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ADVANCES IN REPRODUCTION OF THE LEBRANCHE MULLET *Mugil liza*: MATURATION AND SPAWNING OF F1 BREEDERS IN CAPTIVITY*

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ABSTRACT

This study aimed to evaluate the reproductive response of lebranche mullets (*Mugil liza*) born in captivity (F1) using hormonal induction. Therefore, it was described maturation and induced spawning, which is the first report of this species reproduction in captivity. The males presented viable sperm at 11 months of age, with length of 25.7 ± 0.4 cm and weight of 205.7 ± 11.5 g. In contrast, at three years of age females could be reproduced, with length of 47.4 ± 1.4 cm and weight of 1263.0 ± 64.6 g. Ten spawnings were conducted, and the average diameter of the oocytes at the time of hormonal induction, fertilization rate, total and relative fertility and hatching rate were recorded. Spawning was attained through hormonal induction in females with oocyte diameter greater than 600 µm and in males that released semen with abdominal massage. During this reproductive period, induced females produced a second group of mature oocytes so another hormonal induction was performed. The present study describes the induction of lebranche mullet breeders born in captivity and the possibility of females to be induced more than once during the same reproductive period.

Keywords: Mugilidae; reproduction; spawning induction; mariculture.

AVANÇOS NA REPRODUÇÃO DA TAINHA *Mugil liza*: MATURAÇÃO E DESOVA DE REPRODUTORES F1 EM CATIVEIRO

RESUMO

O objetivo do estudo foi avaliar a resposta reprodutiva de tainhas (*Mugil liza*) nascidas em cativeiro (F1) por indução hormonal. A maturação e desovas induzidas desses reprodutores foram descritas e consistem no primeiro relato de reprodução da espécie em cativeiro. Os machos apresentaram espermatozoides viáveis aos 11 meses de idade, com comprimento de 25,7 ± 0,4 cm e peso de 205,7 ± 11,5 g; enquanto as fêmeas estavam aptas para reprodução aos três anos de idade, com comprimento de 47,4 ± 1,4 cm e peso de 1263,0 ± 64,6 g. A realização das desovas ocorreu mediante indução hormonal, utilizando fêmeas com diâmetro de ovócito maior de 600 µm e machos que liberavam sêmen quando submetidos à massagem abdominal. Foram realizadas 10 desovas, e registrados o diâmetro médio dos ovócitos no momento da indução hormonal, a taxa de fertilização, a fecundidade total e relativa e a taxa de eclosão. Além disso, foi verificado que no mesmo período reprodutivo as fêmeas induzidas a reprodução maturaram um segundo grupo de ovócitos, que atingiram o tamanho recomendado para a indução hormonal, possibilitando uma segunda indução. O presente estudo descreve a indução de reprodutores de tainhas nascidos em cativeiro e a possibilidade de uma mesma fêmea ser induzida mais de uma vez no mesmo período reprodutivo.

Palavras-chave: Mugilidae; reprodução; indução de desova; maricultura.

INTRODUCTION

The *Mugil liza* (Valenciennes, 1836), popularly known in Brazil as *tainha* (Lebranche mullet), belongs to the Actinopterygii class of the Perciformes order and Mugilidae family. It is found from the Caribbean to Argentina (Menezes et al., 2010; Durand et al., 2012; Lemos et al., 2014). They are catadromous fish, with recruitment of juveniles in lagoons and estuaries, followed by a period of oceanic migration for breeding (Vieira, 1991; Vieira and Scalabrin, 1991; Albieri and Araujo, 2010; Lemos et al., 2014). Characterized as a total spawner, this species presents synchronic ovarian development in two groups

(Lemos et al., 2014). The period of reproductive migration is from April to July, with the majority of individuals arriving in South and Southeastern Brazil from Argentina (38°S) and the Lagoa dos Patos lagoon in Rio Grande do Sul (32°S) (Vieira, 1991; Vieira and Scalabrin, 1991; Lemos et al., 2014, 2016). The schools migrate more than 1000 km northwards and group to spawn in the northeast of Santa Catarina state and Paraná (26°S) (Lemos et al., 2014, 2016). Fewer reach as far as São Paulo (24°S) (Vieira, 1991).

