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A new efficient method for the mass production of juvenile spotted rose snapper *Lutjanus guttatus*

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ABSTRACT

The present technical note reports the new method for the mass production of spotted rose snapper *Lutjanus guttatus* juveniles. The source of eggs came from a total of 387 spawns obtained in 2013 and 2014. 85 % of the spawns had floating eggs, and 93.5 ± 2.8 % of them had live embryos. The initial stocking density of eggs in the rearing tanks was 165 ± 69 embryos/l. The hatching rate was 88 ± 4%, with 50 ± 8% larval survival at 48 h post-hatch (hph). After 35 days post-hatch (dph), a total of 706,799 pre-juveniles (0.1 ± 0.05 g of body weight) were harvested, obtaining from first feeding 31 ± 20 % larval survival. The larvae grew from an average of 2.55 mm–23.58 mm in five weeks. From nursery I, a total of 664,301 juveniles with 0.6 g to 0.8 g body weight (BW) were harvested (94 % survival) and transferred to nursery II, growing on average from 0.48 g to 14.78 g BW in seven weeks. Finally, from nursery II a total of 596,382 juveniles (5–15 g BW) were harvested at 90 dph (89.8 % survival) and delivered to farmers for grow-out. The new method was integrated with an older rearing protocol, and a pre-commercial number of juveniles was produced with high efficiency, requiring only four 48 hph larvae to produce every 90 dph juvenile. These results provided an improvement in the state-of-the-art of snapper aquaculture and it can be applied at a commercial scale.

1. Introduction

Fishin the snapper family are highly valued fish for human consumption and the market demand around the world is unsatisfied. However, for the last two decades, wild snapper fisheries are considered to be overexploited in many areas and their aquaculture production is very limited (Davis et al., 2000; Amorim et al., 2019; FAO, 2020). For most facilities that raise snappers, the supply of juveniles to farms has depended primarily on the unpredictable capture of juveniles in coastal waters (Davis et al., 2000; Surtida and Buendia, 2002), keeps them from being sustainable aquaculture.

Conversely, protocols for the production of juveniles of several snapper species are available, but, the only large-scale source of artificially produced juveniles for commercial aquaculture are for red mangrove snapper *Lutjanus argentimaculatus* (Shinn-P. et al., 1991) and spotted rose snapper (Martec, https://www.martec.co.cr/) in Costa Rica (Sardenberg et al., 2014). However, in both cases, a detailed description of their rearing protocols has not been published.

The spotted rose snapper is an emerging species with commercial importance in Mexico and other Latin American countries. We present here the results from research projects carried out at the CIAD-Mazatlan Fish Plant since 2003 in order to develop new and more efficient protocols for juvenile production for growing sustainable aquaculture for this species. Our results show that this newly developed method can be applied as an efficient tool for commercial aquaculture production.

It is important to highlight that, with slight modifications, our methods are currently being applied in the Costa Rican commercial enterprise. Also, this new method allows us to double the number and double the efficiency of juvenile production in the same volume of tank space used by Ibarra-Castro et al. (2020). Hence, the objective of the present work was to introduce these new methods of the traditional rearing protocol used at the CIAD-Mazatlan fish plant to improve production performance and rearing efficiency.

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1.1. Water source and treatment

Seawater was obtained by pumping from a sub-sand system (Alvarez-Lajonchère et al., 2007), filtering it through two parallel pressurized sand filters (Triton 14B4002, Bakes Hidrofiltration Inc.) and a multi-cartridge filtration unit (20 μm relative retention) Jacuzzi Inc. model 2251−515LR6-HK6. The water for larva rearing, then enters a recirculation system with a heat-pump (model AE100, Air energy Heat-Pumps, Inc.) to control the temperature at 26 ± 2.0 °C. Water then passes through a multi-cartridge 10-5−1 μm retention unit (model X-100, FSI Michigan City, Inc.) and finally through four serial UV-lamps (60 mJ/cm^2 each) smart U.V. 02130−2 (2 × 65 W) power supply (Emperor Aquatics, Inc). Water to the nursery tanks was supplied with an open flow system from two 25 m^3 tanks, passed through two pressurized sand filters installed in series (Triton 14B4002, Bakes Hydrofiltration, Inc.), and then delivered to the rearing tanks. With these treatments, mean (± SD) water quality suitable for this species were obtained: temperature 26 ± 2.0 °C, salinity 35 ± 1.0 g/l, dissolved oxygen 6.1 ± 0.6 mg/l (saturation: 88.8 ± 9%), and pH 8 ± 0.2. Concentrations of NH_3 were lower than 0.05 mg/l.

