ICES C.M. 1995

Mariculture Committee
F:9 poster

REPRODUCTIVE EFFICIENCY OF TURBOT (*Scophthalmus maximus* L.) FEMALES TREATED WITH LHRHa

by

Peleteiro J.B.* & Alvariño J.M.R.**

* Instituto Español de Oceanografía
Apartado 1552, 36280 Vigo (Spain)

** Dept. de Producción Animal E.T.S.I. Agrónomos
28040 Madrid (Spain)

ABSTRACT

A broodstock of 25 females with different maturity stages were induced to spawn with a single dosis of 3 µg/Kg of LHRHa. A response was experienced by 100% of the most advanced females (stages II and III). The less advanced females (stages 0 and 1) were induced again two weeks later, using the same dosis divided in two consecutive days, and a response in 50% of females was obtained.

The data obtained were compared with those of females from the same broodstock but naturally matured, not injected. Induced females showed a clear decrease both in the number of ovulations and in the intervals between ovulations, and an increase in the number of ovocytes per ovulation.

Besides, three of the naturally matured females were induced with the same dosis used initially. Two of them were induced at the end of the spawning period and no response was detected; nevertheless another female induced during the spawning period had a very good synchronization of the intervals between ovulations from the day of injection until the end of the spawning period.

1. INTRODUCTION

The response to reproduction control in female turbot by physical mechanisms such as the manipulation of the photoperiod and temperature (Forés et al., 1990) presents no difficulties. These techniques are frequently used by the industrial sector with no problem.

Optimization of these systems requires a greater control of the reproductive cycle in these fish since the natural spawning period in captivity may be extended by up to 7-8 months, and the spawning period with a controlled photoperiod by approximately

3 months. This indeed means an excessive manipulation of the stock which may be avoided by synchronizing the ovulatory cycles (Alvariño & Peleteiro, 1993).

If the method of ovulation prediction makes it possible to obtain very high fecundation rates (McEvoy, 1989), spawning induction with LHRHa also allows us to synchronize ovulations with a 100% success rate if females are in a mid or advanced stage of maturation (Alvariño and Peleteiro, 1993).

The already known effect of induction in other farmable fish species of commercial interest, such as seabass or bream (Zohar, 1988; Prat, 1991; Alvariño, 1992), has still not been sufficiently proven in the case of turbot where treatment with LHRHa may cause the death of the induced female (Prat et al., 1993) or undesirable secondary effects such as the appearance of gonadal hemorrhage (Alvariño and Peleteiro, 1993).

Since the positive response to LHRHa induction in turbot was obtained with one injection of 5 µg/kg (Alvariño, 1993), the purpose of this article is to test a smaller dose of hormone able to synchronize and reduce ovulation time without jeopardizing egg quality.

2. MATERIAL AND METHODS

For the purposes of this experience, a total of 25 females exposed to a natural photoperiod and a constant temperature of 14ºC were used in the presence of 34 ripe males in identical conditions.

The reproducers were kept stabled in two 25 m^3 tanks with a load of 2.3 kg/m^3 with a constant renewal of water and air-lift type aeration. A group of 7 females with an average weight of 5.9 kg was not injected, and these individuals matured normally with no type of induction. The remaining 18 females were divided into 3 groups selection according to their stage of maturity: GROUP A (n=6) comprising females still showing no gonadal development (maturity stage 0) or an initial development of the upper ovaric lobule (maturity stage I). GROUP B (n=8) (maturity stage II), with an advanced gonadal development in the posterior lobule and initial development in the anterior lobule, and GROUP C (maturity stage III), with full development in both ovaric lobules (n=4).

Spawning in the females of groups A, B and C was induced by the intramuscular injection of 3 µg/kg of LHRHa (des-Cly^{10}, [D-Ala^6] LUTEINIZING HORMONE RELEASING HORMONE Ethylamide, Sigma) in the antero-dorsal zone of the body. Those selected as control individuals were also injected with an equivalent dose of physiological serum (n=2, Groups A and B; n=1, Group C).

When no response was obtained in GROUP A, the same was injected once again after 14 days, with a further dose of 3 µg, distributed in two injections of 1 and 2 µg of LHRHa, 24 hours apart.

