered to indicate statistical significance. Results are given as mean SD (n = 15).

III. RESULTS AND DISCUSSION

The present study was conducted to test the possibility of rearing meagre larvae without using the Artemia metanauplii. Results obtained showed that the length of the newly hatched larvae was 2.46 ± 0.1 mm (Figure 1). Larvae from T1 and T2 showed no significant difference in growth rate until 12 dph. At 12 dph, SL was 5,30 ± 0,641 mm and 5,279 ± 0,652 mm in T1 and T2, respectively. Statistical analysis showed that SL was significantly different between the two treatments from 14 to 20 dph ($P < 0.007$). SL was 7,590 ± 0,732 and 8,634 ± 0,833 mm in T1 and T2 respectively.

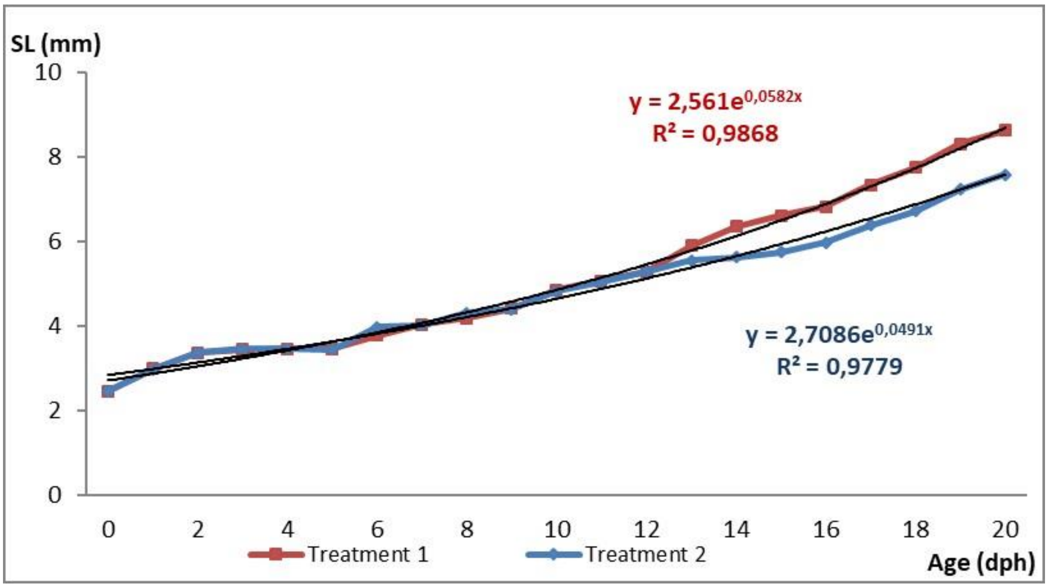

Figure 1: Standard length (SL) of larvae reared using two feeding treatments (T1 and T2).

As indicated in the curves of the linear growth, during the first 12th days no significant difference was recorded following an analysis of variance of one factor. Indeed, until the 12th dah, the meagre larvae of the two treatments fed in the same nutriments which explain their similar and identical growth during this phase.

Since the 12th dah, meagre larvae of the two treatments begin to feed differently, as treatment T1 provided with Artemia metanauplii while treatment T2 receiving microdiets.

During the 12th and the 13th dah, the difference recorded of the linear growth among the two treatments of meagre larvae is not significant (p-value = 0,929).

From the 14th dah, the differences recorded of the linear growth of the two treatments meagre larvae is significant with a p-value = 0,007.

The coefficient of correlation R^2 recorded a large value (above 0,75) of the order of 0,986 for the treatment T1 and 0,977 for the treatment T2, which means that the linear growth is done on a regular basis at the two treatments.

The survival rate (figure 2) at the 5th dah is almost similar for the two treatments (53,98% of larvae survived at the treatment T1 and 53,11% of larvae survived at the treatment T2). T, this result is due to the same larval rearing conditions in both treatments. At the end of the experience, the survival rate is increased to 23.55% for treatment T1 and to 15.32% for treatment T2, which means that there is a great difference in terms of survival between treatment T1 and treatment T2. But without using the Artemia metanauplii might be more cost-effective for larval rearing of meagre. In fact, even with-out using the Artemia metanauplii, T2 showed a relatively interesting and promising survival rate that could be improved by more efforts in further studies and research.

