Broodstock management and induced spawning of flounder *Paralichthys orbignyanus* (Valenciennes, 1839) under a closed recirculated system

Manejo de reproductores e inducción de desoves del lenguado *Paralichthys orbignyanus* (Valenciennes, 1839) en sistema cerrado de recirculación

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Abstract.- The present study was conducted to determine the parameters regulating spawning performance of *P. orbignyanus* wild broodstock. The adaptation to captivity was successfully achieved with minimal stress levels. In three consecutive spawning seasons, the annual photothermal manipulation was not effective for the maturation and natural spawning of females although males were running. Females were HCG injected, releasing non fertilized eggs. Artificial fertilization was carried out after stripping the fish with a total production of 18,000 fertilized eggs in 2001 (egg diameter= 800±10 µm), 55,300 in 2002 (egg diameter= 850±12 µm) and 1,635,000 fertilized eggs in 2003 (egg diameter 818.4 ±30.08 µm, oil drop 98.1 ±2.96 µm). For the study of the ovulation time, females were hand-stripped every 48 h (female 1), 96 h (female 2) and 144 h (female 3), after being induced by hormones. No statistical differences were found for fertilization and hatching rates and SAI. This could be attributed to the small sample sizes, individual variation and/or stripping frequencies. Nevertheless, qualitative differences were evident. Although long-term studies are needed to confirm these observations, 96 h stripping frequency could be considered a good technique to obtain eggs and larvae experimentally. The estimated fecundity by this technique varied from 185,951 to 399,118 total eggs per kg of female.

Key words: Photothermal conditioning, stripping, HCG, Pisces

Resumen.- El presente estudio se realizó para determinar los parámetros que regulan la respuesta reproductiva de adultos salvajes de *P. orbignyanus*. La adaptación al cautiverio fue lograda exitosamente con niveles mínimos de estrés. Durante tres temporadas reproductivas consecutivas la manipulación fototermal no fue efectiva para la maduración y el desove natural de hembras, mientras que los machos estaban fluyentes. Las hembras fueron inyectadas con hormona gonadotrofina humana, liberando huevos no fertilizados. Los reproductores fueron sometidos a masaje abdominal y los huevos fueron fecundados artificialmente. Se obtuvieron 18,000 huevos fertilizados en 2001 (diámetro del huevo= 800±10 µm), 55.300 en 2002 (diámetro del huevo= 850±12 µm) y 1.635.000 en 2003 (diámetro del huevo= 818,4 ±30,08 µm, diámetro de gota oleosa= 98,1±2,96 µm). Para el estudio del tiempo de ovulación, las hembras fueron sometidas a masaje abdominal cada 48 h (hembra 1), 96 h (hembra 2) y 144 h (hembra 3), posteriores a la inducción hormonal. No se observaron diferencias significativas para las tasas de fertilización y eclosión ni para el índice de actividad especifica, lo cual podría deberse al bajo tamaño muestral, variación individual y/o frecuencia de masaje abdominal. Sin embargo, hubo evidentes diferencias cualitativas. La frecuencia de masaje abdominal cada 96 h podría considerarse una técnica apropiada para asegurar la obtención de huevos y larvas experimentalmente. Estudios a largo plazo son necesarios para confirmar estas observaciones. La fecundidad estimada varió entre 185.951 y 399.118 huevos totales por kg de peso de hembra.

Palabras clave: Acondicionamiento fototermal, masaje abdominal, HCG, Pisces

Introduction

The Mariculture Experimental Station of INIDEP is in charge of the research and development of marine finfish aquaculture under closed recirculated water systems since 2001. Among the species studied, red porgy *Pagrus pagrus* (Linné, 1758) and flounder *Paralichthys orbignyanus* (Valenciennes, 1839) are

considered the more promising (Radonić *et al.* 2005[1]). *P. orbignyanus* is one of the most valuable species of the genus *Paralichthys* due to its large size, flesh quality and increasing fishery demand. It is distributed from Río de Janeiro (22° S), Brazil, to Punta Villarino (41° S), Argentina. The species inhabits low salinity shallow waters such as Río de la Plata and Bahía Blanca estuaries, Mar Chiquita Lagoon and Samborombón Bay (Argentina). The ocular side has a dark brown colour with clear spots. Maximum size recorded for females is 103 cm whereas for males is 61 cm (Cousseau & Perrotta 1998).

