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Natural spawning and intensive culture of pejerrey *Odontesthes bonariensis* juveniles

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The pejerrey *Odontesthes bonariensis* is an atherinid fish native of the inland waters of Buenos Aires Province (Argentina). This species has also been introduced in other Argentinean provinces and some countries due to the economic movement generated by pejerrey game fish and aquaculture (Bonetto and Castello, 1985; Grosman, 1995; Mituta, 2001).

Although pejerrey aquaculture is considered of regional importance, this activity has not been fully developed in Argentina. The culture of this species, either in extensive or semi-intensive systems, can be productive alternatives in the Argentinean Pampas where more than 2 millions square hectometers of lagoons have been described (Reartes, 1995; Espinach Ros *et al.*, 1998; López *et al.*, 2001). However, some reports have demonstrated the existence of problems in the feasibility of pejerrey farming: low growth rates, high mortality in the first stages and early sexual maturation before fish reach market size (Luchini *et al.*, 1984; Grosman, 1995; Grosman and González Castelain, 1996; Gómez, 1998; Berasain *et al.*, 2000; Colautti and Remes Lenicov, 2001; Miranda and Somoza, 2001). Then, it is necessary to perform basic and applied studies in order to establish pejerrey culture (Strüssmann, 1989; von Bernard *et al.*, 2002).

A main objective is to control reproduction in order to obtain a massive production of embryos and larvae and, if possible, almost all year round. Up to date, pejerrey seed production in Argentina is being performed only through the capture of wild fish by gill nets and by artificial fertilization, according to traditional methodologies (Ringuelet, 1943), which cause the dead of all parent fish by manipulations.

In the present work we summarize the results of our efforts to control pejerrey reproduction in captivity, for the development of efficient, economically viable and not pollutant techniques for the production of juveniles in an intensive system.

Pejerrey broodstocks in captivity

Pejerrey embryos were obtained from the Kanagawa Experimental Station (Kanagawa, Japan) and transported to IIB-INTECH (Chascomús, Argentina) on

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November, 2001. These embryos are the progeny of an original stock of pejerrey from Chascomús Lagoon sent to Japan in 1965 and kept in captivity for approximately 37 generations. These fish were found to be easier to rear in captivity than those fish obtained from wild parents. The embryos were obtained from natural spawnings, incubated until the stage of "eyes with no pigment", kept in a special thermo box at 17 ± 1°C and transported to Argentina. After 45 h these embryos were immediately transfer to jars to finish the incubation process at the IIB-INTECH aquatic facilities. They were maintained in a close water flow system with a salinity of 0.4%, temperature oscillating between 20 and 23°C and natural photoperiod. The hatched larvae (around 75%) were immediately transferred to indoor 300 l tanks using an open water flow system, salinity of 0.4% and fed *ad libitum* with artemia *nauplii* and artificial food specially designed for pejerrey at the Tokyo University of Marine Science and Technology. After one month the larvae were transferred to indoor 3,000 l tanks and maintained at the same conditions.

Natural spawnings in captivity

On July 2003 two groups of fish were transferred to outdoors 20,000 l tanks with and open water flow system and a salinity of 0.7%. At the beginning of October, all fish were separated by sex, measured (standard length: 25.99 ± 2.14 cm; weight 226.29 ± 63.77g) and distributed in a proportion of 1.5 male per female in 2 tanks with 300 fish each. The gonadal condition of females was evaluated using a catheter following established techniques (Strüssmann, 1989) and in the case of males running milt was verified by stripping.

The first group of eggs was observed on October 3rd (22 months after hatching [MAH]), and in a regular form since October 17th up to December 14th. The eggs were collected daily every morning using a collecting bag at the end of a drain tube with no natural or artificial substrates that may stress parent fish. Water temperature oscillated between 16.2 and 18.1°C, and 599,000 eggs were collected with 52% of fertilization rate.

The second spawning season began on August 17th 2004 (33 MAH). At the end of September all the fish were separated by sex, measured (standard length 30.57 ± 2.2 cm; weight 400 ± 86.6 g) and distributed in a proportion of 1.2 males per female using 2 tanks with 290 fish each. The animals were kept in the same conditions, except that water salinity naturally increased up to 1.5%. In this case the day length was artificially increased up to 15 h (between 6 to 21 hs) because it was already reported that under a long photoperiod regimen the fish presented spawnings distributed uniformely during the reproductive season independently of water temperature (Strüssmann, 1989). In this case, the water temperature oscillated between 17.5 and 18.3°C.

The fish spawned regularly until December 29th, and during that time 2,136,952 eggs with a fertilization rate of 35% were collected. The lower fertilization rate observed in this second compared to the first reproductive period could have been caused either by the lower male:female ratio or by the higher water salinity in the second period. First, it is important to note that pejerrey sperm volume obtained by stripping either in natural conditions or in captivity is scarce (Strüssmann, 1989; Miranda *et al.*, 2001), then it is possible that several males are nedded to fertilize the eggs produced by a

Table I.

