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Research Article

Growth and survival analysis of early stages in the pen shell *Atrina maura* during pilot-commercial production

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ABSTRACT. Hatchery spat of pen shells is difficult to obtain as production runs generally fail. We present a case of commercial pilot spat production of *Atrina maura* in the hatchery, describing each phase in detail and determining the growth of larvae and postlarvae using multi-model inference (MMI). Growth rates of larvae in D-veliger to umbo stage (D-larvae = 65 ± 5.1 µm height and 75 ± 5.1 µm length) increased from 4.33 µm d^{-1} up to 675.6 µm d^{-1} during postlarvae stage (spat = 10.9 ± 2.2 mm height and 28.1 ± 4.4 mm length). Survival presented a significant daily decrease from 22.5% (beginning) to a final absolute survival of 0.042%. Hence, 50,000 commercial spats were produced from 120 million D-larvae. MMI showed that the best-fitting model describing growth corresponds to a Gompertz model for shell length and an exponential model for shell height. Critical phases were transitioning from D-larvae to the umbo stage (>80% collapse of cultures) and high mortality during metamorphosis. Further studies are required to find solutions to these problems. The results of this study may contribute to improving the management of pen shell production in the hatchery, as the production protocol is different from those used for other bivalve species.

Keywords: *Atrina maura*; umbo larvae; metamorphosis; mortality; growth models; hatchery; spat

INTRODUCTION

The pen shell *Atrina maura* (Sowerby, 1835) is a bivalve belonging to the Pinnidae family, which is composed of 55 species divided into three genera; *Pinna* (Linnaeus, 1758), *Streptopinna* (Martens, 1880), and *Atrina* (Gray, 1842) (Lemer et al. 2014). These large bivalves are commonly known as pen shells or razor shells due to their triangular shape; they inhabit open sea bays and coastal lagoons at various depths and can be found semi-buried in sand, mud, or sand-mud sediments (Idris et al. 2008). Natural populations of pen shells in the world, and particularly of *A. maura*, have been overexploited because their exquisite adductor muscles (anterior and posterior), which are highly prized in the markets (US$ ~28 kg^{-1}) (Liu et al. 2009, Fu et al. 2010). Other parts of their bodies are also appreciated for marketing, such as valves, mantle, and even the pearls that they can produce (Baqueiro & Castagna 1988, Beer & Southgate 2006, Ángel-Pérez et al. 2007, Lemer et al. 2016).

Aquaculture practices have been proposed as a logical alternative to address the problem of overexploitation and the consequent worldwide reduction of pen shell wild populations. Sustainable aquaculture management of any bivalve requires a reliable and constant supply of spat and cultivation methodologies permitting the development of organisms to a marketable size (Chávez-Villalba et al. 2022). Unlike most pen shells, *A. maura* is one of the most studied, focusing on broodstock conditioning (Rodríguez-Jaramillo et al. 2001, 2004, Enríquez-Díaz et al. 2003) and production of larvae and postlarvae in hatcheries at pilot and commercial levels (Robles-Mungaray et al. 1996, Melguizo-Robles 2011, Escamilla-Montes et al. 2017). It has served to carry out field culture trials that

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have shown the viability of achieving the production of fully-grown organisms (Miranda-Baeza 1995, Cardoza-Velasco 1998, Mellado-Moreno & Hoyos-Chairez 2008, Leal-Soto et al. 2011, Góngora-Gómez et al. 2016). Despite these encouraging results, no pen shell farm has maintained constant production because spat supply in the field is not guaranteed. These constant spat shortages seem common for all species of pen shells that have been successfully cultivated at some point (Chávez-Villalba et al. 2022).

Spat production in hatcheries has proven essential for developing cultivation activities in some species of bivalves, such as *Crassostrea gigas* (Reynaga-Franco et al. 2020). For instance, the production of Pacific oysters in various countries depends completely on hatchery-grown spat because of low natural or highly intermittent natural availability that does not guarantee the necessary supply (Nel et al. 2014). In the case of *A. maura*, natural spat collection is erratic, intermittent, and seems to decrease yearly; thus, it cannot provide the required amounts to sustain commercial cultivation ventures (Góngora-Gómez et al. 2016). Hence, it has been grown in controlled conditions, but the success rate is low, and most runs fail (Leal-Soto et al. 2011), thus discouraging commercial hatcheries from producing this pen shell. Preliminary studies on early development under hatchery conditions, identification of critical events, and growth performance analyses are necessary to change this situation.