In 1994, the species of the genus *Paralichthys* of the Argentine Sea were identified and conveniently classified by Díaz de Astarloa (1994). Mellito da Silveira *et al*. (1995) studied the structure of the testis and the ovary of *P. orbignyanus* in the estuary of Patos Lagoon (Brazil) indicating some aspects of its reproductive strategy. López Cazorla (2005) recorded that wild *P. orbignyanus* spawning occurs from November to January in the estuary of Bahía Blanca. Macchi & Díaz de Astarloa (1996) studied the reproductive cycle and fecundity of flounder *Paralichthys patagonicus* (Jordan, 1889) found in Argentine waters.

By the time the present studies were carried out (2001-2003), no information was available on broodstock management of *P. orbignyanus* under a closed recirculated water system, nor on photothermal control to induce spawning. Bambill *et al.* (2000)[2] acclimated and maintained in captivity a wild broodstock of the close species *P. patagonicus* subjected to photothermal manipulation and closed recirculated water system. These conditions allowed natural tank spawning of fertilized eggs. In a previous research, different responses of five *P. orbignyanus* females to LHRH-A cholesterol pellets implants (luteinising hormone releasing hormone analogue) were recorded. Two of them did not produce any ova at all after being stripped every 48 h, whereas the other three

ovulated after 4 to 6 days. Total number of eggs produced was 21.813, mean egg diameter was 814±77 μm and mean hatching rate was 9.5±18.2% (range 0-56.2%) (unpublished data). In 1997, Cerqueira *et al*. caught mature individuals from Conceição Lagoon (Brazil) that were maintained in 150 L tank with a daily water exchange of 20%, at 16-18ºC. The collected eggs were fertilized after stripping HCG-induced females. These females ovulated 46 to 69 h after hormone induction. These data as well as those of *P. olivaceus* (Tsujigado *et al.* 1989, Kumagai 1999, Fushimi 2001, Kikuchi & Takeda 2001) were used as models to initiate broodstock management of *P. orbignyanus*.

Abundant literature exists on broodstock management and culture technology of other flatfish of the genus *Paralichthys* such as *P. dentatus* and *P. lethostigma* in the United States of America (Daniels 2000, Lee & Ostrowski 2001, Watanabe & Carroll 2001) and *P. adspersus* and *P. microps* in Chile (Silva 2001). Other successfully cultured flatfish are turbot *Scophthalmus maximus* in the United Kingdom, France and Spain (Bromley *et al.* 1986, Devauchelle *et al.* 1988, McEvoy 1984, Forés *et al.* 1990, Rodriguez Alvariño & Díaz Martínez 1990), and Atlantic halibut *Hippoglossus hippoglossus* in Norway and United Kingdom (Olsen *et al.* 1999, Shields *et al.* 1999).

The present study was conducted to determine the spawning performance of a wild broodstock of *P. orbignyanus* kept in captivity under a closed recirculated water system, and subjected to photothermal control since 1999. The results of three consecutive spawning seasons, 2001 to 2003, are presented in terms of number of eggs obtained by two techniques (natural spawning and hand-stripping), egg diameter, fertilization and hatching rates and larval tolerance to starvation. The optimum timing for stripping was also studied to determine the ovulatory rhythms and to estimate the fecundity of this species.

Material and methods

Broodstock management

The fish were captured by a bottom-net from 1998 to 2001 in Mar Chiquita Estuary and Samborombón Bay. Fish were examined at arrival at INIDEP and put in a quarantine tank for two weeks. One oxytetracycline bath per week (50 ppm, 1h) was applied to avoid bacterial diseases. After two weeks acclimation period, all fish

[1] Radonić M, GA Bambill, O Mazakazu, AV López, MI Müller & JJ Boccanfuso. 2005. Broodstock management and induced spawning of wild black Argentine flounder *Paralichthys orbignyanus* in captivity. Aquaculture America 2005. 17-20 January 2005, New Orleans, USA.