1.2. Procedures for mass production of eggs and larvae

In the present work, broodstock feeding and management as well as the procedures for egg production and handling were similar to those described by Ibarra-Castro and Alvarez-Lajonchere, 2011. Briefly, three groups of ten females and 20 males (approximately one kg body weight per fish); were maintained undisturbed in three 18-m^3 shaded communal maturation and spawning tanks with open water flow (8 tank volumes/day) and strong aeration (Fig. 1). A total of 387 spawn were obtained between 2013 and 2014 reproductive seasons, 85 % of these eggs were floating, and 93.5 ± 2.8 % of the floating eggs had live embryos (viability).

Embryos were transferred to twelve 6-m^3 cylindrical fiberglass larval rearing tanks, starting with a volume of 3-m^3, with an average stocking density of 165 ± 69 embryos/l. Hatching occurred at 20 h after spawning and the hatching average was measured to be 88 ± 4%. Survival at 48 h post-hatch (hph) was 50 ± 8%. This resulted in an average of an initial larval density of 82 ± 39 larvae/l in 6 m^3 larval rearing tanks.

1.3. Larval rearing procedures

We established a protocol for water and feeding management during the rearing process (Fig. 2). Behavioral observations, water control, cleaning, and feeding practices were similar to those previously described by Alvarez-Lajonchère et al. (2002) and Ibarra-Castro et al. (2020). The feeding regime was based on providing live food and commercial feeds by larvae demand. We measured live food density every four hours in the rearing tanks from 6:00 am to 10:00 pm. The first live food supplied to 2–20 days post-hatch (dph) larvae was laboratory-produced and enriched rotifers *Brachionus* sp. (Rojo-Cebreros et al., 2017), followed by newly hatched *Artemia* nauplii (SEP-Art GSL Artemia®, INVE Aquauclture Great Salt Lake, Uta USA) from 15–20 dph, and enriched *Artemia* metanauplii from 19 to 35 dph (Fig. 2).

Fig. 1. Broodstock holding conditions.

Weaning onto formulated dry food: Otohime ® (A, A1, A2, B1, B2, C1, and C2; Marubeni Nisshin Feed Co. Ltd., Japan), was initiated with excellent acceptance on 12 dph supplied twelve times per day, starting with 35 % of juvenile BW-biomass and combining different food sizes as larvae grew, substituting 25 % of the new diet the first introduction day and increasing that by 25 % every day after (Fig. 2). The small juveniles were fed with a mix from between 0.5 mm–3 mm, from day 35–90 dph, using two different brands of formulated food: Otohime® and Skretting® (Skretting Inc., Norway) (Fig. 2).

Larval rearing was carried out in twelve cylindrical 6-m^3 fiberglass tanks (non-reflective black walls and white bottoms) (Fig. 3). Special attention was given to increase success of the first feeding and to decrease cannibalism with several procedures: a) detailed monitoring of the morphological development of the larvae the first 120 hph, b) an initial high stocking density (≥100 eggs/l) in larval tanks following Moretti et al. (1999) and Schipp et al. (2007); c) an intensive nursery, adapting many of Schipp et al. (2007) practices, that included a high stocking density, a continuous food supply, regular size grading to optimize feeding rates and reduce cannimalism, low water depth, and turbulent and strong water current in rearing tanks. As in other marine fish larvae, high mortality occurred during first feeding, probably due to biological characteristics of this species, including limited reserves of yolk and oil droplet, small mouths as well as possible differences in the size and quality of the first live food supplied. Therefore, we found that small larvae started to feed between two and four hours after their mouths opened, when their jaws were fully functional. The second critical stage with high mortality rates was at metamorphosis, defined as the beginning of the change from larva to juvenile. Primarily due to important morpho-physiological changes such as final inflation of the swim bladder, dorsal and pelvic fins appearance, notochord flexion and the gradual change in the profile of digestive enzymes. Also, cannibalism, which is stimulated by normal variation in size hierarchies. To reduce these problems, *Artemia* nauplii were introduced every four hours, we co-fed with a mixture of live and dry food (from 12 dph to 35 dph), and reduced the daily tank cleaning schedule to every other day to avoid stress on the larvae.