Feeding schedule during the indoor phase

Period	Time (hs)	7.30	8.30	9.30	10	11	12	13	14	15	17	18	19	20
From 19/11 to 17/12/03	Artificial food	X	X		X		X		X			X	X	X
	Artemia nauplii			X		X		X		X	X			
From 18 to 22/12/2003	Artificial food	X	X		X		X		X			X	X	X
	Artemia nauplii			X		X		X		X	X			
	Zooplankton									X				
From 23 to 28/12 /2003	Artificial food	X	X		X		X		X			X	X	X
	Artemia nauplii			X		X		X		X	X			

single female as already shown in a related species *Menidia menidia* (Conover and Kynard, 1984) and suggested in pejerrey (Calvo *et al.*, 1977). Another possibility is that the increase in salinity altered the pejerrey sperm motility, because it was already demonstrated that pejerrey spermatozoa are motile in hypoosmotic to slighty hyperosmotic media in relation to the seminal fluid (Renard *et al.,* 1994). It is important to consider that in our study the osmotic pressure of pejerrey seminal fluid was around 320 mOsm/kg and the osmotic pressure of the water was around 480 mOsm/kg.

In Buenos Aires Province, pejerrey shows an intense natural spawning period during the spring, from the end of August to the beginning of December (Calvo and Morriconi, 1972). This data are in accordance with the reproductive season observed in captivity, though we observed that the breading season can be extended in captivity.

During the two breeding seasons reported in this study the mortality rate was almost negligible, showing that it is possible to control pejerrey reproduction in captivity. However, it is important to work on the synchronization of spawning and to get higher fertilization rates by the manipulation of environmental clues such

TABLE II.

Frequency and feeding schedule during the outdoor phase

Date	*Artemia nauplii*	Artificial food	Zooplankton
30/12	5		
31/12	5		
1/1	5		
2/1	4	1	
3/1	3	3	
4/1	3	3	
5/1	3	3	
6/1	2	3	
7/1	2	3	
8/1	2	4	
9/1	1	3	1
10/1	1	4	1
11/1	1	3	1
12/1	1	3	1
13/1	1	2	2
14/1	1	2	2
15/1	1	2	2
16/1	1	2	2
17/1	1	2	2
18/1	1	3	2
19/1	1	3	2
20/1	1	3	2
21/1	1	3	2
22/1	1	3	2
23/1	1	2	2

as photoperiod, salinity and/or through hormonal treatments.

Juveniles production

Groups of pejerrey embryos obtained at the IIB-INTECH aquatic facilities were transferred to the "Estación Hidrobiológica de Chascomús" (EHCh) in order to set up the production of juveniles in an intensive system. This activity was performed in two phases: one indoor phase lasting for approximately 36 days and an outdoor phase of 25 days.

Indoor phase

In this phase, 25,000 larvae hatched between November 16 and 22 and 14,000 larvae hatched between November 22 and 28, were seeded in two 1,800 l tanks (1 and 2 respectively). During the first 10 days a close water flow system was used with a salinity adjusted to 1%. Then, the system was changed to an open water flow system and the salinity was lowered to 0.3%.

Larvae were fed with *nauplii of Artemia sp.,* pelleted artificial food and zooplankton (rotifers *Bracchionus spp.*, filtered from a previously fertilized pond) follow-

TABLE III.

Pejerrey measures during indoor and outdoor phases

	DAH	Total length ± SEM (cm)	Standard length ± SEM (cm)	Weight ± SEM (g)	n
Indoor phase					
Tank 1	12	1.33 ± 0.019	1.16 ± 0.096	0.0099 ± 0.0005	25
	26	2.07±0.046	1.75 ± 0.76	0.0391 ± 0.0115	25
	39	2.79±0.270	2.31 ± 0.212	0.0957 ± 0.0055	13
Tank 2	12	1.43 ± 0.153	1.26 ± 0.118	0.0140 ± 0.0046	25
	27	2.43 ± 0.216	2.03 ± 0.177	0.0639 ± 0.0161	25
	33	2.74 ± 0.094	2.28 ± 0.075	0.0956 ± 0.008	13
Outdoor phase					
	61	4.52 ± 0.067	3.75 ± 0.053	0.4750 ± 0.018	35

TABLE IV.

Density, survival rate and production during indoor and outdoor phase

	Initial density (i/m^2)	Final density (i/m^2)	Survival rate (%)	Production (Kg/ha/phase)
Indoor phase				
Tank 1	7,962	6,729.3	84.52	6,328.5
Tank 2	4,470	3,699.4	82.76	3,474
Outdoor phase				
	1,651	1,208.6	73.19	4,166.9

ing the schedule shown in Table I. During this period the total amount of *Artemia* eggs offered was 4,850 g (hatching rate around 90%) from 5 g to 250 g. Rotifers were 9,753,194 individuals for tank 1, and 4,378,848 individuals for tank 2. From this total amount of *nauplii* and rotifers, 64% was offered to tank 1 and 36% to tank 2 due to the differences in pejerrey number in each tank. Since day three, 250 µm pelleted food (Kyowa, Co, Japan) was also supplied; 969 g to tank 1 and 635 g to tank 2. During this phase water temperature oscillated between 18.5 and 20.8°C.