The growth of bivalve larvae, including that of *A. maura*, has been characterized by size (height and length of the shell) and age parametrization. Obtained growth rates are one of the main attributes of selecting a species for breeding purposes in commercial aquaculture (Castillo-Durán et al. 2016, Reynaga-Franco et al. 2019, Hoyos-Chairez et al. 2020). The growth analysis in living organisms requires mathematical models that can be selected according to the Akaike information criterion (AIC) (Aragón-Noriega 2013). Therefore, it would be pertinent to determine the growth models that best describe growth during the species' early development.

Our laboratory has produced bivalve spat of several species for many years, *A. maura* being one of the most difficult to produce. In this scenario, the general aim of this study was to analyze a pilot-commercial run for the spat production of this species at Centro de Reproducción de Especies Marinas (CREMES) in Sonora, Mexico, allowing us to establish several more concrete objectives: a) describe growth and survival of larvae and postlarvae during commercial hatchery operations; b) evaluate the cultivation phases showing critical aspects; and c) use multi-model inference to assess the early growth of *A. maura*.

MATERIALS AND METHODS

Broodstock conditioning and spawning

The collection of adult breeders of *A. maura* was carried out in October 2008 near the coast of Bahía de Kino and Bahía Kunkaak in Sonora, Mexico (28°45'-28°55'N and 112°05'-111°58'W). Pen shells were extracted from wild populations using a hookah diving system; 50 individuals with >23 cm shell height and >15 cm shell length were selected. According to Camacho-Mondragón et al. (2014), *A. maura* is hermaphrodite and reaches its first maturity at 23.3 cm for females and 22.8 cm for males. Autumn is when pen shells are reproductively active (Ángel-Pérez et al. 2007), so an advanced stage of gametogenesis was expected. Extracted pen shells were enfolded with paper (newspaper type), humidified with seawater, and then placed in coolers containing commercial cold freezer packs to maintain a temperature of around 15-20°C. The animals were transported to CREMES, a commercial hatchery run by the Instituto de Acuacultura del Estado de Sonora (IAES) at Bahía de Kino (Mexico). Once in the hatchery, pen shells were cleaned of epibionts and sediment. Then two specimens were opened to take a smear sample from the gonads to determine their sexual maturity. It was determined that the gametes were still immature, so the broodstock conditioning protocol was implemented.

Pen shells were placed within 500 L circular fiberglass tanks under the following conditions: filtered (5 µm) and UV-treated seawater flowing at 10 L min^{-1}, temperature of 22-24°C, salinity of 36 and 5-6 mg L^{-1} of dissolved oxygen. The daily diet was a mixture composed of three microalgae species; *Isochrysis* sp. (clon T-ISO), *Chaetoceros calcitrans* (clon CCAL), and *Dunaliella tertiolecta* (clon DUN) in a proportion of 3:3:1 and equivalent to 3-5% soft tissue dry weight. Mature gonads were detected after three weeks of conditioning, and after this, spawning was induced using thermo-stimulation. The seawater used for spawning had the same filtering and treatment conditions as that used for conditioning. Pen shells were first placed for 45 min into a tank (500 L) containing filtered seawater at 18°C, then the organisms were moved to another tank (35 m^3) containing seawater at 28°C and remained for 45 min; pen shells were moved between tanks until the release of gametes initiated. All individuals that began to spawn were quickly placed in the large tank, and

fertilization occurred there. Pen-shell breeders were kept in the conditioning system for five months and two weeks.

Larval and postlarval culture

The larval culture was carried out in 35 m^3 circular fiberglass tanks using filtered (5 µm) and UV-treated seawater at 27 ± 1°C, a salinity of 36, and a gentle aeration to maintain oxygen concentration of 5-6 mg L^{-1}. Seawater was replaced after 24 h of culture (first day), and seawater changes (100%) were conducted every 48 h. During water changes, larvae were retained in circular fiberglass sieves (45 cm diameter × 30 cm high) with bottom mesh openings ranging from 40 to 315 µm. Larvae culture density was adjusted in every seawater replacement from 4 larvae mL^{-1} at the beginning to 0.3 larvae mL^{-1} by the end of the culture. Feeding consisted of a monoalgal T-ISO diet the first 10 days after spawning (DAS), and then it changed to a bialgal diet of T-ISO and CCAL (1:1 ratio) until 29 DAS; microalgae concentration was 30×10^3 cells mL^{-1} the first three days of the culture, and then it was gradually increased to 70×10^3 cells mL^{-1} by the end of the culture. Larvae samples of 100 mL were taken before each water change to register the height (dorsal-ventral axis) and length (anterior-posterior axis) in 30 individuals using an ocular micrometer incorporated into a compound microscope.

