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Research Note

Generation cycle closure of the spotted rose snapper, *Lutjanus guttatus*, in captivity

Cierre del ciclo generacional del pargo flamenco, *Lutjanus guttatus*, en cautiverio

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Abstract.- The spotted rose snapper, *Lutjanus guttatus*, is culture in several Latin American countries. The present study reports the generation closure with juveniles obtained from wild adults which matured and spawned at a Mazatlan laboratory (Mexico). F1 fish were sexually matured at 2 years of age, and spawning was induced in 2 successive years. F2 juveniles were reared until their sexual maturity and spawned at the same laboratory. Result indicate that a reliable supply of laboratory reared spawners can be achieved, avoiding the wild spawners dependence and allow the development of selection and genetic improve programs.

Key words: Lutjanidae, F1 broodstock maturation and spawning, generation closure

Introduction

The spotted rose snapper *Lutjanus guttatus* (Steindachner, 1869), is an important food and recreational species with high market prices of US$ 5-8 kg^{-1} and strong demand in America (Avilés-Quevedo *et al.* 2008, Boza-Barca *et al.* 2008). It is a batch spawner with asynchronous ovarian development and a long reproductive season (Cruz-Romero *et al.* 1996). During the last two decades, culture trials of the spotted rose snapper have been started in several Latin American countries along the Pacific Ocean (Avilés-Quevedo & Mazón-Suástegui 1996, Avilés-Quevedo *et al.* 2008, Herrera-Ulloa *et al.* 2010). Their culture, however, is still mostly dependent on the collection of wild juveniles of various sizes (50-200 g), as has been reported in Mexico (Avilés-Quevedo *et al.* 2008, Alvarez-Lajonchère & Puello-Cruz 2011), although artificially produced juveniles are reared in Costa Rica (Herrera-Ulloa *et al.* 2010). With limited, unpredictable and unstable supplies, this practice, especially with larger juveniles, can affect fishery recruitment from natural populations and result in conflicts between fishermen and aquaculturists.

Research projects on artificial reproduction techniques have been carried out in several countries mostly based on collecting mature wild fish (Ibarra-Castro & Duncan 2007, Boza-Abarca *et al.* 2008, Ibarra-Castro & Alvarez-Lajonchère 2009). Recently spawning has been achieved with wild fish acclimatized and matured in captivity (Cano 2003, Boza-Abarca *et al.* 2008, Herrera-Ulloa *et al.* 2010, Ibarra-Castro & Alvarez-Lajonchère 2011). However, in order to make aquaculture a sustainable activity, seed stock must be obtained from captive broodstock, as is the case with several other marine fish species (Moretti *et al.* 1999, Schipp *et al.* 2007). The overall goal is to control the entire life cycle of a given species from eggs to adults which can then mature and spawn in captivity in order to reproduce successive generations (Liao 1977). Controlling the life cycle is the only way of carrying out selection and genetic improvement programs, which would lead to a notable increase in aquaculture production, as found in agriculture and terrestrial livestock. The life cycle of the spotted rose snapper was closed in Panama (Cano 2003), although details of this study are limited.

This paper presents the results of the generation cycle closure of the spotted rose snapper, at the pilot-scale hatchery of the Research Centre for Food and Development (CIAD), at Mazatlan (Mexico), with data on their first sexual maturation and induced spawning results for 2 consecutive years.

Materials and methods

Establishment and spawning of F1 broodstock

The establishment and spawning of a broodstock with wild adult spotted rose snappers and their successful spawning was described by Ibarra-Castro & Alvarez-Lajonchère (2011). Briefly, a group of wild adults were caught at Sayulita Beach, Nayarit (Mexico), during May and June 2004 and transported to a pilot-scale hatchery at Mazatlan. Fish were reared in 18-m^3 fiberglass tanks supplied with seawater flow-through of 6 volumes day^{-1} and strong aeration. Fish were fed a mixture of oily fish flesh, squid and shrimp in equal proportions to satiation once a day for 6 days per week. In June 2005 the first wild broodstock fish reaching maturity in captivity with 0.80 ± 0.13 kg body weight (BW, mean ± SD), were induced with 204 ± 11 µg kg^{-1} GnRHa ([D-Ala6 Pro9 NEt]-GnRH) EVAc implants. After the hormone treatments were applied, each female with 2 males were placed in 3-m^3 spawning tanks with overflow egg collector baskets with 500-µm mesh. The collectors were checked for the presence of eggs with a flashlight every hour, starting at 1800 h.

