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Effects of stocking density and natural food availability on the extensive cage culture of pejerrey (*Odontesthes bonariensis*) in a shallow Pampean lake in Argentina

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Summary

An experiment was conducted for 80 days at La Salada de Monasterio Lake (Buenos Aires, Argentina) to assess the effect of stocking density and natural food availability on the growth and production of zooplanktivorous juveniles of pejerrey (*Odontesthes bonariensis*) in extensive cage culture. Ten cages were installed and stocked with 33-day-old fish, at three density treatments: 25, 50 and 75 ind. m^{-3}. Zooplankton was analysed in terms of abundance, biomass and diversity considering three size classes. Caged pejerrey diet was assessed regularly. The pejerrey gut contents composition was clearly different from cage zooplankton, showing a trend to contain bigger components. Reared fish exhibited a tendency to diversification of the diet and to change the mean prey size depending on fish length and stocking density. Regression models showed a positive and direct effect of the bigger zooplankton biomass on fish growth rates, which were also inversely affected by the availability of smaller zooplankton. The results demonstrate that stocking density and available zooplankton, in both quantity and quality, are key factors in regulating extensive cage culture of pejerrey. Handling stocking densities in a dynamic way it is possible to maximize fish growth, biomass harvest or fish number according to the goals of production.

Keywords: cage culture, pejerrey, stocking density, zooplankton quality, selective feeding, growth performance

Introduction

The pejerrey (*Odontesthes bonariensis* (Valenciennes 1835)) is considered the most important commercial and sport native fish species inhabiting the inland waters of the Pampean region of Argentina (Bonetto & Castello 1985; Grosman 1995; Reartes 1995; Somoza, Miranda, Berasain, Colautti, Remes Lenicov & Strüssmann 2008). While the pejerrey intensive culture had its beginnings in the early twentieth century (López & García 2001), the technique took a real boost in 1925 with the installation of a breeding station in the city of Chascomús, Province of Buenos Aires (Somoza *et al.* 2008).

In recent years, it has been possible to successfully complete the production cycle of the species under intensive culture in tanks (Berasain, Velasco, Shiroyo, Colautti & Remes Lenicov 2006; Velasco, Berasain & Ohashi 2008). Nevertheless, the pejerrey culture has not yet reached a productive scale. Although eggs and larvae can be produced in a massive way, one of the main constraints is the acquisition of large juveniles for stocking or fattening. In addition, other factors have contributed to the historical stagnation of pejerrey aquaculture development in Argentina and other South

American countries, such as those of biological nature, techno-scientific knowledge gaps and those of cultural or socio-economic origin (Somoza *et al.* 2008).

As an alternative to traditional rearing techniques, cage culture has been envisioned as a suitable tool to overcome some of the current constraints. This method has been implemented in the production of many fish species worldwide (Beveridge 2004), having found that extensive cage culture has the biggest potential for low-cost fish production when it is carried out in productive environments using fish species that feed on the lower levels of the food chain (Little & Muir 1987). In the case of pejerrey, cage culture has been also successfully applied for juvenile production in shallow Pampean lakes (Colautti, Garcia de Souza & Miranda 2009; Colautti, Garcia de Souza, Balboni & Baigún 2010). These authors found that the pejerrey extensive cage culture can also reduce costs by not demanding constant attention from staff and not relying on artificial energy supplies, but being mainly dependent on the natural food, in quantity and quality. Since pejerrey feeds on zooplankton (Destefanis & Freyre 1972; Ringuelet, Iriart & Escalante1980; Freyre, Colautti, Maroñas, Sendra & Remes Lenicov 2009), extensive cage culture can take advantage of natural high productivity exhibited by most of the Pampean lakes (Quirós & Drago 1999; Escalante 2001; Quirós, Rennella, Boveri, Rosso & Sosnovsky 2002; Claps, Gabellone & Benítez 2004), avoiding the provision of artificially cultured living food, which is essential for weaning and for optimal development in early stages.

Although the relationship between pejerrey biology in Pampean lakes and the zooplankton community has been documented by Baigún, Colautti and Grosman (2009), Colautti, Remes Lenicov and Berasain (2003) and Freyre *et al.* (2009), more information is still needed about the links between qualitative and quantitative zooplankton data and its influence on pejerrey production.

The structure of the zooplankton community is mainly controlled by fish predation (Stein, DeVries & Dettmers 1995) and according to the size efficiency hypothesis (SEH) (Hrbácek 1962; Brooks & Dodson 1965), the size of zooplankters tends to be maximal when the density of their predators is low, but decreases as the density of predators increases. According to these postulates, the stocking density becomes a key factor to guarantee optimal food availability for the caged fish in extensive pejerrey cage culture.

