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EFFECT OF STRIPPING FREQUENCY ON TURBOT SPAWN QUALITY

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ABSTRACT

In production operations the application of daily examination of each female of broodstock should be a determinant progress in hatchery management in terms of fry output.

The eggs expelled shortly after ovulation by means of a systematic fivefold a week stripping attempt of females, presented a high viability rate (72%) compared to that of eggs collected twice a week (46%) whereas the treatments did not have any effect on female fecundity.

Increase of the stripping rhythm brought the hatching rates and broodstock fertilities respectively from 11% to 35% and from 20,000 to 65,000 larvae/kg of spawning female.

The enhancement of egg quality and of the resulting larva production is discussed.

INTRODUCTION

Among the causes of low egg quality in captive fish broodstocks, rearing conditions and nutrition were pointed out in the literature as determinant parameters (rev. in Billard, 1981; Watanabe, 1985). In species which do not usually spawn spontaneously in captivity, oocytes retained inside the ovarian or coelomic cavity may be damaged by overripening (Rev. in Kjorsvik, 1990).

This phenomenon has been characterized by morphological (Hirose, 1974; Nomura, 1976), physical (Mac Evoy, 1984; Howell and Scott, 1989) and biochemical modifications (Craik and Harvey, 1984; Devauchelle *et al*, 1982; Zhukinskyi and Kim, 1981). However, most of the descriptions concerned the biological consequences of overripening, i.e. the evolution of fertilization and development abilities of the eggs (Billard and Gillet, 1975; Escaffre and Billard, 1979; Hay, 1986; Mac Evoy, 1984; Sakai *et al*, 1975).

The development of overripening was found to be related to the time of egg retention and to be temperature dependant in Salmonids (Billard and Gillet, 1975) and turbot (Mac Evoy, 1984); at last, female size or age might influence the deterioration of unlaid eggs in salmonids (Escaffre and Billard, 1979).

Egg quality was identified as an important bottleneck for turbot aquaculture development since fry production is unregular and unsufficient (Jones,1981). It remains true today. As the overripening may be involved in poor broodstock performances (Mac Evoy,1984), this work aimed at assessing its impact on larva production in an usual farmer's management and at finding out a practical way to overcome it in production conditions. So, the egg quality and the resulting larva production were compared using different rhythms of female examination.

MATERIAL AND METHODS

Six broodstocks of 12 females and 15 males weighing between 2 and 7 kg were used in the experiment. They were placed into six 16 m^3 tanks.

The temperatures were controlled to obtain a maximum of 14°C from mid july to september and a constant decrease to 9°C from August to January (recovery period) followed by an increase from 9 to 12°C from January to May (vitellogenesis period). Then a plateau was maintained at 12° C from May to July (ovulation period) .

The tanks received a 10%/h seawater renewal for a biomass of 5-6 kg/m^3. Broodstocks were subjected to the natural photoperiod..The fish were fed *ad libitum* on fresh trash fish.

The group B3 accidentally underwent a heat shock of 5°C for 15 days just as the ovulation period started.

During the whole ovulation period, 2 groups (B_1, B_2) were subjected to a twice a week examination of the females (treatment S_2), whereas the others (B_3, B_4, B_5, B_6) were observed on five occasions a week (treatment S5). The examination consisted of a systematic stripping trial of each female of the tanks. When eggs had been ovulated, they were exhaustively extracted by abdominal pressure and immediately counted before fertilization, on a 500 μl aliquote through a dissecting microscope .

The spawn quality was assessed on the same sample by the percentage of viable eggs. The eggs were considered viable when they presented a perfect rotondity, a tanslucent aspect and a lack of perivitelline space.

Artificial insemination was carried out using a constant empiric volume ratio egg/sperm/activation seawater (100/ 0,5/ 50). Sperm was allowed to fertilize eggs for 10 mn.

Batches of 100.000 eggs were then transferred into 40 l net bottom incubators gently moving up and down in 300 l raceways receiving a twice an hour seawater renewal at 13°C. (Devauchelle, 1986). Dead eggs or embryos were daily removed from the net bottom. Hatching occured on the fifth or sixth day of incubation.

Larvae were counted on 2 aliquotes of 200 ml after manual homogeneization of the incubators.

Data were subjected to one way ANOVA and a posteriori test of TUKEY when significant differences were found for a 95% confidence interval.

RESULTS

Occurrence of ovulations: the intervals between the ovulation dates are presented in table 1 for the females subjected to treatment S5. An ovulation was considered to have occurred when a *minimum* of 50000 eggs was collected and showed a high viability rate (>75%).

