Induced spawning of the thick-lipped mullet (*Chelon labrosus*, Mugilidae, Osteichthyes)

La reproduction induite du muge à grosses lèvres (*Chelon labrosus*, Mugilidae, Osteichthyes)

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Résumé

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Des essais sur la reproduction induite du muge (*Chelon labrosus*, Mugilidae, Osteichthyes) ont été effectués dans le but d'obtenir des alevins de bonne qualité. Les expériences se sont déroulées dans une ferme d'élevage de poissons située en Italie centrale pendant la saison de reproduction de l'espèce (mars-avril). Seules les femelles ont reçu les traitements hormonaux pour stimuler ou inhiber. Ceux-ci ont induit la reproduction, complète ou partielle, de 9 femelles sur 11 traitées. Cinq femelles ont pondu au total 6810 g d'œufs ayant une haute vitalité (59-88 % de fertilisation) avec un bon pourcentage d'éclosion. Des quatre traitements physiologiquement plus efficaces, le protocole le moins invasif a été obtenu à partir de la combinaison suivante : 7 hypophyses de carpe/kg de poids frais et 24 h plus tard de 50 µg LHRH-a/kg de poids.

MOTS CLÉS :

Mugilidae, *Chelon labrosus*, reproduction induite, domperidone, LHRH-a

Abstract

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Trials to induce spawning of thick-lipped mullet (*Chelon labrosus*, Mugilidae, Osteichthyes) to obtain highly viable seed were carried out in a hatchery of central Italy. Females received one of five different inhibiting and stimulating hormone treatments during the natural spawning season of March - April. The treatments induced spawning completely or partially in 9 of the 11 females selected. Five females spawned a total of 6810 g eggs with high viability (59 - 88% fertilisation rate) and subsequent hatching rate. Of the four most physiologically effective treatments which were all similar in results, the least invasive protocol was the combination of a first injection of a homogenate of 7 carp pituitaries followed 24 h later by a second injection of 50 µg LHRH-a per kg female body weight.

KEY-WORDS :

Mugilidae, *Chelon labrosus*, induced spawning, domperidone, LHRH-a

Introduction

The large family of mullets (Mugilidae) plays an important role in aquaculture in Italy, and production contributes around 3000 mt annually to the domestic market. Two species of mullet are particularly appreciated by producers in Italy. These are the thick-lipped mullet (*Chelon labrosus)*, which is noted for the quality of its flesh, and the leaping grey mullet (*Mugil cephalus*), which has some processed products with high added value, such as dry roe and smoked fillets. Much is raised in the extensive systems of the North Adriatic 'valli,' in ways which have been a tradition for centuries. Such traditional culture of mullets depends on the harvest of wild fry which school in estuaries and coastal waters in large numbers and the need to obtain a steady supply of fry, independent of conditions in the natural environment, have been stressed for years in order to increase the mullet output. Artificial propagation has never reached a commercial scale, and culture still remains dependent on the collection of wild fry.

Upon time various hormonal approaches have been tested on different mullet species in many regions of the world, with particular emphasis on the genus *Mugil,* and especially on the cosmopolitan species *M. cephalus* (Crosetti, Cataudella, 1995; Kuo, 1995).

The first experiences on *C. labrosus* were carried out by Cassifour, Chambolle (1975), who injected female fish with 20 mg/kg/d progesterone for 6 days, and subsequently obtained eggs both by natural spawning and manual stripping. Later Cataudella *et al.* (1988a, 1988b) induced three females to spawn after injection with 5000 IU hCG, fertilized the eggs, and successfully mass-propagated larvae for the first time.

More recently, the effects of a range of inhibiting and stimulating substances were investigated, in different combinations and dosages, on 50 female thick-lipped mullet over a period of seasons (Crosetti, 1998; Crosetti *et al.*, 1998). Spawning was induced, at various levels of success, both with homoplasic and heteroplasic pituitaries (mullet and carp), hCG, LHRH-a, and domperidone, a dopamine antagonist.

