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New approaches for growth improvement in pejerrey *Odontesthes bonariensis* (Valenciennes, 1835) culture (Atherinomorpha: Atherinopsidae)

Patricio J. Solimano[1], Javier R. Garcia de Souza[2], Tomás Maiztegui[2], Claudio R. M. Baigún[3] & Darío C. Colautti[2]

The pejerrey is the most important recreational species in shallow temperate lakes and reservoirs of Argentina and the attempts to develop its culture have started a century ago. A common constraint of pejerrey aquaculture is its poor growth under traditional intensive rearing techniques. The aim of this study was to evaluate the possibility to achieve and maintain high growth rates of pejerrey throughout the rearing process by semi-intensive culture method . Four floating cages were installed in La Salada de Monasterio Lake and each one was stocked with 300 juveniles (10.22 ±0.38cm; 6.52 ±0.82g). From January through March all fish were exposed to natural zooplankton as food source, whereas from April to September two cages were supplied daily with artificial food. The fish exposed to artificial supplementary diets exhibited significantly higher growth (17.5 ±0.98cm; 41.05 ±8.55g) than those in the control cages (15.02 ±0cm ; 23.5 ±0.84g), and exceeded the known values in pejerrey culture. The results suggest that the species potential growth is not fully achieved by common intensive methods and it can be improved by semi-intensive techniques. Accordingly a better understanding of the species nutritional requirements is needed to improve growth rates and enhance pejerrey culture.

El pejerrey es la especie de mayor importancia deportiva en lagos someros templados y embalses de Argentina y el desarrollo de su acuicultura data de cien años atrás. Un impedimento común para el desarrollo de la acuicultura del pejerrey es el pobre crecimiento que manifiesta bajo cultivo intensivo. El objetivo de este estudio fue evaluar la posibilidad de alcanzar y mantener altas tasas de crecimiento en el pejerrrey mediante cultivo semi-intensivo. Se instalaron cuatro jaulas flotantes en la laguna La Salada de Monasterio, sembrándose cada una con 300 juveniles (10.22 ±0.38cm; 6.52 ±0.82g). Desde enero hasta marzo los peces se alimentaron con el zooplancton natural de la laguna, mientras que desde abril hasta septiembre dos jaulas fueron suplementadas diariamente con alimento balanceado, dejándose las otras dos como controles. Los peces bajo dietas suplementadas mostraron crecimientos significativamente superiores (17.5 ±0.98cm; 41.05 ±8.55g) a los de las jaulas control (15.02 ±0cm ; 23.5 ±0.84g), excediendo incluso los conocidos en el cultivo de pejerrey en períodos similares. Los resultados obtenidos sugieren que el potencial de crecimiento del pejerrey no está siendo explotado en su totalidad bajo las técnicas de cultivo tradicionales y puede mejorarse mediante el cultivo semi-intensivo. En consecuencia es necesaria una mejor comprensión de los requerimientos nutricionales del pejerrey para incrementar sus tasas de crecimiento y así mejorar su cultivo.

Keywords: Aquaculture, Fish growth, Floating cages, Growth rate, Zooplanktivorous.

Introduction

The domestication of native species for aquaculture purposes has been stressed as an objective of high priority (Ross *et al.,* 2008). The common practice of the introduction of exotic species for culture is fraught with several problems for the environment (Naylor *et al.,* 2000). Nevertheless, the development of native-fish aquaculture is relatively recent since 97% of the species that have ever been cultured were domesticated during the last one hundred years (Duarte *et al.,* 2007). In the case of Argentina attempts to develop aquaculture for native species had been started a century ago

[1]Escuela de Producción, Tecnología y Medio Ambiente. Universidad Nacional de Rio Negro. Sede Atlántica. Av. Don Bosco y Leloir s/n. Viedma CP 8500. Provincia Río Negro, Argentina. psolimano@unrn.edu.ar

