Aquaculture 450 (2016) 328–334

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

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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ARTICLE INFO

Article history:
Received 11 June 2015
Received in revised form 8 August 2015
Accepted 9 August 2015
Available online 10 August 2015

Keywords:
Spondylus limbatus
Spondylus calcifer
Embryonic development
Larval development
Larval growth
Algal diets

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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http://dx.doi.org/10.1016/j.aquaculture.2015.08.012
0044-8486/

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

a test tube and 1-ml of Lugol (5% solution) was added to preserve the sample for later measurements.

Survival was calculated by extrapolation of 2-ml count using an optical Microscope at 40×. The same sampling procedure was performed for each experimental replicate. Larval growth (μm) was determined by measuring the shell length. Samples were photographed with a digital camera coupled to an optical microscope at 100×. Images of 30 randomly selected larvae were analyzed with Adobe Illustrator 10® software. Daily growth rate (*DGR*, expressed in μm day^{-1}) was calculated for each treatment according to Abaloso-Pacheco et al. (2009): $GR = (T_{final} - T_{initial}) / t$, where T = size of larvae at the beginning ($T_{initial}$) and at the end (T_{final}) of the experiment and t = time (days).

2.5. Statistics

Normality and homoscedasticity of the data were tested using a Kolmogorov–Smirnov test and Levene's test, respectively. One-way ANOVA was used to test significant differences in growth and survival rate of larvae to diet treatments at the end of experiments (day 12). Post hoc Scheffé's pairwise multiple comparisons were performed afterwards to detect significant differences between treatment means. Data were analyzed with the statistical software SPSS® 16.0. Statistical significant differences were considered at a 95% confidence level (type I error 0.05).

3. Results

3.1. Embryonic, larval and postlarval development

The first polar body appeared 10–15 min post-fertilization of oocytes (60.2 ± 1.3 μm). Fertilization rate was >99%. The first cell cleavage took place within 30–40 min after fertilization. The second, third, and successive segmentation cleavages continued during the succeeding 3 h of development reaching the morula stage. Blastula (63.6 ± 2.5 μm) was registered after 5 h. Early gastrula was observed at 6 h (59.4 ± 2.4 μm), while at 9 h the late gastrula turned into a motile early trochophore (62.5 ± 2.5 μm). At 18 h, D-shaped larvae with straight hinge commenced to be noticeable in late trochophore (79.9 ± 4.0 μm); and at 26 h, a calcified D-shaped veliger larva with the prodissoconch-I (PI) was completely developed (98.0 ± 2.2 μm in shell length).

Larva with prodissoconch-II was identified 4 h after prodissoconch-I (30 h post-fertilization), initiating the larval shell growth. A provinculum was highly noticeable at 3-day old larvae sizing 119.4 ± 3.7 μm in shell length. On day 8, veliger larvae turned markedly umboned when the shell length was 154.7 ± 7.0 μm. The eyespots were noted first on day 10 in veligers sizing 166.3 ± 8.2 μm. The appearance of crawling pediveligers with a double ring on the shell edges was identified on day 12 in larger sized larvae (185.2 ± 3.9 μm). During the subsequent days, metamorphosis occurred and first post-larvae were eventually observed on day 16 (>200 μm), characterized by a dissoconch appearance. We did not observe byssus production and postlarvae were able to remain free until a maximum size of 1.2 ± 0.1 mm in shell length, with crawling movements until settlement by cementation. The latter took place on hard substrates (*Spondylus* shell pieces) in two–three months under laboratory conditions. The cemented postlarvae continued their growth, reaching a mean shell length of 6.1 ± 3.1 mm after 3 months and of 14.3 ± 4.9 mm after 6 months from post-larvae settlement. Temperature along embryonic and larval development remained at 25.2 ± 0.5 °C. Fig. 1 provides a description of the *S. limbatus* life cycle until reaching the juvenile stage.

3.2. Algal diets on larval development

The algal diet experiment ended at day 12 when larvae in the pediveliger stage were firstly observed. Fig. 2 shows the daily growth rate (DGR; μm day^{-1}) of different microalgal diets at different time periods (days 0–6, 6–12 and 0–12). The highest DGR was recorded from days 0 to 6 for all treatments, regardless of diet. The 3CG:1IG treatment showed the higher DGR between days 6–12 and throughout the experimental period (days 0–12). Larval DGR ranged between 5.5 ± 0.2 μm day^{-1} for the faster growth (3CG:1IG) and 3.1 ± 0.1 μm day^{-1} for the slowest one (IG). At day 12, shell length was significantly higher ($p < 0.05$) in the 3CG:1IG treatment (164.0 ± 1.8 μm) followed by 1CG:1IG (154.1 ± 1.0 μm), 1CG:3IG (142.2 ± 3.1 μm), CG (136.5 ± 1.4 μm) and IG (135.5 ± 1.3 μm).