The present study concentrates on further refinement of the spawn-inducing experimental protocol on the thick lipped mullet in order to identify the optimal effect use of hormones to obtain the mass production of high quality eggs.

Materials and methods

Source of breeders, selection, handling and marking

Experimental trials were carried out during the natural spawning season of thick mullet in central Italy (March-April) in the hatchery of a fish farm located on the Orbetello Lagoon about 150 km North of Rome.

Breeders selected for our trials came from the farm's broodstock of 65 *C. labrosus* adults which had been captured over a number of years and held in an outdoor pond (400 m^3); moreover mature wild individuals of the same species were caught at the lagoon barriers during the period of our tests.

A few days before the beginning of the experimental trials, the captive broodstock were harvested from the outdoor pond and transferred to two circular PVC tanks (10 m^3), each shaded by tarpaulin, where they were held until the treatment. Wild females selected for hormonal treatment were given the first dose the same day they had been captured. The trials lasted from March 24th to April 11th 1997.

Each fish was identified individually on site by an electronic passive integrated transponder (PIT), injected into the muscle. All fish subjected to handling were first anaesthetised with 2-phenoxy-ethanol (0.3 $mL.L^{-1}$), and about one third of this dose during any transportation (Woynarovich, Horvath, 1980).

Observations on the ovaries of wild females sampled during the natural spawning season showed that females below 950 g in weight were immature. This criterion was thereafter taken to be the basic minimum for female selection. All males, on the other hand, were selected as broodstock, indipendent of their weight.

Other diagnostic criteria for female ripeness were more subjective, such as 'fullness of belly' or redness of genital opening (Kuo *et al.*, 1974). However, the most reliable criterion for ovarian maturity which could be obtained in the field by observation alone, as identified by Shehadeh *et al.* (1973), was an extrusion of oocytes sampled with an intraovarian catheter.

Induced spawning

Three small circular PVC tanks (6 m^3) were located in a greenhouse, partially shaded with dark tarpaulins, in preparation for injection and follow-up spawning. Natural ambient conditions maintained both water temperatures and diurnal light variations. Water temperatures ranged from 12 - 18°C in the tanks containing the experimental pools of fish, and subsequently from 14.5 - 18°C in the spawning tanks containing the

treated fish. On the days the females spawned, water temperatures ranged from 16.5 - 17°C, and salinity 30 - 35 ppt in all tanks.

A total of 12 females (5 from the wild pool and 7 from the captive pool, and identified #1 - #12) and 16 males were then selected from the two experimental pools and divided among the spawning tanks. In each tank the males always out-numbered the females by at least one. The weights of the selected females ranged from 950 - 3000 g and 400 - 2000 g for the males.

Hormone treatments were only given to females, whereas males were selected for running milt and therefore not treated. Each experimental treatment was a combination of the following hormones:

- carp pituitary homogenate,
- LHRH-a (luteinising hormone-releasing hormone analogue, synthetic peptide realising gonadotropin (hCG) and produced by the hypothalamus),
- Domperidone (synthetic dopaminergic blocking agent).

These particular hormones, in a range of doses and combinations, had proven to be the most promising for induced spawning of thick-lipped mullet by Crosetti (1998) and Cataudella *et al.* (1988b).

As shown on **table I** each hormonal treatment was given to females either in 2 or 3 injections 24 hours intervals apart. The treatments with 3 injections were performed for comparison with the results of Yashouv *et al.* (1969), who injected leaping grey mullet (*M. cephalus*) with 3 doses of carp pituitaries and LHRH-a. As control, one female was given three injections of physiological solution 9 ppt (2 mL each). Due to the small number of mature females available, treatments were not replicated.

The commercial dry carp pituitaries were ground in a pestle and mortar, and homogenized in saline solution 9 ppt (2 mL). The homogenate was left to deposit for 15 minutes at room temperature before the supernatant was decanted for use. Domperidone (DOM), commercially available in 10-mg tablets under the trademark of Motilium® (Janssen-Cilag, Cologno Monzese, Italy) was prepared according to Fermin (1991). LHRH-a DAla^6 was a commercial preparation (Sigma-Aldrich, Milan, Italy), diluted in saline solution in the laboratory and stored in liquid nitrogen until used.