Both the induced females and natural spawning females were controlled daily from the day after the injection. Since the start of spawning, the ovocytes were extracted by abdominal pressure and were fertilized the sperm from 2-3 males from the same stock, also obtained by the same method of abdominal pressure.

All spawnings with a volume of over 50 cc were fertilized. It was considered that volumes smaller than this amount were overmature ovocytes from the previous ovulation.

Three hours following fertilization, the floating egg (viable) was separated from the non-floating egg (non-viable) and the percentage of fertilization was calculated against the floating fraction. Then the floating egg was transferred to 150 l capacity incubators where, during embryonic development, the dead eggs were daily removed; until the birth of the larvae (2016 grades/day). At this point, the number of larvae (per sampling) and the percentage of eclosion were calculated.

Comparison of averages was made using the non-parametric Mann-Witney test and comparison of proportions using the Kruskel-Wallis test (Ostle and Mensing, 1975).

3. RESULTS AND DISCUSSION

The dose of hormone administered (3 µg/kg PV) to female turbot in this experience has proven to be effective in 100% of the females with average or advanced gonadal development, showing results similar to those attained with a dose of 5 µg/kg (Alvariño and Peleteiro, 1993). Furthermore, secondary effects are considerably reduced, particularly the appearance of blood clotting and pieces of ovary. The response in females in the early stages of maturation, however, is still scarce, although a second treatment two weeks later makes it possible to achieve ovaric maturation in half of the females injected. This technique may be used in females with a marked delay in gonadal development at the end of the spawning period.

Females in Group A (maturity stages 0 and I) showed no response the first injection and only 50% of these responded to the second injection (Table I) performed 14 days following the first, spawnings starting 4 and 9 days after the injection (Table II).

In Groups B and C, however, (maturity stages II and III, respectively), 100% of the females treated responded (Table I). In Group B, response in the females was obtained on days 7, 8 and 9 after induction, and in Group C, response was obtained on days 2 and 4 after induction (Table II). This would clearly indicate the relationship between the reaction time to the hormone and the stage of maturity at which the individual is (3.3 days in Group C, as opposed to 7.7 days in Group b, $p<0:05$). Only 20% (one in 5) of the control individuals matured and commenced spawning 93 days after the start of the experience. This result has been detected in previous experiences (Alvariño and Peleteiro, 1993), and corresponds to the frequent appearance of problems in gamete

emission in apparently mature females, which may affect 27.9% of the stock (Peleteiro et al., 1993, ICES, Norway).

The influence in the interval between ovulations also seems evident as this is considerably reduced in the induced females (Table I). Nevertheless, since there is practically no variation in fecundity and the ovulatory cycles are shortened, ovulations are far more abundant, some spawnings even rising to 1500 cc in a single ovulation.

The overall yield in the number of eggs been observed to be similar in the induced females and the natural spawning females, although the number of larvae produced has been less. This indicates that the acceleration in the ovulatory cycles may have a negative effect on egg quality. Results were particularly low in Group C (advanced age females of over 10 years old) which suggests that induction in LHRHa is applicable to young females. Further studies are necessary, however, to be able to specify the influence of treatment on egg quality.

As shown in the maps of induced and natural spawning females (Table II, Figure 1), there is a considerable reduction in the length of the spawning period. In the case of induced females, the spawning period lasts 23 days, whereas in the case of natural spawning females, it started on 26th May and ended on 11th November, totalling 169 days.

During this experience, two females were injected, numbers 21 and 23 being natural spawners, at the end of their spawning period, with a similar dose of LHRHa (3 µg/kg PV), and no response was obtained.

Female N.15 was also injected (Figure 2) with the same dose, showing irregular ovulatory rhythms. This obtained a marked regularization of rhythms from the time of the injection until spawnings ended and an increase in egg production following the injection of LHRH (2220 vs 785 cc) and an improvement in the proportion of floating egg (82 vs 65%). Thus, whereas treatment with LHRHa does not appear effective once ovaric regression has commenced, it may regularize ovulatory cycles during the spawning period.