Fish in this family are highly important to commercial and artisanal fishing on the Brazilian coast (Santos et al., 2018). Indeed, the lebranche mullet *M. liza* has historically been associated with the livelihood and culture of artisanal fishing communities in coastal regions (Reis and D'Incao, 2000). On the other hand, industrial fishing is responsible for most of the capture, with mullet roe being a highly valued product for exportation (Brasil, 2015). Despite its importance, there is great variation in the amount of mullet captured annually (Brasil, 2015; Steenbock, 2019). It is currently categorized as a Near Threatened (NT) species, according to the International Union for Conservation of Nature and Natural Resources - IUCN. The status of population conservation is being evaluated to define strategies for fisheries management according to the Ministério da Pesca e Aquicultura e Ministério do Meio Ambiente of Brazil (Brasil, 2015).

The species has important characteristics for fish farming, such as wide tolerance to different salinities and temperatures, and good acceptance of inert food sources (Fonseca Neto and Spach, 1998; Sampaio et al., 2001, 2002; Cerqueira, 2004; Miranda-Filho et al., 2010; Cerqueira et al., 2017). In addition, other factors have aroused interest of the productive sector. When mullet are inserted in an integrated multitrophic cultivation system (IMTA) with white shrimp (*Litopenaeus vannamei*) there is a reduction of *Vibrio* spp. colonies in the water, which decreases the load of pathogens often found in shrimp monoculture (Legarda et al., 2019; Borges et al., 2020). Integration of these species in the cultivation environment increases phosphorus retention and optimizes the use of facilities, allowing production of greater biomass in the same area (Legarda et al., 2019; Borges et al., 2020).

Despite the favorable characteristics of cultivation of this species, there are few reports about its reproduction, spawning and larviculture in captivity. Studies have been conducted since the 1980s in South and Southeastern Brazil (Benetti and Fagundes-Netto, 1980, 1983; Godinho et al., 1993; Monteiro-Ribas and Bonecker, 2001; Cerqueira et al., 2017; Carvalho et al., 2019) and in Havana (Cuba) (Alvarez-Lajonchère et al., 1988). Despite those studies, the captive production of mullet larvae still depends on wild breeders.

In this context, this study aimed to describe and evaluate maturation and spawning of lebranche mullets born in captivity (F1).

MATERIAL AND METHODS

Wild Breeders

The first breeding stock was composed of adult specimens of *Mugil liza* caught from April to July 2014 (the period of authorized artisanal fishing in Santa Catarina) in Laguna - SC (28°29'41.449"S; 48°45'1.494"W). Fourteen adult lebranche mullets (4 females and 10 males) were transported to the Laboratório de Piscicultura Marinha (LAPMAR-UFSC; 27°35'8.960"S; 48°26'24.235"W), where they were maintained and used for reproduction from 2015 to 2018. The wild breeders were used to produce the F1 breeders. In 2015 and 2016, one spawning was performed per year, using different breeders each year, at the sex ratio of 1 female and 2 males.

F1 Breeders

From breeding and larviculture conducted in the laboratory, two groups of fish were separated: one born in 2015 and another in 2016. The fish were kept at LAPMAR in two 12 m^3 circular tanks outdoor, with a continuous flow and daily renewal of 2.5 – 3 times tank volume of sea water pumped from the Mozambique beach, Florianópolis - SC (27°34'1.73"S; 48°25'42.663"W) and aeration from a radial blower coupled to a microporous hose ring (Aquadrop Air®, São Paulo, Brazil). During fish culture it was were monitored daily at 10:00h am dissolved oxygen and water temperature using a YSI Pro20 digital oximeter, pH with a YSI pH10A digital pH meter (YSI Inc., Yellow Springs, Ohio, USA) and salinity with optical refractometer Instrutherm RTS-101ATC-03137 (Instrutherm Instruments of Measurement Ltda., São Paulo, Brazil). In 2019, the dissolved oxygen, salinity and pH parameters averaged 6.1 ± 0.4 mg L^{-1}, 34.6 ± 0.7 and 8.3 ± 0.6, respectively. Photoperiod was determined from official data (Dateandtime.info, 2019). Water temperature (max. 30.3°C in January and min. 15.3°C in August) and natural photoperiod (at latitude 27°S, maximum 13h53min. and minimum 10h23min.) varied according to the seasons (Table 1).