[2] Bambill GA, FAR Pérez, MI Müller, AV López, EO Aristizóbal Abud, M Radonić & AG Vega. 2000. Larvicultura del lenguado argentino *Paralychthys patagonicus*. IV de Jornadas de Ciencias del Mar. 11-15 de Septiembre 2000, Puerto Madryn, Argentina.

were individually tagged (PIT taggs) before stocking in an indoor concrete tank (5 m diameter and 80 cm water depth). The recirculating seawater system ($12m^3/h$, 5% exchange/week) consisted on a settler, a foam skimmer, a biological filter, a Heating/Cooler system (automatic, titanium) and four UV lights (160.000 µwatts x seg/cm^2; Bio Light S.A., Chile) (Fig. 1). The settler and the foam skimmer removed solid wastes. Effluent water was biofiltered for a biological conversion of ammonia to nitrate. Salinity ranged from 33 to 36 psu and pH ranged from 7.30 to 8.40.

Female body weight ranged from 894 to 4,398 g (mean ± SD = 2,571±1,200 g) whereas male's varied from 1,068 to 1,948 g (1,528±348.6 g). Total length recorded for females and males was 43.6 - 69.4 cm (57.80±8.39 cm) and 34.0 to 53.2 cm (49.8±3.81 cm), respectively. Sex ratio used for stocking was 1: 0.7 that is ten females: seven males. Broodstock were fed by hand once a day to satiation using fresh squid (*Illex*

argentinus), anchovy (*Engraulis anchoita*) and silverside (*Odonthestes argentinensis*).

Broodstock were induced to mature through the control of water temperature and photoperiod (Fig. 2). The spawning season was scheduled from January to April at 15±1ºC and photoperiod fixed at 16hL: 08hD (hours light: hours darkness). While males were running, final maturation and ovulation of females were achieved after HCG injection (Human Chorionic Gonadotropin, 1,000 IU/kg for females, Tiekoku Co. Ltd., Japan). Gonadal maturation in females was evaluated by canulation, and oocytes diameters were measured using an image analyzer (Nikon Profile Projector V-12B, Japan) and an electronic caliper (Absolute digimatic, Mitutoyo Corp., Japan). Females selected for hormone induction showed oocytes ≥ 500 µm in diameter. In 2001 and 2002, eggs were stripped every 48 h and artificially fertilized (wet method, Bromage 1996a) with sperm of 2-3 running males.
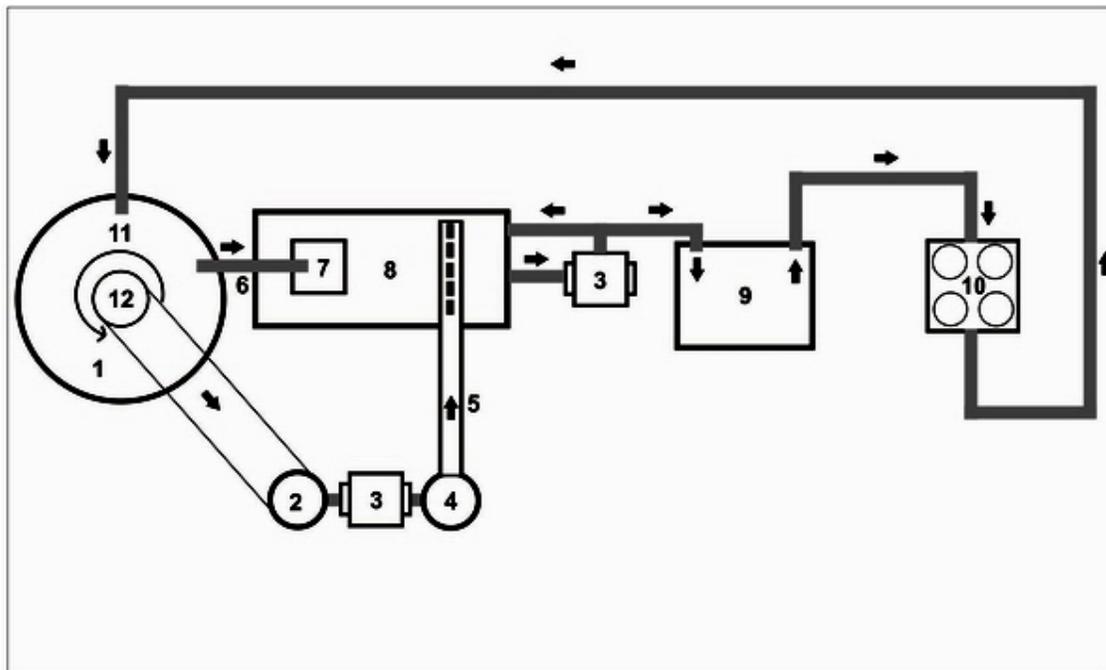

