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Evaluation of Larval Growth and Survival in Mexican Mojarra, *Cichlasoma urophthalmus*, and Bay Snook, *Petenia splendida*, Under Different Initial Stocking Densities

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Abstract

Two experiments were conducted to evaluate the initial stocking density in larvae of Bay snook, *Petenia splendida*, and Mexican mojarra, *Cichlasoma urophthalmus*, using a recirculation system. Five initial stocking densities (0.5, 1, 5, 10, and 20 larvae/L) were evaluated by triplicate for 45 d. Weight and total length (TL) were measured every 15 d, and fish production was calculated for each density. The larvae stocked at the lowest densities (0.5 and 1 larvae/L) presented the highest growth for both species: *C. urophthalmus* (0.78 g and 45-mm TL, and 0.76 g and 45-mm TL, respectively) and *P. splendida* (0.80 g and 52-mm TL, and 0.79 g and 49-mm TL, respectively). However, lowest fish production was recorded (35 and 69 fish per tank, respectively, for *C. urophthalmus* and 34 and 70 fish per tank, respectively, for *P. splendida*) compared with those at densities of 5, 10, and 20 larvae/L (336, 584, and 604 fish per tank, respectively, for *C. urophthalmus* and 341, 679, and 912 fish per tank, respectively, for *P. splendida*). The polynomial model for biomass production related to the stocking density shows that the optimum stocking densities for *C. urophthalmus* and *P. splendida* are 12 and 14 larvae/L, respectively.

Fish culture is important in the production of low-cost food, and carp, catfish, and tilapia occupy the top of the list of freshwater fish species cultivated worldwide (FAO 2006). In Mexico, as in other countries, aquaculture during the 1970s focused on carp and tilapia instead of native fish species (Arredondo and Lozano 2003). The culture of native fish species has been limited by poor knowledge of the biological requirements that are essential to an increased use of the resource (Rojas and Mendoza 2000). As a result of this, the culture of tilapia has been reinforced and the species has become the second most cultivated species in Mexico after marine shrimp (SAGARPA 2006).

The traditional consumption of many native cichlid species throughout the southeastern region of Mexico has shown an increase in both exploitation and economic value for many decades. These species are now either fully exploited or overexploited and must now compete with exotic fish species such as carp, tilapia, and catfish that regularly escape from culture centers to lakes, lagoons, and rivers which affect native fish populations. Among the native cichlids, only the Mexican mojarra, *Cichlasoma urophthalmus*, and Bay snook, *Petenia splendida*, have been studied with a view to introduce them to aquaculture programs. Studies have been carried out on their distribution (Páramo-Delgadillo 1985), ecology, feeding (Reséndez and Salvadores 1983), and reproduction (Chávez-Lomelí et al. 1989; Caro et al. 1994; Valtierra-Vega and Schmitter-Soto 2000), as well as on particular aspects of their feeding habits that suggest they are primarily carnivorous species and only sporadically herbivorous (Martínez-Palacios and Ross 1988; Waltzek and Wainwright 2003).

Basic studies regarding the characteristics needed for the culture of *C. urophthalmus* and *P. splendida* have established the conditions for egg incubation which is around 5000 embryos per female that hatch in 36 h for both species at

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28 C (Martínez-Palacios 1987), larval development that occurs in the first 30-d posthatching (Mendoza and Navarro 1994; Martínez 2004), and protein requirements (45%) for the juvenile stage (Martínez-Palacios and Ross 1994; Uscanga-Martínez 2006). However, fish culture also requires an understanding of the technical conditions needed for the rearing and massive production of larvae (Baskerville and Kling 2000; Gomes et al. 2000; Alvarez-González et al. 2001; Szkudlarek and Zakes 2002; Baras et al. 2003; Ruane and Komen 2003; Maciej and Zdzislaw 2007). In the case of *C. urophthalmus* and *P. splendida*, this knowledge, as well as the optimal larvae density and the water quality derived from the culture systems, is still not adequate. Thus, the purpose of this study was to determine the optimum stocking density for the larval stage of *C. urophthalmus* and *P. splendida* in a recirculation system.

