Maturation, spawning and larviculture of the mullet *Mugil liza* under laboratory conditions

Maturação, desova e larvicultura da tainha Mugil liza em laboratório

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Abstract

The technical feasibility for the sustainable production of a species depends mainly on the control of reproduction in captivity and the availability of juveniles. Studies were performed with the mullet *Mugil liza* in the 1980s, however this species was never produced commercially. With the development of aquaculture, the increasing demand for animal protein and the commercial value of the mullet roe, it was necessary to rear this species in the laboratory to produce juveniles in pilot scale. The broodstock, 4 females and 10 males, were kept in an indoor 12 m³ tank. Hormonal inductions were performed with the hormones pituitary extract of carp (PEC) and luteinizing hormone-releasing hormone analog (LHRHa); spawnings occurred between 54-57 h after the hormonal induction. Embryonated eggs of mullets were pelagic and translucent, with an average diameter of 846.29 ± 14.34 µm. The larvae hatched approximately 48 h after spawning with 2.95 ± 0.12 mm. The survival in the larviculture was estimated at 18.75%. Therefore, this study presents the technical methods for the capture, transport, acclimatization, maturation, induced spawning and hatchery of the mullet *M. liza* in the laboratory.

Keywords: Mugilidae, pelagic, broodstook, larvae; natural fertilization, induced spawning.

Resumo

A viabilidade técnica para a produção sustentável de uma espécie depende principalmente do controle da reprodução em cativeiro e da disponibilidade de juvenis. Estudos foram realizados com a tainha *Mugil liza* nos anos 80, entretanto, essa espécie não chegou a ser produzida comercialmente. Com o desenvolvimento da aquicultura, com a crescente demanda por proteína animal, além do valor comercial agregado nas gônadas dessa espécie, surgiu à necessidade de tentar produzi-la em laboratório, desta vez com o objetivo de produzir juvenis em escala-piloto. Os reprodutores, 4 fêmeas e 10 machos, foram mantidos em tanque de 12 m³. As induções hormonais foram realizadas com os hormônios extrato bruto de hipófise de carpa (EBHC) e um análogo do hormônio liberador do hormônio luteinizante (LHRHa); as desovas ocorreram 54-57 h depois da indução hormonal. Os ovos embrionados das tainhas são pelágicos e translúcidos, com um diâmetro médio de 846.29 ± 14.34 µm. As larvas eclodiram aproximadamente 48h após a desova com 2.95 ± 0.12 mm. A sobrevivência desde a estocagem dos ovos até a despesca dos juvenis foi estimada em 18,75%. Sendo assim, este estudo apresenta os métodos técnicos para a captura, transporte, aclimatação, maturação, desova induzida e larvicultura da tainha *M. liza* em laboratório.

Palavras-chave: Mugilídeos, pelágicos, reprodutores, larvas, fertilização natural, indução hormonal.

Introduction

The mullets (family Mugilidae) inhabit the western Atlantic coast of South America (Menezes et al., 2010; Siccha-Ramirez et al., 2014), form schools and present migratory habits (Vieira and Scalabrin, 1991). The adults are oceanic, whereas juveniles live in estuaries. The feeding habit also differs according to the stage of their life cycle, changing from planktophage juveniles to iliophage adults. The adults feed mainly on vegetable matter from mud or sand in the substrate where they live. Specimens of 50 cm are commonly observed, though they can reach about 1 m in length and weigh 6 kg (Menezes and Figueiredo, 1985; Oliveira and Soares, 1996). Mugilids are important for commercial and artisanal fishing in the Brazilian coast, being *Mugil liza* and *M. platanus* the most abundant species in catches.

Recently, the taxonomic status of the genus *Mugil* was reviewed by several authors (Fraga et al., 2007; Heras et al., 2009; Menezes et al., 2010), who concluded that the mullets *M. liza* and *M. platanus* are the same species, thus renamed *M. platanus* as *M. liza*.

Much has been researched on this species in the 1980s, but it has never been produced commercially. With the increasing demand for animal protein and different demands for the development of marine fish farming, the need arises to investigate others species and intensify the production of marine fish.