Two natural spawns with high quality eggs (100% live embryos, 12 h after fertilization) were obtained. Spawned eggs of wild captive broodstock used to obtain F1 juveniles had 90% viability (defined as percentage floating eggs with live embryos at 12-14 h after spawning) and a hatching rate of 82-93%. Mean ± SD egg and oil droplet diameters were 750 ± 5 µm and 122 ± 2 µm, respectively. The incubation period at 35-36 and 29 - 31°C ranged from 18-20 h. Newly hatched larvae had a total length (TL) of 2.2 ± 0.2 mm. Incubation and larval rearing was carried out in 600-L cylindrical tanks (initial stocked at 20 viable eggs L^{-1}) from which several thousand juveniles were grown after 45-day experimental larviculture (Abdo de la Parra *et al.* 2010).

Nursery rearing of F1 hatchery produced juveniles was carried out in cylindrical fiberglass 5 m^3 tanks, with a seawater flow of 4 volumes day^{-1} and strong aeration. Lansy 1.2 mm (INVE Aquaculture, Mexico) artificial feed was supplied by hand *ad libitum*, four times day^{-1} for 15 days. Subsequently, a formulated pelleted shrimp diet (Alimentos de Occidente, S.A. de C.V., Mazatlan, Mexico) (40% protein, 10% lipid) was supplied with two 12-h automatic feeders per tank. During the 6 months nursery stage, water temperature, salinity and dissolved oxygen varied between 22 and 29ºC, 32 to 35, and 5.0 and 6.0 mg L^{-1}, respectively.

From January to September 2006, a group of 400 juveniles (30-40 g BW each) were selected for the establishment of a broodstock and stocked in four 7-m^3 fiberglass tanks (0.5 kg m^{-3}) with a sea water flow rate of 4 volumes day^{-1} and strong aeration. Fish were fed to satiation once daily, 6 days per week, with a diet of fresh squid and oily fish (mostly skipjack) in equal proportions. At the end of September 2006, 78 fish were selected from the F1 juvenile group, and stocked in equal numbers into two 7-m^3 fiberglass tanks, with a water flow to 6 volumes day^{-1} using the same feeding practices described above. Juveniles were sampled several times during the nursery and grow-out period. In every sampling, 50 fish from each tank were anesthetized with 300 ppm of 2-phenoxiethanol (Sigma, Toluca, México) for morphological observations and determinations of TL to the nearest mm, and body BW to the nearest g.

F1 induced spawning experiments

On July 31, 2007 the TL and BW of F1 fish were determined, as well as their sexual development for their first induced spawning trial. Female gonad stage was assessed by a biopsy technique adapted to the rose spotted snapper, briefly described by Ibarra-Castro & Alvarez-Lajonchère (2011). Sexual maturity of males was based on the presence and fluidity of milt determined by light abdominal pressure, and examination of sperm motility. F1 females with a mean oocyte diameter of at least 350 µm and males with ≥ 80% motile spermatozoa were selected for the induced spawning experiments.

Selected fish were anesthetized with 300 ppm 2-phenoxiethanol and tagged with 1.2 cm Passive Integrated Transponder (PIT) tags (Biomark, Idaho, USA) implanted into the dorsal muscle, for individual identification. Afterwards, selected females and males were implanted with GnRHa ([D-Ala6 Pro9 NEt]-GnRH) EVAc implant (Zohar & Mylonas 2001), between 1000 and 1100 h. Each female was treated individually, according to its stage of sexual development (oocyte mean diameter) and BW. All males received an implant dose of approximately 75 µg kg^{-1}. The effective doses of GnRHa were estimated based on an induced spawning protocol developed for captive fish of this species (Ibarra-Castro & Alvarez-Lajonchère 2009). After spawning, the fish returned to their 7-m^3 fiberglass tanks and left undisturbed for one year under the same rearing conditions described above.

In June 2008 the F1 broodstock fish were again sampled, for TL and BW determinations, as well as their

sexual development, and 3 females and 6 males were selected for a second induce spawning trial.