[2]Laboratorio de Ecología de Peces, Instituto de Limnología "Raúl Ringuelet" ILPLA. (CONICET-UNLP) Boulevard 120 y 62, CC712 (1900) La Plata, Provincia de Buenos Aires, Argentina. (JRGS) javiergds@ilpla.edu.ar; (TM) t.maiztegui@gmail.com; (DCC) colautti@ilpla.edu.ar

[3]Laboratorio de Ecología y Producción Pesquera, Instituto Tecnológico Chascomús (INTECH), (CONICET-UNSAM), Camino Circunvalación Laguna, km8.2 CC164 (B7130IWA) Chascomús, Provincia de Buenos Aires, Argentina. cbaigun@gmail.com

with the pejerrey *Odontesthes bonariensis* (Valenciennes, 1835), but there are still several constraints to be solved to establish its culture as a productive activity (Somoza *et al.,* 2008).

The culture of pejerrey has been considered a main priority, since in the shallow Pampean lakes and reservoirs of Argentina this species represents the main target for most recreational and commercial fishing (Baigún *et al.,* 2006, 2009; Somoza *et al.,* 2008). Because in the last century the natural abundance of the species has changed over time, in accordance with the limnologic and hydrologic conditions (Colautti *et al.,* 2003), governmental research programs have been directed to develop different culture techniques for the pejerrey. The main technologies currently in use comprise the use of tanks and ponds under intensive and semi-intensive rearing conditions, respectively. Both are mostly directed to produce larvae or fingerlings (Luchini *et al.,* 1984; Berasain *et al.,* 2001; Miranda *et al.,* 2006), being these fish mainly used for restocking and population enhancement (Schenone *et al.,* 2011).

Currently, a genuine interest exists from farmers and governmental agencies in developing pejerrey commercial aquaculture. Nevertheless, culture of the species must overcome certain problems to arise from its historical status of developmental stagnation and becomes a productive activity (Somoza *et al*., 2008). According to the same authors, the main constraints can be tentatively classified as those of biologic nature, gaps in scientifically based technology, and others of socio-cultural and economic origin. One of the main biologic constraints is the poor growth rate exhibited under traditional methods of culture, pointing out that reaching a minimum commercially viable size, of 25cm and 200g, could take more than 2 years. In addition, Somoza *et al.* (2008) stated that the slow growth of the pejerrey is a fundamental biologic limitation, unlikely to be completely or readily solved. In turn, the technologic gaps include the absence of species-specific culture methods, and the pejerrey's inability to adapt easily to the technology developed for other widely cultured species, such as salmonids, cyprinids, and cichlids.

However, in search of alternative culture techniques for pejerrey, Colautti *et al.* (2010) and Garcia de Souza *et al.* (2013) have experimentally tested pejerrey extensive cage culture in Pampean shallow lakes obtaining encouraging results. This method takes advantage of the natural water supply and benefits from the natural zooplankton production, which is the main source of food for pejerrey populations in the wild (Freyre *et al.,* 2009; Diovisalvi *et al.,* 2010). Moreover, through this method, Colautti *et al.* (2010) obtained the best mean daily growth rate of 0.86mm day^{-1} ever recorded for juveniles of 45-60 days of age, and survival rates between 59-65%, which are within the range registered by intensive and semi-intensive culture practices. Nevertheless, those authors pointed out that during the months of autumn and winter, when the productivity of these lakes decreases (Torremorell *et al.,* 2009), pejerrey growth in extensive cage culture becomes limited by low temperatures and by the zooplankton availability. As extension of this findings, Colautti *et al.* (2010) suggested that those rates could be improved by supplying artificial food during the periods when the lakes productivity diminish. This statement assumes that poor growth rate in pejerrey is not an intrinsic biologic limitation of the species and that growth could likely be improved by means of alternative approach involving different culture systems. Thus, the aim of this study was to evaluate the possibility to achieve and maintain high growth rates of pejerrey throughout the rearing process by semi-intensive culture method.

