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Effect of age on weaning success in totoaba (*Totoaba macdonaldi*) larval culture

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ABSTRACT

The totoaba (*Totoaba macdonaldi*) is an endemic marine fish of the Sciaenidae family that has been overfished to the risk of extinction, and after three decades of protection and preliminary culture results, it is now considered an excellent candidate for commercial aquaculture due to its fast growth and good adaptation to culture conditions. However, as with most marine fish species, larval culture and adequate weaning are still one of the main bottlenecks to successful commercial production. Thus, the objective of this study was to evaluate weaning success of totoaba larvae onto a microdiet at various stages of development utilizing growth, survival and condition factor as response variables. Weaning onto a microdiet was evaluated at 17 (17 W), 22 (22 W) and 27 (27 W) days post-hatch (DPH). At the end of the experiment (35 DPH), growth was significantly higher (i.e., two-fold increase) in the weaned treatments compared to their respective controls (i.e., feed with live prey exclusively), except for the 22 W treatment that resulted in no significant differences. Comparing final weight and final length among the different weaned treatments did not result in any significant differences. However, survival rate was significantly lower in the weaned treatments compared to their control groups. Although the experiment was not design to quantitatively evaluate cannibalism, this behavior was observed towards the end of the weaning trial and may have a significant contribution to the mortality observed in the weaned treatments. Based on these results and despite of the lower survival, it appears that totoaba larvae possess a sufficiently developed digestive system as early as 17 DPH and can be weaned onto commercial diets before the stomach is fully functional. Thus, it is possible to perform an early weaning (17 DPH) in totoaba larvae, as a strategy to reduce juvenile production costs and increase profitability of the production system, by reducing the use of *Artemia* nauplii without affecting growth.

1. Introduction

Totoaba (*Totoaba macdonaldi*) is an endemic fish of the Gulf of California considered to be the largest member of the Sciaenidae family (Cisneros-Mata et al., 1995). Totoaba has been protected by the Mexican government since 1975 (Flanagan and Hendrickson, 1976) and is included in the list of endangered species by the International Union for Conservation of Nature (IUCN) (Baillie et al., 2004; Barrera-Guevara, 1990; CITES, 2005). One strategy to recover natural populations is to develop culture techniques for restocking hatchery-produced juveniles. Based on this premise several thousand juveniles are released in the Gulf of California every year (López et al., 2006; True et al., 1997). In addition, several fish farms are currently evaluating the potential of this species under commercial culture conditions (Rueda-López et al., 2011) with promising results.

In the culture of marine fish, the larval stage is considered one of the main bottlenecks due to the high mortality typically obtained during this stage that can be as high as 85%. This high mortality is associated among other things to problems related to adequate feeding and nutrition (Alarcón et al., 2002; Avilés Quevedo and Castelló Orvay, 2004; Lazo, 2000; Valverde et al., 2002a).

Live food such as rotifers (*Brachionus*) and *Artemia* are typically used to feed larvae of marine fish under culture conditions (Burke et al., 1999; Evjemo et al., 2003; Hamre et al., 2013). Nevertheless, live food production is costly, unpredictable and laborious (Koven et al., 2001), frequently vary in nutritional quality, and the prey size may not be appropriate for larvae in some stages (Burke et al., 1999; García-Ortega et al., 2003; Hamre et al., 2002; Takeuchi et al., 2003).

During the last three decades, great efforts have been made in the development of microdiets to replace live food in larval culture of marine fishes with variable degree of success (Kolkovski, 2001; Koven et al., 2001; Muguet et al., 2011). Of the different weaning protocols used in the culture of marine fish larvae, those applying a "co-feeding" period using both live and formulated feeds, have been quite successful and have significantly decreased time needed to complete weaning, allowing a gradual adaptation to the physical and biochemical characteristics to the new microdiet (Chu and Ozkizilcik, 1999; Curnow et al., 2006; Hamza et al., 2007; Herrera et al., 2010; Kolkovski et al., 1997; Ma et al., 2014; Rosenlund et al., 1997).

