

Production Protocol for Healthy Larviculture of Meagre (*Argyrosomus regius* Asso1801)

Abstract

This investigation compared the suitability of different diets for larviculture of meagre (*Argyrosomus regius*). For this purpose, wild fish were caught with longline and subsequently fed either a commercial diet or a mollusc diet. After two years, there was a significant difference between the two diets in the body weight but not the survival rate of the fish ($P<0.05$). Both the weight gain and the FCR were significantly higher in fish that were fed the commercial diet compared with those fed the mollusc diet. The broodstock was separated into two groups for spawning. Eggs averaged 800 μm in diameter in a group that spawned naturally, but no spawning occurred in a group injected with GnRHa. Three alga diets were used for the initial feeding of larvae: A₁, A₂ and A₃, consisting of 30/70, 60/40 and 50/50 *Nannochloropsis* sp./*Isochrysis* sp., respectively. There was a significant difference in larval survival rate among the alga diets ($P<0.05$). These results indicate that meagre readily tolerates commercial production systems, where it can grow rapidly and easily reproduce.

Keywords: *Argyrosomus regius*, larvae protocol, GnRHa, algal diets, survival rate

Introduction

Meagre (*Argyrosomus regius* Asso1801) is a member of the Sciaenidae family, which lives in the subtropical waters of the eastern Atlantic, Mediterranean Sea, Black Sea and West Coast of Africa (65°N - 6°S, 23°W - 36°E) (Poli et al. 2003). The fast growth rate of meagre makes it an attractive alternative to other fish for aquaculture. Larval production is very important for commercial aquaculture, and there are multiple factors affecting the growth and survival of fish larvae. One important factor affecting larvae is photoperiod (Barnabe 1990; Chatain & Ounais-Guschmann 1991; Battaglione 1995; Hart et al. 1996; Boeuf & Le Bail 1999; Fielder et al. 2002). Photoperiod affects feed intake, nutrient digestion and, hence, the growth of fish larvae, such as *Siganus guttatus*, *Dicentrarchus labrax*, *Lates calcarifer*, *Rhombosolea tapirina*, and *Pagrus auratus* (Duray & Kohno 1988; Barahona-Fernandes 1979; Cerqueira & Chatain 1991; Barlow et al. 1995; Hart et al. 1996; Fielder et al. 2002). Another important factor for larval production is the size of the mouth opening because mouth size can be used to predict the appropriate time to initiate feeding and the proper feed size and type. Additionally, the mouth size and total body length of fish are linearly related; for example, this relationship occurs in grass carp (*Ctenopharyngodon idella*), silver carp (*Hypophthalmichthys molitrix*) and bighead carp (*Aristichthys nobilis*) (Dabrowski & Bardega 1984).

The aim of this study was to compare the efficacy of commercial and mollusc diets for rearing meagre to provide a foundation for creating a production protocol and improving meagre larviculture.

Material and methods

Broodstock management and feeding

Fish ranging in weight from 50 to 100 g and averaging 66.13 ± 0.70 g were caught with longlines in the Menderes Delta in Turkey. The fish were stocked at an average density of 12 kg m^{-3} in 30 m^3 cylindrical fibreglass tanks kept filled with marine water using 50-mm PVC inlet and outlet pipes and a flow rate that averaged 6 L per hour. After stocking, the fish were separated into two groups; one group was fed the commercial diet (Table 1) (Gültepe et al. 2011; 2012) and the other group was fed the mollusc diet (octopus, shrimp, squid). Feeding of both groups was performed twice a day by hand to apparent satiety until gonadal maturation at approximately two years of age. Satiety was evaluated visually.