Materials and Methods

During the first fortnight of January 2009, four floating cages (3.45m in length, 3.45m in width and 1.4m in depth -1m effectively submerged), totaling 11.9m^3, constructed following the design of Colautti *et al.* (2010), were set up in La Salada de Monasterio Lake (35.8331°S, 57.8871°W) which is an eutrophic shallow water body located in the Pampean plain, Argentina. The cages were randomly distributed in a macrophyte-free area of around 5 hectares leaving at least 50 meters of distance between them. On January 15, each cage was stocked with 300 individuals taken randomly from a pool of fish reared extensively from larvae, obtained from the Estación Hidrobiológica Chascomús, until 111 days old, in a common cage, placed in the same lake. Stocking density was 25.2fish/m^3 and 0.16kg/m^3, with a total length (TL) of 10.22 ±0.38cm (mean ±SD) and total wet weight (W) of 6.52 ±0.82g, respectively.

The experiment was divided into two periods. During the first one, named pretreatment period (P), from January 15 until March 31, which is the most appropriated period for the extensive culture of pejerrey (Colautti *et al*., 2010), fish were left under extensive culture conditions. The second one, called the treatment period (T), elapsed from April 1 to September 15, was carried out during the period of the year when unfavorable conditions for pejerrey growth under extensive cage culture prevail (Colautti *et al.,* 2010). For this stage of the experiment, the cages were separated randomly in two groups, referred to as the fed cages (FC Group: FC1 and FC2) and the non fed control cages (CC Group: CC1 and CC2). Before the beginning of the T period all cages were sampled and statistically tested by nested ANOVA for differences in length and weight.

The FC Group cages were supplied daily with a fixed ration of 36g of artificial food, divided into four portions per day, through the use of artisanal automatic feeders. Such starting amount represented around the 5% of stocked fish biomass as is suggested for intensive pejerrey culture (Toda *et al*., 1995; Velasco *et al*., 2008). The balanced food supplied was Shulet™ (Shulet S.A., General Las Heras, Buenos Aires, Argentina), presented as pellets and consisting of 42.9% crude protein, 1.5% crude fat, 43.8% carbohydrates, 7.5% ash, and around 4% vitamins, phosphorus, and minerals.

The ration remained unchanged all over the experiment to find out if the supply portions of artificial food similar or less than 5% of fish biomass could induce relevant growth rate changes by synergistic effects between natural and artificial food, and to assess its potential value as a management tool for improving production in pejerrey cage culture.

The physicochemical parameters of the water and the zooplankton community were sampled every fortnight. Water conductivity, pH, and temperature were measured with a multi parameter sensor (Horiba U10, Kyoto, Japan) and dissolved oxygen by an oxygen meter (Lutron DO-5508, Taipei, Taiwan). The transparency was estimated by a Secchi disk and the depth registered by immersion of a graduated bar. Zooplankton samples were obtained by filtration through a 50µ mesh plankton net, 60 liters of water taken in 20 liter subsamples from the upper, middle and lower layers of the water column. Samples of zooplankton were counted to obtain the mean densities per liter (ind.L-1) of the different taxonomical groups of Rotifera, Copepoda and Cladocera, while the individuals from the subsamples of each group were measured to estimate their size (length) distribution.

The biomass per liter by dry weight (µg dw.L^{-1}) of the zooplankton was calculated according to Dumont *et al.* (1975) and Bottrell *et al.* (1976). Limnological measurements and zooplankton samples were obtained within the immediate surroundings of the cages.

At the end of period P and T, twenty fish per cage were weighed (W) and measured (TL) at a respective precision of 0.01g and 0.1cm. Nested analysis of variance (ANOVA) (Sokal & Rohlf, 1995) was performed at the end of both periods following the recommendations of how to treat individual measurements inside of a treatment (Ruohonen, 1998) to avoid pseudoreplication (Hurlbert, 1984). The normality and homogeneity of variance were checked by the tests of Kolmogorov-Smirnov and Levene. The first comparison was made to assess if the culture conditions during period P (e. g., cage location) had affected significantly the fish growth in TL and W between the cages FC and CC after its random separation and just before the start of period T. The second comparison was made to assess differences due to only the food treatments, as cage location was the same during all the experiments.