Figure 1

Recirculating water system used for *P. orbignyanus* broodstock. 1. Broodstock tank, 2. Settler, 3. Electromagnetic pump, 4. Foam skimmer, 5. Foam skimmer outlet, 6. Overflow, 7. Egg collector, 8. Biological filter, 9. Heating/Cooler system, 10. UV lights, 11. Tank inlet, 12. Water direction

Sistema de recirculación de agua para reproductores de *P. orbignyanus*. 1. Tanque de reproductores, 2. Decantador, 3. Bomba electromagnética, 4. Espumador, 5. Salida del espumador, 6. Salida por rebalse, 7. Colector de huevos, 8. Filtro biológico, 9. Intercambiador de calor/frío, 10. Lámparas ultravioleta, 11. Entrada de agua al tanque de reproductores, 12. Circulación del agua
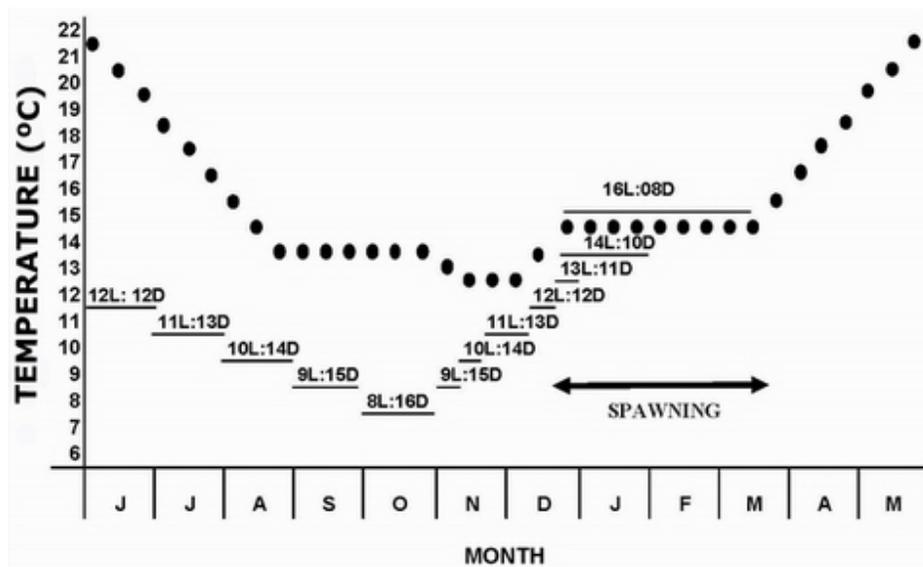
Figure 2

Temperature and photoperiod changes used for *P. orbignyanus* broodstock during 2001, 2002 and 2003 spawning seasons. Photoperiod expressed as number of hours of light:hours of darkness (L:D)

Cambios de temperatura y fotoperíodo aplicados a los reproductores de *P. orbignyanus* en las temporadas reproductivas 2001, 2002 y 2003. Fotoperíodo expresado como número de horas luz: horas de oscuridad (L:D)

Ovulation time

In 2003, a study to estimate the precise timing for stripping was carried out. By 17 March, females were biopsied and only three presented oocytes ≥ 500 μm in diameter, therefore they were HCG injected. These females were monitored after hormonal injection as follows: Female 1 (59.7 cm and 3,405 g): every 48 h; Female 2 (60.4 cm and 3,744 g): every 96 h; Female 3 (69.4 cm and 4,091g): every 144 h.

The eggs collected from each female were artificially fertilized with sperm of 2–3 running males, and total fecundity (number of eggs per kg of female) estimated.

Egg collection

The egg net (300 μm) suspended beneath the overflow outlet and inside the biological filter was daily checked to collect the eggs obtained from natural spawning. The eggs were washed and placed in graduated cylinders to separate buoyant and non buoyant. Buoyant layer of eggs contain both fertilized and non fertilized eggs. Fertilized eggs were considered as viable. Sinking eggs

were considered non-viable (Aristizábal *et al.* 1997, Bromage 1996b). The two layers of eggs were separated using a fine mesh, their numbers estimated from three 1 mL sub-samples and the fertilization rate was recorded (FR). The diameter of the egg and the oil droplet was measured from a sub-sample of 30 eggs using an image analyzer (Nikon Profile Projector V-12B, Japan) and an electronic caliper (Absolute digimatic, Mitutoyo Corp., Japan). Diameters are presented as mean ± SD. The embryonic stage and estimated spawning time were determined according to Oka & Bambill (2003).