1.4. Juveniles rearing procedures (nursery I and II)

The duration of the larval rearing period was as short as possible (between 28–35 dph), and as soon as pre-juveniles were able to be handled, they were harvested. At this time, partially metamorphosed pre-juveniles (only the scles are missing) (>1.5 mm head width) were nursed in two stages: nursery I (up to 45 dph) in 1.5 m^3 fiberglass raceways (Fig. 3) and nursery II (another 45 days) in 5 m^3 cylindrical fiberglass tanks (Fig. 4).

A total of 706,799 pre-juveniles 28–35 days post-hatch (dph) were harvested from the larval tanks (58,900 ± 35,370 average per tank), with 31 ± 20 % survival (10 ± 6/l) after first feeding (10 ± 6/l). The average body weight (BW) at harvest was 0.1 ± 0.05 g BW with a mean total biomass of 6 kg/tank. The larvae grew at an average of 2.55 mm–23.58 mm in length in the first five weeks (Fig. 5).

In the nursery I raceways (Fig. 3), water was supplied with an open-flow system (52 L/min) with a water current of 12−21 cm/s through oblique holes of 2.0–2.5 mm every 20−25 mm of a 25 mm diameter PVC pipe placed near the bottom along the first two-thirds of the raceway. During the transfer to nursery I, pre-juveniles were size-graded using a Mohn Aqua Group® grader and separated into three groups (< than

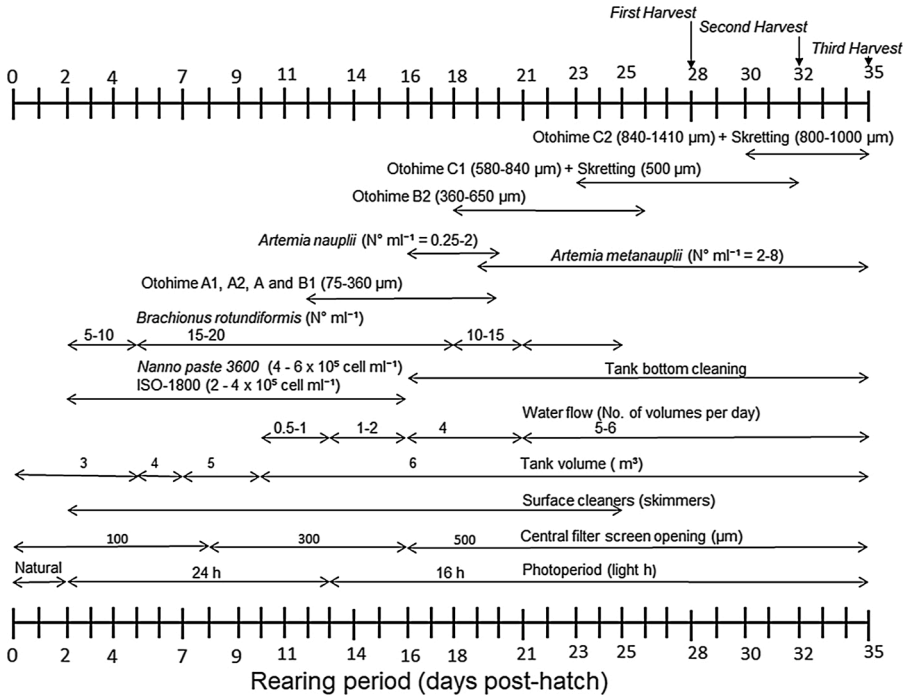

Fig. 2. Water quality and environmental management protocol during incubation, larval, and pre-juvenile rearing of the spotted rose snapper, *Lutjanus guttatus*.