By the end of larval culture (29 DAS), pediveliger larvae were recovered in a 315 µm mesh sieve to calculate the total number of larvae following a gravimetric method using the equivalence of 30 g per million individuals (Robles-Mungaray et al. 1996). Pediveligers were placed within square fiberglass units (50×50×10 cm) with a 200 µm mesh in the bottom for the settlement and metamorphosis phase. The culture units were arranged on a table. In each one, a flow of seawater of 3 L min^{-1} was maintained in a downwelling direction, creating a recirculation system that distributes the water with a PVC pipe of 3/4" diameter and 1/8" bottom outlet for each unit. The system is complemented with a fiberglass reservoir (500 L) and a 1/6 Hp submersible pump. The same culture conditions as in the larval phase were used for this stage, but seawater changes were 150% daily. Larvae density in each unit was maintained at 30-40 larvae cm^{-2}, and no additional substrate was used. Feeding consisted of the same mixture and proportion of microalgae (T-ISO and CCAL) but at a concentration of 80×10^3 cells mL^{-1}.

First, postlarvae (spat) showing dissoconch were harvested and maintained in the same system as the initial nursing stage, but density was reduced to 10-20 ind cm^{-2}, and feeding increased to 90×10^3 cells mL^{-1}. Postlarvae remained in this system until reaching 2 mm (40 DAS) and then moved to a nursing section. A sample of 30 spat was taken for size measurements using millimeter paper under a dissecting microscope. The nursing section uses unfiltered and non-sterilized seawater flowing at 10 L min^{-1} in an upwelling direction; density was adjusted to 10 spat cm^{-2}, and feeding consisted of a mixture of T-ISO, CCAL, and DUN (3:3:1 ratio) with an initial concentration of 90×10^3 cells mL^{-1} that was increased up to 150×10^3 cells mL^{-1} by the end of the nursing stage. Seawater featured a temperature of 25 ± 1°C, a salinity of 36, and oxygen levels of 4-5 mg L^{-1}. Samples of 30 spat were periodically obtained to register height and length shell increments using a digital caliper. Survival was estimated as the difference in the number of spats at the beginning and the end of the nursing phase, estimated with the gravimetrical method.

Data analysis

Growth of larvae and postlarvae was described using age, shell height, and length (µm) (mean ± standard deviation; n = 30) and related using linear regression to compute the isometric relationships. Absolute growth rates (AGR) were calculated for both average height and length (mm) according to Hoyos-Chairez et al. (2020): $AGR = V_f - V_i / t_1 - t_0$, where V_f is the dependent variable (height or length) after the experimental period, V_i is the initial value of the dependent variable, t_1 is time (days) at the end, and t_0 is the initial time. Similarly, accumulated survival was estimated as $\%S = N_t \times 100 / N_0$, where S is the survival rate, N_t is the number of postlarvae, and N_0 is the number of "D" larvae. Survival of critical stages was calculated using this equation.

Growth model selection

The statistical package XLSTAT (Addinsoft 2022) was used to analyze, through multi-model inference, different mathematical models of growth applied to the observed age data (DAS) related to the variables of height and length of the shell (µm). Compared models included Gompertz, beta, exponential, logistic, and asymptotic regression. The package selected the best model based on the lowest value of the statistic according to the AIC and corrected AIC (AICC). In addition, it generates the main statistics according to the least squares method and describes equations with their parameters for the selected models. Finally, it provides size-age graphics, including raw data, confidence limit, and 95% prediction interval.

RESULTS

Broodstock conditioning

During this period, eight production runs were attempted, but production of commercial size spat was reached only once. Spawning was achieved in six runs, but larvae reached the early umbo phase and died (8 to 13 days of culture). There was also spontaneous spawning, and larvae were produced but only attained the early umbo phase (10 days of culture), and only on one occasion did pen shells do not spawn (Table 1). Eight initial 50 pen shell breeders were sacrificed to assess gonad maturity, four died, and 38 broodstock survived and were returned to the field.