Cage stocking density and, therefore, the volume of water per fish becomes a significant factor in determining production in cages (Hengsawat, Ward & Jaruratjamorn 1997). Furthermore, the increase in the stocking density results in stress (Leatherland & Cho 1985), implying more energy requirements. High densities produce a decrease in the growth rate of fish, greater standard deviation of the growth parameters and higher mortality (Yi, Kwei Lin & Diana 1996; El-Sayed 2002; Coulibaly, Ouattara, Koné, N'Douba, Snoeks, Goore & Kouamélan 2007; Zeng, Li, Ye, Xie, Liu, Zhang & Duan 2010). Inter-individual contact, competition for food and stress are more important at high densities, although low densities clearly reduce the efficient use of the facilities. Indeed, optimum stocking density is species-specific as has been demonstrated for several species such as *Colossoma macropomum* (Gomes, Chagas, Martins-Junior, Roubach, Akifumi Ono & Lourrenço 2006), *Clarias gariepinus* (Hengsawat *et al.* 1997; Barcellos, Kreutz, Quevedo, Fioreze, Cericato, Soso, Fagundes, Conrad, Krammer Baldfisera, Bruschi & Ritter 2004; Islam, Rahman & Tanaka 2006), *Dicentrarchus labrax* (Papoutsoglou, Tziha, Vrettos & Athanasiou 1998), *Lates calcarifer* (Fermin, Bolivar & Gaitan 1996), *Oncorhynchus mykiss* (North, Turnbull, Ellis, Porter, Migaud, Bron & Bromage 2006), *Sarotherodon melanotheron* (Ouattara, Teugels, N'Douba & Philippart 2003) and *Oreochromis niloticus* (Gibtan, Getahun & Mengistou 2008), among others. Pejerrey stocking density has been partially analysed under intensive culture methods in tanks (Berasain *et al.* 2006; Miranda, Berasain, Velasco, Shirojo & Somoza 2006; Velasco *et al.* 2008), but has not been studied for extensive cage culture yet.

Given this scenario, the aim of this study was to assess the effect of stocking density and natural food availability on the growth and production of pejerrey juveniles under extensive cage culture conditions, in order to provide suitable criteria for the development and management of this culture system.

Materials and methods

Study area

The study was conducted in a shallow lake located in the Pampean plain of Argentina named 'La

Salada de Monasterio' (35°47′ S, 57°52′ W), with an area of approximately 600 hectares and 1.3 m mean depth (Fig. 1). It is covered with abundant patches of rooted emergent vegetation (*Scirpus californicus*), having low human impact, moderated agricultural activity and extensive cattle rearing in the surroundings.

Experimental design

On 4 January 2010, ten floating cages of 12 m^3 each were installed in an area of 2 hectares, randomly distributed. The cage design was made according to Colautti *et al.* (2010) with a 4 × 4 m floating wooden frame, to which one net bag of 1.4 m height (1 m effectively submerged) and mesh size 1.5 × 1.5 mm, was added around the inner perimeter (3.5 × 3.5 m). Such mesh maximized the exchange of water and zooplankton with the environment, avoiding at the same time the fish escapement (initial size).

Likewise on 4 January, juvenile pejerrey of 33 days old, previously reared together in a nursery cage at the same waterbody, was stocked in each cage following an experimental design that included three treatments (T): T1 (a, b, c, d): four cages with 25 ind. m^{-3}, T2 (a, b, c, d): four cages with 50 ind. m^{-3} and T3 (a, b): two cages with 75 ind. m^{-3}. At the beginning of the assay, the fish had an initial mean total length of 2.60 ± 0.26 cm and initial mean weight of 0.12 ± 0.03 g. The experiment lasted for 80 days, until 25 March 2010.

Monitoring and analysis of samples

Water temperature was measured every hour using a programmable automatic thermologger (Thermochron iButton, Sunnyvale, CA, USA), installed close to cage T2c. The depth, transparency, conductivity and pH were measured weekly using a graduated bar, a Secchi disc and a multi parameter sensor (Hanna HI 98130; Smithfield, RI, USA), respectively.

The zooplankton was sampled fortnightly, starting 1 week before the fish were stocked in their respective treatments (late December), through a suction pump inside and outside the experimental units. At each sampling site, 60 L of water was taken as follows: 20 L near the lake or cage bottom, 20 L at an intermediate depth and 20 L next to the surface. The three depths were integrated in one sample representing the whole water column, filtered through plankton net of 50 μm mesh size and fixed in 4% formalin. The samples were analysed qualitatively and quantitatively in Sedgwick-Rafter (APHA 1995) and Bogorov (Gannon 1971) counting chambers. The zooplankters were identified to genus or species level and were counted to estimate their abundance per litre (ind. L^{-1}) inside the cages (Cage Zooplankton abundance, CZa) and outside the cages (Lake Zooplankton abundance, LZa). At least 20 individuals of each species per sample were measured to obtain an estimate of their size (length). The zooplankton components were grouped in three size classes: I: up to 0.3 mm