Fig. 3. Larval rearing (round tanks) and nursery I (rectangular tanks) holding contions.

Fig. 4. Nursery II holding conditions.

1.5 mm; 1.5 mm–2.0 mm; and > than 2 mm head width) before being transfered to one of four raceways. During nursery I, juveniles were size graded with 1.5, 2, 2.5, and 3 mm graders (Mohn Aqua Group® grader) every four or five days. The biomass managed in the nursery I was up to 30 kg $^{-1}$ per m^3. Finally, a total of 664,301 juveniles with 0.6 g to 0.8 g BW (94 % survival) were harvested from the raceways of nursery I and transferred to a nursery II facility consisting of eighteen 5-m^3 cylindrical fiberglass tanks (Fig. 4) where juveniles grew an average from 0.48 g to 14.78 g in seven weeks (Fig. 6).

The tanks in nursery II had a daily water exchange rate of 15–20 tank volumes day^{-1}, through an 80 mm diameter PVC pipe placed near the bottom similar to that used in the nursery I raceways. Size grading was performed as in the previous nursery I raceways, but with 3.5, 4, 5, 6, 7, 8, 10, 12, 14, and 16 mm graders (Mohn Aqua Group®). The biomass managed in nursery II was up to 30 kg $^{-1}$ per m^3. A total of 596,382 juveniles (5–15 g BW) were harvested at the end of 90 dph (89.8 % survival) and delivered to the farmers for growing in cages or shrimp ponds.

2. Discussion

The constant supply and availability of high quality and quantity of eggs and 48-hph larvae are essential to scale marine and estuarine fish juvenile production from a pilot plant to a commercial operation. The results presented showed that these goals were possible with the present methods, and produce juveniles at pilot and commercial scales.

High mortalities at first feeding and metamorphosis have been reported for this species by Boza-Abarca et al. (2008) and Alvarez-Lajonchère et al. (2012), as well as for other snapper species (Tucker, 1998). In contrast, Watanabe et al. (1998) and Leu et al. (2003) reported no mass mortalities during the first two weeks in mutton snapper *Lutjanus analis*, and red mangrove snapper *Lutjanus argentimaculatus*, respectively. Suppling enriched rotifers 2 h after the larval mouth opening increased first feeding efficiency between 80 and 95 % drastically reducing the mortality compared with 40–65% to previous trials at CIAD-Mazatlan fish plant (unpublished data). Cannibalism rates were also severely reduced in larvae or pre-juveniles between 28- to 35 dph, by high stocking density and constant food supply compared to

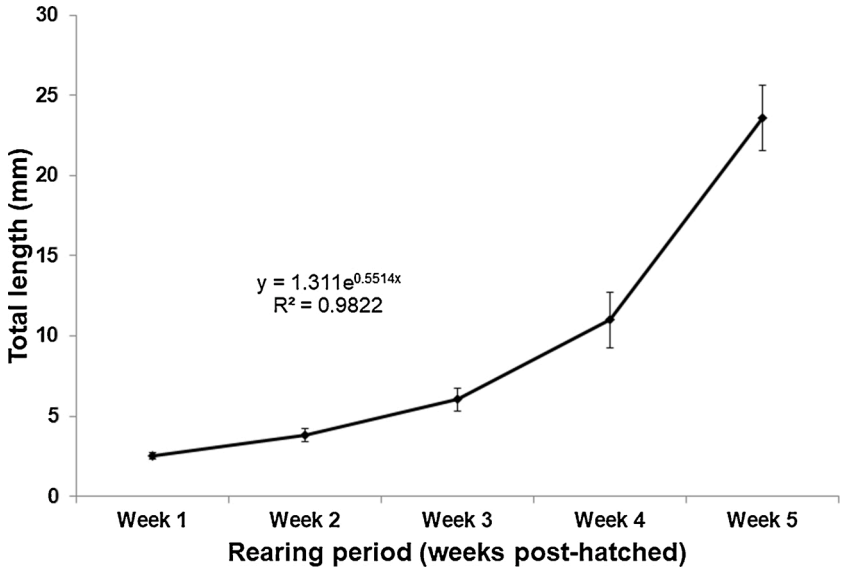

Fig. 5. Growth in total length (mm) curve of spotted rose snapper, *Lutjanus guttatus* larvae. Each point represents the average ± standard error of the average of 25 fish sampled.