Hatching rate and Specific Activity Index

The quality of the different batches obtained in 2003 was evaluated according to the hatching rate (HR) and the Specific Activity Index (SAI) as described by Radonić *et al.* (2005) for *Pagrus pagrus*.

Statistical analysis

Data on fertilization and hatching rates and SAI obtained from the ovulation time study were statistically treated using the Kruskal-Wallis non-parametric test.

Results

Broodstock conditioning and natural spawning

Running males and mature females showing a swollen abdomen were observed during the three spawning seasons using the photothermal conditions cited above.

However, no hydrated oocytes were observed by ovarian biopsy and stripping. Females induced by the use of HCG, spawned naturally non fertilized eggs during the three years. Total egg production obtained by natural spawning and stripping in spawning seasons 2001-2003 are presented in Table 1. Data of 2003 summarizes the egg production obtained from females 1, 2 and 3 subjected to the study of the ovulation time.

Table 1

P. orbignyanus total egg production obtained by natural spawning and stripping in three spawning seasons. ED= Mean egg diameter; HR= Mean hatching rate; NF= Non fertilized eggs; F= Fertilized eggs (100%). Values in brackets are ± SD

Producción total de huevos de *P. orbignyanus* provenientes de desoves naturales y por masaje abdominal durante tres temporadas reproductivas. ED= Diámetro medio del huevo; HR= Tasa media de eclosión; NF= Huevos no fertilizados; F= Huevos fertilizados (100%). Valores entre paréntesis son ± DE

Month	Batches	Natural Spawning Buoyant eggs Nr (x 10^3)	Type	Total eggs (x 10^3)	Batches	Stripping Buoyant eggs Nr (x 10^3)	Type	ED (µm)	HR (%)	Total eggs (x 10^3)
Jan 2001	10	247.6	NF	3,848	-	-	-	-	-	-
Feb 2001	28	193.8	NF	1,235	3	18	F	800 (10.0)	36.1 (13.03)	63.5
March 2001	10	-	-	69	-	-	-	-	-	-
2001 TOTAL	48	441.4	NF	5,152	3	18	F	800 (10.0)	36.1 (13.03)	63.5
Feb 2002	20	-	-	2,980	2	55.3	F	850 (12.0)	19.2 (3.39)	116.5
March 2002	22	-	-	520	-	-	-	-	-	-
2002 TOTAL	44	-	-	3,500	2	55.3	F	850 (12.0)	19.2 (3.39)	116.5
March 2003	9	215	NF	907	12	1,385	F	816.9 (32.55)	9.2 (11.01)	2,697
April 2003	16	192	NF	786	7	250	F	820.9 (28.06)	10 (13.11)	556
2003 TOTAL	25	407	NF	1,693	19	1,635	F	818.4 (30.08)	9.5 (11.52)	3,253

Ovulation time and fecundity

The observations lasted a month. Total number of eggs produced, percentage fertilized eggs, egg and oil droplet diameters and SAI recorded for all egg batches obtained per female are presented in Table 2. The mean oil drop diameter was 98.1±2.96 µm. FR and HR for females stripped at different frequencies are shown in Figs. 3 and 4, respectively. The stripping procedure was stopped for female 1 after 16 days, since it showed injuries all over the body due to repetitive handling. FR average for female 1 was 60.2±33.1% (range 0.0-

95.1%). Female 2 had an average value of 43.0±18.0% (range 19.4-70.3%). Average FR for female 3 was 36.8±16.9% (range 15.4-62.2%). Average HR for females 1, 2 and 3 were 1.9±2.41% (range 0.0-5.1%), 20.5±14.79% (range 0.0-34.5%) and 13.9±11.77% (range 0.0-30.4%), respectively. No significant differences were found with the Kruskal-Wallis test for FR, HR and SAI ($P > 0.05$) for the three females. Female fecundity varied from 185,951 to 399,118 total eggs produced per kg and from 76,146 to 233,765 fertilized eggs produced per kg (Table 3).