In contrast, by day 12, the significantly highest survival ($p < 0.05$) was found in the CG treatment (18.8 ± 4.3%) in comparison to 3CG:1IG (6.3 ± 0.4%), 1CG:1IG (5.5 ± 2.3%), 1CG:3IG (1.2 ± 1.3%) and IG (1.0 ± 0.6%), which did not show significant differences among each other ($p > 0.05$; Fig. 3).

4. Discussion

4.1. Embryonic and larval development

The early trochophore and D-larvae stages of *S. limbatus* in this study were observed at 9 and 26 h after fertilization, respectively, at 25.7 °C. Other works observed faster embryonic development with the same species incubated at 29 °C (Soria et al., 2010) and with *S. tenebrosus*, at 22 °C (Parnell, 2002). Embryonic development in bivalve molluscs, regardless of species, depends mainly on water temperature (Le Pennec et al., 2003; Wright et al., 1983). Differences in elapsed time to reach D-stage larvae among studies might be explained therefore by culture water temperature conditions.

Size and other physical cell characteristics (presence of umbonate larva, eyespot, double ring and foot) of *S. limbatus* larvae obtained in this study closely agree with those reported for *S. tenebrosus* (Parnell, 2002) and other Pectinoidea larvae, such as *Argopecten ventricosus* (Ibarra et al., 1997), *Amusium balloti* (Rose et al., 1988), *Chlamys asperrimus* (Rose and Dix, 1984), *Nodipecten nodosus* (De la Roche et al., 2002; Velasco et al., 2007) and *Argopecten nucleus* (Velasco et al., 2007). Nevertheless, our results diverge partially from those of Soria et al. (2010), which reported longer sizes for pediveligers (234.0 ± 28.03 μm) than those obtained in the present study (185.2 ± 3.9 μm) for the same species. Differences in sizes and timing obtained in larval development might be explained due to the culture conditions such as temperature, food quality and quantity, stocking density, light intensity or the quality of food supplied to broodstock (Brown et al., 1997; da Costa et al., 2011; Farías et al., 2003; Rico-Villa et al., 2006; Uriarte et al., 2001; Yan et al., 2006).

The appearance of first pre-metamorphic larvae (double-ringed pediveligers with an eyespot) observed in this study on day 12 agrees with that reported for *S. tenebrosus* (Parnell, 2002) and the tropical scallop *N. nodosus* (De la Roche et al., 2002). Parnell (2002) recorded a minimum precompetent period for 21 day old larvae (in which settlement was first observed), which was longer than that reported in the present study (16 days, when first post-larvae appeared). Soria et al. (2010), furthermore, found that *S. limbatus* larvae reached the pediveliger stage 15 days after fertilization, but without reporting evidence of settlement or post-larvae stage.

Additionally, the capability of remaining as free post-larva (uncemented and not attached by byssus) for two–three months observed in this study supports the hypothesis from Villalejo-Fuerte and Muñetón-Gómez (2002) that settlement of *S. limbatus* may occur during the post-larvae stage. Similar delayed settling behavior was observed in *S. tenebrosus*, remaining as larvae for at least two months (Parnell, 2002). Other studies agree in that pediveligers may delay metamorphosis for a considerable time until suitable physical substrates for settlement are encountered (Culliney, 1974; Gosling, 2003); however, little is known about the ontogeny of the *Spondylus* spp. settlement process. In the rock scallop *Crassadoma gigantea*, it has