4. BIBLIOGRAPHY

ALVARIÑO, J.M.R.; ZANUY, S., PRAT, F., CARRILLO, M. & MAÑANOS, b. 1992. *Aquaculture* 102:177-186.

ALVARIÑO, J.M.R., PELETEIRO, J.B. 1993. *Actas IV Congreso Nacional de Acuicultura*, 31-36. Illa de Arousa, Spain.

BARTON, L.A. 1981. En: *Egg-quality of turbot (Scophthalmus maximus L.) kept in captive conditions*. PhD thesis, University of Liverpool, U.K.

BROMLEY, P.J., SYKES, P.A. & HOWELL, B.R. 1986 *Aquaculture* 53:287-293.

DEVAUCHELLE, N., ALEXANDRE, J.C., LECORRE, N. & LETTY N. 1986. *Mariculture Commitee, ICES C.M. /F:10*

FORES, R., IGLESIAS, J., OLMEDO, M., SANCHEZ, F.J. & PELETEIRO,

J.B. 1990. *Aquacultural Engineering* 9:357-366
GIRIN, M. 1979. *Public C.N.E.X.O. Rapp. Sci. Tech.* Nº 39.
McEVOY, L.A. 1989. *Cuaderno da Area de Ciencias mariñas, Seminario de Estudos Galegos* 3:9-28. Sada, Spain.
OSTLE, B. & MENSING, R.W. 1975. *Statistics in Research.* Ames I.A.:Iowa State University Press.
PELETEIRO, J.B., RODRIGUEZ-OJEA, G. & IGLESIAS, J., 1993. *ICES Symp. Mass Rearing of Juvenile Fish.* Poster nº 33. Bergen Noruega.
PRAT, F. 1991. En: *Control del ciclo reproductor de la lubina (Dicentrarchus labrax L.) por manipulacion hormonal y ambiental.* Tesis Doctoral, Universidad de Barcelona. Spain.
PRAT, F., MINKOFF, G., ZANUY, S., CARRILLO, M. & BROADHURST, A. 1992. En: *Broodstock Management and Egg & Larval Quality,* Abstract Book. Stirling, Scotland. U.K.
PRAT, F., ZANUY, S., BROADHURST, A., CARRILLO, M., AGUILERA, C. y RAMOS, J. 1993. *Actas del IV Congreso Nacional de Acuicultura* 25-30. Illa de Arousa. Spain.
ZOHAR, Y. 1988. En: *Reproduction in fish. Basic and applied aspects in endocrinology and genetics.* Pp 47-62. INRA. Paris. France.

GROUP	N. ♀	Treatment LHRH (µg)	Av. weight	% spawns	Interval Injection /Spawn	N. Spawns/♀	Interval between Spawns	Egg Production		% Fertilization	Fertility cc/Kg ♀	% Eclosion	Total production	N. Larva/Kg ♀
								Floating	Total					
A	4	3+3 *	4.9	50	20.5 a	3.0	1.80 a	771	1289	25	61 a			
B	6	3	4.9	100	7.7 b	4.33	1.90 b	5.270	7.377	47	205 b	6.0	172.395	4.789 a
C	3	3	7.4	100	3.3 c	5.67	1.43 c	1054	6072	49	274 c	4.5	23.400	1.054 b
Natural Spawns	7		5.9	100		10.13	3.24 b	3931	8793	45	216 b	17.3	474.900	11.450 c

a, b, c: averages followed by different letters indicate significant differences (P<0,05)

* A second dose of 3 µg/kg was administered 14 days after first treatment, in two inyection of 1 and 2 µg/Kg, 24 hours apart.

Table I.- Productive efficiency of females during natural spawning or treated with LHRHa, according to the initial state of gonadal development: A (incipient), B(intermediary) and C (advanced)

GROUP	♀	NUMBER OF DAYS AFTER INJECTING LHRHa (27th June, 1994)																							
		2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25
A	20													*				P		P		P	P		P
	13													*									P		
B	4						P	P		P		P													
	14							P	P	P			P	P											
	29						P		P		P														
	7							P	P		P			P	P			P							
	58						P	P		P			P												
	43								P		P			P	P			P							
C	18			P		P	P		P	P															
	62	P	P	P		P	P	P	P		P														
	96			P	P	P			P																

* These females were injected for the second time 14 days after the same dose administered between two injections of 1 and 2 µg, 24 hours apart.