The mullet *M. liza* is a lower-level consumer (Bennetti and Fagundes Neto, 1991) that tolerates wide variations of salinity and temperature. Such characteristics facilitate its rearing in different environments and with

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other fish (Sampaio et al., 2001; Sampaio et al., 2002; Miranda-Filho et al., 2010; Carvalho et al., 2014; Lisboa et al., 2015). The mullet meat has a tasteful flavor and the roe (female gonad) is quite appreciated in Brazil as well as other countries like Taiwan, France, Greece, Italy and Spain, where it is known as the “Brazilian caviar" (Ferreira et al., 2011).

The industry that uses mullet meat and/or roe depends exclusively on the capture of adult individuals. With the increased commercial interest especially for the roe, the fishing effort to capture adult individuals has grown significantly and the *M. liza* was considered overexploited in 2004 (Garbin et al., 2014).

However, mugilids have been cultivated worldwide: Egypt has been the largest producer of *M. cephalus,* followed by Korea and Taiwan (FAO, 2012). In the United States, juvenile *M. cephalus* are commercially produced in laboratory (Lee and Ostrowski, 2001) for restocking purposes (release of hatchery fingerlings to increase the natural recruitment).

In order to commercially produce the mullet *M. liza*, the knowledge on its cultivation must be improved. The studies of Alvarez-Lajonchere et al. (1988) and Godinho et al. (1993) about this species were experimental and did not lead to a massive production of juveniles.

Thus, our study demonstrates the technical feasibility for the captive production of the mullet *M. liza*. This study presents technical methods for the capture, transport, acclimatization, maturation, induced spawning and juvenile production in the laboratory.

Material and methods

All fish used in this study were handled according to procedures approved by Ethics and Animal Use Comitee of the Universidade Federal de Santa Catarina (UFSC) (PP00861/CEUA/PROPESQ/UFSC/2013).

Capture, transport and acclimatization

The fish used in this study were captured by fishermen during the mullets reproductive season (between April and July) at the Barra channel in Laguna, Santa Catarina, southern Brazil. Casting nets, with a mesh size of 10 cm, were used to capture the fish. After catching, the fish were transported to the shore avoiding loss of scales due to contact with sand. On the beach, each fish was transferred to the 60 L transport bags covered with a black bag to reduce the passage of light and, consequently, stress. Each bag contained 1/3 of seawater (32-35 g/L) and 2/3 of oxygen. Bags are inflated with oxygen, supersaturating the water, eliminating problem with oxygen depletion in transport water caused by animal metabolism. The bags were closed with a resistant rubber and placed on a mattress to reduce the impact during transport. The fish were transported by truck to the laboratory (Laboratório de Piscicultura Marinha - LAPMAR) located at Barra da Lagoa in Florianópolis, Santa Catarina, Brazil. The transport took from 2 to 4 hours.

Reception in the laboratory and pretesting of hormonal induction

In the laboratory, the bags with the fish were placed within 2 m^3 tanks with marine water (32-35 g/L) and aeration. One bag was open at a time and each fish was anesthetized in the bag (70 ppm of benzocaine). Subsequently, the sex was determined by the release of semen after abdominal massage for males and intraovarian biopsy for females. The fish received an intramuscular injection with 0.1 mL kg^{-1} oxytetracycline and a healing ointment was applied for loss of scales or bruises. When the mullets are caught in the sea, they are quite stressed due to the reproductive migration and can be very sensitive to any kind of injury. Therefore, this treatment was essential in newly caught fish. The fish were kept in quarantine and observed for 48 h.

After evaluation, only females that showed oocytes larger than 600 µm and males that released sperm were hormonally induced following the methodologies adapted to *M. liza* (Godinho et al., 1993) and *M. cephalus* (Yousif et al., 2010). Shortly after the evaluation, the first hormonal injection was applied only in females. After 24 hours, we applied the second injection in the females and the first in the males (half of the female dosage). Three males and one female were used in each induction. Three combinations of hormone were tested: pituitary extract of carp (PEC), human chorionic gonadotropin (HCG) and luteinizing hormone-releasing hormone analog (LHRHa). The salinity of the water during the inductions was 35 g/L and the temperature remained between 19 and 21°C. Based on the results of these tests, we determined the best hormone therapy to be used in the following year.

A total of 29 mullets (*M. liza*) were captured (7 females, 16 males and 6 fish with undefined sex). Ten trips were needed to transport all the fish, which took 2 months. The average weight ad length of females and males was 1849.00 ± 399.00 g and 54.50 ± 2.50 cm, and 1125.50 ± 205.85 g and 47.50 ± 3.28 cm, respectively.