After 39 days after hatching (DAH) in tank1 and 36 DAH (tank 2) all juveniles were counted and measured (Table III). During this phase the survival rate was 84.5% in tank 1 and 82.8% in tank 2 (Table IV).

Outdoor phase

After the indoor phase, 32,400 juveniles were transferred from tanks 1 and 2 to an outdoor 20,000 l tank with and open water flow system and a salinity of 10 g/l. Juveniles were fed with *nauplii*, artificial food (Kyowa, Co, Japan) and zooplankton. The total quantity of *nauplii* supplied was obtained from 7,535 g of *Artemia* eggs (mean hatching rate 75%). During the first 13 days 400µm pelleted food (a total of 1,275 g) was offered to the fish and then changed to 700 µm until the end of the period (3,790 g, Table II). In this case, the zooplankton supplied was mainly composed by cladocerans (16,867,387 individuals) and copepods (18,843,220 individuals). The water temperature oscillated between 18.7 and 23.7°C. After 25 days in outdoor conditions (61-64 DAH), juveniles were counted and measured (Table III). The survival rate during this phase was 73% (Table IV) and the overall survival rate during the 61-64 days was obtained 60.76%. However, it is important to note that the mortality rate was increased by the transfer of fish from indoor to outdoor tanks, probably caused by manipulation stress.

The data obtained for standard length in this study (Table III) was similar with those obtained by Berasain *et al.* (2000) using an intensive system, and higher than those obtained by Colautti and Remes Lenicov (2001) using a cage system, and those obtained by Grosman and González Castelain (1996) using a combination of natural and artificial food (Fig. 1). However, the weight

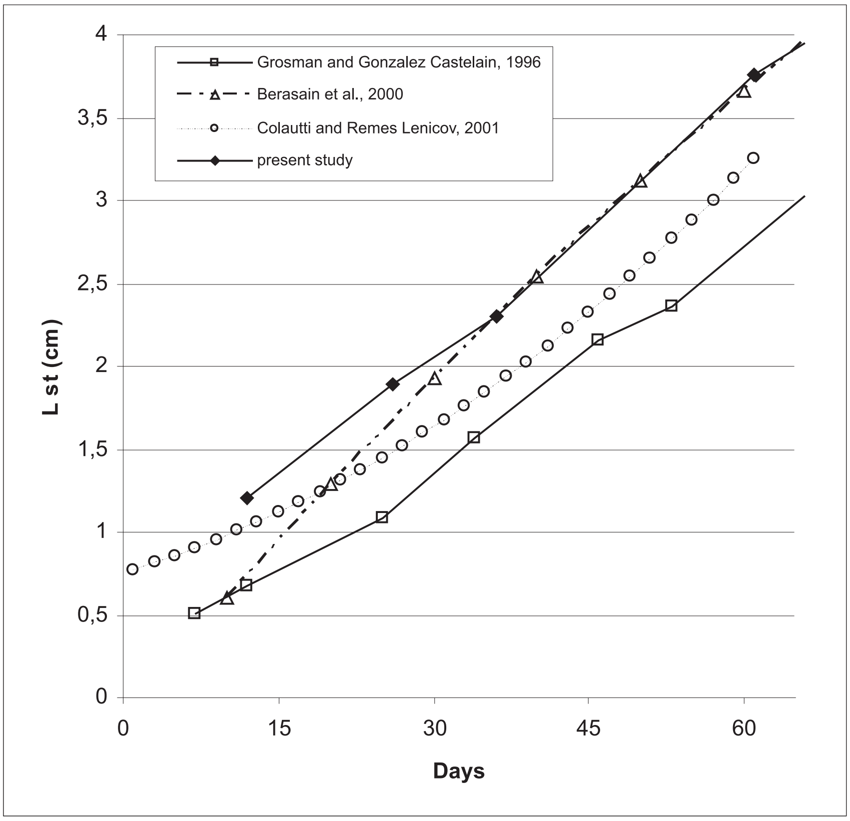

FIGURE 1. Comparison of standard length (L st) obtained in different studies

values obtained in this study (Table III) were lower than those obtained by Reartes (1995) and Berasain *et al.* (2000), both using lower densities.

The survival rate obtained in this study (60.7%) is higher to those reported using different methodologies and systems (Luchini *et al.*, 1984; Grosman and Gonzalez Castelain, 1996; Berasain *et al.*, 2000; Colautti and Remes Lenicov, 2001) and it was lower than the obtained by Reartes in 1995 (89.2%). However, it should be pointed that in all cases the initial densities were significantly lower than the one used in this work (see Berasain *et al.*, 2000).

Taking together all these data show that it is possible to get massive productions of pejerrey fingerlings (Table IV) starting from a high initial density using an intensive system. However, it is necessary to develop cheaper ways of production by replacing both the *nauplii* of *Artemia* and the artificial food by the use of natural zooplankton.

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