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Early life cycle and effects of microalgal diets on larval development of the spiny rock-scallop, *Spondylus limbatus* (Sowerby II, 1847)

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ABSTRACT

The *Spondylus limbatus* fishery in most of the eastern Pacific is restricted due to a decline in natural populations. The aquaculture production of seedlings applying proper larviculture techniques could contribute to the restoration programs of this valuable fishing resource. However, literature of proper culture techniques for *S. limbatus* is scarce. We describe the embryonic, larval and post-larval development of *S. limbatus* raised in the laboratory, and the effect of two monoalgal (*Chaetoceros gracilis*, CG; and *Isochrysis galbana*, IG) diets and a combination of different cell ratios (3CG:1IG, 1CG:3IG and 1CG:1IG) on growth and survival of *S. limbatus* larvae. Fertilized eggs had a diameter of 60.2 ± 1.3 μm. Swimming D-larvae, with a shell length of 98.0 ± 2.2 μm, were obtained within 26 h; and pediveligers (185.2 ± 3.9 μm) in 12 days. At day 16 (>200 μm) pediveligers metamorphosed into postlarvae and the dissoconch appeared. After metamorphosis, we did not observe byssus production and post-larvae were capable of remaining free (in plantigrade stage) for 2–3 months, with crawling movements until settlement by cementation took place on hard substrates. The post-larval settling behavior is suggested to be characteristic of the genus *Spondylus*. The algal diet experiment revealed significantly larger larvae (164.0 ± 1.8 μm) and faster growth (5.5 ± 0.0 μm day^{-1}) with 3CG:1IG treatment, while survival was higher when fed CG alone (18.8 ± 4.3%). The embryogenesis and larval development of *S. limbatus* are similar to other Pectinidae species. The combination of *C. gracilis* and *I. galbana* (3:1) could be used as an appropriate diet for *S. limbatus* larval culture. Future research will be focused on improving survival of competent *S. limbatus* larvae by implementing water treatment systems and consequently testing preference substrates that potentially stimulate attachment and cementation on this species.

Statement of relevance

This study presents a complete description of the embryonic and larval development of *S. limbatus*. Additionally, we determined an appropriate diet (*C. gracilis*, *I. galbana*; 3:1) for enhancing larval growth and survival of this commercially and ecologically important species. Finally, we provide unpublished observations about the settling behavior and physiological characteristics of *S. limbatus* postlarvae and their implication for aquaculture.

1. Introduction

The spiny rock-scallop, *Spondylus limbatus* (Sowerby II, 1847) (Order: Pectinoida; Family: Spondylidae), formerly referred to as *Spondylus calcifer*, is distributed along the Eastern Tropical Pacific coast from the Baja California Peninsula and the Gulf of California, Mexico, to Tumbes, Peru (Coan and Valentich-Scott, 2012). This species has played an important economic, political, and cultural role in coastal communities of the Eastern Tropical Pacific for thousands of years (Cudney-Bueno and Rowell, 2008). The attributes of *Spondylus* shells have a long past in Andean prehistory as symbols of the oracles, ceremonial offerings and currency that were important integrative mechanisms in the evolution toward large-scale societies as to the later Huari and Inca empires (Paulsen, 1974).

At present, *S. limbatus* represents an important economic resource along its geographical distribution range due to its meat for human consumption. In Ecuador, this species made a comeback as a commercially important resource since 1990 after centuries of being disregarded (Mackensen et al., 2011). As a result, stocks of *S. limbatus* in natural beds declined in some intense fishing zones along the Ecuadorian coast (MAGAP, 2010). In this regard, the Undersecretary of Fishing Resources of the Agriculture, Livestock, Aquaculture and Fishery Ministry (MAGAP) of Ecuador announced the closure of the *S. limbatus* fishery (Acuerdo Ministerial 136, October 02, 2009). Consequently, the Undersecretary of Aquaculture of the MAGAP and the National Aquaculture and Marine Research Center of the Litoral Polytechnic University of Ecuador (CENAIM-ESPOL), initiated a project for the production of

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seedstock and juveniles of *S. limbatus* in laboratory with the final aim of potential restocking of overexploited natural fishing grounds. The feasibility of rebuilding overexploited natural *S. limbatus* grounds using hatchery-reared individuals will be evaluated through the deployment of cages containing *S. limbatus* juveniles (3–5 cm) on selected seabeds. Growth and survival will be monitored seasonally until reproductive conditions are reached and then settling of new stock will be evaluated. Several studies are currently been carried out to achieve proper larviculture techniques. A similar scenario has been observed in Mexico, where permission for fishing *S. limbatus* is also restricted (Soria et al., 2010).

To date, most studies carried out with *S. limbatus* have described its biology, ecology and population structure (Mackensen et al., 2011; Soria et al., 2012; Villalejo-Fuerte and Muñetón-Gómez, 2002; Villalejo-Fuerte et al., 2002). Base-line information about its early life cycle and larvae culture has been initiated in recent years. Soria et al. (2010) provided information about spawning and rearing of *S. limbatus* larvae using *Isochrysis galbana* and *Chaetoceros calcitrans* (1:1) as microalgae source in feeding experiments. This study, however, did not provide further information about larval or post-larval settlement. On the congeneric species, *Spondylus tenebrosus*, Parnell (2002) described its larval development and stated that this species can delay settlement and remain as larva for at least 2 months.