Statistical analysis

Results are expressed as means ± standard error of the mean (SEM), unless otherwise indicated. All percentage data were normalized by arcsin square transformation prior to statistical analyses. Homogeneity of variances and testing for normality were conducted. Parametric data were subjected to one-way analysis of variance (ANOVA) and when significant, a multiple comparison test (Tukey) was applied. Non parametric data were analyzed using a Kruskal-Wallis ANOVA test, and when significant, the multiple comparisons Dunn's test was performed. Differences were considered significant at $P < 0.05$ unless otherwise indicated. The data were analyzed using SigmaStat 3.1 (SYSTAT Software, Inc., Point Richmond, California, USA) statistical software package.

Results and discussion

F1 juveniles 45 days post-hatch (dph) produced in 600-L larval rearing tanks had a TL of 4.5 ± 0.3 cm and a BW of 0.5 ± 0.3 g. They grew to 30.5 cm TL ($y = 9.9131\ e^{0.0015x}$, $R^2 = 0.957$) and 583 g BW ($y = 12.183e^{0.0052x}$, $R^2 = 0.959$) in 25 months (Fig. 1), in 5-m^3 and later 7-m^3 tanks, when the sexual development was considered adequate for induced spawning.

The laboratory tank growth of artificially produced juveniles was slow compared to the growth of commercially reared wild juveniles in floating cages (Avilés-Quevedo *et al.* 2008) as well as significantly slower ($P < 0.05$) than siblings of this progeny reared in floating cages with similar temperature and salinity conditions (Abdo de la Parra *et al.* 2011). The water quality and flow rate, in addition to the vital space effect of being reared in small tanks could have been the cause of slower growth in the laboratory. In Costa Rica, growth of wild spotted rose snapper has been reported faster than in Mexico, and sexual maturity is attained at a similar size, but at 2 years of age (Rojas 1997) as in the captive F1 fish at the present study, instead of the 4 years required for fish in Mexico (Amezcua *et al.* 2006). Also, captive fish growth is faster in Costa Rica (Boza-Abarca *et al.* 2008) compared to those reared in Mexico (Avilés-Quevedo *et al.* 2008). This suggests that there may be growth differences between the populations, as in the case of other the striped mullet, *Mugil cephalus* (Tamaru *et al.* 1993).

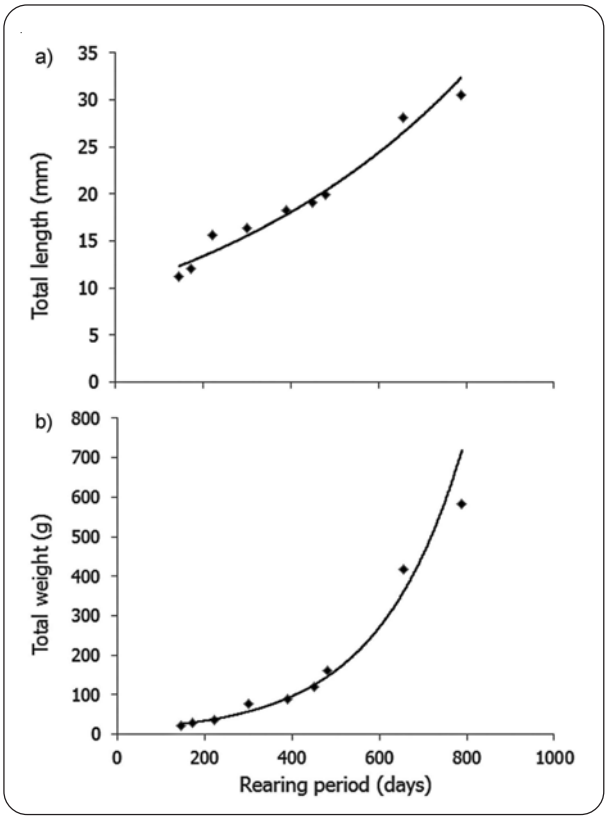

Figure 1. Growth of F1 juveniles before induced spawning experiments: a) total length; b) total weight / Crecimiento de los juveniles F1 antes de los experimentos de inducción del desove: a) largo total; b) peso total

On July 31 2007, 5 females (BW = 687 ± 74 g; TL = 32.0 ± 1.6 cm) with a mean oocyte diameter of 360 ± 19 µm were induced with a mean GnRHa EVAc implant dose of 239 ± 20 µg kg^{-1}. All males had sperm with > 80% motility, and 10 were selected (BW = 553 ± 82 g; TL = 30.0 ± 1.6 cm) and implanted with a mean dose of 72 ± 26 µg kg^{-1} GnRHa. During the second night, 2 females spawned (36 and 43 h after hormone-treatment), but few floating eggs were collected (90% viable eggs) (Table 1). Mean egg and lipid droplet diameters were 739 ± 17 µm and 117 ± 12 µm, respectively. These eggs had 66% hatching (Table 1).