Fish were weighed monthly after a 2-day fast while anaesthetised with 1/10000 of tricaine methanesulphonate (MS-222; SIGMA, St Louis, MO, USA). After each monthly sampling period, the amount of feed given was adjusted according to the mean weight of the fish in each tank. From the results of the first and last sample, we calculated the weight gain ($\text{WG} = \text{final weight} - \text{initial weight}$) and the food conversion rate ($\text{FCR} = \text{feed consumption} / \text{WG}$). The water temperature was stable for the entire period, ranging from 25.5 to 18.5 °C. Salinity, dissolved oxygen and pH of the water were $36 \pm 0.5 \text{ ‰}$, $11.5 \pm 0.5 \text{ mg L}^{-1}$ and 7.8, respectively. After the final weight determination, the fish were disinfected with 75 mg L^{-1} oxytetracycline and 0.1 mL L^{-1} formaldehyde for seven days and stocked at 1♂/3♀ in the hatchery system for spawning. Additionally, gonads were dissected from some of the fish at the end of the 2-year maturation period and weighed for calculation of the gonadal somatic index ($\text{GSI} = \text{gonadal weight} / \text{body weight}$). Once disinfected, the transferred fish were separated into two groups for ovulation and spawning. One group was allowed to spawn naturally, whereas the other group was injected intramuscularly with gonadotropin-releasing hormone ($[\text{D-Ser}(\text{tBu})^6, \text{Pro}^9\text{-NEt}]\text{-GnRH}_a$, Syndel Lab. Ltd., CA) for spawning (Arabacı et al. 2004). Before the injection, the fish were anaesthetised with 1/10000 of tricaine methanesulphonate (MS-222; SIGMA, St Louis, MO, USA). Fertilised eggs were gathered from the collection sump, measured (Figure 1) using a light microscope (Olympus® BH-50), and transferred to 1000 L cylinder conical tanks containing sterilised seawater (20 mg L^{-1} sodium hypochlorite added to the seawater for 24 h and then neutralised with sodium thiosulphate) for incubation in darkness (Fielder et al. 2002; Moksness et al. 2004).

69 *Larval feedings and photoperiod*

70 Hatching occurred approximately 72 h after fertilisation of the eggs, and then feeding
71 of the larvae was started with algae. At this stage, the size of the mouth opening and the total
72 length of the larvae were measured using a light microscope (Olympus® BH-50) (Figure 2).

73 One of three alga diets was initially fed to the larvae. These diets, designated A₁, A₂
74 and A₃, consisted of 30/70, 60/40 and 50/50 *Nannochloropsis* sp./*Isochrysis* sp., respectively.
75 Later, Rotifera and then Artemia were added to the larval diet. The larvae were stocked at an
76 average density of 55±5 per L., and their survival rates were recorded daily. The water
77 parameters and photoperiod are given in Figure 3.

78 *Statistical analyses*

79 The mean and standard deviation of each measured parameter were calculated. All
80 data were statistically analysed using one way analysis of variance (ANOVA) with SAS,
81 version 6.07. Differences between means were compared with Duncan's multiple range test
82 using $P<0.05$ to indicate significant differences.

83 **Results**

84 Table 2 shows the growth performance of fish fed the two experimental diets. At two
85 years, there was a significant difference between the commercial diet and the mollusc diet
86 ($P<0.05$) in the body weight of the fish but not in their survival rate. Mean individual body
87 weight ranged from 4.700,00 to 2.100,00 g. The weight gain and FCR were significantly
88 lower in fish fed the commercial diet compared to those fed the mollusc diet:
89 3.742,48±106,13 g and 1,84±0,01 for the commercial diet and 2.895,73±100,13 g and
90 2.10±0.05 for the mollusc diet, respectively. Gonad weight ranged from 294,00 to 658,88 g.
91 The GSI was 0,13±0,01 in all diet groups.

92 The diameter of eggs averaged 800 µm in the naturally spawned group, but the GnRHa-
93 injected group did not spawn. There was a significant difference in larval survival rate among
94 the alga diets ($P<0.05$), with survival rates of 45, 65 and 57% for the A₁, A₂ and A₃ diets,
95 respectively. Figure 4 shows feeding regime by day after egg hatch for larvae fed the A₂ alga
96 diet.

97 **Discussion**

98 In the last decade, the developing aquaculture industry has sought alternative fish
99 species appropriate for culture. Meagre has a high growth rate and a low mortality under
100 culture conditions and is tolerant to a wide range of aqueous environments; for these reasons,
101 it can be adapted easily to culture conditions (Quéro & Vayne 1997; Quémèner 2002; Poli et
102 al. 2003; Chatzifotis et al. 2010). The present study demonstrates that larviculture produces