The variations of periodicity ranged from 2 days for the more regular cycles to 10 days . Those intervals were related neither to the number of spawns per female nor to the ovulation position in the spawn sequence. The number of spawns per female appeared to be different from a broodstock to another (table 2).

Egg production: The total production of eggs obtained respectively from the different groups averaged 6.03×10^6 eggs with a high variability(sd: 4.31×10^6)

The variations were mostly due to the number of spawns occuring in each tank (r=0.82). No significant difference appeared between the treatments S_2 and S_5 (table 2).

Fecundity: Individual female fecundities were highly variable (mean: 1.27×10^6 egg/kg of female, sd: 0.86×10^6) Nevertheless broodstock fecundities represented by the ratio of total egg production on total weight of spawning females were similar except that of tank B3 (table 2). No significant difference appeared between treatments S_2 and S_5.

"Egg quality": The "egg quality" characterized by the viability rates (number of viable eggs/total egg number) are presented in table 3. A highly significant difference ($P < 0,01$) was found between treatments S_2 and S_5. The twice a week examination yielded lower viability rate spawns (46.62%) than the daily stripping of females (72.56%).

When comparing the viability rates of the spawns of the different broodstocks only that of group (B3) subjected to treatment S_5 was neither significantly different from that of tanks B4 and B5 (S5), nor from that of B_1 and B_2 subjected to the treatment S_2, although it presented a tendency to be higher than the latter.

Hatching rate: The hatching rates (number of larvae/number of viable eggs) obtained from the different broodstocks and treatments are reported in table 4.

A highly significant ($P < 0,01$) increase of the hatching rates was found in treatment S_5 (34.45%) when compared to treatment S_2 (11.47%).

The mean hatching rate of broodstock B_1(subjected to a twice a week examination) could not be considered lower than that of broodstocks B_3, B_4, B_5 (five times a week) although its value was half that of the latter.

Influence of egg quality on hatching rate: The egg viability and the hatching rate were correlated($r= 0,63$).

Comparing mean hatching rates obtained from 20 % interval viability classes allow to point out evidence that hatching rate moved up to the higher values whereas the viability rate increased; the 80-100 % viability class yielded a significantly ($P < 0,01$) better hatching rate than other viability classes even for a confidence interval of 99 % (figure 1).

Larva production: The production of larvae appeared to be similar within treatments S_2 and S_5.The comparison of mean larvae production of broodstocks between treatment revealed a significant ($P<0,05$) increase in the case of treatment S_5. (Tab 5)

This significant difference between treatments was also found in female fertility (number of larvae/spawner weight) (Tab 5).

DISCUSSION

The fecundities observed in the different broodstocks which were used in this experiment, were of the same order as previously reported in the case of stripped females (Howell and Scott, 1989; Jones, 1981) and higher than those of females allowed to spawn naturally (Bromley *et al*, 1986; Devauchelle *et al*, 1988).They were not altered by the increase of the stripping rhythm since the egg productions per kg of female were similar whatever the examination frequency. This result tends to show that the increase of handling allowed to collect exhaustively the ovulated eggs without altering the ovulation. The handling stress did not modify the process although it might prevent fish from natural spawning as stated by Bromley *et al* (1986). The ovulation was found stress sensitive in pike (de Montalembert *et al*, 1971) but it was not altered in the case of rainbow trout (Maxfield *et al*, 1971).The poor fecundity obtained after a heat shock suggests that, in turbot, high temperature stress may be deleterious for the ovulations as it is the case in rainbow trout and goldfish (Billard *et al*, 1981).

Mac Evoy (1984) studying the evolution of the egg viability, showed that overripening began to develop on 10 hours after ovulation and concerned the whole of oocytes in a delay of 24 hours. In the present experiment, the viability rates obtained by the daily stripping (72%) were quite higher than those yielded by the twice weekly routine (46%). In the former condition, ovules appeared to be expelled in a postovulatory delay sufficiently short to limit the development of overripening.As it is the case in salmonids (Billard and Gillet, 1981), the phenomenon was probably slowed down by the temperature (12°C) since Howell and Scott(1989) only obtained 42% of apparently viable eggs when they stripped every day females subjected to 15°C.