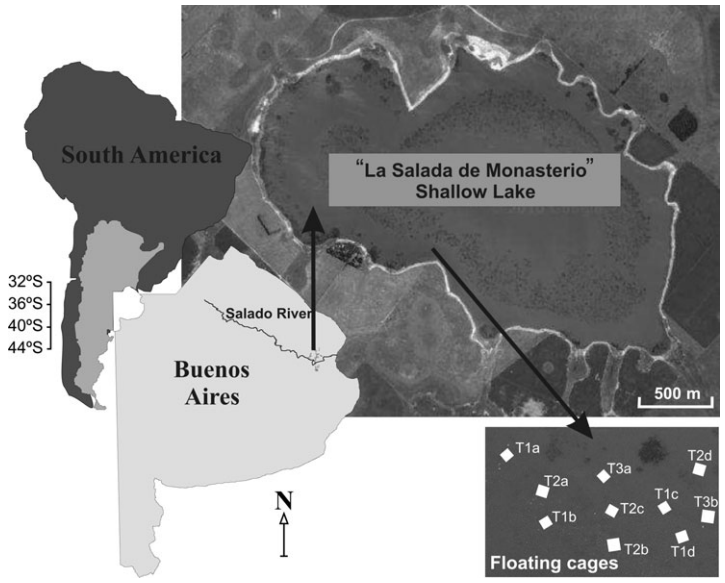

Figure 1 Geographical position of 'La Salada de Monasterio' Shallow Lake and cages location for this study.

(rotifers and nauplii larvae of copepods), II: 0.3–0.7 mm (small copepods and cladocerans) and III: more than 0.7 mm (large copepods and cladocerans), and mean size (ZMS, in mm) at each sampling date and treatment was estimated as the average of mean value of each size class according to its respective abundance.

Dry weights of the zooplankton components were estimated for each sampling date and for each treatment: in the case of the rotifers, these were estimated from volume measurements using geometric approximations (Ruttner-Kolisko 1977; McCauley 1984), and for the microcrustaceans from length–weight regressions available for morphologically similar species (Dumont, Van De Velde & Dumont 1975; Bottrell, Duncan, Gliwicz, Grygierek, Herzig, Hillbricht-Ilkowska, Kurasawa, Larsson & Weglenska 1976; Lawrence, Malley, Findlay, Maciver & Delbaere 1987). Using these values, the dry weight per litre (μg dw L^{-1}) for zooplankton was calculated inside the cages (CZdw) and outside the cages (LZdw).

The reared fish were sampled monthly, starting 1 week after they were stocked in their respective treatments. In each sampling date, 15 individuals per cage were anaesthetized [10 mL of benzocaine solution (1 g:100 mL alcohol) in 1000 mL of water] and were measured in length *in situ* (total length in cm). Another five fish were slaughtered in the cold to avoid food regurgitation, and were taken to the laboratory where they were measured and weighed (total weight in g) and then fixed in 10% formalin for subsequent diet analysis. The gut contents were transferred to counting chambers and treated as described above for zooplankton samples. At the end of the experiment, all fish in each cage were counted.

Data analysis

Zooplankton community (natural food supply)

To test if zooplankton structure differed among treatments, ANOSIM (non-parametric analysis, permutation-based one-way ANOSIM) was applied (Clarke & Warwick 2001). Similarity percentages (SIMPER) were used to identify the species ('discriminating species') that were most important to account for the observed similarity (or dissimilarity) between samples. The method uses the Bray–Curtis measure of similarity, comparing in turn, each sample in one group with each sample in another. Prior to both analyses, the rare species were discarded from the general matrix and then the data were transformed to log $(x + 1)$ to reduce the contribution of high abundant species.

Feeding of cultured pejerrey

The specimens in the gut contents (zooplankton in the gut contents, ZGC) were treated as those in the zooplankton samples, and mean size was also calculated as 'prey mean size' (PMS, in mm). Zooplankton in the gut contents were analysed together with CZa using ANOSIM and SIMPER to evaluate the differences and similarities between what was offered by the environment (natural food supply) and consumed by fish. In turn, the trophic breadth and food selectivity of the reared fish at each age, fish size and stocking density treatment were evaluated by means of the application of two indices:

a) Levins breadth trophic index

$$H' = \left(\sum p_i^2\right)^{-1}$$

where p_i is the proportion of each item in the diet (Levins 1968).

This index retrieves values between 1 and $+\infty$. A H′ value of 1 means that the specimen is monophagous and values higher than 1 mean that the diet is diverse.

b) Foraging rate food selectivity index

$$FR = pC_i/pO_i$$

where pC_i is the proportion of each consumed item, and pO_i is the proportion of each item 'offered' by the environment.

This index can take values between 0 and $+\infty$. If the FR value is 0, it means that this item is not part of the diet; between 0 and 1 represents a negative selectivity; 1 means indifference; and FR values larger than 1 indicate positive selectivity.