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The ontogeny of the digestive tract in totoaba larvae can be characterized in four main stages: initial (< 3 DPH, mouth opening), early (4 to 17 DPH, liver, intestine and pancreas well differentiated), medium (17- 24 DPH, beginning of stomach development and pepsin secretion) and mature (> 24 DPH, fully functional stomach) (Galaviz et al., 2013; Valverde et al., 2002b). To date, most totoaba larvae protocols recommend initiating weaning around day 24 post-hatch (Rueda-López et al., 2011; True et al., 2001; Valverde et al., 2002b). In order to develop an adequate weaning protocol for the totoaba (*T. macdonaldi*), the overall objective of this experiment was to evaluate weaning success of totoaba at various stages of development using growth, survival and condition index as response variables.

2. Materials and methods

2.1. Larval rearing conditions

Totoaba eggs were collected from natural spawns of domesticated broodstock held at the Biotechnology and Fish Culture Laboratory of the Universidad Autónoma de Baja California (UABC) in Ensenada, B.C., México. Upon collection, eggs were transferred to the Marine Fish Culture Laboratory of the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE), in Ensenada, Mexico.

Eggs were incubated at 22 ± 1 °C in 2000 L light-blue fiberglass conical tanks at a density of 40 eggs L^{-1}. Typically, eggs hatch after 24 h of incubation, and this was considered as 0 DPH. Newly hatched larvae were kept in the same tanks (holding tanks) at a constant temperature of 24 ± 1 °C before being transferred to the experimental tanks. Seawater was passed through an expandable bead-biofilter (Bubble Bead 2000, Aquaculture Systems Technologies, New Orleans, USA), 10 μm cartridge filter, carbon filter unit and a UV unit. Water from the recirculated system was allowed to flow through the tanks at 3 L min^{-1}. Water quality was monitored daily; in order to keep constant salinity at 35‰, dissolved oxygen was close to 6.5 ± 0.05 mg L^{-1} and total ammonia nitrogen <1.0 N-NH3 mg L^{-1}. Larvae were exposed to an artificial photoperiod of 12 L:12D using fluorescent lights with a light intensity of 500–1000 lx at water surface.

In the main holding tanks, larvae were feed HUFA enriched rotifers from 3 to 20 DPH, and enriched *Artemia* nauplii from 17 to 35 DPH, with a rotifer and Artemia overlap of 4 days (i.e., 17 to 20 DPH). Commercial enrichments were used to improve the nutritional quality of the live prey (Palmtag et al., 2006). Rotifer concentrations were maintained in the larval tank at densities between 3 and 10 prey mL^{-1} and *Artemia* between 1 and 5 prey mL^{-1} (Lazo et al., 2000b). To ensure that enough food was provided and that live prey maintain adequate nutritional value (i.e., n-3 HUFAs), twice a day (8:00 and 15:00 h) prey counts were performed daily from each tank to maintain the required density of enriched food and provide freshly enriched live prey.

Rotifers (*Brachionus plicatilis*) were cultured in 500 L tanks, maintained at 26 °C, with a salinity of 32 ‰, and illuminated with fluorescent lights. Rotifers were fed continuously with *Nannochloropsis* sp. (Instant Algae®; Campbell, CA, USA) through a peristaltic pump connected to the tanks to achieve a density of 35×10^6 cells mL^{-1}. Rotifers were collected each morning, concentrated in a nylon mesh (60 μm), washed with seawater and enriched with Algamac® 3050 (Hawthorne, USA) for 12 h according to the manufacture recommendations and then used to feed the larvae.