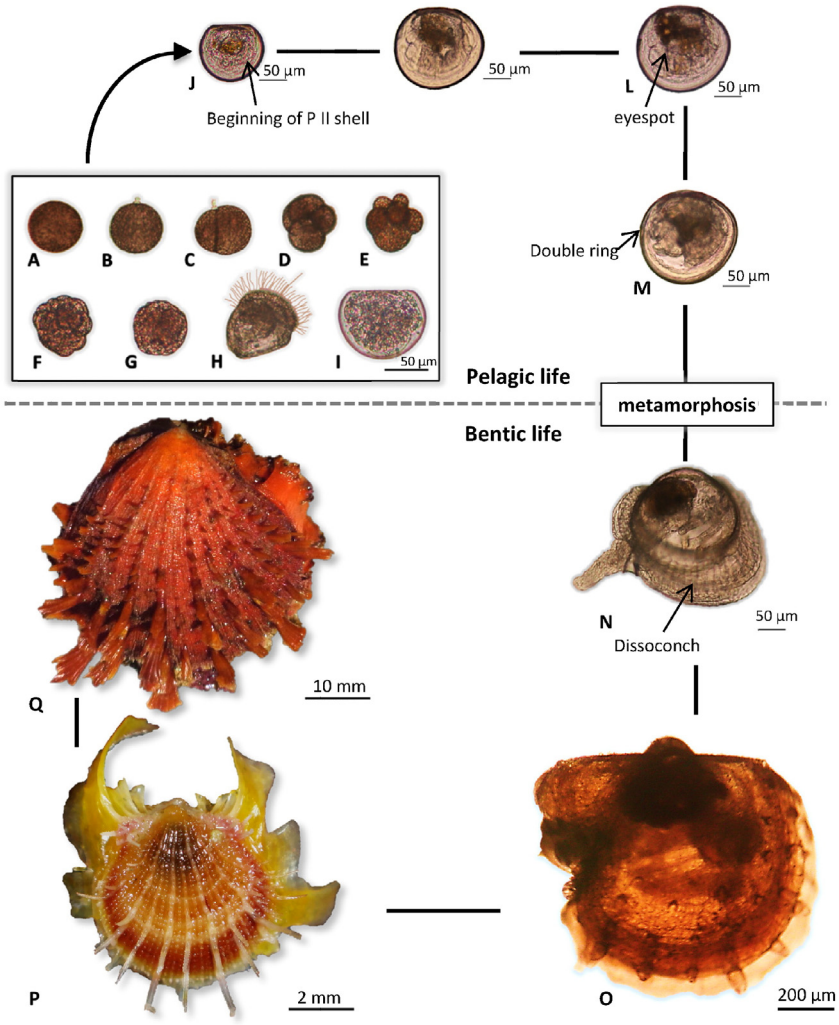

Fig. 1. Life cycle of *Spondylus limbatus*: A) spawned oocyte, B) expulsion of polar bodies, C) first cleavage, D) second cleavage, E) third cleavage, F) morula, G) ciliated gastrula, H) trochophore larva, I) straight-hinge D-larva, J) veliger, K) umboned veliger, L) eyed veliger, M) pediveliger, N) unattached post-larva, O) unattached juvenile, and P and Q) attached juveniles.

been observed that postlarvae can attach to substrates through a byssus and then eventually release themselves to become free until about 25 mm in shell length when cementation occurs, suggesting that cementation takes place when suitable substrates are available (Culver et al., 2006). The observed delay of settlement by cementation in the present study can be hypothesized as a characteristic of the ontogeny and life cycle of *Spondylus* genus. Unfortunately, this characteristic can considerably affect the hatchery production. Studies of larval and

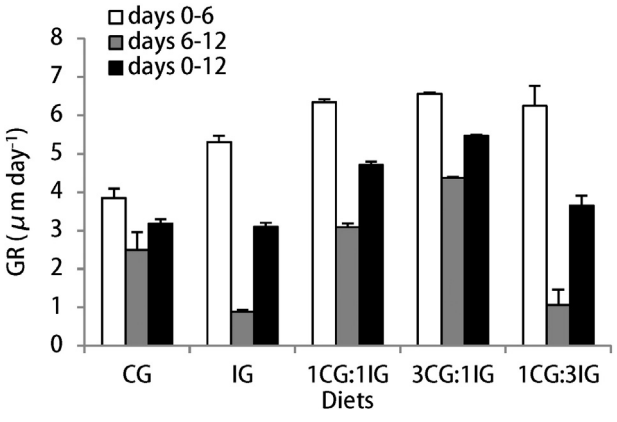

Fig. 2. Daily growth rate (DGR) of *Spondylus limbatus* larvae fed different microalgal diets at distinct time periods. Error bars represent SE ($n = 3$). Diets consisted of two monospecific diets (CG = *Chaetoceros gracilis*, IG = *Isochrysis galbana*) and mixtures of CG and IG at three ratios 1:1, 3:1 and 1:3.