Growth, survival and production of cultured pejerrey

A two-factor nested (or hierarchical) ANOVA (Sokal & Rohlf 1995; Ruohonen 1998; Quinn & Keough 2002) was performed to assess differences in fish length, between treatments, for each sampling date. In this design, where the levels of the nested factor were different within each level of the main factor, we used the stocking density as the main factor, replicate cages within stocking density treatments as the nested factor and replicate fish sampled from each cage as the residual. An SNK

post hoc test was performed to explore differences among individual cages if the ANOVA was significant (Underwood 1997).

The specific growth rate (SGR) (Weatherley & Gill 1987; Hopkins 1992) of reared fish was calculated using the measurements obtained for TL (SGRL) and W (SGRW), as follows:

$$\mathrm{SGRL(cm\ \ day^{-1})} = (\ln \mathrm{TL}_2 - \ln \mathrm{TL}_1)/(t_2 - t_1)$$

where TL_2 and TL_1 are the mean total lengths at time 2 (t_2) and at time 1 (t_1) respectively.

$$\mathrm{SGRW(g\ \ day^{-1})} = (\ln W_2 - \ln W_1)/(t_2 - t_1)$$

where W_2 and W_1 are the mean total weight at t_2 and at t_1 respectively.

One-way nested ANOVA was used to search for differences in SGR between treatments.

The SGR were regressed against the different stocking density treatments and against the mean zooplankton dry weight in lake and cages (LZdw, CZdw) of each size classes, both separate and combined (LZdw I, LZdw II, LZdw III, LZdw II+III, CZdw I, CZdw II, CZdw III and CZdw II+III), to evaluate the relationship between these variables by using multiple regression (Sokal & Rohlf 1995). The LZdw and CZdw values used in regressions were the means from zooplankton estimates obtained along the time intervals corresponding to the respective SGRL and SGRW (MLZdw and MCZdw respectively). Also, growth rates were regressed against ZGC, as a percentage of the different size classes by separate and combined values (ZGC I, ZGC II, ZGC III, ZGC I+II), by means of linear and non-linear models.

The mean survival rate per cent (S%) and mean final biomass per treatment (B) were calculated using the O'Conell and Raymond equation (Fex de Santis 1991)

$$\mathrm{S\%} = \{[(\mathrm{Lc/K}) + \mathrm{Sf}]/\mathrm{Ls}\} \times 100$$

where Lc is the number of fish slaughtered in the samples, Sf the final fish number (at the end of the experiment), Ls is the initial fish number (the stocking density treatment) and K is a constant equal to Sf/100.

$$\mathrm{B(g)} = \mathrm{M_{fw}} \times N_\mathrm{f}$$

where $\mathrm{M_{fw}}$ and N_f are the mean final individual fish weight and the final number of fish respectively.

The significance level used in all statistical test performed was the universal $P \leq 0.05$.

Results

Physicochemical parameters

The mean temperature registered along the experiment was 23.9°C with a SD 3.1°C ranging from 32.5°C at the end of January to 16.5°C in the middle of March. Mean and SD of daily temperature range were 3.81 ± 2.37°C, with maximum and minimum values of 12.5 and 0.5°C respectively. Conductivity was increasing throughout the experiment ranging from 1.9 to 2.8 mS $\mathrm{cm^{-1}}$ with an average and SD of 2.18 ± 0.38 mS $\mathrm{cm^{-1}}$. Means and deviations registered for other variables were: pH: 9.49 ± 0.28, depth: 1.04 ± 0.03 m and transparency: 22 ± 4 (Secchi disc cm).

Zooplankton community (natural food availability)

The zooplankton community of the lake and cages was dominant in terms of abundance by rotifers during the whole experiment (Lake: 1278, T1: 751, T2: 1055 and T3: 1025 ind. $\mathrm{L^{-1}}$ mean), with maximum values registered in March (5237 ind. $\mathrm{L^{-1}}$). The microcrustaceans (Copepoda and Cladocera) abundance never surpassed 230 ind. $\mathrm{L^{-1}}$ during the experiment. The total zooplankton biomass followed a close variation pattern in the lake and cages during the whole experiment (Fig. 2). Two biomass peaks were observed, one in January (222 μg $\mathrm{L^{-1}}$ mean, mainly due to copepods in lake and T1) and the other in March (258 μg $\mathrm{L^{-1}}$ mean, mainly due to rotifers in lake and T3).

The zooplankton community was significantly different between treatments in terms of abundance by species in all of the sampling dates except in the first of them (ANOSIM, $P \leq 0.05$). The 'discriminating species' identified with the SIMPER analysis were the rotifer *Brachionus havanaensis* (Size Class I) and the Cyclopoida copepod *Acanthocyclops robustus* (Size Class III).