excellent growth and survival of healthy meagre; thus, this fish species is suitable for commercial production under aquaculture conditions. The commercial feed was more effective than the mollusc diet for the growth of meagre cultured in tanks or in cages. Chatzifotis et al. (2010) used the same commercial feed for rearing meagre juveniles, but these authors obtained the best results with provision of a 17% crude lipid level for 110 days. Over a period of approximately two years, we obtained better results with a commercial diet containing a lipid level of 12,51 g kg⁻¹ than with a mollusc diet. These contrasting results are difficult to compare, partly because of the difference between the studies in the duration of feeding. Interestingly, Jimenéz et al. (2005) reported that sea cages and indoor tanks produced similar growth rates, but the FCR was higher in cages than in tanks. The study by Jimenéz et al. (2005) and the present study observed similar growth rates, but the FCRs obtained in the present study with both the commercial and the mollusc diet were better than the FCRs reported by Jimenéz et al. (2005). In the present study, the GnRHa-injected group did not spawn because meagre broodstock is very sensitive to manipulation during the maturation period. In contrast, GnRHa, alone or in combination with other treatments, produced spawning in numerous other species, including *Salmo trutta* (Billard et al. 1984); *Oncorhynchus mykiss* (Billard et al. 1984; Breton et al. 1990; Arabacı et al. 2004); *Salvelinus namaycush* (Erdahl & McClain 1987); *Labeo rohita* and *Cirrhinus mrigala* (Halder et al. 1991); *Heteropneustes fossilis* (Alok et al. 1993); *Cyprinus carpio* (Dorori et al. 1994; Yaron 1995; Kulikovsky 1996); *Oncorhynchus nekra* (Slater et al. 1995); *Chondrostoma nasus* (Szabo et al. 2002); *Chalcalburnus tarichi* (Arabacı & Sarı 2004); and *Clarias gariepinus* (Sharaf 2012). Anaesthesia was used in the present study to protect against stress from the injection of GnRHa, although this group did not spawn despite the anaesthesia. Zohar and Mylonas (2001) reported that species and environmental factors may affect the ovulatory response of fish to GnRHa. The survival rate of the larvae fed the A₂ diet was better than that of the larvae fed the other alga diets (A₁ and A₃). Compared to the A₁ and A₃ diets, the A₂ diet is richer in *Nannochloropsis* algae, which contain a relatively large quantity of eicosapentaenoic acid (EPA) (Suklenik 1991), an omega-3 polyunsaturated fatty acid. This result suggests that EPA may be effective in increasing the survival rate of the larvae.

In conclusion, meagre is very tolerant of aquaculture conditions and rapidly grows and reproduces in commercial production systems. For these reasons, dietary practices, and possibly hormone treatments, should be further researched for application in meagre aquaculture. EPA-rich feeds should be used for larviculture. Producers must be careful to

avoid cannibalism during the larval feeding stage because the size of the mouth opening in meagre larvae is larger than that of other cultured fish.

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Table 1. Formulation and chemical composition of the commercial feed

Table 2. Growth parameters of fish after 2 years on experimental diets

Figure 1. Photographed and measured eggs

Figure 2. Mouth opening and total length of larvae

Figure 3. Water parameters and photoperiod application during the initial days of larval development

Figure 4. Feeding regime by day after egg hatch for larvae fed the A₂ alga diet.

Table 1. Formulation and chemical composition of the commercial feed

<i>Ingredients (g kg⁻¹)</i>	
Fish meal	400
Soybean meal	220
Corn gluten	80
Wheat gluten	40
Wheat	158
Fish oil	79.75
DL-methionine	2
Antioxidant	0.25
Carboxymethyl cellulose	3
Vitamin mix ^a	3
Mineral mix ^b	2
Bio-Mos ^c	2
Chromic oxide ^d	10
<i>Chemical composition (g kg⁻¹)</i>	
Dry matter (DM)	90.01
Protein	44.24
Lipid	12.51
Ash	8.20
Carbohydrate	24.39
Gross energy (kJ)	1.620

^aProvided per kg of diet: 4500 IU retinyl acetate (Vit. A), 3600 IU cholecalciferol (Vit. D), 90 IU DL- α -tocopheryl acetate (Vit. E), 1.8 mg menadionesodiumbisulphate (Vit. K), 0.04 mg cyanocobalamin (Vit. B12), 90 mg ascorbylpolyposphate (ascorbic acid), 25.2 mg D-biotin, 1800 mg cholinechloride, 1.8 mg folic acid, 18 mg niacin (nicotinic acid), 36 mg pantothenic acid, 9 mg pyridoxine, 10.8 mg riboflavin, 1.8 mg thiamin.