Oocyte sampling and observation

For identification of the stage of development, or to follow maturation after hormone treatment, oocytes were sampled with a polyethylene cannula (3 mm i.d.) inserted into the genital opening. Sampling was carried out immediately before the first and second injection, and subsequently 48 h and 192 h after the first injection. Sampling was not performed when females appeared to be overly stressed, in particular if injured or about to spawn.

A sub-sample of the extracted oocytes were placed in 9‰ physiological solution and examined *in vivo*, with the naked eye or low-magnification binocular, to determine their quality and wether females were ready for the hormone treatment. Another sub-sample was fixed in 70% ethanol and saline solution for measurement, and another was fixed in Bouin's liquid for 4 h, rinsed in 90% ethanol, and then stored in 90% alcohol for later histology; to prepared the slides, the samples were inclused in paraffin making sections of 6 ?m in thickness; the sections were stained in Mayer's haemalum solution.

The exact stage of development of the oocytes was determined in the field. About 10 eggs were mounted on a slide and observed under the microscope. Application of gentle pressure on the cover glass and viewing the number and size of oil droplets in each oocyte enabled identification of the maturation level. Oocyte development stage were recorded according to Kuo *et al.* (1974) and later confirmed by histology. Only females bearing oocytes at least at the secondary yolk globule stage were selected for hormone treatment.

Spawning and egg collection

Females spawned naturally in the tanks, and the eggs were fertilized naturally by the males. Eggs were collected with a framed hand net and transferred to a container (25 L) of high-saline water (45 ppt salinity) and without aeration. Fertilised and buoyant eggs were collected from the water surface, weighed, and transferred to one or more incubation tanks. Here, the fertilised eggs were observed regularly to follow early cell divisions, and later embryonic development.

Results

Oocyte maturation stages

Histological observations are shown on **table II**: at first injection, most females had oocytes at the secondary yolk globule stage. At the time of the second injection, 24 h later, the oocytes of some fish showed no change, whereas others had oocytes developed to the tertiary yolk globule stage. Two females (#3 and #8) had oocytes at the tertiary yolk globule stage at first injection, which developed to the migratory nucleus

Table I

Hormone treatments and spawnings. / *Traitements hormonaux et pontes*.

date		n.	weight (g)	hormone treatment	1st inj	2st inj	3st inj	spawning			fertilisation rate %	hatching rate
								time from 1st inj (h)	eggs weight (g)	% on total female wt		
24-mars	W	1	3000	cp 7/kg+LHRH 50 mg/kg	7 cp	50 LHRH		71				
	D	2	1900	cp 7/kg+LHRH 200 mg/kg	7 cp	200 LHRH		63	4230*	57	85	+++
	D	3	2500	d 10 mg+LHRH 100 mg/kg	10 d+30 LHRH	70 LHRH		50				
	D	4	1150	cp 7/kg+LHRH 50 mg/kg	2 cp	5 cp	50 LHRH					
	W	5	1100	cp 7/kg+LHRH 200 mg/kg	7 cp	50 LHRH	150 LHRH	65	435 partial		42	+
	W	6	1200	d 10 mg+LHRH 100 mg/kg	10 d	30 LHRH	70 LHRH					
	W	7	950	control (saline solution)	2 mL	2 mL	2 mL					
1-avr	D	8	2500	cp 7/kg+LHRH 50 mg/kg	7 cp	50 LHRH		57	1440	57	88	+++
	D	9	1800	cp 7/kg+LHRH 100 mg/kg	7 cp	100 LHRH		91	350 partial		0	
11-avr	D	10	1050	d 10 mg+LHRH 100 mg/kg	10 d+30 LHRH	70 LHRH		60	330 partial*		69	+
	D	11	1800	cp 7/kg+LHRH 50 mg/kg	7 cp	50 LHRH		53				
	W	12	1750	cp 7/kg+LHRH 100 mg/kg	7 cp	100 LHRH		70	1140	65	59	+++