Materials and Methods

Fish Specimens

Wild mature males and females of *C. urophthalmus* and *P. splendida* were collected in the surrounding lagoons of Villahermosa, Tabasco, Mexico. Broodstock was maintained in an open system with 9 × 2000-L circular plastic tanks in a ratio of two females per male (250 and 300 g, respectively, for both species), in the Aquaculture Laboratory of the División Académica de Ciencias Biológicas (DACBiol), Universidad Juárez Autónoma de Tabasco (UJAT), Mexico. Broodstock was maintained in natural photoperiod (12:12 light : dark), with daily 200% water exchange and continuously fed with freeze tilapia for 30 d until the reproductive behavior began. Thirty days after the first spawn was recorded, two spontaneous spawns (one of each species) were obtained from the tanks. After 36-h postfertilization (embryos hatching), yolk-sac larvae of each species (around 5000) were collected by siphoning and placed in a small plastic tray. Then the yolk-sac larvae were placed in 5 × 100-L circular plastic tanks connected to a recirculating system and incubated for 5 d (30 ± 0.5 C, 5.2 ± 0.3 mg O_2/L) until yolk absorption.

Experimental Conditions and Rearing System

Five-day-old *C. urophthalmus* and *P. splendida* larvae were used for individual experiments conducted at the intensive freshwater hatchery of the DACBiol-UJAT. Larvae of both species were randomly placed in five groups with experimental densities of 0.5, 1, 5, 10, and 20 larvae/L, and this was performed by triplicate using 15 tanks. Larval rearing was performed for each species in the same recirculation system consisting of 15 × 70-L cylinder–conical fibreglass tanks with continuous aeration and filtration of water (10 μm mesh size) with a biological (polyethylene mesh) and sand sieve (20 μm) at a rate of 100% for every 4.8 h. Water parameters in each tank were monitored once in a day. Water quality was maintained for an average of 90 d of testing for both species at 31.1 ± 1.2 C, 6.3 ± 0.2 pH and 7.5 ± 0.2 mg/L of oxygen content, which was measured with YSI Model 55 digital oxymeter (Yellow Springs Industries, Yellow Springs, OH, USA), and at 0.4 ± 0.1 mg/L of total ammonia and 0.29 ± 0.1 mg/L of total nitrates, which were measured with spectrophotometric techniques according to AOAC (1995).

Feeding

Larvae of both species were fed *Artemia nauplii* for the first 15 d of the experiment, and then switched to 3 d of co-feeding of a commercial diet for trout (Silver Cup®, 45% protein and 16% lipid) for the remaining 30 d. The live prey feed consisted of 100 *A. nauplii*/larvae, whereas the artificial feed was adjusted to 10% of biomass considering the total number of larvae per tank. The commercial diet was ground and adjusted to 0.5-mm particles, suitable for the size of the mouth of the larvae. Feeding frequency was adjusted to four provisions, one every 4 h, starting at 8 AM and finishing at 8 PM.

Fish Biometry and Survival

Samples of more than 30% of the total population in each tank were randomly collected every 15 d from both experiments. The wet weight of individual fish was recorded with an

analytical balance (OHAUS-Phoenix GH-300), and the total lengths (TL) were measured with a digital caliper (Neiko-HKMUND473). Dead larvae were removed and counted daily from all the tanks, in order to estimate the daily food rations. Survival was calculated by harvesting and counting the remaining juveniles per tank.

Statistical Analyses

Normality was verified with the Kolmogorov–Smirnov test for every sampling. Homogeneity of variance (Levine test) was used to determine the difference among replicates. Weight, TL, survival (arcsine transformation), and fish-produced data were analyzed with one-way ANOVA to determine the differences between densities for each species, and the Tukey test was applied when significant differences were detected. With the weight data, the coefficient of variability was calculated as $CV = (SD \times 100)/\text{mean}$, and statistically compared between initial stocking densities. In order to compare the growth rates, the weight data were transformed into natural logarithm, adjusted to an exponential model $Y = ae^{bX}$ (Everhart et al. 1953), where Y is the estimated weight, a is the initial weight, e is the exponential base, b is the specific growth rate, and X is the time (d), and compared with a covariance analysis, followed by a Tukey test when significant differences were detected (Zar 1996). Additionally, a polynomial regression model $Y = y_o + aX + bX^2$ was applied to relate the density with the total biomass production, where Y = estimated biomass, y_o = initial biomass, a = growing constant, b = asymptotic constant, and X = stocking density. All statistical analyses were conducted with STATISTICA v.7 (StatSoft, Tulsa, OK, USA) using a significance value of 0.05.