Feeding was conducted four times a day until apparent satiety (Calixto et al., 2020) with commercial feed (45% crude protein and 9.0% ether extract) of 0.8 - 1 mm (for fish of 1 to 15 g) and 1.3 - 1.5 mm (15 to 100 g fish) during the juvenile phase, and 2.6 mm ration (45% crude protein and 8.0% ethereal extract) for fish over 100 g. Maximum fish biomass was 3.0 kg m^{-3} in the grow out phase and 1.5 kg m^{-3} in the gonadal maturation phase.

Selection of fish for hormonal induction

Fish were subjected to previously approved and authorized procedures by the UFSC ethical use of animals committee (Protocols CEUA n° PP00861 and n° 7759170919). To assess their gonadal maturation stage, fish were anesthetized with benzocaine at 50 mg L^{-1} (Braz et al., 2017). From April to November the analysis of maturation of females was performed through intraovarian biopsy, with a non-toxic PVC probe (tracheal probe nº 04) inserted in the oviduct and aspiration of an oocyte sample. Fifty oocytes were measured with a stereoscopic loupe (Leica EZ4HD) and LAZ EZ 2.1.0 software (Leica, Switzerland). The reproductive period began when females presented average oocyte diameter greater than 600 µm, value considered suitable for hormonal induction (Cerqueira et al., 2017). Males were mature and able

Table 1. Water temperature and photoperiod (means ± SD) in tanks of F1 lebranche mullet breeders (*Mugil liza*) in 2019.

Month	Temperature (°C)	Photoperiod (minutes)
January	27.9±1.7	821.6±8.38
February	25.6±1.2	777.0±15.9
March	25.5±1.9	735.4±14.7
April	24.7±1.7	687.3±14.3
May	22.2±1.4	646.8±9.2
June	20.4±3.0	624.0±8.6
July	18.7±1.2	639.1±5.9
August	17.9±1.6	682.0±11.3
September	19.1±1.6	718.4±15.1
October	21.4±1.1	780.3±7.2
November	22.5±0.4	807.5±9.1
December	23.2±1.3	829.7±2.6

to reproduce when they released semen after abdominal massage (Magnotti et al., 2018a, 2018b; Castro et al., 2019).

In the first gonad verification procedure breeders were identified with external numbered markers, model T-bar anchor tag FD-94 (Floy tag Inc., Seattle, Washington, USA) inserted in the dorsal region for individual monitoring.

Hormonal induction methodology

The first hormonal injection was intramuscular in the dorsal region and standardized at 16h pm. Females received two doses: 20 µg kg^{-1} of carp pituitary extract (CPE) and after 24 h 200 µg kg^{-1} LHRHa (des-Gly^{10}, D-Ala^{6} LH-RH *ethylamid acetate salt hydrate*, L4513, Sigma-Aldrich, St. Louis, USA) (Yousif et al., 2010; Carvalho et al., 2019), while males received 100 µg kg^{-1} LHRHa when females were first injected. For each spawning, one female and two males were induced.

Spawning and egg collection

During the period females presented mature oocytes, ten spawning inductions were performed. For spawning of females F1 01 (June), F1 04, F1 05 and F1 08, males from the same parents were used. For the other spawnings, females born in 2016 were crossed with males born in 2015, and the female born in 2015 (F1 01) with males from 2016 (spawning in September).