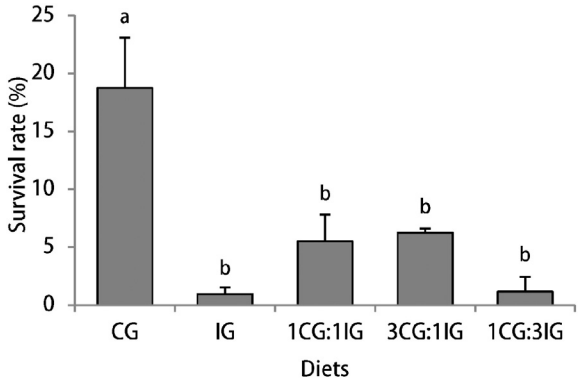

Fig. 3. Survival rate of *Spondylus limbatus* larvae at different microalgal diets on day 12. Means with different letters differ significantly ($p < 0.05$). Error bars represent SE ($n = 3$). CG = *Chaetoceros gracilis*, IG = *Isochrysis galbana*; 1:2, 3:1, 1:3 = cell mixture ratio.

post-larval development and its behavior should be performed in *Spondylus* species for the understanding of this physiological characteristic.

The growth rate reported for settled *S. limbatus* juveniles in this study (80.0 ± 2.7 μm day^{-1}) was relatively lower than those recorded for tropical juvenile pectinids in laboratories and suspended cultures, such as *Nodipecten subnodosus* (125–380 μm day^{-1}; Koch et al., 2005; Saucedo et al., 2013), *N. nodosus* (167–333 μm day^{-1}; Lodeiros et al., 1998) and *A. nucleus* (300 μm day^{-1}; Lodeiros et al., 1993). Growth rate in juveniles has been shown to vary due to culture conditions such as temperature, food availability and nutritional value of microalgae (Chauvaud et al., 1998; Farías, 2001; Saucedo et al., 2013). Further research on nutritional and physiological requirements of *S. limbatus* juveniles is needed for proper management and culture.

4.2. Algal diets on larval development

Larval development of *S. limbatus* varied among algal diets. Treatment containing the diatom CG and the flagellate IG at a cell ratio of 3:1 presented the best growth rate. Combination of *Isochrysis* and *Chaetoceros* species has been reported to be successful for larval bivalve rearing (De la Roche et al., 2002; Gonzalez-Araya et al., 2012; Marshall et al., 2010; Pernet and Tremblay, 2004; Rico-Villa et al., 2006). Levels of polyunsaturated fatty acids (PUFA) are essential for bivalve larvae growth (Brown et al., 1997). It has also been reported that the presence or absence of PUFA in diets might affect pectinid larvae by promoting or failing to complete metamorphosis (Delaunay et al., 1993; Tremblay et al., 2007). Additionally, fatty acid requirements appear to be species-specific for some animals (Milke et al., 2008; Pernet and Tremblay, 2004). Both of the most important n-3 fatty acids, i.e. docosahexaenoic acid (DHA, 22:6n-3) and eicosapentaenoic acid (EPA, 20:5n-3), are present in high proportion in *Isochrysis* spp. and *Chaetoceros* spp., respectively (Brown and Blackburn, 2013; Pettersen et al., 2010).

The highest growth rates observed with diets 3CG:1IG, 1CG:1IG and 1CG:3IG during the 0–6 day period suggest that the combination of IG and CG is efficient for larval growth. Nevertheless, the higher larval growth rate obtained between days 6 and 12 with the 3CG:1IG treatment (4.4 ± 0.0 μm day^{-1}) suggests that *S. limbatus* requires higher amounts of CG (rich in EPA) to supply all of the nutrients necessary to reach the maximum growth rate. Demand of more EPA content than DHA on growth of bivalve species is in agreement with previous observations reported for the pectinid *N. subnodosus* (Cerón-Ortiz et al., 2009; Lora-Vilchis et al., 2004) and the oyster *Crassostrea gigas* (Rico-Villa et al., 2006). These results suggest specific nutritional requirements for particular larval stages. Unfortunately, the fatty acid profile of the tested microalgae species was not analyzed in the present study.

Conversely, poor larval growth using a single microalga, either CG or IG, suggests deficiency of specific complementary nutrients among algal species. Low nutritional value with mono-specific diets has been reported for other bivalve species (Milke et al., 2008; Rico-Villa et al., 2006; Robert et al., 2001). However, this is not always the case. Other studies demonstrated better growth and survival with monoalgal diets such as *C. calcitrans* in *N. subnodosus* (Cerón-Ortiz et al., 2009; Lora-Vilchis et al., 2004) and *Crassostrea corteziensis* larvae (Rivero-Rodríguez et al., 2007), relating these results to higher EPA content in the algal strain. Similar results were observed by Farías et al. (2003) when *I. galbana* (rich in DHA) was supplied to *Argopecten purpuratus* larvae. An alternative explanation to lower growth rates obtained in this study with CG might be related to lower ingestion rates caused by the size and pronounced spine structure characteristic of *Chaetoceros* species, such as those described with *C. calcitrans* and *Chaetoceros muelleri* in *Pteria sterna* (Martínez-Fernández et al., 2004). Finally, the hypothesis that the relatively higher larval density observed in the treatment with CG, produced lower algal feed concentration per larvae, and as a result, may have lead to a deficiency in the diet and subsequent low growth rate is not discarded.

