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Induced Spawning, Fertilization Rate and Hatching Rate of Brill, *Scophthalmus rhombus*

Fatih BASARAN[1*] Bilhan FILIZKAN[2] Hayal BOYACIOGLU[3] Osman OZDEN[1] Sureyya OZKIZILCIK[4]

[1] Ege University, Faculty of Fisheries, Department of Aquaculture, 35440, Urla, Izmir, Turkey
[2] AKVA-TEK Fish Products Farm, Zeytindag, Bergama, Izmir, Turkey
[3] Ege University, Faculty of Science, Department of Statistics, Bornova, Izmir, Turkey
[4] NUTRA Feed Ltd. Isıkkent, Izmir, Turkey

[*] Corresponding Author:
e-mail: fatih.basaran@ege.edu.tr

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Abstract

An experiment was performed to obtain ovulation in female brill, *Scophthalmus rhombus*, to fertilise the oocytes, to describe the hatching rate and to assess their potential for aquaculture development. Broodfish were captured between 6 to10 meters depths by the gill nets then transported to a commercial hatchery in Çanakkale, in the northwest cost of Turkey, where the experimental trials took place. The females (31 specimen) weighed between 498 and 1620 g and the males (25 specimen) between 218 and 644 g.

The Broodfish were divided into two groups and treated with: a) a saline injection (control group), b) single injections of luteinizing hormone-releasing hormone analog (LHRH-a; single injection: 25 μg kg^{-1}, induced group). Females successfully ovulated with a hormone dose of 25 μg kg^{-1}. The eggs and the sperm were obtained by hand-stripping and then fertilized artificially. Mean egg fertilization varied between 1.0 % and 21.2 % in control groups and 12.5 % and 37.5 % in induced groups, where hatching ranged from 0 % to 6.82 % in control group and 8.7 % to 46.2 % in the induced group. Hatching occurred approximately 110-115 h after fertilization at 14 ± 1°C. There were significant differences in the fertilization and hatching rates between the hormone induced and the control group ($p<0.05$, Pearson's and Yates' χ^2 test, respectively).

Key words: The brill, *Scophthalmus rhombus*, induced spawning, fertilisation, hatching.

INTRODUCTION

Brill, *Scophthalmus rhombus*, is a highly prized fish usually caught by bottom trawls as well as by gill nets [1, 2, 3, 4]. The brill is a very large, broad bodied, left-eyed flatfish that belongs to the Scophthalmidae family [5]. Its geographical range extends from Icelandic seas to the Mediterranean including the Sea of Marmara, the Aegean and the Black Sea [5, 6]. This species is distinguished by the absence of bony tubercles on both sides of the dorsal sections along with the position of the dorsal fin that extends to just above the large curved mouth. The brill usually lives on sandy seabeds but occasionally encounteres gravel or mud [6]. It feeds on demersal fish, crustaceans, and bivalves [5, 6]. The brill is often confused with turbot (*Scophthalmus maximus*) however the latter has no frilly edge at the front of the dorsal fin.

One of the requirements in improving a new species for aquaculture is obtaining reliable amounts of viable eggs [7, 8, 9] and it is often necessary to use exogenous hormone treatment to stimulate ovulation [10] under artificial conditions. The physical injury and physiological stress of capturing, handling, injecting and holding brood fish can individually and collectively have negative effects on spawning success [2, 11, 12, 13]. When female fish are stressed or injured, they may undergo rapid physiological changes that can result in the break-down of the oocytes in the ovary [7, 14, 15, 16]. Suboptimum conditions may stress brood fish, resulting in spawning failure or perhaps in mortality [13, 16].

Successful manipulation of reproductive processes is dependent on a good level of understanding reproduction in the target species. Induction of ovulation by gonadotropin-releasing hormone analogue (GnRH-a) is a general procedure for controlling reproduction in commercial fish farming [9, 17]. Among the different types of GNRH-a, [D-Ala^6-Pro^9-Net]-luteinising hormone releasing hormone (LHRH, an analogue of mammalian luteinising hormone releasing hormone) is efficient and practical in inducing ovulation in fish [18, 19, 20]. Under captivity, final oocyte maturation and spawnig has been achieved in other species of the genus Scophthalmus, including the Black sea turbot *Psetta maxima maeotica* [2, 21, 22] and Atlantic turbot *Scophthalmus maximus* [24, 25, 26]. However, little information is presently available on spawning of the brill [3, 23]. The aim of this study is to investigate the induction of spawning, fertilization and hatching of eggs of the brill in culture conditions.

MATERIALS AND METHODS

Experimental Animals

Experimental brill broodstock were captured by gill nets from 6-10 meters depths in Çanakkale Bay (the Sea of Marmara, Turkey) by small commercial fishing boats between February and March 2004. A total of 3 operations were performed. After fishing operation, only live fish were taken in two 200-l plastic tanks in each boat, transferred to the shore and numbers were recorded.

Table 1. Weight (W., g.) and Total Length (T.L., cm.) of experimental animals in each group.