After the first injection, fish were transferred to a 2 m^3 circular tank, supplied with a continuous flow of saltwater (250% rate) and coupled to two 35 L conical cylindrical egg collectors (Cerqueira et al., 2017; Carvalho et al., 2019). Water temperature was controlled by a 1000W titanium heater connected to a digital thermostat (Coel tlz-11). While selecting fish, temperature was the same as in the breeding tanks (19.7 ± 1.1°C), and was gradually increased to 22.5°C at the second hormone injection and up to 24.5°C at the time of spawning. Fertilization was natural in all spawning.

Evaluation of reproductive indexes

When embryos reached the blastospore closure stage (approximately 16 h after spawning), three 5.5 mL samples were collected to quantify the total number of eggs (fertility rate) and fertilization rate, using a stereoscopic loupe (Leica EZ4HD). Relative fertility was later calculated, where: Relative fertility (eggs g^{-1}) = (Total number of eggs) / (Initial female weight (g)).

To verify the hatching rate, three samples of 200 eggs were counted by the naked eye in 10 mL glass pipettes and transferred to a 2 L beaker with thermostatic bath at 24.5ºC and aeration. A larvae count was performed 12 h after the first hatching. To determine the hatching time, the interval between the time of spawning and the hatching of the first larvae was considered.

RESULTS AND DISCUSSION

Full gonadal maturation in male lebranche mullet (*M. liza*) was verified in all fish produced at LAPMAR, which released sperm after an abdominal massage. The 15 males born in 2014 presented sperm releasing at 11 months of age, and semen was collected, evaluated and classified as able to spawn (Magnotti et al., 2018b), while 45 born in 2015 were capable of reproduction at 25.7 ± 0.4 cm in length and 205.7 ± 11.5 g in weight (Castro et al., 2019).

Maturation of F1 female oocytes was first observed in 2018. The thirty fish born in 2015, which were from the same spawning used by Castro et al. (2019), presented all males matured, one female had oocyte maturation and three fish were undifferentiated. However, oocytes of this female did not have sufficient diameter for hormonal induction (<600 µm). The groups of fish used as breeders in 2019 are described in the Table 2.

In April 2019, the initial development of oocytes was verified in all females. One female born in 2015 (F1 01) and seven females born in 2016 (F1 02 to F1 08) were selected and evaluated during the reproductive period of 2019, when they initially presented oocytes of 260 ± 23 µm in diameter. The complete development of gonads occurred in the next months until June, when all females presented oocytes diameter above the recommended values (>600 µm) and spawning induction was initiated. Considering the ten spawnings performed, relative

Table 2. Year of birth, sex, weight and total length (means ± SD) of lebranche mullet breeders (*Mugil liza*) used in 2019.

Year of birth	Number of fish	Sex	Weight (g)	Total length (cm)
2015	12	male	1198.9±146.7	48.2±1.6
	2	female	1722.0±39.6	55.0±1.4
2016	17	male	967.3±107.4	44.5±1.8
	13	female	1263.0±64.6	47.4±1.4

fecundity of F1 females was 924 ± 238 eggs g^{-1} (min. 638, max. 1426) (Table 3).

In all spawning using fish from the same parents there was high mortality during embryonic development, resulting in hatching rates below 5% (Table 3). It is possible that this event might be related to the inbreeding of embryos from siblings, although more specific studies must be performed in order to better understanding of the process. Within a batch, inbreeding causes depression in productive variables, reducing the growth rate and affecting reproductive performance (Fessehaye et al., 2009).

When analyzed, the time between first hormonal application and spawning in F1 females was shorter (41 ± 2 h at 24.2 ± 0.4°C) than in wild females (54-57 h at 23°C) observed by Cerqueira et al. (2017) and Carvalho et al. (2019). It is known that adaptation to captivity conditions facilitates reproductive management procedures, favors animal welfare and consequently efficiency and effectiveness of hormone doses in obtaining spawning. Stress in fish negatively affects hormonal production related to oocyte maturation and spawning (Fuzzen et al., 2011), causing negative feedback that may affect response to hormonal induction (Rivier and Rivest, 1991).