Feeding and maturation in a recirculating aquaculture system (RAS)

The broodstock (4 females and 10 males) were selected and carefully transferred and kept in a 12 m^3 maturation tank for a year. When handling was needed, the water level in the tanks was reduced to 500 L and an anaesthetic (benzocaine) was diluted in the water to calm the fish (light anesthesia, 10 ppm). Subsequently, the fish

were transferred to a box of 30 L with freshwater, where they remained for about 5 minutes (prophylactic treatment against ectoparasites of marine fish) and were transferred back to the maturation tank.

The maturation tank had a volume of 12 m^3 (3,20 m diameter and 1,50 m height) and connected to a recirculating aquaculture system with marine water (protein skimmer and mechanical filter, biological filter and UV disinfection filter), with natural temperature and photoperiod.

The captured broodstock readily accepted commercial feed (45% crude protein, 12% ether extract) with 4 mm in diameter, fish were fed twice a day until apparent satiation. We followed the reproductive maturation in captivity for a year, and as the reproductive season approached, fish reduced feed intake on their own until they stopped feeding, indicating the start of their breeding season.

Results and Discussion

Maturation and spawning

After capture, the broodstock were kept in a 12 m^3 maturation tank for a year. After eight months in tank the fish were evaluated. In this opportunity, males did not release semen and the oocytes were collected from two of the four females. Female 1 (Fe1) presented oocytes with an average diameter of 384.85 ± 70.97 μm, whereas female 2 (Fe 2) had oocytes with average of 125.00 ± 35.00 μm. Two months after that assessment (start of their breeding season in the environment), all males were releasing semen and three of four females were cannulated. The oocyte diameters of Fe1, Fe2 and Fe3 were 630.95 ± 42.89 μm, 623.75 ± 42.92 μm and 390.00 ± 39.24 μm, respectively. At that point, Fe1 and Fe2 had attained the average diameter of oocytes recommended for hormonal induction of the species.

The female hormonal induction was performed with the hormones PEC and LHRHa, according to the previous results performed in the laboratory. Fe1 received the first hormone induction (20 mg kg^{-1} of PEC) and the four fish were transferred to a 2 m^3 tank with marine water (35 g/L), at the same temperature (23°C) of the maturation tank. The temperature was controlled with a titanium heater of 2000 W and a thermostat. After 24 h, Fe1 received the second application with 300 μg kg^{-1} of LHRHa and all males received an injection with 150 μg kg^{-1} of LHRHa.

The same procedures for management and induction were used in all male and female. The spawning data of each induction are presented in Table 1.

The first induced female presented a new development of vitellogenic oocytes (rematuration) two months after spawning (diameter of oocytes 490.00 ± 72.02 μm). In the following month, the oocytes had an average diameter of 670.83 ± 67.57 μm, being subjected to a new spawning induction. According to Tamura et al. (1989), some *M. cephalus* females that mature at the beginning of the reproductive season can mature and spawn a second time within the same reproductive season. In this study, we observed that the rematuration can also occur with *M. liza*.

Spawning, collecting and hatching eggs

The hormonal induction of the broodstock, spawning and sampling of the eggs were carried out following the same protocol in all procedures. After the hormonal induction fish were kept in 2 m^3 tanks with an egg collector in the water outlet on the surface of the tank. The four natural spawns occurred between 54-57 h after the first hormonal injection. Embryonated eggs of mullets were pelagic and translucent, with an average diameter of 846.29 ± 14.34 μm.

All the eggs were removed from the collectors after the blastopore enclosure (approximately 16 hours after spawning) at the stereoscopic microscope. The eggs were transferred to incubators with daily water renovation of 200% and gentle and constant aeration. Due to water renewal, the water temperature of the incubators has decreased gradually from 23°C to 20°C.

Egg counts were performed in triplicate in a stereoscopic microscope with a Bogorov pipette. We calculated the fertilization rates based on the ratio between fertilized and unfertilized eggs (Tab. 1).

In the first spawning, when the embryos began to move inside the eggs, 400,000 embryonated eggs were transferred to larviculure tanks of 5 m^3 with marine water (35 g/L), constant aeration and temperature of 20°C.

The hatching rate of each spawning was determined through the count of the larvae that hatched from the embryonated eggs stocked in three beakers with 500 mL of marine water (water of incubators). In each beaker, 50 eggs were stocked. The larvae hatched approximately 48 h after spawning with 2.95 ± 0.12 mm.

