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Efficiency of co-feeding red porgy (*Pagrus pagrus* L.) larvae with live and compound diet

Eficiencia de la alimentación mixta con dieta viva y alimento balanceado en larvas de besugo (*Pagrus pagrus* L.)

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Resumen.- El besugo (*Pagrus pagrus*) es una importante especie comercial y deportiva con buenas perspectivas para acuicultura. Debido a que importantes mortalidades han dificultado la producción masiva de esta especie, en este trabajo se describen tres ensayos de destete para investigar sus efectos sobre la supervivencia y el crecimiento de las larvas de besugo nacidas en el laboratorio: A) destete tradicional de tres pasos (rotíferos - *Artemia* - dieta) (control); B) destete temprano con alimentación mixta en un paso (rotíferos - *Artemia* + dieta) y C) destete temprano directo de un paso (rotíferos - dieta). Se registraron diferencias significativas entre los tres tratamientos (P<0,05). Las mejores tasas de supervivencia (45,6 ± 7,6%) y crecimiento larval (7,4 ± 0,5 mm) se encontraron en el tratamiento B. Los resultados indican que la alimentación simultánea de las larvas de besugo con dieta viva e inerte a partir del día 20 después de la eclosión es una prometedora estrategia de destete para la cría de esta especie.

Palabras clave: Crecimiento, desarrollo larval, destete

Abstract.- The red porgy (*Pagrus pagrus*) is an important commercial and recreational species with very good prospect for aquaculture. Because significant mortalities, the mass production of this species is limited. This paper describes three weaning strategies to investigate their effects on the survival and growth of red porgy larvae reared in laboratory: A) traditional three-step weaning (rotifers - *Artemia* - diet) (control); B) one-step early weaning by co-feeding (rotifers - *Artemia* + diet), and C) one-step direct early weaning (rotifers - diet). Sigificative differences were recorded among treatments (P<0.05). Best survival rate (45.6 ± 7.6%) and growth (7.4 ± 0.5 mm) were found in treatment B. Results indicate that co-feeding red porgy larvae with inert and live diets from day 20 after hatching is a promising weaning strategy in the hatchery of this species.

Key words: Growth, larval development, weaning

Introduction

The red porgy (*Pagrus pagrus*) is an important commercial and recreational species found in the Argentinean waters (Cotrina & Cousseau 1977, Cotrina 1989), with very good prospect for aquaculture due to its suitability for reproduction in captivity (Mylonas *et al*. 2003, Aristizábal in press). For this reason, the red porgy is intensively studied as a potential aquaculture candidate at the National Fisheries Research and Development Institute (INIDEP) since 2000, with the support of the Overseas Fisheries Cooperation Foundation (OFCF) of Japan.

Red porgy larvae, like other marine fish larvae, are characterized by small size and early stage of development at hatching, being very vulnerable to the rearing conditions and demanding live prey during that period (Sargent *et al*. 1997). Any kind of diet or rearing method that may reduce the production of live feed will be of great technical and economical interest. Fernández-Díaz *et al*. (1994) found that gilthead seabream (*Sparus aurata*) larvae previously fed live feeds would preferentially select live feed during co-feeding with inert feed. Co-feeding may improve larval nutrition and may condition the larvae to more readily accept the manufactured diet when live feeds are withdrawn (Munilla-Moran *et al*. 1990, Person le Ruyet

et al. 1993, Rosenlund *et al*. 1997, Cañavate & Fernández-Díaz 1999). The quality and quantity of both live and manufactured diets are also of considerable importance for growth, survival and success during weaning (Bromley & Howell 1983, Person le Ruyet *et al*. 1993).

Red porgy larvae are normally fed *Artemia* nauplii from day 20-22 after hatching (AH), rotifers density gradually decreases from that time on (Stephanou *et al*. 1995, Roo *et al*. 1999, Mihelakakis *et al*. 2001), while weaning usually starts after day 30 AH (Mihelakakis *et al*. 2001). Because significant problems have prevented the mass production of this species (Conides & Glamuzina 2001, Hernández-Cruz *et al*. 1999), we considered the possibility of an early weaning based on both a good osteological-cartilaginous development of the mouth of red porgy larvae by day 20 AH (Aristizábal 2003), and also the presence of the necessary enzymes for digestion in related species, like the red seabream *Pagrus major* (Kanazawa *et al*. 1989) and the gilthead seabream *Sparus aurata* (Kolkovski 2001), at early stages of development. This report details a successful attempt to increase survival rate at larval rearing of *P. pagrus*.