^bProvided per kg of diet: 2.46 mg sodium chloride (NaCl), 0.05 mg ferrous sulphate (FeSO₄), 0.02 mg copper sulphate (CuSO₄), 0.07 mg manganese sulphate (MnSO₄), 0.008 mg potassium iodide (KI), 0.01 mg zinc sulphate (ZnSO₄).

^cBio-Mos, Alltech Inc., Nicholasville, KY, USA.

^dSupplied by SIGMA, St Louis, MO, USA.

Table 2. Growth parameters of fish after 2 years on experimental diets

	Diet	
	Commercial diet	Mollusc diet
Initial body weight (g)	66.13±0.70	66.13±0.70
Final body weight at 710 th day (g)	3.808,61±40,00 ^a	2.961,86±34,00 ^b
Weight gain (g)	3.742,48 ±106,13 ^a	2.895,73 ± 100,13 ^b
Food conversion ratio	1,84 ± 0,01 ^a	2.10 ± 0.05 ^b
Survival rate (%)	97.7	98.1

*The values are expressed as the means±SD (n=300 for growth parameters). Within rows, means with different superscripts are significantly different ($P < 0.05$)

Figure 1. Photographed and measured eggs

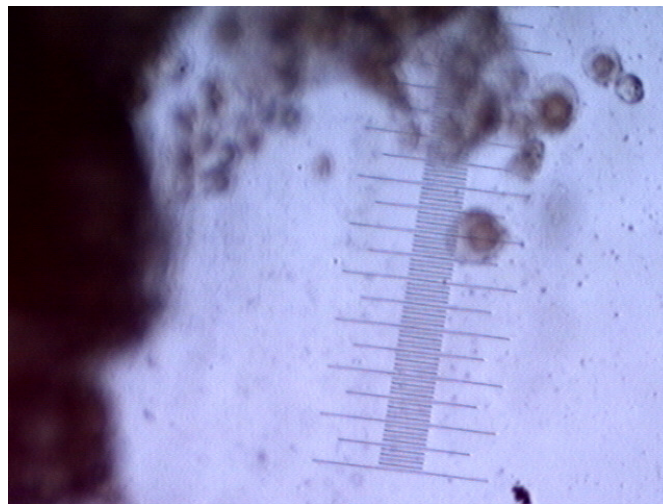


Figure 2. Mouth opening and total length of larvae

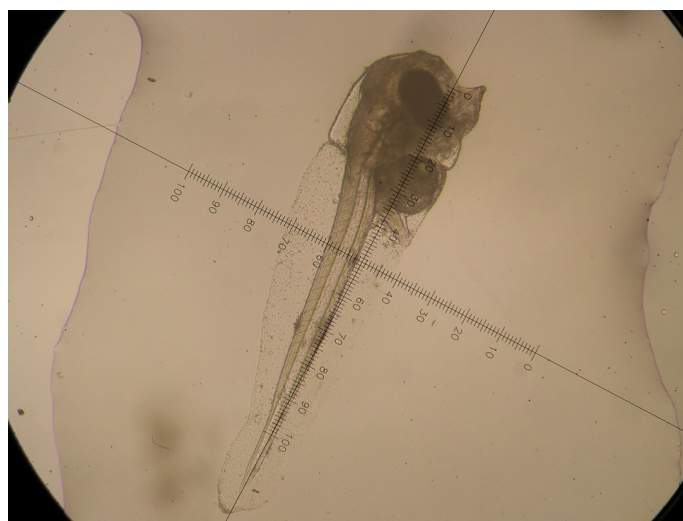


Figure 3. Water parameters and photoperiod application during the initial days of larval development