Table II.- Map showing spawns (P) after injecting 3 µg/Kg of LHRHa.

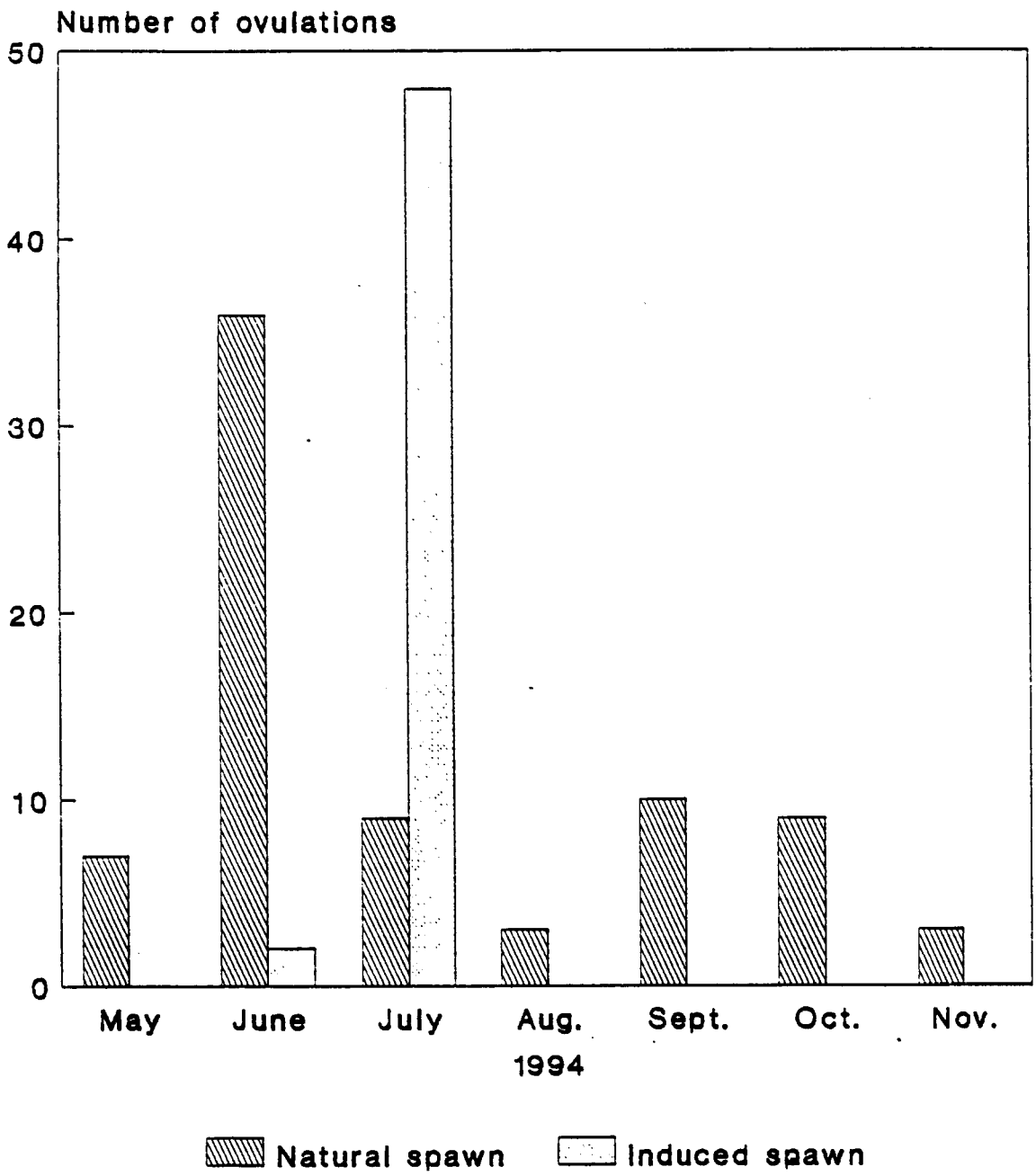

Figure 1: Distribution of ovulations in natural and induced spawns for 1994.