Feeding of cultured pejerrey

The diet composition, at a specific level, was significantly different between treatments only in the last sampling date, when the reared pejerrey were 113 days old (ANOSIM, $P \leq 0.05$). In turn, ZGC

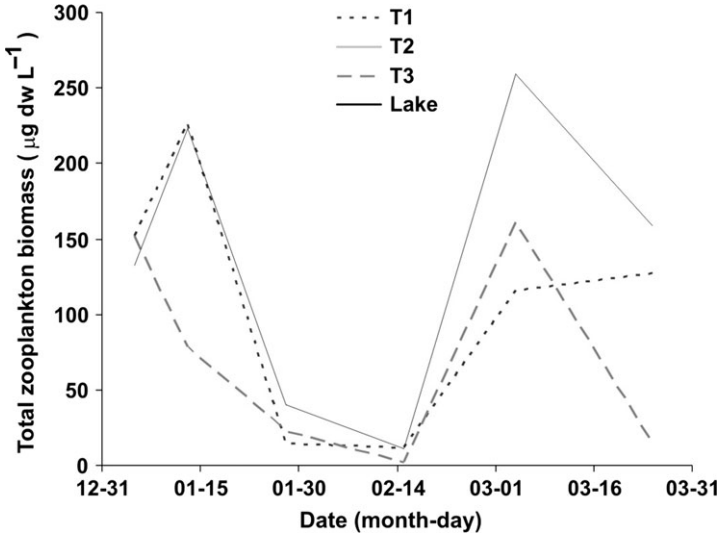

Figure 2 Average zooplankton biomass (μg dw L^{-1}) from samples taken from the lake and cages (of all treatments, T1, T2 and T3) respectively, along the study period.

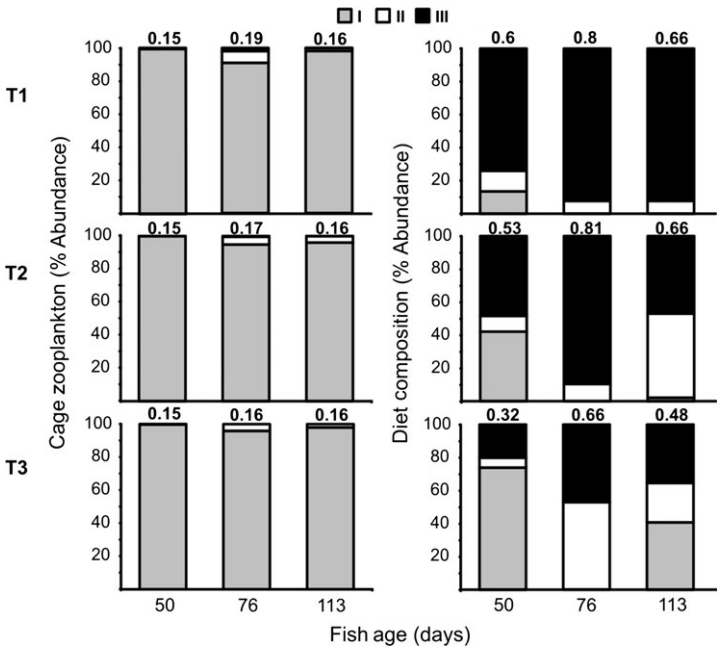

Figure 3 Percentage zooplankton abundance (%) (size classes I, II and III) in samples taken from cages stocked with different fish densities (25, 50, 75 fish m^{-3}) and percentage diet composition (%) of cage fish of each treatment based on three moments of the experiment (age of fish: 50, 76 and 113 days old). Zooplankton mean size (ZMS, in mm) and prey mean size (PMS, in mm) are indicated, in bold numbers, above the left and right columns respectively.

composition was significantly different from CZa, in all treatments (ANOSIM, $P \leq 0.05$; Fig. 3). The SIMPER analysis showed a dissimilarity of 87.4 between what was found in the lake and what was eaten by the reared fish, with the rotifer *B. havanaensis* followed by the adult copepod *A. robustus* being the species that mostly contributed to this dissimilarity (Table 1). The referred differences are also reflected by the PMS observed in the treatments at different fish ages. Fish stocked at the lowest density strongly preferred large size zooplankton, independent of their age. However, at the highest density, small and intermediate size zooplankton items were selected by fish at all fish variable ages (Fig. 3).