Results

Experiment 1 (C. urophthalmus)

The increase in stocking density decreased growth in weight and TL of the *C. urophthalmus* larvae from Day 15 post-yolk-sac absorption until the end of the experiment (Fig. 1A, B). Significant differences ($P < 0.05$) were detected for larvae seed at the lowest densities (0.5 and 1 larvae/L) that were higher (0.78 g and 46.0 mm, and 0.76 g and 45.6 mm, respectively) than that seed at the highest densities (5, 10, and 20 larvae/L) (Fig. 1C, D).

The growth rates in the exponential models of the *C. urophthalmus* larvae seeded at different densities (Fig. 2A) showed significant differences ($P < 0.05$) that the lowest densities presented the highest growth rates (0.051 g/d for 0.5 larvae/L, 0.050 g/d for 1 larvae/L, and 0.047 g/d for 5 larvae/L) and the highest densities presented the lowest growth rates (0.017 g/d for 10 larvae/L, and 0.024 g/d for 20 larvae/L). The polynomial regression model showed a high correlation ($R = 0.83$) and significance ($P < 0.05$) between density and total biomass produced that the maximum biomass (175.1 g) was estimated for an optimum stocking density of 12 larvae/L (Fig. 2B). Table 1 shows that there are significant differences ($P < 0.05$) in survival only between the densities of 0.5 and 20 larvae/L (100 and 43%, respectively). No differences were detected among the other densities. On the other hand, the production of juveniles at each density showed significant differences ($P < 0.05$), with the highest densities (10 and 20 larvae/L) obtaining the highest productions (584 and 604 juveniles) in comparison with the other treatments, notwithstanding that the highest statistical differences for the CV were detected for larvae seed at 5 and 10 larvae/L compared with 20 and 1 larvae/L; the lowest CV was calculated for 0.5 larvae/L.

Experiment 2 (P. splendida)

The *P. splendida* larvae presented a differential growth in weight and TL from Day 30 post-yolk-sac absorption that the increase in stocking density decreased growth (Fig. 3A, B). A comparison at the end of the experiment of the weight and TL of the larvae at different stocking densities (Fig. 3C, D) showed significant differences ($P < 0.05$), where the larvae at lower densities (0.5 and 1 larvae/L) were similar (0.81 g and 52.6 mm, and 0.80 g and

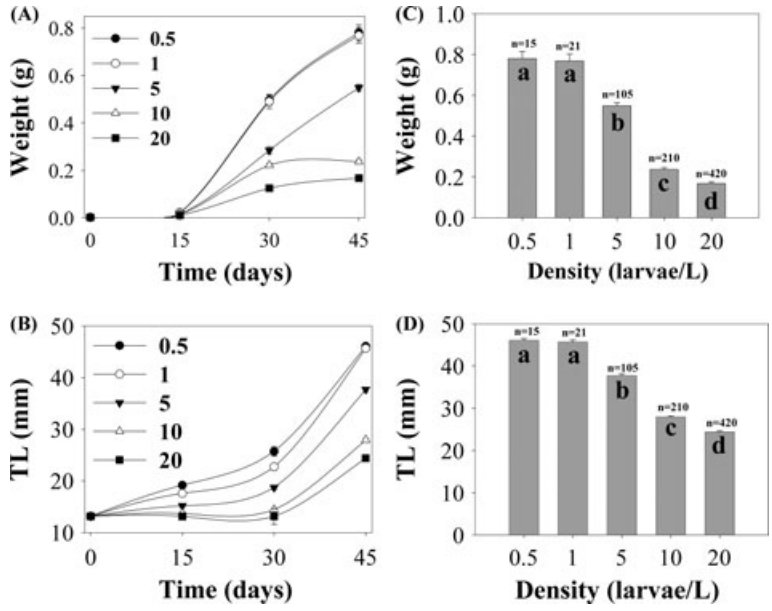

FIGURE 1. *Growth in weight (A) and total length (TL) (B) of* Cichlasoma urophthalmus *larvae during 45 d. Comparison of the weight (C) and TL (D) at the end of the experiment with larvae seed, with five initial stocking densities. (C) and (D) show significant differences between treatments* ($P < 0.05$). *Box and whiskers show the mean and standard deviation, N = number of sampled larvae.*