The next year in June 2008, 3 females (BW = 687 ± 9 g; TL = 32.0 ± 0.2 cm) and six males (BW = 745 ± 121 g; TL = 35.7 ± 2.8 cm) were selected for a second induced spawning trial. Females had a mean oocyte diameter of 410 ± 20 µm and received a GnRHa dose of 188 ± 55 µg kg^{-1} while males received a mean implant dose of 70 ± 12 µg kg^{-1}. Two of the three females had multiple spawns (Table 1). One fish initially released a few eggs with a low percent

Table 1. Induced spawning results of F1 broodstock in July 2007 and June 2008 / Resultados de la inducción del desove de los reproductores F1 en julio de 2007 y junio de 2008

Date	Female	TL (cm)	BW (g)	Initial oocyte diameter (µm)	Dose (µg kg^{-1})	Latency (h)	Total fecundity	Floating eggs	Viability (%) / Hatching (%)	Mean diameter ± SEM[a] (µm)	
										Egg	Oil droplet
July 31, 2007	1	30.5	628	388 ± 22	239	36	81,851	2,640	90 / 66	751 ± 17	125 ± 11
July 31, 2007	2	30.5	642	347 ± 17	234	--	--	--	0	--	--
July 31, 2007	3	34.8	830	355 ± 21	271	43	55,582	--	0	727 ± 18	108 ± 12
July 31, 2007	4	31.5	646	341 ± 17	232	--	--	--	0	--	--
July 31, 2007	5	32.5	688	371 ± 18	218	--	--	--	0	--	--
June 28, 2008	1	36.5	876	325 ± 21	228	--	--	--			--
June 28, 2008	2	45.1	1308	400 ± 22	156	63	20,300	3,400	0	711 ± 5	93 ± 3
June 29, 2008						--	88,000	14,700	0	735 ± 3	87 ± 4
June 27, 2008	3	47.2	1496	400 ± 20	181	39	80,000	6,000	1 / --	717 ± 4	122 ± 3
June 28, 2008						--	296,000	148,000	89 / 75	734 ± 5	91 ± 2
June 29, 2008						--	43,000	36,000	90 / 86	765 ± 4	125 ± 1
June 30, 2008						--	127,000	125,000	70 / 71	759 ± 4	93 ± 2
July 1, 2008						--	353,000	353,000	98 / 90	682 ± 8	125 ± 1
July 2, 2008						--	202,000	202,000	97 / 86	726 ± 5	125 ± 4

[a] = Standard error of the mean

viability 39 h after the hormone treatment and then continued for another 5 consecutive nights, spawning a mean of 172,800 ± 121 eggs with high viability of 81.6 ± 8.4%. The other female spawned during the third and fourth night after the hormone treatment and none of the eggs were fertilized. Diameter of egg and high viability percentage were 725 ± 5 µm and 80.4 ± 10% of buoyant eggs (Table 1). F2 juveniles from these spawns were reared in the same hatchery to maturity with the same methods described above, and were spawned with 2 years of age, following the same procedures (Ibarra-Castro *et al*. unpublished).

Although the spawning results from first sexual maturation of the hatchery-born broodstock showed poor results, the larvae produced a progeny that was able to grow and mature in captivity. The results of the second spawning experiment with three-year adults were significantly more productive than in the first experiment ($P < 0.05$), as found in other species (Carrillo *et al.* 2000) and were not significantly different ($P > 0.05$) from those previously reported for captive wild broodstock spotted rose snapper in terms of fecundity, viability, and egg and lipid diameter characteristics (Ibarra-Castro & Alvarez-Lajonchère 2011). Survival rate achieved was satisfactory, compared to other marine fish hatcheries (Moretti *et al.* 1999) agreeing with other reports (Schipp *et al.* 2007).

Results of the present study indicate that broodstock should initially be reared from hatchery produced juveniles to adult size in floating cages, for maximizing growth rates.

The successful sexual maturation and spawning of the spotted rose snapper F1 fish indicates that controlled reproduction of the complete generation cycle in captivity can be achieved. These procedures could be used with broodstock fish for mass production of juveniles, allowing the extension of culture technology to a commercial scale without depending on wild fish.

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