SHORT COMMUNICATION

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IMPACT OF OOCYTE SIZE ON LHRHa INDUCED OVULATION AND FERTILIZED EGG QUALITY IN SADDLED BREAM *Oblada melanura* (LINNAEUS, 1758)

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ABSTRACT

The objective of this study was to evaluate the effects of oocyte size and luteinizing hormone releasing hormone analogue (LHRHa) on ovulatory success in artificial fertilization. Vitellogenic females with maximum oocyte diameters 400-550 μm were repeatedly injected with LHRHa (20 μg kg^{-1} per injection). Fish with maximum oocyte diameters <500 μm failed to ovulate. In contrast, all fish with maximum oocyte diameters >500 μm spawned within 48-54 h. These results demonstrate that injected LHRHa is effective for ovulation of saddled bream with maximum oocyte diameters >500μm.

INTRODUCTION

Saddled bream *Oblada melanura,* (Linnaeus, 1758), is recently considered as a potential candidate for mariculture in the Adriatic Sea. The reports on characteristics of the species in captivity are scarce. There is also no data on spontaneous spawning in captivity. Control of reproductive function in captivity is essential and can be achieved by manipulating photoperiod, water temperature or spawning substrate (Mylonas et al., 2010). In captivity females of most fish species fail to undergo final oocyte maturation (FOM), ovulating and spawning (Zohar, 1989). Similar to most wild animals held in captivity, many fish of commercial interest to aquaculture industry exhibit reproductive dysfunctions (Zohar and Mylonas, 2001). Among other hormones, luteinizing hormone releasing hormone analog (LHRHa) has been successfully used to induce ovulation in fish (Mylonas et al., 2010; King and Pankhurst, 2007; Berlinsky et al., 2005; Firat et al., 2005).

MATERIALS AND METHODS

Broodstock of saddled bream were maintained under natural ambient conditions (water temperature 12-26 °C, salinity 37 gL^{-1}). During the spawning studies (June), photoperiod was held at 15L: 9D and water temperature at 19-23 °C. Females were selected for induced ovulation based on their ovarian development. Ovarian biopsy tissue was obtained using tygon cannula (1.88 mm o.d. x 1.11 mm i.d.) as described by Shehedeh et al. (1973) and measured to the nearest 25 μm using stereo microscope (Wild Heerbrugg) fitted with an ocular micrometer (Wild Heerbrugg type 325400). The experiment was conducted at the Institute for Marine and Coastal Research (June, 2008). Temperature of water was 21 °C, females were biopsied and 25-30 of the largest oocyte present were measured. Fish (n= 24; 210-260 g body weight (BW)) that exhibited pre-spawning morphology (distended abdomens; Mylonas et al., 1995) were divided into two groups: females with the oocyte size <500 μm (range 400-500 μm) and females with oocyte size >500 μm (range 500-550 μm). Fish were anesthetized in solution (1 mg l^{-1}) of benzocaine, LHRHa was administered intramuscularly with injection (priming injection), 20 μg kg^{-1} BW. After hormonal treatment the fish were placed in separated 1500 l tank and beginning 24 h post injection. After 24 h fish received second injection (resolving injection) of 20 μg kg^{-1} BW. The eggs were fertilized by adding pooled milt (0.5 ml) from three anesthetized males and 35 ml of filtered ambient sea

water and gently mixed. Hormone treatments were evaluated using the following criteria: egg production, buoyancy, fertilization rate, hatching rate. The total number of ovulated eggs produced by each female was calculated by counting eggs in 1 ml subsample of egg mass. Total egg count per female was derived by multiplying the number of eggs in a subsample with the total egg volume. Buoyancy, fertilization and hatching rates were determined for each female (tree replicates of 300 eggs from each fish were randomly taken and used to estimate fertilization rate and hatch rate). These percentages were calculated using the following formula:

Buoyancy (%) = 100 x no. of buoyant eggs/ no. of ovulated eggs

Fertilization (%) = 100 x no. of fertilized eggs/ no. of buoyant eggs

Hatching (%) = 100 x no. of hatched larvae/ no of fertilized eggs

The percentage of fertilized eggs was determined 1h and 20 min after spawning (4-8 cell stage) by stereo microscopic examination.

Statistical methods

All data sets were tested for normality using Kolmogorov-Smirnov normality test, while ANOVA model was used to examine the effects of LHRHa on oocyte size of females within each 6 hours. Multiple regression was used to model the relation of egg diameter to LHRHa treatment.

