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Reproduction of hatchery-produced meagre *Argyrosomus regius* in captivity III. Comparison between GnRHa implants and injections on spawning kinetics and egg/larval performance parameters

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ABSTRACT

Hatchery-produced meagre (*Argyrosomus regius*) were induced to spawn using either controlled-release delivery systems (implants) loaded with gonadotropin-releasing hormone agonist (GnRHa) or GnRHa injections. Over two consecutive years, individual females were paired with single males after treatment with a GnRHa implant (40–104 μg kg^{-1}) or a GnRHa injection (15–25 μg kg^{-1}). Three consecutive GnRHa implants were given every 20–30 days, while five (in 2012) or seven (in 2013) consecutive GnRHa injections were given every 10 days. Fecundity and fertilization success was evaluated the day of spawning, and embryo/larval development was evaluated using 96-well microtiter plates. The first implantation with GnRHa produced up to 23 almost daily spawns, but most females failed to spawn repeatedly after the second implantation, even though their ovaries contained significant numbers of post-vitellogenic oocytes. Most of the eggs were produced in the first 3–4 spawns after GnRHa implantation, while the remaining batches were of low fecundity, which related directly to low fertilization. On the other hand, GnRHa injections produced consistently two spawns on days 2 and 3 after each treatment. Fecundity also decreased over time in response to the subsequent injections, but the response was more gradual and less dramatic than in GnRHa implanted females. Although both methods produced similar overall results in terms of total fecundity and egg/larval quality, multiple GnRHa injections resulted in more consistent spawning results and better egg production control, and this method may offer significant advantages over the use of controlled-release GnRHa implants.

Statement of relevance: We present an efficient spawning induction method for meagre, which is the result of extensive experimentation both in previously published works, as well as in this one. The resulting method is proposed for its effectiveness and efficiency to the aquaculture industry.

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1. Introduction

The meagre *Argyrosomus regius* (Sciaenidae) is a species with great potential for the diversification of aquaculture production in the Mediterranean region (Duncan et al., 2013; Monfort, 2010). Its aquaculture production has increased rapidly in the last decade, though reproduction in captivity still remains a problem (Duncan et al., 2013). With few exceptions, females do not mature in captivity (Duncan et al., 2013; Gil et al., 2013; Mylonas et al., 2013b), and exogenous hormones need to be used to induce ovulation and spawning (Duncan et al., 2012).

Meagre have been classified to exhibit an asynchronous (Gil et al., 2013; Schiavone et al., 2012) or group-synchronous oocyte development pattern (Duncan et al., 2012; Schiavone et al., 2012). Experiments with GnRHa treatments were effective in inducing maturation of both wild-caught (Duncan et al., 2012) and hatchery-produced broodstocks (Fernández-Palacios et al., 2014; Mylonas et al., 2013a). Both liquid injections and controlled-release delivery systems (implants) that release GnRHa for a prolonged period of time (Mylonas and Zohar, 2001) have been shown to be effective in inducing maturation in females (Duncan et al., 2012, 2013; Fernández-Palacios et al., 2014; Mylonas et al., 2013a). In the case of a single injection of GnRHa, lower total fecundity was obtained due to the fact that a GnRHa injection induced only 2–3 spawns (Duncan et al., 2012, 2013; Fernández-Palacios et al., 2014), while the species has the capacity for multiple spawns (Gil et al., 2013; Mylonas et al., 2013b). On the other hand, GnRHa implants

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produce multiple daily spawns (Mylonas et al., 2013a), as described in various other fishes with a protracted spawning season and asynchronous or group synchronous ovarian development, which undergo many cycles of oocyte maturation in the course of many weeks (Guzmán et al., 2009; Kokokiris et al., 2005; Larsson et al., 1997; Zohar et al., 1995). However, only the first few spawns were of high fecundity and there was a large number of subsequent spawns of low fecundity, which may not be incorporated efficiently in the production cycle of today's large commercial hatcheries. Therefore, there is still a need to optimize spawning induction methods to make them not only effective, but also efficient for commercial aquaculture applications.

The present study follows two recent ones on the reproduction of hatchery-produced meagre broodstocks (Mylonas et al., 2013a, 2013b), and compares the spawning kinetics and egg production and quality characteristics of multiple GnRHa injections vs implants. The information obtained is expected to be useful for improved implementation of GnRHa induction therapies in meagre and optimization of egg production in commercial aquaculture operations.

2. Materials and methods

2.1. Broodstock maintenance

Rearing was undertaken at the facilities of the Institute of Marine Biology, Biotechnology and Aquaculture (previously Institute of Aquaculture) of the Hellenic Centre for Marine Research (HCMR), Iraklion, Crete, Greece. Fish came from eggs produced in the hatchery in 2004, 2006 and 2007. Feed was given 5 days per week to apparent satiation with industrial feed (Excel XL, Skretting S.A., Spain or Genesis, IRIDA, S.A., Greece). Fish were starved 2 days prior to any handling. During the year and outside the period of the spawning induction experiments, fish were maintained in large communal tanks (10–35 m^3) exposed to a simulated natural photo-thermal regime (Fig. 1). For spawning induction, single pairs of fish (one male and one female) were transferred to 5000-l Recirculation Aquaculture System (ACE, the Netherlands) supplied with seawater from a well, under simulated natural photoperiod but controlled temperature ranging between 19 and 20 °C (Fig. 1). Measurements of temperature and water quality (dissolved oxygen, NH_3-N and NO_2-N) throughout the year were conducted once per week. Due to limited facilities (a total of 4 tanks), 2 pairs were used per treatment and the experiment was repeated over two years, in order to ensure the validity of the results. The females used in the two years were the same individuals, randomly allocated to the GnRHa treatment.