Table 1. Data on the inductions and spawns made with mullet broodstock in captivity.

Spawns	Female	Oocyte diameter (μm)	Fertilizing rate (%)	Total fecundity (eggs)	Hatching rate (%)
I	Fe1	703.12 ± 37.37	98%	1827000	96.18 ± 0.15
II	Fe2	675.00 ± 19.36	96.15%	805526	48.94 ± 5.24
III	Fe3	600.00 ± 25.00	78.60%	930750	47.45 ± 7.30
IV	Fe1	670.83 ± 67.57	94.96%	2249017	92.16 ± 4.80

The lowest total fecundity and hatching rate of the second induction may be related to the variation in water temperature of the spawning tank. The thermostat controlling the heaters had a problem and the water temperature fell 5°C after hormone injection. In the third induction, the problem can be related to the oocyte diameter, which was close to the recommended size limit for the induction of mullets.

Larviculture

In each tank, 40 eggs L^{-1} were stocked. The feeding protocol used during the larviculture was based on the work of Yousef et al. (2010) and adjusted according to the development of the larvae (Fig. 1).

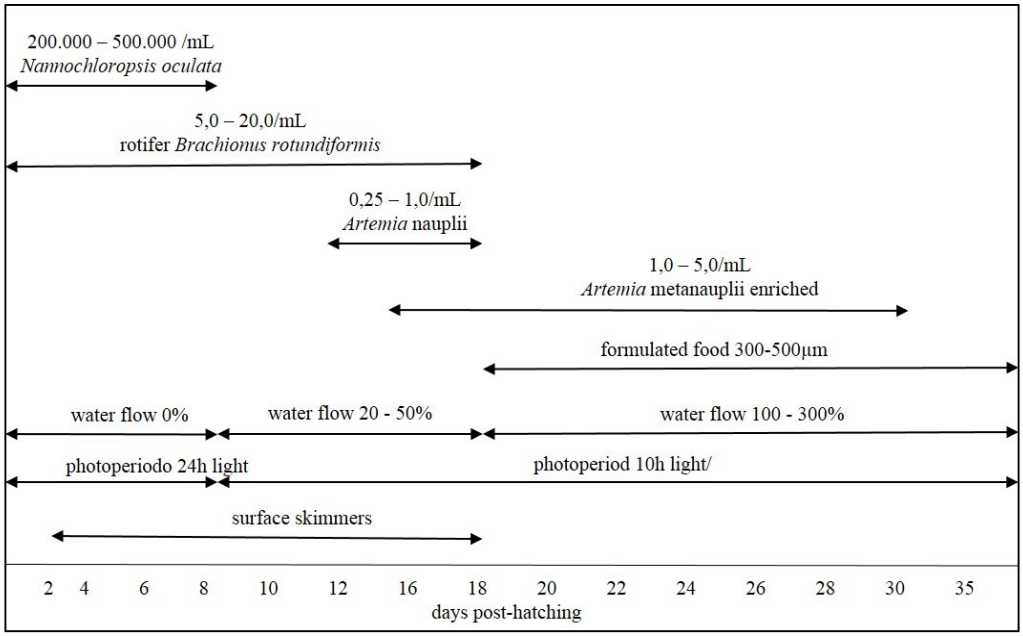

Figure 1. Protocol used during the larviculture of *Mugil liza.*

In the first 7 days after hatching (dah), the larviculture tanks were kept in a green water system, with the microalgae *Nannochloropsis oculata* (Instant Algae®, Reed Mariculture) at a density of 300,000 cells mL^{-1}. From 8 to 19 dah, 20 to 50% of the water of each tank was daily renewed. After 20 dah, the larviculture tank was kept in continuous flow (100% to 300% of the total volume per day).

In the early days, the larvae fed on the rotifer *Brachionus rotundiformes*, followed by *Artemia* nauplii and *Artemia* metanauplii enriched with Selco spresso (INVE Aquaculture Inc., Belgium). Subsequently, larvae were weaned, which is the transition from live to inert food. From 18 dah onwards, they were fed a commercial feed (Orange Grow Small 300 to 500 µm – INVE Aquaculture Inc., Belgium) and the particle size was adjusted according to the larvae development. After 35 dah, the larvae were feeding exclusively with inert feed.