*: eggs from females 1-2-3 and 10-11 were pooled together
hatching rate: +++: high, ++: medium, +: low
cp: carp pituitaries - d: domperidone - LHRH: LHRH-a
W: wild females captured at the fish barrier - D: domesticated females from previous years, kept in the farm

Table II

Oocytes maturation stage. / *Stade de maturation des ovocytes.*

female	1st inj	2nd inj		spawning	192 h
	0 h	24 h	48 h	(h)	
1	II	nd	nd	71	
2	II	III	nd	63	
3	III	M	nd	50	
4	II	II	III	---	atresia
5	II	III	III	65	
6	II	II	III	---	atresia
7	II	II	II	---	atresia
8	III	M	nd	57	
9	II	III	nd	91	
10	II	II	nd	60	
11	II	III	nd	53	
12	II	III	nd	70	

"II: secondary yolk globule stage; III: tertiary yolk globule stage"
M: migratory nucleus stage
nd: no disturbance as female preparing to spawn
Oocytes stages according to Kuo *et al.*, 1974

stage by the time of the second one. Both spawned 50 h and 57 h later, respectively.

Oocytes sampled from females receiving a third injection, two days after the first, showed further progressive maturation and these fish were not disturbed again as they prepared to spawn. The oocytes of the control female (#7), which was a recently captured fish at the secondary yolk globule stage, did not change throughout the experiment (Table II).

Spawning and egg yields

Spawning success and egg yields are given in **table I**. Between 50 and 91 h after receiving their first injections, 9 of the 11 females induced with various treatments completed maturation and ovulated, either completely or partially. These 9 females yielded 7925 g of eggs. Yields from individual females were not always accurately recorded as some fish spawned in the same tank at about the same time. Therefore, for example, eggs from females #1, #2 and #3, and females #10 and #11 were pooled.

The 5 females (#1, #2, #3, #8, #12), which spawned out completely, yielded 6810 g of eggs within 2 h, or 86% of the total quantity of eggs obtained during in this study. Fertilisation rate ranged from 42 – 88%. The other 4 females spawned only partially, and the eggs were judged to be of poor quality as they had a low hatching rate. These fish never completely emptied their ovaries, and at 192 h after the first injection still bore degenerated eggs which could be expelled manually with slight pressure. One female, #9, spawn out but the eggs proved to be not viable and were discarded.

Two (#4 and #6) of the 11 treated females, together with the control (#7), showed no sign of oocyte maturation or hydration, and did not spawn. When sampled 192 h after their first injections, all had oocytes exhibiting signs of atresia.

Eggs taken from all sources measured 1.2 - 1.4 mm in diameter and contained between 2 - 12 oil droplets.

Discussion

As a rule, the advantage to use the carp pituitary homogenates is that it contains, besides hCG, also others hormones with synergic effect on the gametogenesis, so that it is much used in the induced spawning of fishes. Similary, combined treatment with LHRA-a and dopamine antagonist, domperidone, induces ovulation and spawning. Several trials carried out in previous years with different hormonal substances, such as mullet and carp pituitary homogenates, hCG, LHRH-a, and domperidone, gave poor results in spawning and production of highly viable eggs of mullets (Cataudella *et al.*, 1988b; Crosetti, 1998). However, they provided considerable information on the reproductive biology of *C. labrosus*, and some of the basic protocols for induced breeding of the species. Prior to the natural spawning season, vitellogenesis of both wild and captive female thick-lipped mullet proceeds to the secondary or tertiary yolk globule stage, but is then blocked until final maturation leading to ovulation is triggered by the environmental stimuli of seaward migration and spawning grounds, or the artificial stimuli of hormone treatment. Consequently, female mullets migrating from the lagoon, and captured at a fish barrier, have attained the same level of maturity. Therefore, to reduce handling, oocyte sampling of these fish is optional, and selection criteria can be confined to females which have soft and full abdomens, and no external injuries.