RESULTS

Prespawning females contained vitellogenic oocytes varying in oocyte diameters containing 400-550 μm. The ovulatory response to LHRHa was affected by oocyte size. No fish with the maximum oocyte diameters <500 μm ovulated. In contrast, 12 of 12 fish with uniformly, fully vitellogenic oocyte >500 μm ovulated substantial numbers of eggs. After 24 h of prime injection of LHRHa at the sea temperature of 21.8 °C, maximum oocyte diameters were 700 μm, oocyte range was from 600-750 μm with the mean diameter 718± 21μm and the formation of oil drop began. After 48 h of resolving injection, maximum oocyte diameters were >800 μm and range from 755-928 μm, oil drop was formed with range of 166-185 μm and fish were ready to spawn. All injected females with oocyte maximum diameters >500 μm were spawned at 48 – 54 h after the priming injection. The number of ovulated eggs, rate of buoyancy, fertilization and hatching for different females are shown in Table 1. The mature unfertilized eggs were pelagic and transparent with one oil globule, with the mean diameter 833±55 μm. Significant increase in egg diameter was shown (ANOVA, Wilks-lambda, 0.00027; $p<0.001$) after administering LHRHa.

This study shows that oocyte with diameter of 500 μm or more grows to 833±55 μm 48h after the first injection of LHRHa at the mean temperature of 21.8 °C.

Table 1. Body weight (g), eggs production (x g $^{-1}$ fish), buoyancy (%), fertilization rate (%) during the induction spawning of saddled bream *Oblada melanura*

Spawned female number	Body weight (g)	Number of ovulated eggs (x g $^{-1}$ fish)	Buoyancy[a] (%)	Fertilization rate[b] (%)	Hatching rate[c] (%)
1	254	605	35	46	52
2	209	589	54	54	58
3	236	608	49	49	61
4	214	702	57	52	72
5	238	625	62	62	59
6	246	730	38	71	64
7	205	506	39	72	58
8	223	608	46	58	63
9	246	654	52	64	68
10	209	723	57	75	58
11	218	568	52	48	54
12	248	502	29	52	70
Mean ± SD	228.8 ±17.7	618.3 ± 74.8	47.5 ±10.1	58.5 ± 10.2	61.4 ± 6.2

[a]Buoyancy (%) = 100 x no. of buoyant eggs/ no. of ovulated eggs
[b]Fertilization (%) = 100 x no. of fertilized eggs/ no. of buoyant eggs
[c]Hatching (%) = 100 x no. of hatched larvae/ no. of fertilized eggs

DISCUSSION

Synthetic analogues of LHRHa are successful in spawning induction of saddled sea bream. LHRHa has been effectively used to induce ovulation and spawning in a number of commercially important finfish (Rasines et al., 2013; Tucker, 1994; Mylonas and Zohar., 2001; Glamuzina et al., 1998). In some species, such as spotted seatrout (*Cynoscion nebulosus*) and red drum (*Sciaeops ocellatus*) with fully grown oocytes, a single injection of LHRHa is sufficient for ovulation induction (Thomas and Boyd, 1989). In the present study, ovulation was induced in saddled bream just prior to the natural spawning season. Under this condition LHRHa administered with two injections of 20 μg kg^{-1} BW was sufficient to induce ovulation in saddled bream. Our study is in accordance whit Berlinsky et al., (2005) who suggested that oocyte diameters >550 μm have high potential for response to LHRHa. The latency period after hormonal injection was related to the initial oocyte diameter and water temperature. Fertilization percentage was 58.5% when eggs were fertilized 0-6 h after ovulation. Fertilization percentage of saddled bream, if compared with other sparids, was smaller. For example, in red porgy *Pagrus major* fertilization percentage was 70% (Mylonas et al., 2004), 88% for gilthead sea bream and 82% for sharpsnout sea bream, *Diplodus puntazzo* (Papadaki et al., 2008). Overall, saddled bream exhibits good fertilization percentages in captivity, but further studies are needed in order to examine the effect of various environmental and physiological parameters on egg quality variation.

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Sažetak

DJELOVANJE LHRHa ZA IZAZIVANJE OVULACIJE PRI RAZLIČITIM VELIČINAMA OOCITA I KVALITETE OPLOĐENIH JAJAŠACA U UŠATE *Oblada melanura* (LINNAEUS, 1758)

Cilj ovog rada bio je utvrditi veličinu oocita za uspješno djelovanje hormona LHRHa i uspješnost umjetne oplodnje nakon hormonskog tretmana. Vitelogene ženke s promjerom oocita 400-550 μm injektirane su hormonom LHRHa 20 μg/kg težine. Ženke s najvećim brojem oocita promjera <500 μm nisu ovulirale, dok su ženke s najvećim brojem oocita promjera >500 μm ovulirale u roku 48 -54 sati nakon tretmana s LHRHa. Ovaj rad pokazuje da je hormon LHRHa djelotvoran za izazivanje ovulacije kod ušate kada se injektira pri veličini oocita iznad 500 μm.

Ključne riječi: ušata, *Oblada melanura*, LHRHa, umjetna oplodnja

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