In previous studies, turbot successive ovulations were shown to occur at regular intervals of 2 to 4 days depending on endogenous spawner cycles. The determination of individual periodicity was proposed to allow a schedule of the stripping times for each female in order to get eggs short after ovulation, hence to prevent overripening (Mac Evoy, 1984). In the present work, the five fold weekly examination revealed that the ovulation cycles were variable not only between but also within most of the spawners. These variations did not seem to be strictly temperature dependant as it was observed by Howell

and Scott (1989).In production operations, the systematic stripping trial appeared to be simpler and more reliable than ovulation prediction since it allowed to avoid the biological constraints of individual cycles..

In the present work, a correlation was found between the viability of the eggs and hatching rate. The rather low coefficient of correlation may be explained by the wide deviations of survival due to fertilization and incubation conditions which, though repetitive, may not be perfectly suitable for the embryo needs. However it appeared that the percentage of viable eggs reaching the larval stages decreased when overripening progressed. This is consistent with the result of HOWELL and SCOTT (1989) who found that only 60% of the viable eggs were fertilized when the viability rate was around 40% . The decrease of fertilization rates may be due to a loss of the egg viability before the development of morphological modifications (KJORSVIK,1989) or to an alteration of coelomic liquid which could not maintain its buffer effect necessary for an optimal motility of the spermatozoa (Billard and Cosson, 1990; Fauvel *et al* unpublished).

CONCLUSION

Overripening appears to be largely involved in low egg quality in turbot and to hide other causes limiting larva production. Overcoming the phenomenon by a systematic daily examination of female subjected to adequate temperatures will allow to study and improve vitellogenesis, maturation, fertilization and incubation conditions. Moreover the method described can be easily applied in industrial turbot hatcheries to reduce the problem of "egg quality", hence to enhance fry production.

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TABLE 1: Successive time intervals between ovulations for females which spawned more than six times during the experimental period.

Ovulations	*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	*7th*	*8th*	*9th*	*10th*	*11th*	*12th*	*13th*
Females	Time (days) elapsed between ovulations												
A	3	3	3	5	1	1	2	3	1	2	1	3	
B	4	2	2	3	2	6	2	4	6	4	2	1	
C	1	1	1	1	3	4	3	3	11	*1*			
D	2	3	3	3	3	1	3	4	3				
E	4	4	1	4	5	4	7	1	4				
F	2	4	3	4	2	2	4	2					
G	1	3	4	3	1	1	5						
H	4	4	1	7	4	4	3						
I	2	1	4	1	1	5	4						
J	4	1	2	4	8	6							

TABLE 2: Egg production data

Treatments	**S2**		**S5**			
Broodstocks	*B1*	*B2*	*B3*	*B4*	*B5*	*B6*
Mean egg production ($x10^6$)	**9.62**	**---**	**5.96**			
Total egg production ($x10^6$)	*7.24*	*12.04*	*4.34*	*6.15*	*7.38*	*6.0*
Mean fecundity($x10^5$ Eggs/kg)	**2.89**	**---**	**2.33**			
Fecundity($x10^5$ Eggs/kg)	*2.69*	*3.10*	*0.95*	*2.89*	*2.87*	*2.64*
Spawn number/female	*5*	*11*	*4*	*9*	*4*	*8*
Total spawn number	*25*	*75*	*26*	*44*	*25*	*32*

(---: Homogeneous groups)

TABLE 3 : Effect of stripping on egg viability

Treatments	S 2			S 5			
Broodstocks	*B1*	*B2*		*B3*	*B5*	*B4*	*B6*
Mean viability (%)	**46.62**		**	**72.56**			
	43.64	*47.62*		*59.59*	*71.17*	*74.42*	*83.31*
Homogeneous groups					----------		

(**: highly significant difference ,p<0.01)

TABLE 4 : Effect of stripping rhythm on hatching rate

Treatment	**S 2**			**S 5**			
Broodstock	*B2*	*B1*		*B3*	*B4*	*B5*	*B6*
Mean hatching rates(%)	**11.47**		**	**34.45**			
Homogeneous groups	*9.88*	*16.78*		*30.02*	*31.12*	*32.57*	*50.46*
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(**:highly significant difference)

TABLE 5: Larva production data

Treatments	**S2**			**S5**			
Broodstocks	*B1*	*B2*		*B3*	*B4*	*B5*	*B6*
Mean larva production($x10^6$)	**0.7**		**	**1.49**			
Total larva production($x10^6$)	*0.76*	*0.64*		*1.16*	*1.68*	*1.71*	*1.44*
Number of larvae/kg ($x10^5$)	**0.22**		**	**0.66**			
	0.28	*0.17*		*0.43*	*0.81*	*0.70*	*0.72*

(** :Highly significant difference ,$p<0.001$)

FIGURE 1: Relation between viability and hatching rate