The larval growth rates ranged between 3.1 and 5.5 μm day^{-1}, depending on the diets provided. Growth rates between 3.1 and 5.5 μm day^{-1} were considered moderate for *Pecten maximus* (Cochard and Gérard, 1987). Daily growth rates reported in this study are lower than those cited by Soria et al. (2010) for the same species (12.4 μm day^{-1}), which can be partially explained by higher water temperature reported by these authors (30.3 ± 0.8 °C). Nevertheless, larval growth rates recorded in the present study are comparable with studies on larvae growth in pectinid species such as *A. purpuratus* (3.5–7.3 μm day^{-1}; Cantillánez et al., 2007), *P. maximus* (4.8 μm day^{-1}; Magnesen et al., 2006), *Patinopecten yessoensis* (4.3 μm day^{-1}; Thompson et al., 1985), *A. balloti* (6.3 μm day^{-1}; Rose et al., 1988), *C. asperrimus* (5.6 μm day^{-1}; Rose and Dix, 1984), and *Placopecten magellanicus* (2.5–4.0 μm day^{-1}; Dadswell et al., 1987). The nutritional value of microalgae is not only related to its biochemical composition, but also to its size, digestibility, and even larval and microalgae concentrations (Brown et al., 1989; Marshall et al., 2010). Further studies need to be carried out to address the effects of these factors on *S. limbatus* larvae.

The survival of *S. limbatus* larvae at the end of the experiment was considerably low (between 1.0 ± 0.6% and 18.8 ± 4.3%, fed with IG and CG, respectively). Factors that may contribute to high mortalities are related to larval origin, rearing density, microalgae quality and quantity, and water conditions (Liu et al., 2006; Magnesen et al., 2006). Daily mortalities obtained in this study might be attributed to bacterial load associated with algal cultures that were introduced in rearing tanks, as reported in other studies (Magnesen et al., 2006; Nicolas et al., 2004). Soria et al. (2010) attributed larval mortalities of *S. calcifer* to a higher input of pathogens associated with higher algal cell concentrations (70 cell μl^{-1}). Similar low survival has been reported for *Aequipecten tehuelchus* larval (approximately 10% at day 12), citing a bacterial infestation as the main cause (Narvarte and Pascual, 2003), and *Pinctada margaritifera*, with less than 10% during larval culture (Doroudi et al., 1999). In contrast, better survival obtained in larvae fed on CG alone could be attributed to a lower energy demand in smaller larvae (136.5 ± 1.4 μm) in comparison with those pre-metamorphic larvae recorded in other diets at day 12. It has been reported that prior and during metamorphosis of bivalves, the energy reserves are considerably consumed, resulting in many cases in high mortalities (Labarta et al., 1999; Videla et al., 1998). Studies to identify pathogens and their control on the larval development of *S. limbatus* should be performed in order to optimize spat production techniques.

In conclusion, the embryogenesis and larval development of the *S. limbatus* are similar to other Pectinidae species. Nevertheless, the non-production of byssus and the no cementation observed throughout the metamorphosis and early postlarval stage, similar to that observed in other *Spondylus* species, suggest that the delay in post-larval settlement is characteristic of the genus *Spondylus*. In regard to larval development, the algal diet based on *C. gracilis* and *I. galbana* at a 3:1 ratio provided the best growth for *S. limbatus* until day 12, while the *C. gracilis* diet (CG) resulted in the best larval survival rate. Future research will be focused on improving survival rates of competent *S. limbatus* larva by controlling or reducing bacterial load in culture tanks using probiotics and water treatment systems. Focus should also be given to determine the biological, chemical and physical cues that potentially stimulate metamorphosis, attachment and cementation on this species.

Acknowledgments

We thank Daniel Rodríguez, Ramiro Solórzano, Adrian Márquez, and the staff of the Mollusc and Phytoplancton departments of CENAIM-ESPOL for their help during the experiment and José Alió for the English revision of the manuscript. This study was performed as part of the

Research Project “Maricultura y Piscicultura para el Fomento Acuícola en el Ecuador” financed by the Undersecretary of Aquaculture, Ministry of Agriculture, Fisheries and Agriculture (MAGAP). Cesar Lodeiros participated in the study during his linkage to CENAIM-ESPOL, through the Prometeo Project of the Secretaría Técnica de Educación Superior, Ciencia, Tecnología e Innovación of the Republic of Ecuador (SENESCYT).

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