Treatments	Groups	*N*	*Value*	Min	Max	Mean±Se
Control Groups	Females	13	W	588	1568	1001.2 ± 84.2
			TL	34.2	50.5	40.8 ± 1.2
	Males	12	W	218	644	370 ± 39.7
			TL	26.1	36.3	30.3 ± 1.0
Induced Groups	Females	18	W	498	1620	867.8 ± 72.1
			TL	31	50.5	39 ± 1.1
	Males	13	W	262	508	362.2 ± 22.7
			TL	27.2	42.3	31.6 ± 1.2

Upon shore the fish were separated into two one ton tanks. At this stage, the fish were weighed to the nearest 0.01 kg, and their total lengths (TL) were measured to the nearest 0.1 cm. The stocking densities in these tanks were 19 kg/ton approximately. Weight of the experimental animals varied between 498 and 1620 g for 31 females and between 218 and 644 g for 25 males. Table 1 presents information on the number and size of broodstock.

The broodstock were transported to the IDA-GIDA Fisheries Company within two hours. The oxygen was recorded between 7.0-7.3 mgl^{-1} and the water temperature was kept at 11.5±0.5 °C during transportation. In order to reduce the stress and the microbial contamination, the antibiotic (furozolidone) was applied to tanks at the level of 5 mg l^{-1}. The wild broodstock were acclimated to the experimental conditions as described previously Basaran and Samsun (2004).

Experimental Setup

Total of fifty-six mature fish including thirty-one females and twenty-five males were kept in a 4000-L rectangular polyester tank supplied with filtered sea water and aeration. Additionally, salinity (30 ppt), photoperiod and temperature (13-15°C) were kept natural conditions. After 3 days acclimatization period, the female and the male fish were randomly separated into two groups: control (13 females and 12 males) and LH-RHa treated (18 females and 13 males). Both experimental groups were maintained in the 1500-L circular polyester tanks with the same conditions as in the stock tank.

Prior to the trial, the broodfish were anaesthetized with 2-phenoxyethanol (0.2 ml L^{-1}), then, they were placed on a hygiene towel. LHRH-a (D-Ala^6-Pro^9-NET, single injection, 25 μg kg^{-1} body weight) hormone were injected to female fish in the induced group and only saline solution were injected to female fish in control group. The males and females of each group were placed together after the hormone treatment. Hand-striping was carried out by using dry or semi-dry fertilization methods by Maslova (2002). In order to obtain the eggs and milt, a gentle pressure was applied to dorsal part of the gonads. After the urinary bladder was emptied, eggs were collected in a sterile 500-ml beaker and weighed immediately. Quantity of the eggs produced by each female was calculated by egg mass weights. Sperm was collected by stripping ripe males after a gentle abdominal pressure. Milt was collected in a silicone tube with syringe. Then sperm was poured onto the eggs, slowly diluted with sea water, mixed, and gradually diluted further with sea water [3, 7, 11, 21]. After 3–5 min, eggs were placed in 10-L transparency cylinder beakers to calculate the rate of the fertilized eggs.

The fertilized eggs were weighed immediately and incubated in 40-L incubators placed in 4000-L cylinder polyester tank to determine the hatching rates. A continuous flow of 13–15°C water was circulated (50% h^{-1}) through the incubators. During the incubation period, dead eggs were removed and weighed daily from the incubators by siphoning. When hatching was completed, the unhatched eggs of each incubator were weighed to calculate the hatching rates.

Statistical Analysis

The significance tests of the normal distribution of the total weights of females and males were done by using one-sample Kolmogorov Smirnov's, and homogeneity tests were done by using Levene's statistical analysis. The significant tests of the differences between the total weights of (these fish) were compared by using independent sample *t*-test. Pearson's Chi-Square test was applied to find whether there is statistical difference (between) in the weight of fertilized eggs between the control and induced groups. Yates' Chi-Square test was used to determine if there is a significant difference in the mean weight of obtained eggs (from one kilogramme female fish) between the control and induced groups. Yates' Chi-Square test was also used to determine the significant differences in the hatching rates based on fertilized eggs and in the hatching rates based on total eggs between groups. All statical analysis were performed by using the Statistical Package of Social Sciences (SPSS 9.0) and significance was accepted at p=0.05.

RESULTS

Table 1 gives information on the number and size of broodstock. The females were up to two times bigger than the males, and positive relationship was found between weight and length (Fig. 1). The mean weights of the females between the control and induced groups were not significant differences (independent sample *t-test,* df = 29, $p>0.05$) and the mean weights of the males were also not significant differences (independent sample *t-test*, df = 23, $p>0.05$).