Results of selectivity indices by age (Table 2) using the Levins index (H′) showed that the reared fish diet had greater breadth in the older fish and in those stocked at higher densities. In turn, the foraging rate index (FR) indicated that rotifers and Nauplii larvae (zooplankton size class I) were not part of the diet or even where negatively selected

Table 1 Multivariate analysis results from comparisons between cage zooplankton abundance (CZa) and zooplankton found in the digestive contents of cultured fish (ZGC) per treatment

Treatment	T1	T2	T3
ANOSIM $P < 0.01$ (R value)	0.878	0.967	0.775
SIMPER (dissimilarity percentages)	86.58	90.37	85.86
SIMPER (species contribution to dissimilarity)	*Brachionus havanaensis*	*B. havanaensis*	*B. havanaensis*
	Acanthocyclops robustus	*A. robustus*	*Bosmina huaronensis*

Table 2 Levins index (H′) and foraging rate index (FR) for the different fish ages and length at each stocking density

Treatment	Age (days)	Fish length range (cm)	H′ index	FR index		
				I	II	III
T1	50	5–6	1.42	0.21	1.93	18.01
	76	7	1.24	0.00	1.49	46.62
	113	8–9	1.69	0.15	21.35	405.78
T2	50	5	1.39	0.15	3.03	20.43
	76	7	1.27	0.00	2.47	97.77
	113	8	1.92	0.04	15.41	740.10
T3	50	4–5	1.72	0.32	5.61	31.67
	76	6	1.45	0.00	14.48	5650.04
	113	7–8	2.01	0.30	83.53	51.29

by reared fish; meanwhile the size classes II and III were always positively selected.

Growth of cultured pejerrey

The two-factor nested ANOVA showed that, at the first sampling date (50 days old), mean lengths were not significantly different between treatments ($P > 0.05$), but were significantly different ($P \leq 0.05$) on the other two sampling dates (76 and 113 days old). The mean weights showed significant differences between treatments in the three sampling dates ($P \leq 0.05$; for details about the *post hoc* comparisons see Fig. 4).

Specific growth rates showed their maximum values at the first sampling date (Table 3). No significant differences were observed between treatments, except for the first sampling date when the T3 value of SGR in weight (*) was significantly different from the other treatments ($P \leq 0.05$).

Multiple linear regression analysis selected the mean zooplankton dry weight of size categories III and I, found in the lake (MLZdw; $R^2 = 0.935$; $n = 22$; $F = 136.97$; $P \leq 0.05$, in length and $R^2 = 0.93$; $n = 22$; $F = 128.82$; $P \leq 0.05$, in weight), as significant independent variables that account for SGR variation. The fitted model is detailed below:

$$\mathrm{SGR} = a + x_1 \mathrm{MLZdw\ III} + x_2 \mathrm{MLZdw\ I}$$

where, for length: $a = 0.011806$; $x_1 = 0.000134$ ($P \leq 0.05$); and $x_2 = -0.000069$ ($P = 0.000246$); and for weight: $a = 0.020284$; $x_1 = 0.000425$ ($P \leq 0.05$); and $x_2 = -0.000083$ ($P = 0.039049$).

The simple regression made between SGR of caged pejerrey and percentage abundance of zooplankton size III of gut contents (ZGC III), showed the best and most significant fitting to an exponential model ($R^2 = 0.80$; $P \leq 0.05$). The fitted model is detailed below:

$$\mathrm{SGR} = 0.001503 \exp\ (2.36085\ \mathrm{ZGC\ III})$$

The maximum mean final fish number was obtained in T3 (655 ind. per cage), mean survival was higher in T1 (84.9%), and the highest final mean biomass was recorded for T2 (1145.5 g). The results of these variables for each treatment are shown in Table 4.

Discussion

Planktivorous fish cultured in cages and feeding only on naturally available food would probably have maximum growth and productivity in

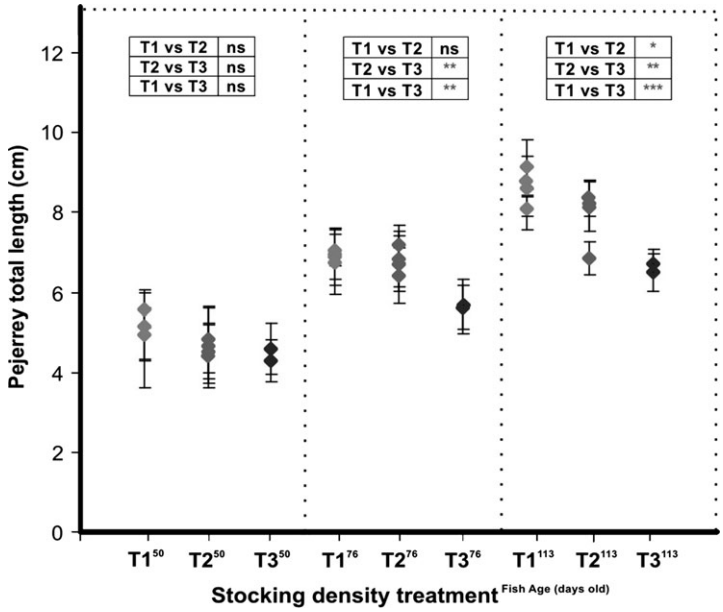

Figure 4 Pejerrey total length (mean and SD) for each cage in each treatment at the three fish ages. Significant differences between treatments are indicated (ns, not significant; $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