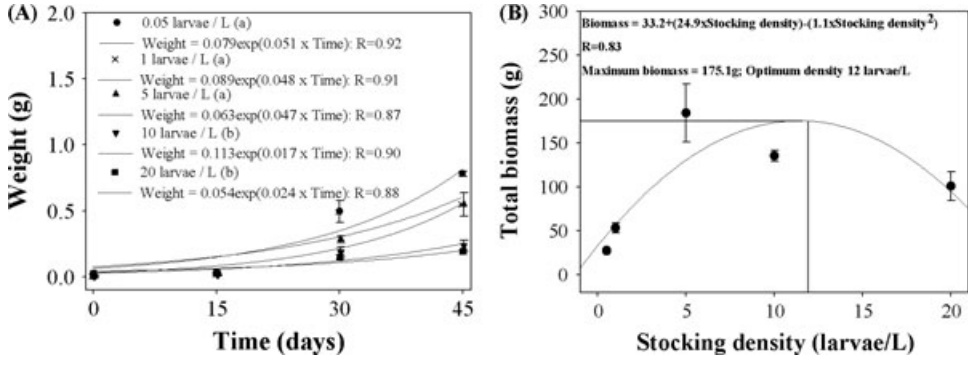

FIGURE 2. *Growth exponential model (A), biomass polynomial regression (B) of* Cichlasoma urophthalmus *larvae. Box and whiskers show the mean and standard deviation.*

50.0 mm, respectively) and higher than those at the densities of 5, 10, and 20 larvae/L (0.56 g and 39.2 mm, 0.24 and 28.2 mm, and 0.19 g and 24.7 mm, respectively), which were also significantly different from one another.

The exponential models of the *P. splendida* larvae presented significant differences ($P < 0.05$) at different densities (Fig. 4A) that the highest growth rates were detected at the lower densities (0.050 g/d for 0.5 larvae/L, 0.048 g/d for 1 larvae/L, and 0.046 g/d for 5 larvae/L) in comparison with the larvae at higher densities that presented lower growth rates (0.018 g/d for 10 larvae/L, and 0.032 g/d for 20 larvae/L). A highly significant correlation ($R = 0.94$, $P < 0.05$) was detected between stocking density and total biomass produced that the maximum biomass estimation (195.29 g)

TABLE 1. *Survival, total fish production, and coefficient of variability of* Cichlasoma urophthalmus *and* Petenia splendida *larvae at different stocking densities (mean ± SD)*[1].

Density (larvae/L)	Survival (%)	Fish produced	Coefficient of variability of weight
C. urophthalmus			
0.5	100 ± 0^{a}	35 ± 0^{d}	2.8^{c}
1	99 ± 1^{ab}	69 ± 1^{c}	10.2^{b}
5	96 ± 2^{ab}	336 ± 7^{b}	16.6^{a}
10	83 ± 2^{ab}	584 ± 106^{a}	17.3^{a}
20	43 ± 8^{b}	604 ± 108^{a}	10.6^{b}
P. splendida			
0.5	99 ± 1^{a}	34 ± 0^{e}	7.9^{b}
1	100 ± 0^{a}	70 ± 0^{d}	9.7^{b}
5	97 ± 1^{a}	341 ± 3^{c}	15.5^{a}
10	98 ± 1^{a}	679 ± 5^{b}	15.5^{a}
20	65 ± 5^{b}	912 ± 70^{a}	4.7^{c}

[1]The mean values in each column followed by different superscript letters differ significantly ($P < 0.05$).

was calculated for an optimal stocking density of 14 larvae/L (Fig. 4B). Table 1 presents the significant differences detected for survival that the *P. splendida* larvae seeded at densities of 0.5, 1, 5, and 10 larvae/L obtained the highest values, and these differed from the larvae seeded at 20 larvae/L which produced the lowest survival. Significant differences among all densities were also obtained in the case of the production of juveniles, with the highest density (20 larvae/L) producing the highest number of fish. Similar to the first experiment, the highest statistical differences for the CV were detected for larvae seed at 5 and 10 larvae/L compared with 0.5 and 1 larvae/L, and the lowest CV was calculated for 20 larvae/L.