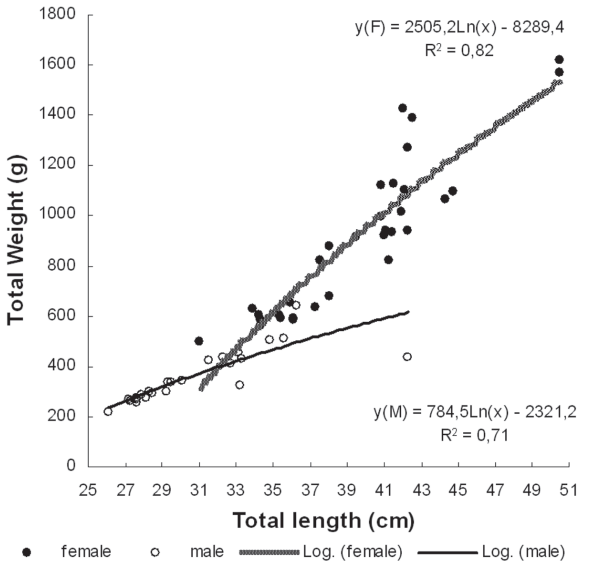

Figure 1. Total weight and total length relationship of the brill breeders.

All females were ready to spawn when transported to the hatchery. All the brill females successfully ovulated with a dose of 25 µg LHRH-a kg^{-1} body weight. Prior to hormonal treatment (at day zero), eggs and milt were obtained from both of the two experimental groups by hand-stripping. The following days, amounts of egg production of female fish in the control group decreased drastically, but this was not observed in the induced group (Table 2). However, the group treated with hormones had larger amount of eggs, exceeding 71.3 g kg^{-1}, in contrast with control group which produced 33.2 g kg^{-1} body weight. There were statistical differences in quantity of eggs obtained from the two groups (p<0.05). The spawning lasted 72 hours in the induced group at nearly the same proportions of egg mass, but higher fertilization rates and hatching rates took place between hours 24 and 48. Additionally, higher fertilization and hatching rates were observed in induced group than the control group (p<0.05). Hatching occurred approximately 110-115 hours after fertilization at 14±1°C.

Data in the same column and between the induced and control groups with the same small letter in the superscription are significantly different (Yates' χ^2 test, p<0.05).
Data in the same column and between the induced and control groups with the same trace (*) in the superscription are significantly different (Pearson's χ^2 test, p<0.05).

Statistical differences were found on the survival rates of three fish groups after the fishing operations (χ^2 = 91.703, df=2, p<0.05).

DISCUSSION

Speed and gentleness during fish capture and handling are of utmost importance. Broodfish must be handled carefully to minimize physical injury and stres [13, 19]. Females that have eggs in a sufficiently advanced stage of development for successful hormone-induced spawning should be injected as soon as possible [4, 18, 19, 20, 25]. Any delay in manuplation greatly diminishes the chance for a successful spawn [8, 27]. Dissolved oxygen content of the water, proper temperature, and absence of disturbance to the fish following hormone injection(s) are believed to play an important role for a successful induced-spawning [12, 15, 18, 28].

Many researchers studied different spawning methods to supply healthy fertilized eggs before they started to produce the larval fish [2, 18, 21, 22]. The present study was the first attempt on the capture of brill broodstock by gill nets to investigate its spawning features in the culture conditions. The wild broodtock brill was successfully ovulated by hormonal injection under culture conditions. Final ovulation treatment showed that a single injection of hormonal applications was effective for inducing ovulation in the brill. Although the wild-caught brill spawned by hand-stripping without hormonal treatment during the first day. In the following days, the quantity, fertilization and hatching rates of the eggs significantly decreased (p<0.05). It appears that LHRH-a treatment had manifested positive effect on ovulation success and egg quality in the brill. The similar

Table 2. Egg production, fertilization and hatching rates of the experimental groups. (TT: Treatment time; WSE: Weight of stripped eggs; WFE: Weight of fertilized eggs; FR: Fertilization rates; HRFE: Hatching rates based on fertilized eggs; HRTE: Hatching rates based on total eggs; TV: total values).

TT (hour)	Groups	WSE (g)	WFE (g)	FR (%)	HRFE (%)	HRTE (%)
0	Control	254	54	21.2	6.48	1.38
	Induced	343	54	15.7	8.7	1.37
24	Control	138	22	15.9	6.82	1.09
	Induced	287.5	75	26.1	36	9.39
48	Control	25	0.8	3.2	3.75	0.12
	Induced	283	106	37.5	46.23	17.31
72	Control	15	0.15	1	0	0
	Induced	200	25	12.5	12	1.5
TV	Control	432	76.95*	17.81	6.5[a]	1.16[a]
	Induced	1113.5	260*	23.4	32.2[a]	7.5[a]

results on the spawning of other turbot species were found by Mugnier *et al.* (2000), Maslova (2002) and Hara *et al.* (2002).

In conclusion, the current results showed that LHRH-a treatment was an effective and reliable method for inducing ovulation in ripe broodstock of the brill. According to the current data, inverse relation was obviously seen between the induced and control groups in their egg production, fertilization and hatching rates. However, it becomes increasingly difficult to obtain the required quantity of spawners in succession-ripe breeder from wild stocks because of overexploitation and environmental pollution. Therefore, reproduction in the brill still requires further study, particularly regarding some peculiar aspects, such as setup of the methods of induced ovulation and identification of ejective minimal hormonal doses. The present work may be considered as a preliminary contribution to this topic.

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