Table 2

***P. orbignyanus* egg batches obtained from females 1, 2 and 3 stripped every 48, 96 and 144 h, respectively, after hormonal (HCG) induction on 17 March 2003. TE= Total number of eggs; FE= Number of fertilized eggs; ED= Egg diameter (mm); OD= Oil droplet diameter (mm); SAI= Specific Activity Index; ND= No data available**

Camadas de huevos de *P. orbignyanus* obtenidas de hembras 1, 2 y 3 (inyectadas con hormona HCG el 17 de marzo 2003) mediante masaje abdominal cada 48, 96 y 144 h, respectivamente. TE= Número total de huevos; FE= Número de huevos fertilizados; ED= Diámetro del huevo (mm); OD= Diámetro de la gota oleosa (mm); SAI= Índice de actividad específica; ND= Datos no disponibles

	Female 1					Female 2					Female 3				
Date Day-month	TE (x 10³)	FE (x 10³)	ED (µm)	OD (µm)	SAI	TE (x 10³)	FE (x 10³)	ED (µm)	OD (µm)	SAI	TE (x 10³)	FE (x 10³)	ED (µm)	OD (µm)	SAI
19-3	0	0	-		-	-									
21-3	378	138	766	ND	1.4	584	330	811	ND	-					
23-3	243	82	762	ND	-						3.4	1.2	832	ND	0.3
25-3	252	178	822	ND	13.5	170	33	801	ND	13.5					
27-3	90	78	839	94.9	-										
29-3	258	196	858	94.7	-	147	60	801	100.5	19.9	354	142	833	ND	13.0
31-3	54	44,8	860	92.9	19.6										
2-4	82	78	846	95.3	-	64	45	788	99.8	20.4					
4-4											164	102	851	99.4	20.0
6-4						87	33	799	99.6	17.8					
8-4															
10-4						82	27	792	100.4	-	189	59	849	100.2	5.5
12-4															
14-4															
16-4											52	8	821	101	-
Total	1,357	794,8				1,134	528				762,4	312,2			
Mean	169,6	99,4	828.3	94.5	11.5	189	88	791.3	100.1	17.9	152,5	62,4	837.2	100.2	9.7
SD	130.7	66.6	34.3	1.1	9.3	197.8	119.1	13.0	0.4	3.1	136.4	60.5	12.6	0.8	8.6

TE= Total number of eggs   FE= Number of fertilized eggs   ED= Egg diameter (mm)
OD= Diameter of the oil droplet (mm)   SAI= Specific Activity Index   ND= No data available
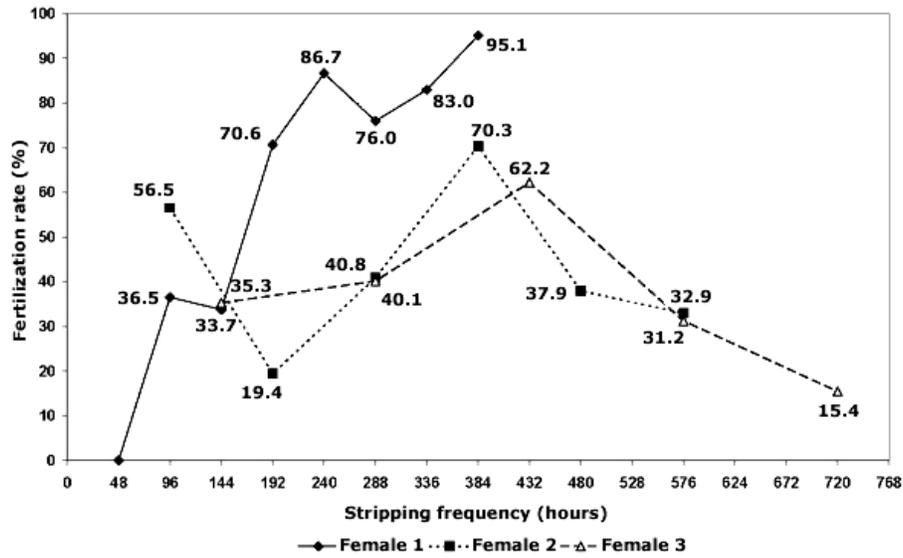
Figure 3

Fertilization rates of *P. orbignyanus* eggs obtained from females 1, 2 and 3 stripped every 48, 96 and 144 h after hormonal (HCG) injection