Table 3 Specific growth rates (SGR), in length and weight, for each treatment and fish age (days old). Mean values and its SD are shown

		Fish age (days old)		
	Treatment	**50**	**76**	**113**
SGRL (cm day^{-1})	T1	0.031 ± 0.004	0.010 ± 0.004	0.005 ± 0.001
	T2	0.027 ± 0.002	0.015 ± 0.001	0.004 ± 0.003
	T3	0.025 ± 0.002	0.008 ± 0.001	0.005 ± 0.001
SGRW (g day^{-1})	T1	0.100 ± 0.004	0.024 ± 0.005	0.010 ± 0.005
	T2	0.095 ± 0.001	0.022 ± 0.006	0.011 ± 0.009
	T3	0.065* ± 0.008	0.021 ± 0.001	0.014 ± 0.005

*T3 value of SGR in weight (*) was significantly different from the other treatments ($P < 0.05$) at 50 days old.

eutrophic lakes, assuming appropriate environmental conditions (temperature, dissolved oxygen) and an abundance of suitable plankton (Bayne, Joshi, Rai & William 1991; Rai 2000). Nevertheless, there have been few systematic attempts to determine optimum stocking densities for cultured species in agreement with aquaculture conditions (Beveridge 2004). Hence, the importance of evaluating the natural environment where the culture units are placed, not only in terms of the limnological parameters but also in terms of availability of zooplankton in quantity and quality, can be seen.

During the present experiment, important variations were observed in lake and cage zooplankton biomass, but followed a similar pattern inside and outside the cages (Fig. 2). This similarity has shown that inside the culture units, the fish would have similar zooplankton availability to that registered in the lake. Moreover, the zooplankton community, in terms of composition by size classes, was always dominated by the size I individuals, and nonetheless, the gut content composition was clearly different from cage zooplankton composition (Fig. 3).

Table 4 Experimental final parameters: Mean final fish number, mean survival and mean biomass per treatment

Treatment	**T1**	**T2**	**T3**
Mean final fish number (ind. per cage)	254.7	432.2	655
Mean survival (%)	84.9	72	72.7
Mean biomass (g)	876.3	1145.5	982.5

The results showed that reared fish had a tendency to the diversification of prey size depending

on fish length and on its stocking density, despite a subjacent trend to reduce the percentage of smaller prey items ingested as fish increases in age and size.

For the early stage of the experiment (50 days old), the fish mouth size appears to influence the size of the food particles that fish are able to ingest, because the three zooplankton size classes were present in gut contents, with class III being the best represented in fish from T1 which have had, on average, the maximum growth, and therefore better skills to access and consume individuals of the biggest size class at the end of period. Similar observations were made by Dabrowski and Bardega (1984) and Kozlowski, Mamcarz, Poczyczynski and Dostatni (2000) for cyprinid and coregonid fish respectively. Although fish density could also be influencing this situation, the low fish biomass in the cages at the first sampling date determines a minimal inter-individual competition for zooplankton prey in quantity but not in quality determining the observed differential growth.

As fish increased their size they were able to ingest zooplankters of any size class, which was possible to observe at 76 and 113 days old. Furthermore, at these ages, the T1 and T2 prey mean size had almost exactly the same value, and for the T3 cages PMS was lower, indicating that competition affected access to food. In fact, PMS was negatively related to stocking density and class I zooplankters are therefore an alternative resource when competition increases (Fig. 3). This observation is consistent with the Optimal Foraging Theory (Pyke 1984; Stephens & Krebs 1986; Beauchamp, Wahl & Johnson 2007).

The assertions about the diversification of prey size depending on fish length and on its stocking density were confirmed not only by the diet composition, particularly at 113 days in T3 (Fig. 3), but also by the Levins breadth trophic index (H′). The expansion of breadth trophic niche was related to the increasing stocking density likely associated with inter-individual fish competition for available prey (implying the decrease of prey size consumed). Moreover, although the size I zooplankton was found in digestive contents, the FR values indicated that such size of prey was negatively selected. Nevertheless, the low FR values for this kind of alimentary items could be linked to its high abundance in nature, which was significantly different from the amount of zooplankton size I consumed, despite its relative importance for fish. In addition, the higher surface-volume quotient of small prey facilitates the energy assimilation, despite the reduced gastric efficiency of post-larval fish (Mills, Confer & Ready 1984; Zagarese 1991), being apparently insufficient to satisfy the energy demands of juvenile pejerrey.

However, the selective feeding decreased as the fish grew (those cultured at high density), and a shift in their diet occurred, resulting in the consumption of smaller zooplankton at the same time that large prey (class III) began to disappear.