Discussion

This study shows that the increase in stocking density decreased the growth for *C. urophthlamus* and *P. splendida* larvae. The growth and survival recorded here for the larvae of these species are similar to those obtained by Melika et al. (1997) for Macquarie perch

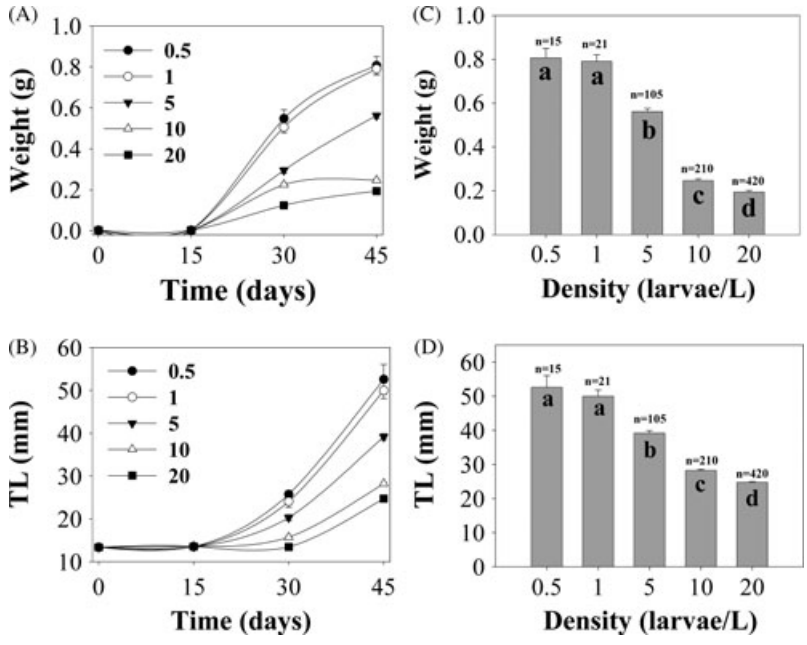

FIGURE 3. *Growth in weight (A) and total length (TL) (B) of* Petenia splendida *larvae during 45 d. Comparison of the weight (C) and TL (D) at the end of the experiment with larvae seed, with five initial stocking densities. (C) and (D) show significant differences between treatments ($P < 0.05$). Box and whiskers show the mean and standard deviation, N = number of sampled larvae.*

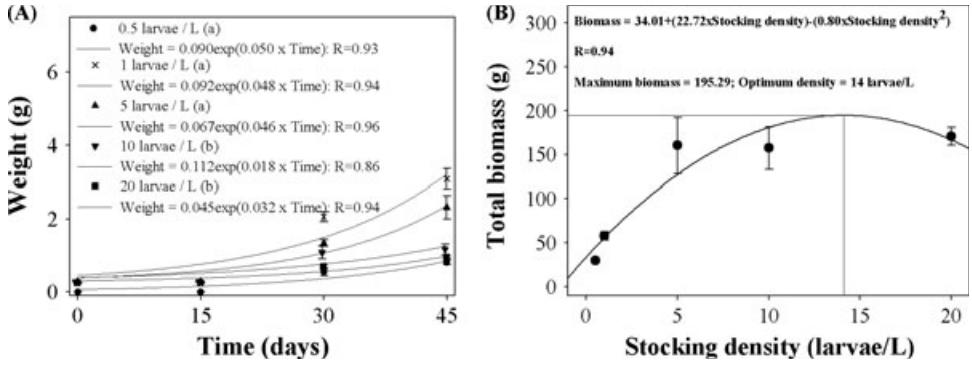

FIGURE 4. *Growth exponential model (A), biomass polynomial regression (B) of* Petenia splendida *larvae. Box and whiskers show the mean and standard deviation.*

larvae, *Macquaria australasica* (Cuvier, 1830), for which an optimum stocking density of 8 larvae/L, a weight of 9.30 mg, an average length of 9.75 mm, and a survival of 78% were recorded. El-Sayed (2002) experimented with larvae of tilapia, *Oreochromis niloticus*, at stocking densities of 3, 5, 10, 15, and 20 larvae/L for 30 d in a closed recirculation system. Results showed that the highest weight (0.89 g) was recorded for larvae seeded at a density of 3 larvae/L. The author concluded that the optimum density for this species was around 5 larvae/L, and pointed out that density has a limiting effect on survival, and that growth decreased when density increased.