Artemia cysts (Salt Creek Inc., Salt Lake City, UT, USA) were disinfected and decapsulated with a hypochlorite solution and hatched in 20 L tanks at 25 °C with continuous aeration based in the procedures described by Sorgeloos et al. (1986). After 10 h post-hatch, in a different 20 L tank, the *Artemia* metanauplii were enriched for 12 h with the commercial emulsion Algamac® 3050 (Hawthorne, USA) following the manufacturer protocol as described by Zacarias-Soto et al. (2006). After enrichment, metanauplii were washed with UV disinfected seawater and fed to the larvae.

2.2. Experimental design of the weaning trial

Based on the development of digestive tract (Galaviz et al., 2013; Valverde et al., 2002b), three larval ages were selected to initiate weaning: early weaning 17 DPH (17 W), when the stomach development had not started and larvae have only been feed rotifers; mid-weaning 22 DPH (22 W), at the start of stomach development and during the feeding transition from rotifers to *Artemia*; and late-weaning 27 DPH (27 W), when the larvae possess a functional stomach. For each weaning date, a control group fed exclusively live feed was used. Three replicate tanks were used for each of the three weaning treatments (17 W, 22 W and 27 W) and the three controls (17C, 22C and 27C). Larvae for each treatment were randomly allocated into eighteen circular 150 L light-blue flat-bottom fiberglass tanks connected to a recirculation system (similar system and ambient conditions as described in larval rearing section but with a water recirculation rate of 1.56 L min^{-1}). To reduce stress, 3 days before the start of each treatment, 1100 larvae were randomly selected from the holding tanks and placed into each of the experimental tanks (Muguet et al., 2011). Larvae were stocked at a density of 9 larvae L^{-1}.

In the experimental tanks before the weaning phase of each treatment and depending on weaning age, the experimental and controls groups were fed on rotifers until 17 DPH, with rotifers and *Artemia* nauplii during feeding transition from 17 to 20 DPH and only *Artemia* nauplii from 20 to 35 DPH. However, larvae in treatment W17 did not receive Artemia and were co-fed with rotifers during the weaning phase. For the weaning treatments, the live food was reduced to 75%, 50%, 25% and 0% of the original ration to obtain a gradual weaning during a period of 4 days, from this point larvae were fed solely uniquely with microdiet. The control tanks received only live food until the end of experiment (35 DPH) based on modified version of rearing protocol described by Lazo et al. (2000b).

A commercial microdiet was used for weaning (Othohime®, Inc., Japan). Larvae were fed "in excess" with a daily ration of 1 g, distributed continually during an 8 h period using automatic feeders (Rainbow, Lifegard Aquatic) (Lazo et al, 2000a). Depending on the larvae size and mouth opening, a gradual increase in the microdiet particle size was performed during the experiment (B_1 = 200–360 μm; B_2 = 360–620 μm; C_1 = 580–840 μm) following the procedure described by Holt (1993). Once a day, each tank was cleaned (i.e., siphoned to remove feces and uneaten-feed).

2.3. Sampling

To quantify the exact number of larvae in each treatment and to obtain an accurate final survival, counts were made at the beginning and at the end of the experiment (35 DHP). To achieve this, larvae were placed in 2.5 cm deep rectangular fiberglass containers with shallow water. Subsequently, a digital photo was taken (C-5060. OLYMPUS, USA) and recorded. Finally, the larvae were counted with the help of an image scan process using the "Sigma Scanpro 5" software (SPSS Inc., USA). Growth in terms of length and weight was determined by taking 10 freshly anesthetized (using MS-222) larvae out of each tank at the beginning and the end of the experiment period. Dry weight (g) was measured using an analytical scale (92SM-202A, Precisa Instruments Ltd., Switzerland, ± 0.01 mg precision), and standard length (mm) was measured using a stereo microscope equipped with micrometer scale (Wild M8. Wild-Heerburgg, Switzerland).

Finally, to assess the welfare or condition of the larvae, the Fulton condition factor was calculated for each treatment (Williams, 2000), based on the following formula;

$$K = (100,000 \times W)/L^3$$

where W is the dry weight in grams and L is the standard length in millimeters.