During the natural spawning season of thick-lipped mullet, all wild females selected for the trials at the fish barrier and weighing over 950 g were presumably ripe, as shown by previous observations on 50 females (Crosetti, 1998). This weight can be considered the lower limit at which females reach maturity, and can be used as broodstock. On the other hand, the smallest mature males weighed only 400 g.

Successful spawning was obtained in 9 out of 11 females receiving the experimental treatments. This high percentage was obtained with females receiving hormone treatments of (i) 7 carp pituitaries +50, +100 and +200 µg LHRH-a per kg body weight, and (ii) domperidone 10 mg +100 µg LHRH-a per kg body weight, given in two injections within a 24 h interval. Eggs with high viability, determined by fertilisation and hatching rates, were obtained from only 5 large females, whereas small batches of poor quality eggs were obtained from females which spawned incompletely.

These minimum dosages for the thick-lipped mullet are only slightly less than those required for the *Mugil cephalus*. Lee *et al.* (1987, 1988) found the combination of 20 mg carp pituitary homogenate (equivalent to approximately 7 pituitaries glands) and 200 µg LHRH-a was the minimum dose required to induce spawning success (94.1%) in that fish.

Benefits of applying hormone treatment in 3 injections instead of 2 were not evident, and only subjected the fish to additioned handling and stress, and possibly unsuccessful spawning (Zohar, Mylonas, 2001).

There is an on-going debate on whether induced oocytes with more than one oil globule produce the most viable larvae. Stand here the successful results with thick-lipped mullet in this study, and also by Cataudella *et al.* (1988b) indicate that eggs containing multiple (2-13) oil droplets produce viable larvae. Similarly, some other authors, which studied other Mugilidae species, assert this opinion (Cassifour, Chambolle, 1975; Van der Horst, Lasiak, 1989). On the other hand, these results disagree with the statement of Kuo *et al.* (1973) for *M. cephalus*, which suggested multiple oil droplets to be a sign of premature inducement by stripping manually, and Nash *et al.* (1974), which asserted that spontaneous release by the female produced eggs with a single oil droplet.

There was no clear difference in spawning success between wild broodstock females which had been in captivity for a year or more, or wild females captured shortly before induced breeding. Captive broodstock were invariably fatter and easier to handle than wild specimens captured at the fish barrier.

Our results confirmed that the thick-lipped mullet spawns a large volume of eggs. Spawned eggs can amount to 57 - 65% of the female's body weight, and may be as high as 77% (Crosetti, 1998).

There are no records of mullets spawning spontaneously in captivity, and this continues to be confirmed by the records from this paper. There have, however, been reports of 'spontaneous' spawning in previous trials. Crosetti (1998) recorded one female which spawned 8 days after being injected with 5000 IU hCG as a third injection, following failure with two previous treatments 13 days earlier. The oocytes of the female in question were as perfect at day 13 as at day 0. The long time-gap between the last injection and spawning suggests these events to be spontaneous and not induced. It is thought that the hormone prevents oocytes from undergoing atresia, brought on by captivity and handling stress, thus enabling the female to mature slowly and eventually spawn. On the other hand, the female controls start undergoing atresia between 72 - 120 h after the first injection. Cataudella *et al.* (1988b) reported a similar reaction in three *Chelon* females, which spawned 9, 11, and 14 days after a single dose of 5000 IU hCG.

Conclusion

The dosages of hormones to induce spawning of thick lipped mullet in captivity are typical for the family Mugilidae. Current practices with a variety of hormones, such as carp pituitary homogenate, luteinizing hormone releasing hormone analogue, and domperidone and are all physiologically effective to some degree. The least intrusive protocol, in terms of the quantity of hormone used and the number of injections, is the combination of (i) a first injection of a homogenate of 7 carp pituitaries followed 24 h later by a second injection of 50 µg LHRH-a per kg female bodyweight. Anyway all tested protocols seem to be reliable for the production of highly viable eggs from carefully selected females, with the best chance of high early larval survival.

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