The choice of an optimum stocking density depends on various factors such as the species, the type of culture system, and the number of fish that one wants to produce. With respect to the species, this depends heavily on the feeding habits (carnivores, herbivores, omnivores, or detritivores) that characteristically vary throughout the life cycle, particularly during the larval stage when most fish larvae are carnivorous, and during the juvenile stage when feeding habits may change (Balon 1984). Thus, most freshwater larvae eat small planktonic organisms such as copepods, cladocerans, aquatic larvae, and rotifers. (Hepher 1993). Also present during the larval stage of most fish species is cannibalism, that is defined as a special type of predation (intraspecific) that consists in partly or totally eating an individual of the same species (co-specific), independently of the developmental stage (Kestemont et al. 2003). The main reason for this is associated with the availability of food that, in the case of wild larvae, becomes a survival strategy (Atencio-García and Zaniboni-Filho 2006). Cannibalism was not observed during our experiments, and this may be because of the feed being provided in adequate amounts to all stocking densities, apart from the fact that *C. urophthalmus* is an omnivorous species (Anonymous 1983). This is confirmed as cannibalism would have been observed in the experiment had the amount of food not been adequate, particularly in the case of *P. splendida*, a species that is carnivorous and fish-eating after its larval stage (Reséndez and Salvadores 1983). In spite of this, an increase in the aggressive behavior from Day 16 onward was observed when the stocking density increased for both species previous to food supply. The increase in the aggressive behavior could be related to the increase for the food competition and the size differences observed for the highest densities when the coefficient of variability for weight was compared.

From a behavioral point of view, cichlids are very territorial and aggressive fish. Considering this, the above-stated behavior is not related to the environmental conditions or the design of the system, but to the fact that at a certain size, the fish mature and seek territory for

reproduction, whereas during the larval stage the fish use their energy to complete morphological changes and are thus less aggressive. Similarly, high densities may increase tension and generate aggressive interactions among individuals (Greaves and Tuene 2001). The lower survival recorded at the high densities may have resulted from a possible interaction of two factors, the increase in pressure from intraspecific competition for space, and the change in behavior that occurs when larvae become juveniles. This causes the fittest individuals to feed more efficiently and develop faster than the others. Other aspects to consider include that when a difference in size goes hand in hand with an increase in density, there is more competition for food and a higher expenditure of energy, and hierarchies are established among individuals with the bigger ones eating first and preventing the lower ones from eating. This can be observed in the growth and survival and through an increase in dispersion (Teng et al. 1981; Kuronuma and Fukusho 1984; Alvarez-González et al. 2001). The mortality observed at the high densities may be associated with this effect, as has been recorded for other species such as *Dicentrarchus labrax* (L), *Gadus morhua*, *Sander lucioperca*, *Perca fluviatilis*, and *Cyprinus carpio* (Coves et al. 1991; Baskerville and Kling 2000; Szkudlarek and Zakes 2002; Baras et al. 2003; Ruane and Komen 2003).

Another aspect to consider is that the recirculation system worked appropriately during both experiments, as was evidenced by the water quality parameters. An adequate system plays an important part in larviculture and controls all exogenous factors such as temperature, dissolved oxygen, ammonium, etc., particularly under intensive production (Blancheton 2000). An excellent handling and a good system design are also necessary in order to increase the efficiency of any closed recirculation system, where efficiency in the rearing of fish larvae is improved by the addition of sophisticated technological elements such as sand filters, biological filters, ultraviolet light, active carbon filters, oxygen injectors, and sensors for temperature control with coolers, titanium heaters, etc. (Timmons et al. 2002). Consequently, a better control of the environmental conditions, a higher knowledge of the species under culture, and an adequate aquacultural management (stocking density, among others), may lead to a maximization of fish production (Schipp and Gore 2007). The choice of an optimum stocking density depends on technical and biological aspects, as well as on economical considerations, with a view to obtain a maximum number of fish. Although it has been proved that low densities result in higher growth and survival, from the productive point of view, this may not be the best scenario if the amount of fish produced is not profitable. For this reason, a cost–benefit analysis that allows one to obtain the maximum yield possible for a culture system should be included, especially if one has invested in the construction of a recirculation system that is highly technical for the control of water quality (Maciej and Zdzislaw 2007). Thus, growth and survival at higher densities will also depend on the time required to recover the investment made. In this sense, if we want to calculate the optimum stocking density during larviculture, the use of an appropriate decision tool such as the polynomial model estimation could be useful; thus, the optimum stocking density for *C. urophthalmus* and *P. splendida* (12 and 14 larvae/L, respectively) are similar that use for other species as previously mentioned, but calculated using the total biomass production that allows to relate the growth and fish production for each density tested.

It is concluded that the optimum initial stocking densities for *C. urophthalmus* and *P. splendida* larvae in our recirculation systems are 12 and 14 larvae/L, respectively, because high biomass could be produced in spite of lower growth. However, if we want to increase the initial stocking density, the improvement of the filtration system will allow to increase the number of larvae per liter in the same system used in these experiments.

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