Growth

The initial growth of *A. maura* larvae from D-veliger (65 ± 5.1 µm height and 75 ± 5.1 µm length) to umbonate (145.7 ± 16.3 µm height and 135.7 ± 16.3 µm length) was relatively slow with an AGR of 5.76 µm d^{-1} for height and 4.33 µm d^{-1} for length. During this phase, it was observed that height was smaller than the length in the first five DAS but tended to be similar from day 7 to 13 DAS, and by the end, (15 DAS), height growth exceeded that of length (Fig. 1, Table 2). At the next stage, from umbo larvae to pediveliger (400.3 ± 32.6 µm height and 415.7 ± 39.1 µm length), AGR increased to 18.2 and 20.0 µm d^{-1} for height and length, respectively. During the first 10 days of this stage, shell height was always greater than length, but at the end of the stage (24-29 DAS), the morphological relationship shifted again, and shell height became smaller than before. From 29 DAS and after, individuals developed a prominent foot that reached around 300 µm and allowed them to crawl actively. Part of the larval population tends to "float" on the seawater surface of the tanks; most are non-viable larvae that do not grow properly and end up dying, beginning to be observed from the third DAS and gradually decreasing as the pediveliger larvae appear-for instance, none of the larvae that float and those that do not have an eyespot.

The third stage (metamorphosis) was the shortest since it only lasted six days. Still, it was characterized by substantial morphological changes in pen shell larvae, loss of cilia and development of branchial arches, and disoconch formation with protruding growth lines (Fig. 2, Table 2), resulting in newly formed postlarvae or spat (566.7 ± 112.4 µm height and 875 ± 127.1 µm length). Compared to the previous stage, the AGR increased again, especially for shell length that reached 79.1 µm d^{-1}, whereas 25.2 µm d^{-1} was recorded for shell height. By the end of this period, postlarvae featured greater shell length than height. In the last stage, AGR showed greater values (256.52 µm d^{-1} for height and 675.6 µm d^{-1} for length) than in previous phases. At the postlarvae stage, the dorsal-ventral axis corresponding to height continued to be smaller than the anterior-posterior axis (length). Spat reached the commercial size at 70 DAS, showing 10,933.3 ± 2180.4 µm of height and 28,100 ± 4373.5 µm of length.

The height-length relationship for the shell of *A. maura* is shown (Fig. 3). It is notable that, although the linear model of this relationship underestimates the values for height during the intermediate stage and overestimates them in the final part of the early development, the correlation coefficient still explains more than 99% of the variation.

Survival rates

Survival concerning age and developmental stages of *A. maura* showed that the percentage of live larvae presented a significant daily decrease. From D-larvae to the 9th DAS, survival was only 22.5% of the initial population (Fig. 4). In the phase of umbo larvae (9-15 DAS), absolute and relative survival was 13.7% (Table 3). For the stage from umbo to pediveliger (15-29 DAS), survival continued to decrease but to a lesser extent, with 4.2% absolute survival and 26.5% relative survival. The metamorphosis period showed the study's lowest relative survival (1.2%). Finally, the greatest relative survival was observed in the postlarval stage (84.0%), with a final absolute survival of 0.042%, which means that out of 120 million D-larvae, 5 million pediveligers were achieved. Still, after the metamorphosis, only 60,000 postlarvae were alive and available for the beginning of the nursing stage, with a final production of 50,000 commercial spat.

Model fitting and selection

Table 4 presents the most important statistics of the five non-linear growth models proposed to describe the increase in shell height and length and their relationship with age in early individuals of *A. maura*. For shell height, the selected model corresponds to an exponential equation since it showed the lowest value of AIC and AICC (6841.62), followed by the Gompertz model (6842.91), while the logistic model was the least recommended (8493.45). Figure 5a represents the resulting curve, with confidence intervals and prediction intervals at 95%, according to the selected model and the following equation:

$$\text{Height } (\mu m) = 50.4856 \times \exp(0.076849 \times \text{age (DAS)})$$

Table 1. Runs attempted to produce spat in *Atrina maura* in the hatchery (CREMES).