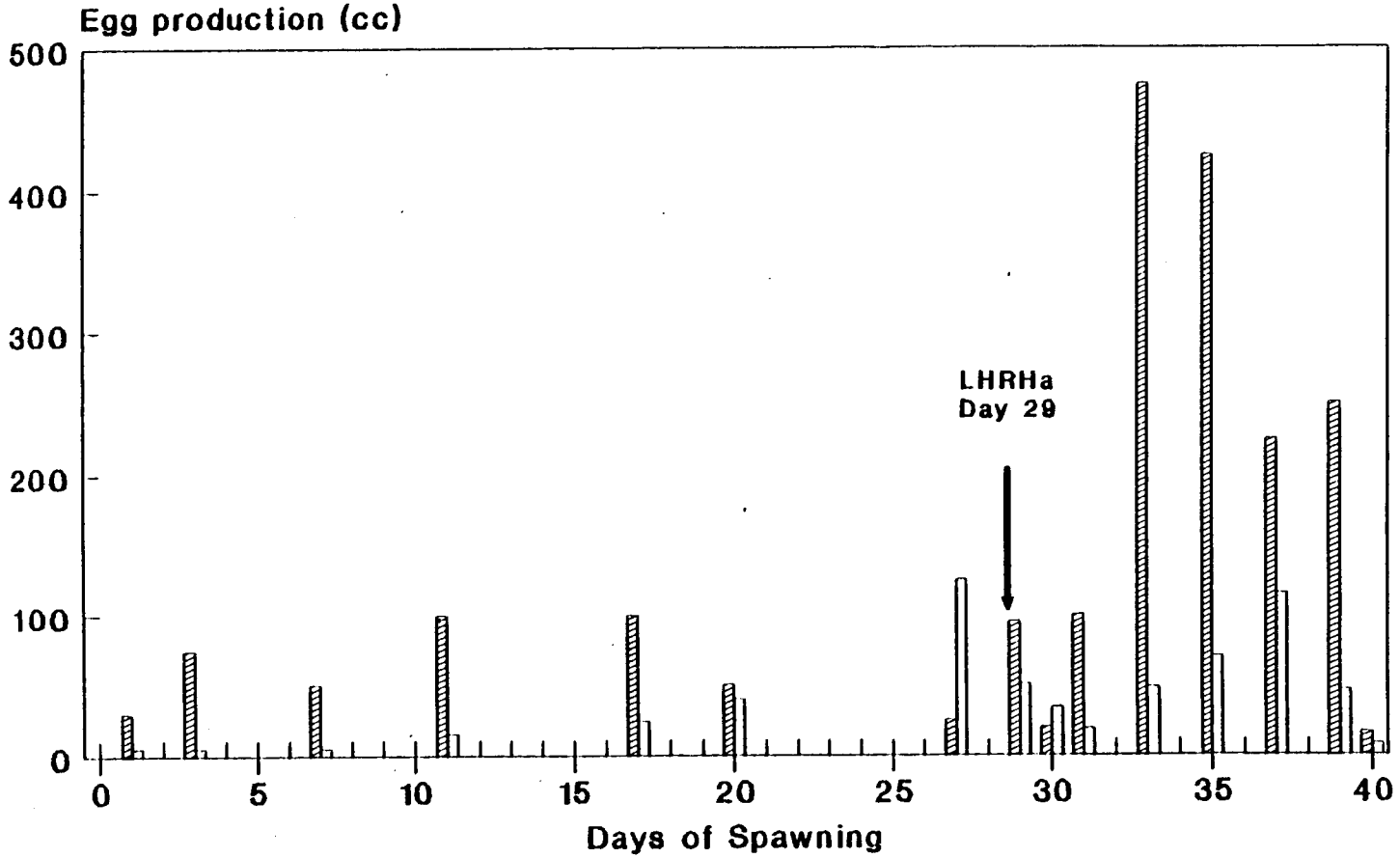

Figure 2: Reproductive efficiency of female nº 15, before and after treatment with LHRHa.