Materials and methods

Larvae supply

Red porgy (*P. pagrus*) eggs were collected from spontaneous spawnings from a broodstock kept under a recirculation system at the National Fisheries Research and Development Institute (INIDEP), Argentina. Eggs were incubated at 19.5 ± 0.5ºC in 200-L cylindrical-shaped nets inside a 2000-L recirculated tank with gentle aeration. Newly hatched larvae were used to seed nine 0.4 m^3 circular fiberglass tanks at a density of 30-40 larvae/L^{-1}.

Table 1

Description of the feeding regimes during the larval rearing of *P. pagrus*

Descripción del régimen alimenticio durante la cría larval de *P. pagrus*

Feeding regime (days)	Algae (x10^6 cells mL^{-1})	Rotifers (ind mL^{-1})	Artemia (nauplii mL^{-1})	Dry feed (mg mL^{-1})
A- Traditional (control)				
3-22	0.3	10-20	-	-
20-25	-	-	1-3	-
26-40	-	-	5-10	-
35-50	-	-	-	9
B- One-step early				
3-22	0.3	10-20	-	-
20-25	-	-	1-10	4
26-40	-	-	-	10
35-50	-	-	-	20
C- One-step direct				
3-22	0.3	10-20	-	-
20-25	-	-	-	6
26-40	-	-	-	-
35-50	-	-	-	-

Experimental conditions

The rearing tanks were kept under a flow-through system with an increasing daily water exchange from 10% at hatching (day 0) to 500% at day 50 AH, accordingly to the age of the larvae. During the period of experimentation, the water temperature was kept at 19.5 ± 0.5ºC and the salinity ranged from 33 to 34 psu. A skimmer was used to remove surface oil film and other residuals. Deposits from the bottom of the tanks were carefully removed daily from day 15 AH. Light intensity and photoperiod were 200 lx at the water surface (Digital light meter, Cole-Parmer) and 10 D : 14 L, respectively.

In all treatments, green water containing *Nannochloropsis oculata* was supplied to the rearing tanks every morning to achieve an approximate concentration of 1-3 x 10^6 cells/mL. Three experimental feeding designs by triplicate were used (Table 1): A) Traditional three-step weaning (rotifers - *Artemia* - diet) (control); B) one-step early weaning by co-feeding (rotifers - *Artemia* + diet), and C) one-step direct early weaning (rotifers - diet). The larvae were initially fed with enriched rotifers (*Brachionus* sp.) from day 3 AH. Rotifer density was maintained at 10-20/mL. Red porgy were fed enriched *Artemia* nauplii twice a day from day 20 AH at increasing densities from 1 to 10 nauplii/mL. From day 20 AH, no new rotifers were added to the rearing tank but their presence was detected until day 23 AH. A commercial artificial feed (Otohime B1, B2, C1 and C2, Nisshin Inc., Japan) was used from day 35 AH in trial A, and from day 20 AH in trials B and C. Rotifers and *Artemia* nauplii were enriched with a commercial enrichment media (DHA-Ce, Oriental Ind., Japan) plus *N. oculata*, during 6-14 h.

Larval sampling was performed by dipping the water column at different zones and individually counting the live larvae. Morphological observations and body measurements were carried out in 50 specimens each day at the yolk-sac stage, and every week during the larval and fingerling stage. Larvae were anesthetized by adding a couple of drops of a solution of benzocaine : acetone (2:1 w/v) into a 50 mL beaker.

Data and statistical analysis

Data are presented as means ± SD. Means from the different treatments were compared by One-way ANOVA and factorial analysis (Sokal & Rohlf 1981). When significant differences were detected, Tukey multiple-range procedure was used to determine statistical differences among treatments. A probability level of $P<0.05$ was used to judge whether any effects were significant.

Results

The hatching period at a water temperature of 19.5 ± 0.5ºC lasted 48 h. Total length of newly hatched larvae (day 0) was 2.32 ± 0.12 mm. Hatching rate was 88%. Larvae opened the mouth and anus on day 3 AH and began to feed on day 4 after the absorption of the yolk sac. Larvae from all treatments commenced tail flexion by day 16 AH. No lordosis was detected in juveniles by day 50 AH. On day 20 AH, all individuals had a functional swim bladder. By day 23 AH, no rotifers were detected in the rearing tanks.