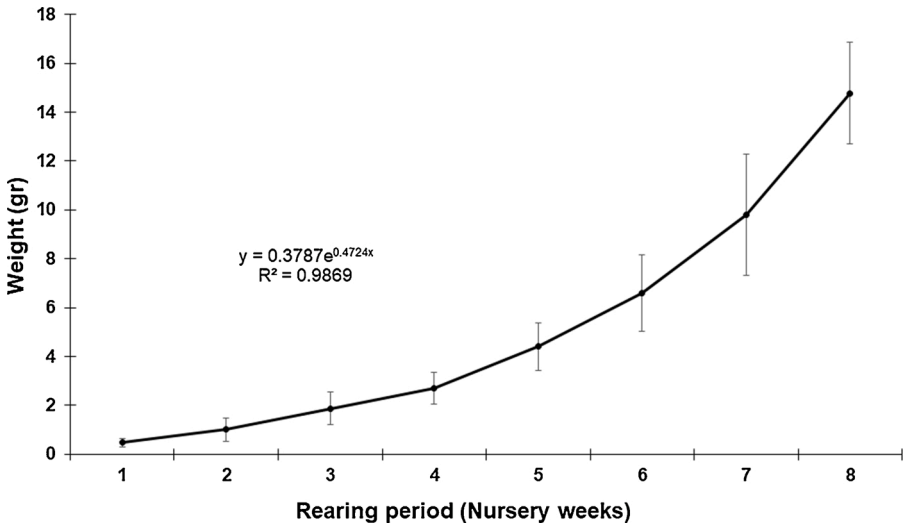

Fig. 6. Growth curve in weight (g) of spotted rose snapper, *Lutjanus guttatus* juveniles. Each point represents the average ± standard error of the average of 25 fish sampled.

previous rearing trials at CIAD-Mazatlan fish plant (Alvarez-Lajonchère et al., 2012; Ibarra-Castro et al., 2020) to only five days period in the present work (from 23 to 28 dph). Major improvements of our raceways increased the chance of contact between the constantly moving food particles and the pre-juveniles, which are kept continuously swimming, made the weaning process more efficient and minimizized cannibalism compared with the previous traditional methods (Ibarra-Castro et al., 2020). Similar rearing techniques have been successfully applied to reduce cannibalism in other finfish species (Hecht and Pienaar, 1993; Ostrowski et al., 1996; Schipp et al., 2007).

Our new methods greatly improved larval rearing performance including the initial stocking density, and larval density at first feeding compared to even the best results obtained with the traditional protocol in CIAD-Mazatlan Fish Plant (IbarraCastro et al., 2020). Juvenile survival, total juvenile harvest density at both 45 dph and at 90 dph, as well as the production efficiency in terms of the number of first feeding larvae required to produce each juvenile at both terms (one juvenile fish was recovered for every four 48 hph larvae generated for an overall 25 % survival ratio) were higher with this new method. Our results also showed higher efficiency than studies with other snappers, which required between five and seven 48 hph larvae for every juvenile, Watanabe et al. (1998) and Leu et al. (2003), respectively. While Alvarez-Lajonchere et al. (2012) and Gutierrez-Sigeros et al. (2018) required 8.3 and seven 48-hph larvae for every juvenile produced, respectively.

3. Conclusion

With our new improvements integrated into the older rearing protocol a pre-commercial number of juveniles was produced with high final density and efficiency. Furthermore, none of our methods have been applied to any other snapper species before. Therfore, our results described here fulfill the objective of generating state-of-the-art of snapper aquaculture that can be applied at a commercial scale.

Statement of relevance

This work has a positive impact on commercial aquaculture

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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