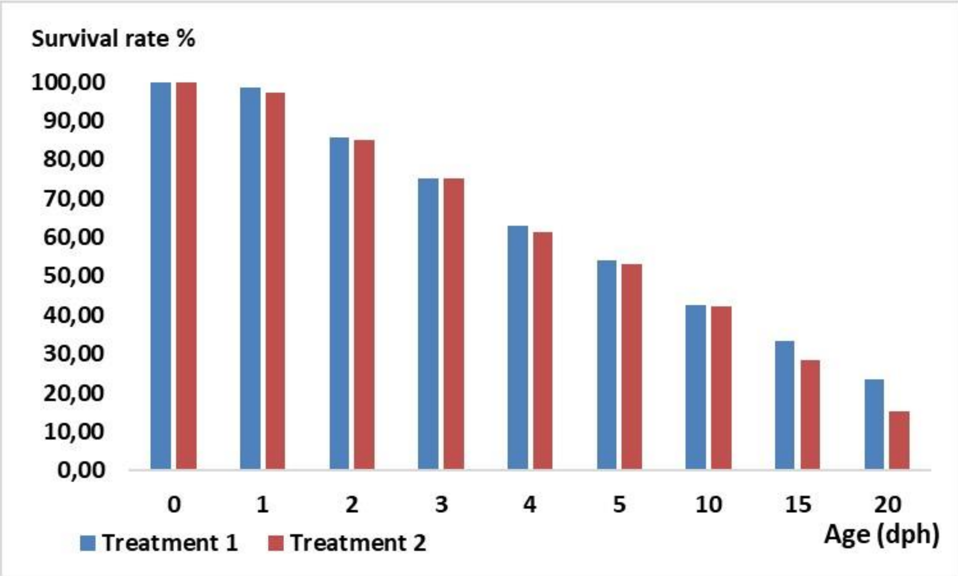

Figure 2: Survival of larvae reared using two feeding treatments (in T_1: rotifers + Artemia nauplii and Artemia metanauplii and T_2: without Artemia metanauplii) in the period up to 20 dph..

IV. CONCLUSION

Meagre is seen as a new candidate for the Mediterranean aquaculture, but for best knowledge, there is little data on its nutrient requirements (Lozano et all, 2017).

Meagre has a high growth rate and a low mortality under culture conditions and is tolerant to a wide range of aqueous environments; for these reasons, it can be adapted easily to culture conditions [3] [38] [2] [39].

This study has showed that meagre larvae could be weaned at early age as 12 dph. Same result has been found by Campoverde et all., in 2017 [36], who showed that the larvae of meagre can feed directly with micro-diets from 12 dph. However, other aspects need to be mastered to have a resistive production, especially the performance and the survival of the larvae. Special care should be taken to avoid cannibalism such as reducing light intensity at the water surface and increasing the frequency of larval feeding.

In 2013, Kolkovski [31] has reported that the substitution of rotifers and Artemia by artificial foods, such as micro-diets, as the first food for marine fish larvae has not yet been commercially realized without a negative impact on larval growth. Other authors in 2017, as Sedki et al. [18], showed that the production of fry of meagre, without the use of rotifers, is possible by introducing the nauplii of Artemia as the first food from 6 dph, and they showed too, that the absence of rotifers in the larval food chain will simplify the larval rearing protocol of the meagre and will also reduce the production costs of fry of this species.

In our study we were able to demonstrate that it is possible to produce the fry of meagre without using the Artemia metanauplii. The absence of Artemia metanauplii in the diet of meagre larvae will simplify the larval rearing protocol of the meagre and will also reduce his fry production costs. At 20 dph, the survival was lower (15.3%) compared to the standard protocol (23.5%) but while simplifying the rearing protocol and reducing costs. However, it is necessary to carry out further studies to produce meagre at low cost and to solve the problem of the lack of fry of this species on an industrial scale. Further studies are still required to investigate the micro-diet quality taking in consideration nutritional needs of meagre larvae.

Results obtained by this study show that there is a significant difference in linear growth from the 14th day after hatching until the end of the experience between the two treatments as well as a clear difference in survival rates respectively. The comparison of regression coefficients showed that the larvae of treatment T1 and treatment T2 are increasing and show that there is no problem in the growth of larvae, then it could be concluded that

- These results are important because it is a first experience of this kind for the meagre and are encouraging the perspective to simplify and optimize the larval rearing process of this species;
- Other studies are still needed in order to master the production of meagre fry without using Artemia and even rotifers;

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Conflict of interest

The authors declared that they have no conflict of interest to declare for this study

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