In order to evaluate the differences in fish growth, the specific growth rate (SGR) (Ricker, 1979) and the thermal-unit-growth coefficient (TGC) (Iwama & Tautz, 1981; Cho, 1992) were estimated according to the following formulas:

$$SGR = 100\ (LnW_2 - LnW_1)\ D^{-1}$$
$$TGC = 1000\ (W_2^{1/3} - W_1^{1/3})\ (T°\ D)^{-1}$$

Where W is the mean weight of the fish in the cage either at the end (W_2) or the beginning (W_1) of the period evaluated, D the total amount of days elapsed, and T° the mean temperature registered.

To test for differences in SGR and TGC between the mean values of the groups, Student t test was used (Sokal & Rohlf, 1995).

In order to make comparable the present results with those of previous studies, transformations from standard length (SL) to TL were made, when was needed, by means of the equation proposed by Berasain *et al.* (2000).

Results

The study periods P (pretreatment period) and T (treatment period) involved different ranges of temperature, with a decrease occurring over the course of the experiment. During period T, the depth of the lake became shallower followed by an increase in the conductivity, pH, and turbidity. The dissolved-oxygen concentration remained high during the whole experiment. The zooplankton abundance was higher during period P, but the biomass became greater in period T (Table 1). Therefore, during the summer (period P) this community was dominated by smaller-sized individuals (mainly rotifers, being the most common species found *Brachionus havanaensis* and *B. caudatus*, and nauplii larvae of Copepoda) than during the autumn and winter (period T) (with increasing values of abundance of the Cyclopoida copepod *Acanthocyclops robustus* and the Cladocera *Bosmina huaronensis*, towards the end of winter).

Table 1. Water-quality parameters and zooplankton availability in Period P (January through March) and in Period T (April through September). SD = standard deviation. Max indicates the maximum value and Min the minimum value for the variables considered in each period.

Period	P				T			
	Mean	SD	Max	Min	Mean	SD	Max	Min
Depth (cm)	123.0	7.5	135	120	97	7.9	110	90
Secchi-disk reading (cm)	37.5	4.9	43	32	22.4	5.8	31	15
Conductivity (mS.cm^{-1})	1.5	0.2	1.7	1.3	1.77	0.05	1.8	1.7
pH	8.7	0.4	8.9	8.1	9.1	0.1	9.3	9.0
Dissolved-oxygen concentration (mg.L^{-1})	8.2	1.4	9.7	6.1	10.7	1.8	13.3	9.4
Temperature (°C)	24.0	0.7	27.5	23.4	12.9	4.7	22.1	7.2
Zooplankton (Ind.L^{-1})	1341.4	1862.5	4647.5	181.0	478.9	270.5	968.8	152.3
Zooplankton (mg dw.L^{-1})	44.6	12.8	62.6	26.7	271.3	133.6	415.9	34.1

At the end of period P, the growth of the fish in terms of TL and W showed no significant differences (p>0.05) among the randomly grouped cages. The respective mean values and standard deviations of TL and W for the fish before the beginning of period T were 12.19 ±0.75cm and 11.6 ±2.3g in the FC cages, and 11.9 ±0.72cm and 10.9 ±2.3g in the CC cages. At the end of period T the total TL and W values obtained by the fish in the FC cages, were significantly higher (p<0.05) than those recorded for the fish in CC cages (Table 2).

The average SGR and TGC during period P were 1.02 and 0.30, respectively, for the FC group and 0.93 and 0.27, respectively, for the CC cages. No significant differences in these rates, were detected between the cage groups (p>0.05). In contrast, the SGR and TGC for the period T were significantly higher in the FC cages (p<0.05). A comparison of the results from both periods indicated a remarkable 50% decrease in the SGR of the CC cages during period T over the values seen in period P. The TGC values in the CC cages were similar during both periods P and T, but in the FC cages were approximately 50% higher in period T than in period P (Table 2).