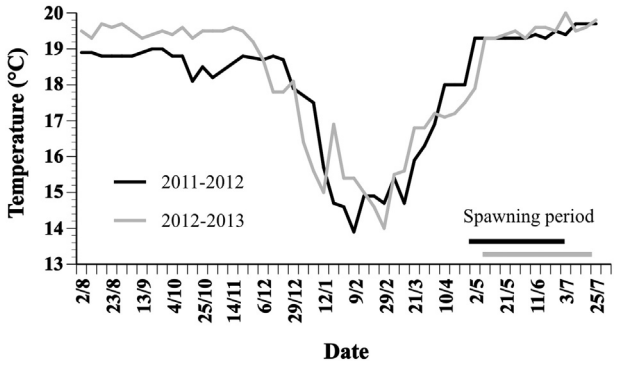

Fig. 1. Annual water temperature profile for the meagre broodstocks used in the spawning induction experiments in two consecutive years. The horizontal bars at the bottom of the graph indicate the approximate duration of the spawning induction experiments each year.

2.2. Evaluation of reproductive stage and broodstock selection

To select the broodstocks for the spawning experiments, fish were tranquilized initially in their tank with the use of clove oil (0.01 ml l^{-1}) and then transferred to an anesthetic bath for complete sedation with a higher concentration of clove oil (0.03 ml l^{-1}) (Mylonas et al., 2005). Ovarian biopsies for the evaluation of oocyte development were obtained by inserting a glass cannula (Natelson tube) into the ovarian cavity, connected to a TygonR tubing and applying gentle aspiration. A wet mount of the biopsy was first examined under a compound microscope (40 and 100×) to evaluate the stage of oogenesis and measure the mean diameter of the largest, most advanced vitellogenic oocytes (n = 10). A portion of some biopsies was fixed in a solution of 4% formaldehyde–1% glutaraldehyde for further histological processing. Females were considered eligible for spawning induction if they contained oocytes in full vitellogenesis with a diameter of >550 μm with very little atresia present (Mylonas et al., 2013a).

Male fish were considered eligible for spawning induction, if they were in full spermiation, releasing substantial amounts of sperm upon application of gentle abdominal pressure (Mylonas et al., 2013b). To obtain sperm for evaluation, the genital pore was carefully blot dried and gentle abdominal pressure was applied to force the sperm out of the testes, avoiding contamination of the samples with feces or urine.

2.3. Year 1 experiments

In the first year, spawning induction trials using injections and implants of GnRHa were conducted between 27 April and 20 June 2012 using two pairs of fish per treatment (n = 2). The GnRHa injections were given approximately every 10 days (a total of 5 injections) and the GnRHa implants approximately every 20 days (a total of 3 implantations). Females (mean ± SD body weight 7.3 ± 0.7 kg) were treated with a GnRHa injection of 20–25 μg kg^{-1} (n = 2) or with an Ethylene-Vinyl acetate (EVAc) GnRHa implant (Mylonas and Zohar, 2001) loaded with 500–710 μg of Des-Gly^{10},D-Ala^{6}-Pro-NEth^{9}-mGnRHa (H-4070, Bachem, Switzerland) for an effective dose of 62–104 μg kg^{-1} (n = 2). There were variations in the effective GnRHa dose applied to each fish with the GnRHa implants due to the fact that implants are loaded with fixed amounts of GnRHa. Even though combinations of two GnRHa implants loaded with different amounts of GnRHa were used when necessary, it was still not possible to adjust the dose exactly to the different body weights of the fish. Four males (6.8 ± 0.6 kg body weight) were treated at the start of the experiment with 68–163 μg kg^{-1} using a GnRHa implant, in order to enhance spermiation. Similar GnRHa implantation of males was repeated at any sampling sperm production was considered low. After treatment with GnRHa, a single female with a single male were placed in separate 5000-l tanks connected to overflow egg collectors and were allowed to spawn. The temperature range during the spawning induction experiments was 19.3–19.4°C. Twelve days after the last GnRHa treatment (54 d after the first GnRHa treatment), fish were removed from their spawning tanks, evaluated using ovarian biopsies and sperm collection, and returned to a large communal tank (10 m^3) to recover.

2.4. Year 2 experiments

In the second year, spawning induction trials using injections and implants of GnRHa were conducted between 8 May and 22 July 2013 using two pairs of fish per treatment (n = 2). The females were the same individuals used in the previous year's experiment, but were randomly re-allocated to the GnRHa treatment. The GnRHa injections were given approximately every 10 days (a total of 7 injections) and the GnRHa implants approximately every 30 days (a total of 3 implantations). A slightly different frequency of GnRHa implantations and more GnRHa injections were used in 2013, based on the results of the

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