Table 2

Survival and total length of 24 and 50-days old larvae and fries reared by three different weaning methods. Different letters within a column indicate significant differences between treatments ($P<0.05$)

Supervivencia y largo total de larvas y alevines de 24 y 50 días sometidos a tres estrategias de destete. Las diferentes letras dentro de una misma columna indican diferencias significativas entre tratamientos ($P<0,05$)

Weaning method	Survival (%) mean ± sd		Total length (mm) mean ± sd	
	24-day old	50-day old	24-day old	50-day old
A	9.8 ± 3.3^a	3.2 ± 2.4^a	6.7 ± 0.6^a	50.8 ± 6.9^a
B	45.6 ± 3.6^b	41.8 ± 4.3^b	7.4 ± 0.4^b	56.3 ± 5.1^b
C	16.5 ± 4.2^c	-	5.1 ± 1.1^c	-

Best survival rate and growth were found in treatment B (Table 2). The larvae fed *Artemia* nauplii and inert diet showed a significantly larger total length (7.4 ± 0.5 mm) and better survival rate (45.6 ± 7.6%), when compared with the other treatments. Individuals from treatment C, massively died between days 25-30 AH. No food items were detected in the gut of these larvae.

Discussion

This study demonstrated that the one-step early weaning by co-feeding (treatment B) resulted in a increased survival and growth rate in red porgy larvae. Growth and survival was lower in larvae fed an inert diet alone compared to those fed *Artemia* or a combination of *Artemia* and inert diet. Similar results were obtained by Lazo (1999) in red drum larvae (*Sciaenops oscellatus*).

Stephanou *et al*. (1995) and Mihelakakis *et al*. (2001), reported the starting of the weaning phase of red porgy by day 32-33 AH, but no data about survival rate were presented. Kentouri *et al*. (1995) utilized the traditional three-step larval rearing, starting the weaning phase of red porgy larvae approximately by day 26 AH, with a low final survival rate of 6% (Table 3).

Weaning conditions clearly influenced the growth and survival of red porgy. Assimilation of ingested rotifers, or other live feeds, by fish larvae can occur rapidly (Lubzens *et al*. 1989). Consequently, larvae from all treatments faced similar feeding conditions during the first 20 days of development. The faster larval growth in treatment B showed low individual size variation and high survival rate. High size variation usually occurred in the larvae that apparently experienced adverse growth (Dou *et al*. 2003).

Traditionally, it has been assumed that the inability of marine fish larvae to grow well with inert diets was a consequence of their low enzymatic activity (Lazo 2000). It has also been suggested that the real cause of the poor growth and low survival of marine fish larvae was directly related to nutritionally unbalanced inert diets (Cahu & Zambonino-Infante 1997). Although inert diets provide sufficient nutrient in a digestible form, it is more likely that these diets failed to stimulate ingestion (Allen Davis & Dinis 2002). This may be an explanation of the lack of food items in the gut of red porgy larvae in treatment C. By co-feeding red porgy larvae with *Artemia* nauplii and inert diet (treatment B), survival increased significantly and positively correlated with the increase of growth rate. The ingestion of *Artemia* nauplii may stimulate the ingestion of inert diet and its digestion. *Artemia* is a good carrier of HUFA as it incorporates them to its body, but it is poor in other nutrients, such as proteins. Consequently, the traditional three-step larval rearing (treatment A) may restrict the incorporation of some essential elements reducing growth rate. Results indicate that co-feeding red porgy larvae with inert and live diets by day 20 is a promising weaning strategy in the hatchery of this species. Adding of inert diet to the feeding strategy in treatment B may supply the necessary elements to promote a good larval development and survival. Further studies will be needed to improve the transferring of these nutrients from live food to red porgy larvae during the hatchery.

Table 3

Survival rates (%) of red porgy larvae reported by different authors. DAH = days after hatching

Tasas de supervivencia (%) en larvas de besugo señaladas por varios autores. DAH = días después de la eclosión

Survival rate (%)	DAH	Culture density	References
6.7	8	-	Conides & Glamuzina (2001)
17.7	17	100-150 eggs/L	Hernandez-Cruz *et al*. (1999)
6.0	50	50-70 larvae/L	Kentouri *et al*. (1995)
15.0	50	1 larva/L	Kentouri *et al*. (1995)

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