The comparison of growth achieved in other studies with pejerrey indicated that the semi-intensive technique in cages applied in this work, allowed to obtain the highest length and weight ever recorded for pejerrey of similar age, reared during similar periods (Table 3).

Table 2. Length and weight and growth rates of *Odontesthes bonariensis* obtained in Period T (March-September). FC1 and FC2 cages with provision of artificial food, CC1 and CC2 cages without provision of artificial food. TL, total length; W, total wet weight; Max, maximum value for the variable; Min, minimum value for the variable; SGR, specific growth rate; TGC, thermal-unit-growth coefficient.

Cage	TL (cm)			W (g)			SGR	TGC
	Mean	Max	Min	Mean	Max	Min		
FC 1	18.2	21.8	15.5	47.1	83.6	25.9	0.67	0.52
FC 2	16.8	18.1	14.3	35.0	47.9	20.0	0.64	0.46
CC 1	15.2	16.9	13.9	24.1	32.9	19.2	0.41	0.27
CC 2	15.2	17.8	13.6	22.9	38.6	17.0	0.39	0.26

Table 3. Length and weight reached by pejerrey *Odontesthes bonariensis* in different studies, under several culture methods, after a rearing period of approximately 1 year. Lt, range of mean total lengths; W, range of mean wet weights; Age, of the fish at the end of the cultivation period.

Reference	Culture method	Lt (cm)	W (g)	Age (days)
Berasain *et al*., 2000; 2001 Several experiments	Intensive in tanks	10.7-12.6	17.6-24.8	350-415
Luchini *et al*., 1984 Several experiments	Semi-intensive in ponds	10.9-17.2	9.6-37.0	310-434
Colautti & Remes Lenicov, 2001	Extensive cages	9.7	9.61	242
Colauti *et al*., 2010	Extensive cages	15.0-15.8	20.9-25.4	315
This work	Semi-intensive cages	16.8-18.2	35.0-47.1	354
This work	Extensive cages	15.2-15.2	22.9-24.1	354

Discussion

The Pampean lakes where pejerrey originally evolved (Heras & Roldán, 2011) are highly productive temperate environments (Claps *et al.,* 2004; Torremorell *et al.,* 2009). The values of limnologic parameters registered during the experiment were in agreement with the natural environmental variation described for those lakes (Quirós *et al.,* 2002) and centered around the median values within the pejerrey´s tolerance limits (Gómez *et al.,* 2007).

The respective mean values of the temperature during the periods P and T were 24°C and 12.8°C in agreement with the range expected for this type of lake. The reduction in temperature from April through September could explain the poor growth registered in the fish of the CC group during the period T but would hardly have predicted the results observed in the FC cages, where the fish showed similar growth rates during both periods P and T. The TGC recorded values (two times higher in the FC cages than in the CC cages during period T) confirmed this difference in growth kinetics and furthermore indicated that growth could proceed unimpeded within that lower temperature range.

Observed results also confirm the critical influence of zooplankton on pejerrey food preferences. Moreover, the zooplankton abundance during period P was about ten times higher than during period T, suggesting an increase in the probability of the fish encountering prey during that period. Although the zooplankton in period T was not registered at high densities, the higher biomass at that time can be accounted for by the presence of larger-sized individuals (mainly Cyclopoida copepods) than those recorded in period P. Thus, this larger zooplankton could be more able to escape from planktivorous fish (Drenner *et al.,* 1978; Walls *et al.,* 1990) and despite being lower in density, the encounter probability could also decrease. These latter observations would explain the low growth rate observed in the fish in the CC cages during the period T. In fact, the fish in the FC cages grew significantly more during the period T, thus demonstrating that the natural food supply as the sole source of nutrients was insufficient to maintain high a growth rate during the unfavorable period. According to Jensen (1985), temperature and food availability are the main variables affecting fish growth. However, in the case of pejerrey, the influence of temperature can be assumed to be secondary, whereas the food availability can be considered the main growth regulator in cage culture, at least during the low-temperature periods. This particular hierarchy of variables is not in agreement with the suggestions of Somoza *et al.* (2008) that the pejerrey is a temperate fish with narrow temperature optimum for growth. In fact, the atherinids are a group of fish that adapt easily to new and changing environments and as such are abundant in temperate areas of the world where fluctuations in the environmental temperature are common (Bamber & Henderson, 1988). Therefore, the availability of appropriate food could act as a critical factor in regulating growth performance in environments where the pejerrey has evolved.