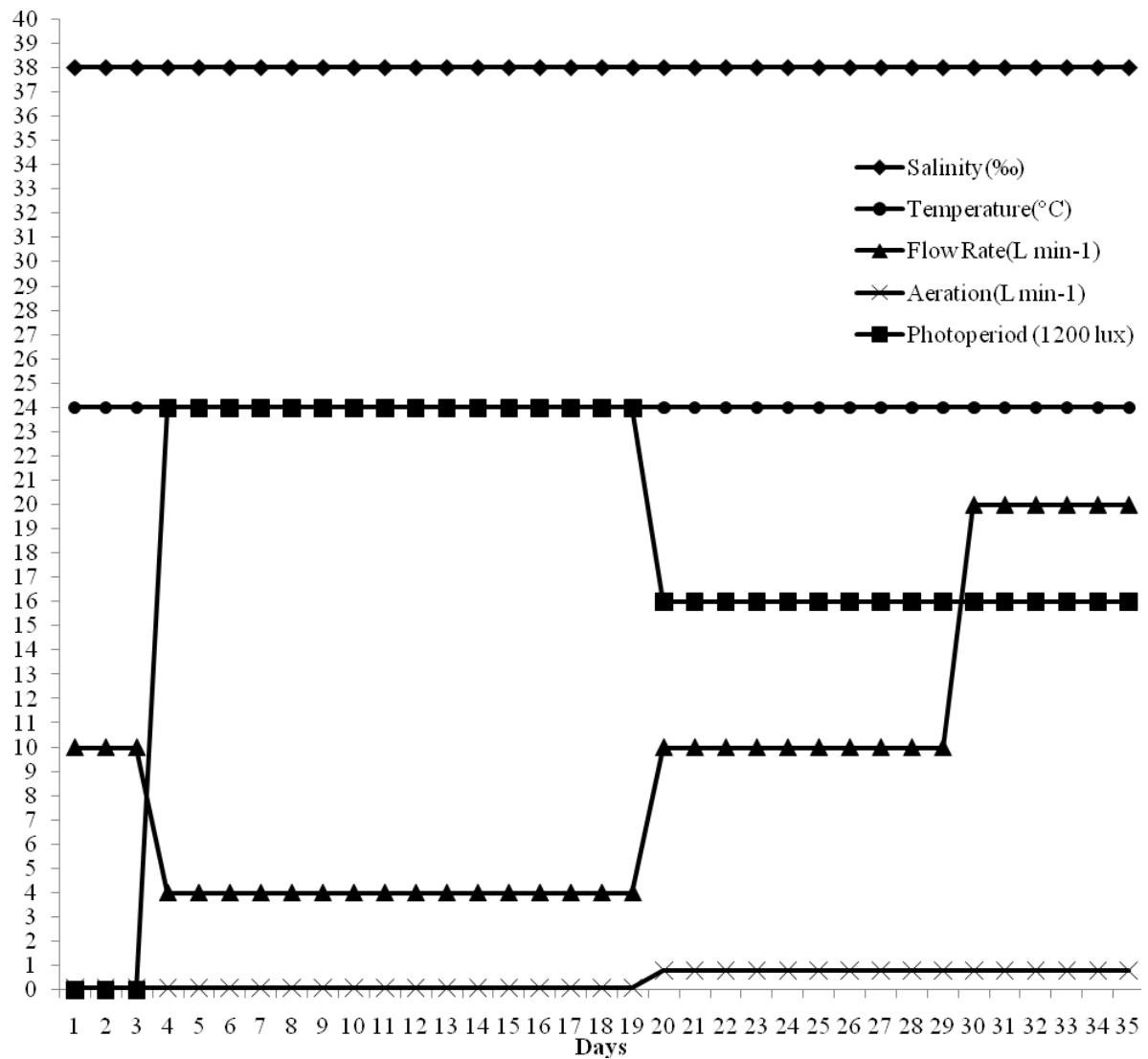
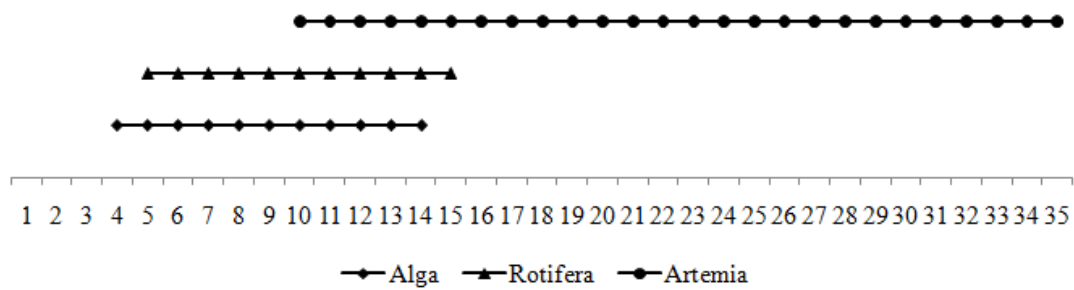


Figure 4. Feeding regime by day after egg hatch for larvae fed the A₂ alga diet



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