Spawning dates (2008-2009)	Total number of breeders (spawning ♂/♀)	Number of D-larvae obtained	Cultivation days	Stage reached, size
November 2	42 (3/2)	150×10^6	12	Early umbo, 130 µm
November 18	42 (6/7)	360×10^6	8	Early umbo, 120 µm
December 2	41(10/6)	120×10^6	70	Spat >10 mm
January 16	40 (6/7)	210×10^6	9	Early umbo, 120 µm
January 30	40 (0/0)	-	-	-
February 2	Spontaneous spawning	320×10^6	10	Early umbo, 125 µm
February 16	39 (7/7)	125×10^6	13	Early umbo, 130 µm
March 20	38 (6/7)	390×10^6	8	Early umbo, 110 µm

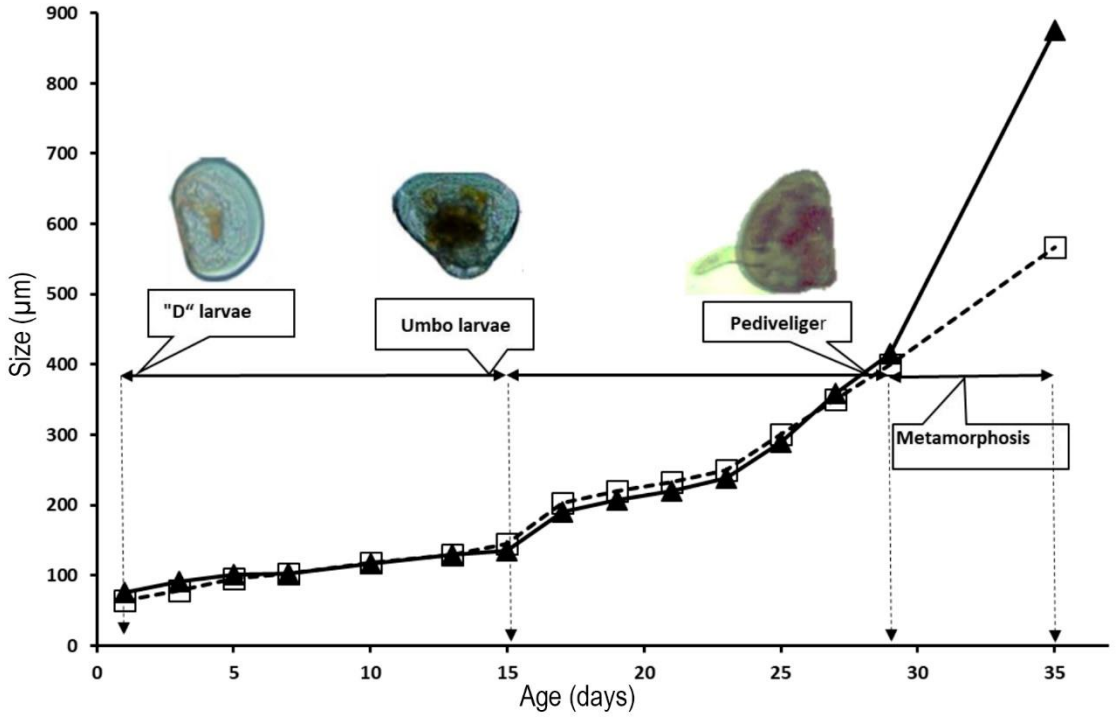

Figure 1. Variation of mean shell height (dotted line) and length (solid line) in *Atrina maura* during larval and metamorphosis stages. Images of developmental stages are shown according to age.

In the case of shell length, the Gompertz model was selected as the one with the best fit to the observed data since the values of the AIC and AICC (7622.25) correspond to those of lesser magnitude, followed by the beta model (7622.34), while the logistic model is the least adequate (9536.61). Figure 5b shows the generated curve, with confidence intervals and prediction intervals at 95%, according to the selected model and the following equation:

Length (µm) = 180192 × exp (-exp (-0.0274109× (age (DAS)-92.6132)))

DISCUSSION

Descriptions of larval development for *Atrina maura* have been reported in various contributions (Serrano-Guzmán 1996, Goldchain-Goldin 2010, Melguizo-Robles 2011, Ángel-Dapa et al. 2015), as well as the spat production of the species (Mellado-Moreno & Hoyos-Chairez 2008, Leal-Soto et al. 2011). These last reports, however, only provide a general biological context and do not offer details of the biological stages or the technical cultivation processes. In this study, the production processes of each phase are described in

Table 2. Absolute growth rates (AGR) for developmental stages in *Atrina maura* during hatchery production.