Tasas de fertilización de *P. orbignyanus* obtenidas para hembras 1, 2 y 3 sometidas a masaje abdominal cada 48, 96 y 144 h después de ser inyectadas con hormona HCG
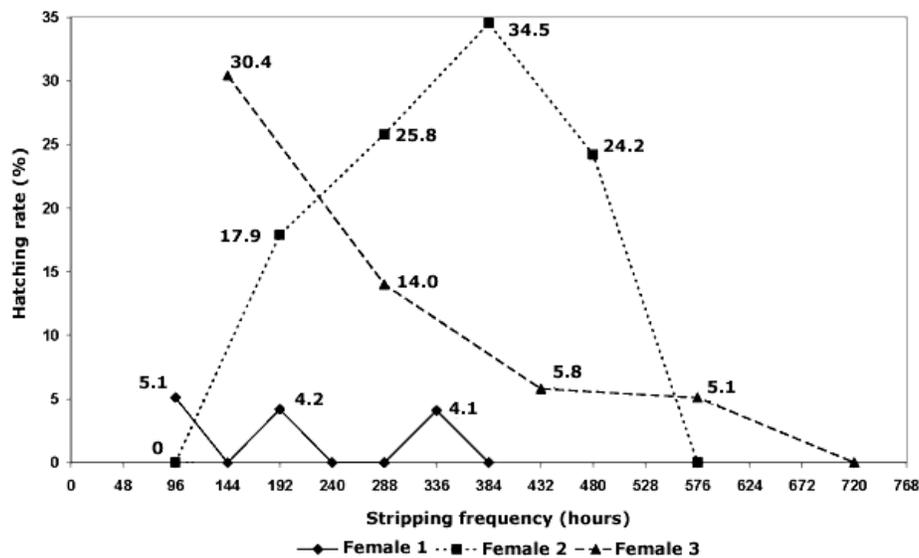

Figure 4

Hatching rates of *P. orbignyanus* obtained for females 1, 2 and 3 stripped every 48, 96 and 144 h

Tasas de eclosión de *P. orbignyanus* obtenidas para las hembras 1, 2 y 3 sometidas a masaje abdominal cada 48, 96 y 144 h

Table 3

Fecundity of *P. orbignyanus* expressed as total number of eggs per kg of female (TE/kg) and as fertilized eggs per kg of female (FE/kg), for females 1, 2, and 3, subjected to different stripping frequencies (48, 96 and 144 h). TE= Total eggs; FE= Fertilized eggs

Fecundidad de *P. orbignyanus* expresada como número total de huevos por kg de hembra (TE/kg) y como huevos fertilizados por kg de hembra (FE/kg), para hembras 1, 2 y 3 sometidas a diferentes frecuencias de masaje abdominal (48, 96 y 144 h). TE= Huevos totales; FE= Huevos fertilizados

Female	Fecundity	
	TE/kg	FE/kg
1	399,118	233,765
2	306,486	142,703
3	185,951	76,146

Discussion

Many factors such as tank dimension, light intensity, nutritional status and husbandry of broodfish have been proved to greatly influence the reproductive behaviour of the fish and the production of eggs both in quality and in quantity (Furuita *et al.* 2000, Smith & Denson 2000, Izquierdo *et al.* 2001, Schreck *et al.* 2001). During the years of this study, the adaptation to captivity was successfully achieved with minimal stress evidenced by the active feeding of *Paralichthys orbignyanus* broodstock and the absence of mortality and/or diseases. Management, husbandry conditions and water quality were efficient for the broodfish welfare. Smith & Denson (2000) indicated high feeding rate as an index of adaptation of *P. lethostigma* to captive conditions. Robaldo (2003) reported 64% mortality over a 6.5-month period of keeping *P. orbignyanus* broodfish in captivity, due to starvation and the appearance of the dinoflagellate parasite *Amyloodinium cf. ocelatum* (Abreu *et al.* 2005).