According to Table 3, the pejerrey growth under a facultative feeding regime in cages outperformed the results previously obtained during similar rearing periods by Luchini *et al.* (1984), Berasain *et al.* (2000, 2001), Colautti & Remes Lenicov (2001) and Colautti *et al.* (2010) through different culture methods, based either on artificial or on natural foods. In this study, the maintenance of higher growth rates over the entire culture period was achieved through an adaptation of the rearing methods to the ecology of the species, on the basis of the year-round cyclical environmental fluctuations. Thus, following this approach, the species putative poor growth could not represent a critical biologic limitation for the successful development of its aquaculture. The results have demonstrated that the production of fish of average 18cm up to a maximum of 21.8cm TL in less than one year is totally feasible, breaking the traditional assumption that more than two years are required to reach a proper plate-size of 25 cm TL (Somoza *et al.* 2008). This is in agreement with the high growth performance achieved by several atherinid species (Bervian & Fontoura, 2007) and even observed in wild pejerrey populations (Saint-Paul, 1987; Espinach Ros *et al.,* 1998; Espinach Ros & Dománico, 2006; Freyre *et al.,* 2008). Thus the maximum growth rates obtained in this study contributes to solve one of what was referred to as the "techno-scientific knowledge gaps in pejerrey aquaculture" (Somoza *et al.,* 2008) through the development of species-adapted culture. The benefit of the use of a facultative artificial-food phase could promote a synergistic effect, where the zooplankton supplies the main part of the nutrients for growth (essential aminoacids and lipids) and the artificial food contributes to sustain the growth rate during scarce zooplankton availability periods, providing the extra energy for maintenance of metabolic functions. This is coincident with the statements of De Silva (1993) and Shiau & Peng (1993) for other species. A similar effect on tilapias (*Oreochromis* sp.) in semi-intensive systems was also reported by several authors (Green, 1992; Diana *et al.,* 1994; Tacon & De Silva, 1997; Waidbacher *et al.,* 2006). In view of pejerrey growth results, it is possible to affirm that the poor growth performances registered by previous intensive culture experiments can be attributed to the lack of essential nutrients in artificial diets, that currently only zooplankton can provide. This assertion is supported by the results of Velasco *et al.* (2008), who obtained high initial growth rates in early stages of pejerrey development through intensive culture but involving the provision of live food. Similar results were also reported for extensive culture based on only zooplankton intake resulted in higher length and weight values (Colautti *et al*., 2010; Garcia de Souza *et al*., 2013). However, since nutritional requirements of the pejerrey are still unknown (Gómez-Requeni *et al.,* 2012), growth rates attained under intensive culture conditions without a proper formulation of an artificial food cannot be as higher as the one achieved in cages in natural environments. This statement reinforces the idea that the current situation of pejerrey culture is still far from having reached a state of optimal development and that zooplankton supply represents an essential diet component for obtaining a good growth performance.

Within this context, the problem of the pejerrey growth in extensive culture systems can be overcome by transforming them into semi-intensive ones during low zooplankton availability periods. In turn, and more important, intensive aquaculture limitations can expected to be solved, by enhancing pejerrey growth through a better understanding of the species nutritional requirements, and development of appropriate artificial food formulations.

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