Description of the main characteristics of embryonic, larval and post-larval development of target species is important for investigating larval dispersion, settlement events, levels of recruitment, and growth rates of bivalves (Gribben and Hay, 2003; Soria et al., 2012), and to establish proper hatchery production protocols. Likewise, the identification of the appropriate algal species and/or their combinations to provide the essential nutrients is required for effective growth and survival for *S. limbatus* larvae. Previous studies in other bivalve larvae species have shown that diets based on at least two microalgal species lead to better nutritional profile causing higher growth and survival (Gonzalez-Araya et al., 2012; Marshall et al., 2010; Pernet and Tremblay, 2004; Rico-Villa et al., 2006). Two of the most preferred microalgae species due to their size, biochemical composition and ease of cultivation are the flagellates *Isochrysis* spp. and the diatom *Chaetoceros* spp. (Rivero-Rodríguez et al., 2007; Saucedo et al., 2013). The former has been reported to be rich in docosahexaenoic acid (DHA, 22:6n-3), and the latter in eicosapentaenoic acid (EPA, 20:5n-3); both essential polyunsaturated fatty acids (PUFA; Pettersen et al., 2010; Brown and Blackburn, 2013). These microalgal species are frequently used in larval culture of bivalve molluscs, particularly pectinids (Farías, 2001).

Considering the scarcity of a complete life cycle description and larviculture techniques for *S. limbatus*, the aim of the present study was to describe the embryonic, larval and post-larval development; and to evaluate the effect of the microalgae *Chaetoceros gracilis* and *I. galbana* on larval growth and survival.

2. Materials and methods

2.1. Broodstock conditioning and spawning induction

Several specimens of the spiny rock-scallop *S. limbatus* with an average shell length of 134.1 ± 11.4 mm and a mean weight of 1.3 ± 0.4 kg ($n = 39$) were collected from the shallow coastal waters of Ayangue, Ecuador (01°58′09″S; 80°45′32″W) on September 2013 (23 °C). Individuals were transported in coolers to CENAIM-ESPOL laboratories at San Pedro, Ecuador. Organisms were brushed to remove fouling and maintained in 8000-l tanks at ambient water temperature (24.3 ± 0.2 °C) and salinity (33–34) over an 8-week period. On a daily routine, 25% of the water volume was changed and feces were removed. Constant aeration was provided at all times. Food consisted of an algal mixture (ratio 2:1 in cells number) of *C. gracilis* and *I. galbana* provided daily at a mass dry algal weight equivalent to a 5% of broodstock dry meat weight in tanks (Millican and Helm, 1994).

For spawning induction, all 39 individuals were cleaned, desiccated for 45 min and individually placed in 30-l tanks with filtered (5 μm) seawater at 25.8 °C and a salinity of 34. Spawning occurred 30 min after the first stimuli and the addition of sperm helped to accelerate the spawning process.

2.2. Algae production

Algae used in broodstock conditioning and subsequent experiments were cultured in 1-m^3 tanks and 50-l carboys, respectively, using the f/2 medium (Guillard, 1975), at 20 °C, with permanent fluorescent light (3500–5000 lx) and constant aeration. Algal concentration was estimated using a Neubauer chamber.

2.3. Embryonic, larval and postlarval description

Oocytes were fertilized with active sperm at a ratio of 10:1 (spermatozoa:oocyte). Fertilized eggs were incubated in 1000-l tanks, at a density of 25 eggs ml^{-1}, with 1 μm filtered seawater at 25.7 ± 0.1 °C and a salinity of 34. Four 50-l replicates derived from the incubation tank were prepared in parallel to monitor embryogenesis and trochophore larvae. D-stage larvae were drained and retained on a 40-μm mesh screen and allocated in one 1000-l tank to monitor larval and post-larval development. Larval feeding consisted of *C. gracilis* and *I. galbana* (3:1, in cell number) and was provided progressively at concentrations from 10,000 cells ml^{-1} (day 0) to 50,000 cells ml^{-1} (day 12). The latter concentration was maintained constant throughout the settlement process. Settled and unsettled post-larvae were monitored for six months after providing some *Spondylus* shell fragments as substrate for settlement. Settled juveniles were fed once a day with *C. gracilis* and *I. galbana* in equal proportion at a concentration of 100–150 cells μl^{-1}. Water temperature ranged between 25 and 27 °C throughout the monitoring period. The larval and post-larval stages were photographed with a digital camera (Nikon E995) coupled to an optical Olympus CH-2 Microscope at 100×. A random sample of size $n = 30$ was selected to obtain the average length for all embryonic and larval stages.

2.4. Larval experiment: the effect of algal diets on larval development and survival

Another D-larvae group from the same batch was drained from the incubation tank, retained on a 40-μm mesh screen and allocated in fifteen 50-l cylindro-conical tanks at an initial density of 4 larvae ml^{-1}, containing 1-μm filtered, UV-treated and aerated seawater. Water temperature and salinity were 25.3 ± 0.7 °C and 34, respectively. The influence of algal diets on growth (shell length) and final survival (%) of *S. limbatus* larvae was evaluated. The microalgal species *C. gracilis* (CG) and *I. galbana* (IG) in the exponential growth phase were tested in 5 dietary treatments: two monospecific microalgal treatments (CG, IG) and three treatments consisting of a mixture of both microalgal species at cell ratios of 1:3, 1:1 and 3:1 (CG:IG); each treatment was carried out in triplicate. The initial phytoplankton density was 10,000 cells ml^{-1} (day 0) and was subsequently increased by 3,000 cells ml^{-1} per day. The feeding frequency was divided into two daily rations provided at 9:00 and 15:00 h. The larval experiments were terminated at day 12 when larvae reached the pediveliger stage.

Culture water was renewed 100% every second day. During water exchange, tanks were rinsed with fresh water. Throughout the process, larvae were sieved through a 40-μm mesh screen and transferred into a 2-l beaker. Beakers were aerated with air stones to homogenize the larvae for sampling, which consisted in the extraction of four 0.5-ml samples using a micropipette (0.1–1 ml). The sample was placed into