The larval development was monitored every 7 days during the first 35 dah (Fig. 2). Soon after hatching, the larvae showed the yolk sack with a drop of oil and three days after hatching their mouth was opened and they began to feed on rotifers. Between 9 and 10 dah, the oil drop was totally consumed and the notochord was flexed in every larvae between 16 and 17 dah.

A total of 75,000 juveniles were produced after 80 days. The survival from embryonated eggs in the larviculture tank until their harvest was estimated at 18.75%. By the time of the harvest, the juveniles had 0.67 ± 0.25 g and 3.7 ± 0.58 cm. Few individuals with malformation were observed (less than 50 juveniles), which was considered a negligible abnormality rate.

The parameters of water quality during the larviculture were: temperature 22.91 ± 1.38°C, salinity 35 g/L, dissolved oxygen 6.23 ± 1.12 mg L^{-1}, pH 8.03 ± 0.25, and ammonia lower than 0.02 mg L^{-1}.

The first step in the reproduction of species in captivity is the control of maturation, spawning and production of viable embryonated eggs (Mylonas et al., 2010). In addition, it is important to validate the rearing techniques not only in the laboratory, but also on a commercial scale (Lee and Ostrowski, 2001). Despite the mullet *M. liza* being a migratory species for reproduction in nature, the management adopted in this study proved to be efficient, allowing advanced maturation (gametogenesis) of captive broodstock. However, we needed to use a hormonal therapy to achieve the final maturation and ovulation. The PEC and LHRHa hormones were effectively used to induce ovulation in females. In males, only LHRHa was used to increase efficiency in the release of semen. Both hormones act on the pituitary to induce the release of luteinizing hormone, which will act on the gonad to induce the steroidogeneseis and the process of oocytes maturation of the oocytes and spermiation (Mylonas, 2010).

In addition to hormone therapy, the temperature control after hormone induction allowed the males and the females to naturally spawn and fertilizing the eggs in the tank, without extrusion.

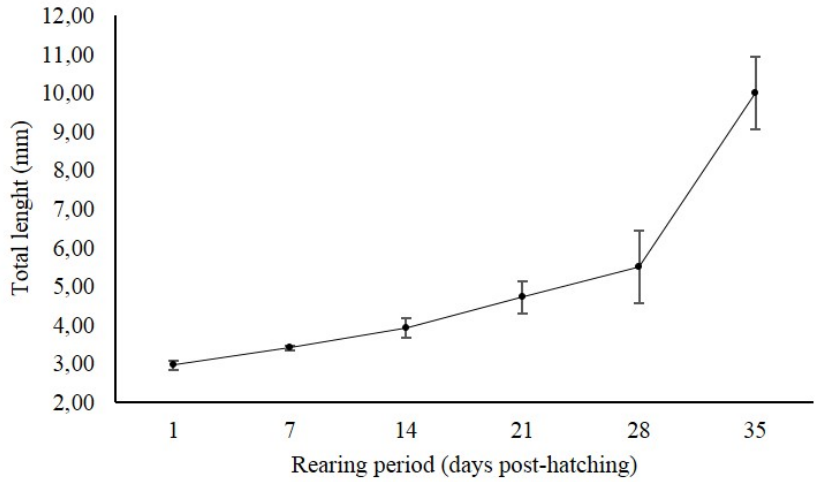

Figure 2. Growth of *Mugil liza* larvae during 35 days. Each point represents the mean ± standard deviation of 15 larvae sampled from each rearing tank.

In relation to larviculture, the development of feeding protocols for the rearing of marine fish still represents one of the barriers to success in the massive propagation of various species of marine fish. The last report on the larviculture of the mullet *M. liza* was published by Alvarez-Lajonchere et al. (1988), with a survival rate of 11% at 26 days after hatching. The results of this study represent an advance in relation to the management and feeding of mullet larvae. Important aspects for larviculture are: water quality, quality control of microalgae added in the tank, supply of live food in the proper size to the mouth of larvae and enriched correctly with appropriate fatty acids for larval development, and quality of inert food. All these factors are essential for the healthy development of the larvae and must be taken into consideration during the larviculture (Cahu and Infante, 2001) to optimize survival and quality of the larvae.

Conclusion

These results demonstrate the feasibility of maintaining the *M. liza* broodstock in indoor tanks, induced spawning and a massive production of juveniles.

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