In this study, the annual photothermal manipulation was not as effective as expected for the maturation and natural spawning of wild female broodstock of *P. orbignyanus* in captivity, although it seemed to be effective for males. Unfertilized eggs were spontaneously released only if females were previously induced by the use of hormones. In species of the same genus, such as *P. dentatus* and *P. lethostigma,* variable fertility success has been attributed to low participation

of males in the spawning events (Smith *et al.* 1999, Smith & Denson 2000, Watanabe & Carroll 2001). Similarly, in *P. orbignyanus* no courtship and spawning behaviour could be observed during three spawning seasons. Moreover, artificial fertilization was only achieved when the sperm of 2-3 running males was used, due to the low milt volume available per fish. According to Robaldo (2003), wild *P. orbignyanus* males kept under captive conditions were always running with good sperm quality and quantity. The absence of fertilized eggs by natural spawning could be attributed to inadequate water spawning temperature and sex ratio. The water temperature variation from 13 to 15ºC would not induce ovulation and oviposition of females, and a sex ratio of 10 females: 7 males might have not favoured courtship. Robaldo (2003) suggested that a water temperature gradient of 6-7ºC along the photothermal induction period would be appropriate to induce *P. orbignyanus* maturation. Some authors have recommended a sex ratio of 1-2 males: 1 female for *Paralichthys* reproduction, although the optimal ratio has not been determined yet (Kumagai 1999, Smith *et al.* 1999, Watanabe & Carroll 2001).

Regarding the ovulation study, no statistical differences found for FR, HR and SAI could be attributed to the small sample sizes (one female/treatment, with no replication), individual variation and/or stripping frequencies. Nevertheless, qualitative differences were evident for the parameters mentioned above. Female stripped every 48 h showed increasing FRs values, HRs less than 5% (linear trend) and poor health condition. FRs for females stripped every 96 and 144 h fluctuated over time, although HRs for the former showed a parabolic trend, and for the latter a decreasing tendency. Moreover, larvae obtained from the 96 h frequency showed more tolerance to starvation and activity. Although long-term studies are needed to confirm these observations, 96 h stripping frequency could be considered a good technique to obtain eggs and larvae experimentally. Robaldo (2003), keeping *P. orbignyanus* females at 26ºC water temperature, reported female ovulation within 1 to 2 days after HCG induction.

Female fecundity is a valuable parameter to estimate the number of available eggs per spawning season. Macchi & Díaz de Astarloa (1996) reported that *Paralichthys patagonicus* is a batch spawner with an estimated fecundity ranging from 20,000 to 160,000 hydrated oocytes in females of 42-53 cm total

length. Cerqueira *et al.* (1997) presented preliminary results on HCG induction and stripping of *P. orbignyanus* wild mature adults (4 males and 4 females). Two females responded to hormone treatment yielding fertilized eggs by stripping and unfertilized eggs by natural spawning (one natural spawn per female). Females weighing 1.78 kg and 2.86 kg showed a fecundity of 224,000 and 280,000 eggs/kg, respectively. In the present study, female mean weight was over 3 kg. All the females used for stripping produced higher number of eggs per kg than the values indicated by Cerqueira *et al.* (1997). The different fecundities recorded are not contradictory considering that broodstock size, age and genotype, as well as the daily and seasonal feeding rates can influence the number of eggs produced (Bromage 1996b). In the case of *P. olivaceus,* females weighing 4-5 kg and measuring 80 cm in length have an estimated fecundity of 200,000-1,000,000 eggs (Kafuku *et al.* 1987).

Cerqueira *et al.* (1997) and Robaldo (2003) recorded egg diameters of 790 and 820 μm, and oil droplet diameters of 116 and 117 μm, respectively. The values presented in this study were similar being 818.4 ±30.08 μm and 98.1±2.96 μm, respectively. Broodstock size, husbandry conditions, quality and quantity of food also influence egg dimensions as stated by Bromage (1996). In species of the same genus such as *P. olivaceus* the size of the egg and the oil droplet ranged from 820 to 960 μm and 130 to 180 μm, respectively (Kafuku *et al.* 1987).

In summary, this work describes the management and conditioning of flounder *Paralichthys orbignyanus* for use as broodstock. The closed recirculated water system is effective for the long-term maintenance and welfare of captive fish. This system minimizes the outbreak of diseases and its application is increasing worldwide to reduce water pollution (Timmons *et al.* 2002). The Argentine coasts are widely opened not allowing the installation of cages or pens. Therefore, baseland farms with recirculated water systems are the only possibility for the development of marine finfish aquaculture. Timing and frequency of hand stripping are presented. Several parameters of the egg morphology such as egg and oil droplet diameters as well as fecundity, hatching rates and larval tolerance to starvation are reported. Further researches are needed focussed on the appropriate photothermal cycle to induce natural spawnings based on natural environmental conditions found in Argentine waters,

sex ratio, reproductive biology, and rate of replacement of older fish by newly caught fish.

Acknowledgments

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