Furthermore, the SEH, which explains the inverse relationship between the density of fish predators and the zooplankton size, as the result of size-selective predation upon the largest prey, is probably modelling the zooplankton community of the whole lake and possibly affects the community inside the cages, where the only fish predators are the cultured pejerrey. The small size zooplankters (rotifers), which are dominant in the Pampean lakes, are a relevant food item for pejerrey during early stage of development (Zagarese 1991), but they are negatively selected by fish bigger than 4 cm (TL). Such dominance could be related in part with top–down control mechanisms exerted by planktivorous species as the pejerrey and the bagarito (*Parapimelodus valenciennis*) (Quirós *et al.* 2002). According to the results, predation inside the cages must be directly related with the stocking density, affecting the zooplankton composition in the cage, functioning like a sieve (in such an aleatory experimental design, it is assumed that the quantity of food that pass through the mesh is similar for all the experimental units).

Moreover, a selective utilization of the resources by the juvenile pejerrey is evidenced by regression models showing a positive and direct effect of zooplankton size III biomass on growth rates (MLZdw III, particularly the Cyclopoida copepod *A. robustus*). On the other hand, growth rates were inversely affected by the availability of zooplankton size I (MLZdw I, particularly Rotifera, mainly *B. havanaensis* as SIMPER analysis has shown). Both results highlight the importance of zooplankton quality for pejerrey production by means of extensive cage culture. Thus, total fish biomass produced in cages appears to be regulated by the available zooplankton resources in quality and quantity, which, in turn, are dependent of planktivorous fish abundance in the natural community.

According to this, a multivariate regression model such as presented in this study could be

useful to predict the growth performance in pejerrey cage culture. In turn, this highlights the importance of selecting lakes with a high abundance of Class III zooplankton for successful cage culture practices. Clearly, high proportions of ZGC III provided better SGR in an exponential way, at least for individuals of 76 and 113 days old. Consequently, the increment in the percentage of zooplankton sizes I plus II in the gut contents implied an exponential reduction in fish growth rate. This indicates that fish feeding on zooplankton size III in high proportions (>80%) tends to maximize their growth rates; meanwhile, those which feed mainly on I+II (>50%) were less efficient, suggesting that this kind of food is almost enough to keep them alive.

The fish grew more in length and weight at lower stocking densities (T1), while the maximum fish number was obtained at maximum density (T3). The intermediate stocking density (T2) proved to be the most appropriate to maximize the production showing that there are different optimum fish densities to maximize growth performance, biomass production or fish number. Indeed, the observed results indicate that the maximum production for juvenile pejerrey in cages was reached at intermediate density, indicating that T2 culture conditions were the most appropriate to profit more efficiently the carrying capacity of the system. These observations differed with authors who found no significant differences between stocking density treatments (Fermin *et al.* 1996; Sagratzki Cavero, Pereira-Filho, Roubach, Rabello Ituassú, Lima Gandra & Crescêncio 2003; Gomes *et al.* 2006); others could not indicate the upper limit of stocking densities (Hengsawat *et al.* 1997; Gomes *et al.* 2006) and still others have obtained better productions at the highest stocking densities. This identifies a positive relationship between stocking density and yield of other species such as one tilapia species (*O. niloticus*) (i.e. Watanabe, Clark, Dunham, Wicklund & Olla 1990; Gibtan *et al.* 2008), other tilapia (*O. spilurus*) (Cruz & Ridha 1991) and for catfish (*Pangasius sutchi*) (Islam *et al.* 2006).

La Salada de Monasterio Lake showed similar limnological and zooplankton community features to other Pampean lakes (Claps *et al.* 2004; Colautti *et al.* 2010; Diovisalvi, Berasain, Unrein, Colautti, Fermani, Llames, Torremorell, Lagomarsino, Pérez, Escaray, Bustingorry, Ferraro & Zagarese 2010). Thus, the results suggest that this extensive method of rearing pejerrey can be applied in other shallow lakes of the region. Our results demonstrate that stocking density and available zooplankton, both in quantity and quality, are key factors in pejerrey extensive cage culture. Hence, assessing the lake zooplankton becomes a critical step for providing a general perspective about food availability (in quantity and quality) in order to determine the suitability of lake for installing cages and the adequate rearing densities. Furthermore, during the rearing process, the analysis of the digestive contents stands out as another key aspect to be considered. Accordingly, proper selection of stocking density and lake (based on zooplankton characteristics) can provide valuable clues for predicting the feasible production. Moreover, by handling stocking densities in a dynamic way, regarding the zooplankton and fish diet features, it is possible to optimize, in 'real-time', the efficiency of extensive aquaculture of pejerrey, and also focus of the system production to maximize growth, production or final number of fish according to the aquaculturist needs. In other words, according to Costa-Pierce and Page (2012), an adaptive management based on ecosystem changes, could be the key to build an ecosystem-based aquaculture for the species.

This work adds new pieces of information and simple predictive models that enhance our understanding about extensive cage culture of pejerrey, supporting the assumption that the method could be incorporated to the pejerrey production cycle, at least to generate juveniles, overcoming some of the current limitations exhibited by the traditional intensive methods.

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