After these spawning events, there was a second maturation of oocytes in F1 breeders (Table 4). The female F1 01, which spawned in June, was ready for another hormonal induction after 82 days, with oocytes at 656 ± 26 µm in diameter. This female was induced in September and had relative fecundity of 932 eggs g^{-1} and a 53% hatching rate. The same was verified for female F1 03, which was induced to a second spawning 75 days after the first one and presented relative fecundity of 862 eggs g^{-1} and a 49% hatching rate (Table 3). Maturation of a second group of oocytes in the same spawning season was also verified in other females from the brood stock. Oocytes of female F1 06, however, were not sampled because its oviduct was not dilated and thus the probe could not be inserted (Table 4). Oocyte rematuration was also observed in a wild female of *M. liza* three months after the first spawning by Carvalho et al. (2019), and for grey mullet (*Mugil cephalus*) by Tamaru et al. (1989).

Table 3. Reproductive indices referring to spawning of F1 lebranche mullets (*Mugil liza*) at LAPMAR during the reproductive period of 2019.

Spawning month	Female identification	Oocyte diameter (µm)*	Fertilization rate (%)	Total fecundity	Hatching rate (%)
June	F1 01	644±24	99	1,080,000	1**
July	F1 02	637±15	98	1,039,000	65
July	F1 03	625±22	99	1,634,000	72
July	F1 04	615±16	50	1,246,500	5**
August	F1 05	641±23	98	897,000	3**
August	F1 06	641±23	99	1,836,200	83
August	F1 07	677±26	93	1,142,818	26
August	F1 08	629±17	90	863,600	2**
September	F1 01	656±26	97	1,539,000	53
October	F1 03	655±33	97	1,120,800	49

* means ± SD; ** Spawn using fish from same parents

Table 4. Date of measurement (day/month) and oocyte diameter (µm; mean ± SD) of F1 female lebranche mullets (*Mugil liza*) during reproductive period in 2019.

Female identification	Oocyte 25/04	Oocyte 21/05	Date of Spawning	Oocyte	Oocyte 16/09	Oocyte 02/10	Oocyte 12/11
F1 01	256±49	-	27/06	644±24	656±26*	-	ł
F1 02	265±46	-	08/07	637±15	544±36	633±44*	ł
F1 03	283±40	405 ± 43	17/07	625±22	590±30	655±33*	ł
F1 04	237±47	-	29/07	615±16	292±38	366±32	ł
F1 05	303±43	454±33	05/08	641±23	431±37	526±23	ł
F1 06	252±35	-	12/08	641±23	#	#	ł
F1 07	235±37	-	22/08	677±26	#	598±39	ł
F1 08	249±51	-	27/08	629±17	188±22	210±33	ł

* Females able to spawn after oocyte rematuration. # Undilated oviduct (gamete collection unfeasible). ł Atresic oocytes in resorption, deformed and/or ruptured (measurement unfeasible).

In November, the beginning of oocyte resorption in all females and the absence of sperm in males were observed, demonstrating that in 2019 the period in which F1 breeders were able to reproduce was from June to October. In these months, the average water temperature ranged from 17.9 ± 1.6°C in August to 21.4 ± 1.1°C in October. In the region studied (25–31°S, Southern Brazil) the reproductive period in captivity was different from that of wild fish, for which reproductive migration and final maturation of gonads occur between April and May, and spawning events in June and July modulated by sea surface temperature between 19-21°C (Lemos et al., 2014, 2016). The extended reproductive period, higher range of water temperature and the re-maturation of gonads within the same period in F1 females are positive characteristics of aquaculture that may facilitate production scheduling and planning and provide availability of young forms for longer periods.

CONCLUSION

Between June and October 2019 lebranche mullet spawnings were obtained using hormonally induced females breeders born in captivity (F1) aged three and four years old. It was also observed that females presented gonad rematuration and were able to spawn more than once in the same reproductive period.

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