Stages	Age (days)	AGR height (µm d^{-1})	AGR length (µm d^{-1})
D-larvae - umbo larvae	1 to 15	5.76	4.33
Umbo larvae - pediveliger	15 to 29	18.18	20.00
Pediveliger - postlarvae (disoconch)	29 to 35	27.33	76.55
Postlarvae (disoconch) - comercial postlarvae	35 to 70	296.18	790.98

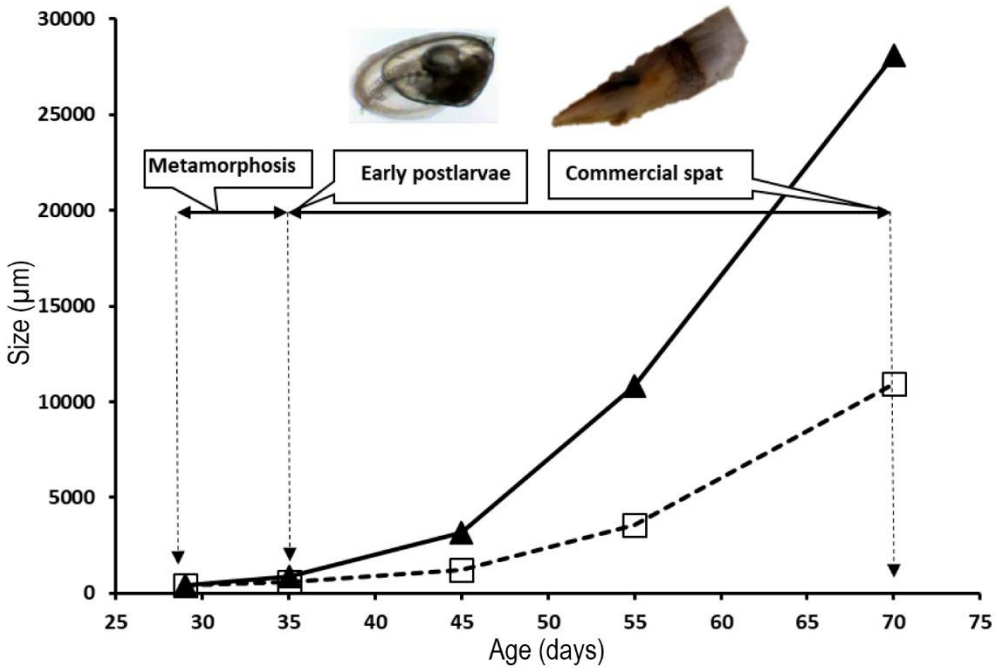

Figure 2. Variation of mean shell height (dotted line) and length (solid line) in *Atrina maura* during the metamorphosis and nursing stages. Images and developmental stages are shown according to age.

detail, and specific information on larval and post-larval development stages is provided. This information is relevant to continue working on technical aspects to improve survival during critical biological stages needed to advance spat production in pen shell species.

It is well known that, among bivalves, pen shells feature the highest growth rates from the young to adult stages (Narváez et al. 2000, Aucoin & Himmelman 2011). Nevertheless, it is unclear whether this also occurs during the early development of these organisms. According to the results of our hatchery, the larval development for *A. maura* is much faster than for any other bivalve under similar culture conditions. For example, the average daily growth for shell height of cultured *A. maura* during larval and postlarval development is 0.155 mm d^{-1}, which is higher than those reported for the pearl oyster *Pteria sterna* (0.096 mm d^{-1}) (Hoyos-Chairez et al. 2020), and for the smooth Venus clam *Chionista fluctifraga* (0.030 mm d^{-1}) (Hoyos-Chairez 2014, Castillo-Durán et al. 2016). However, survival at the end of spat production goes the opposite; 40.0% for *C. fluctifraga*, 0.25% for *P. sterna*, and 0.04% for *A. maura*. The review by Marshall et al. (2010) about the effect of nutrition on larval growth in several species of bivalves shows that the highest growth rates of *Ostrea edulis* (0.012 mm d^{-1}), *Venerupis philippinarum* (0.014 mm d^{-1}), *Crassostrea gigas* (0.006 mm d^{-1}), and *Pecten maximus* (0.008 mm d^{-1}), are still lower than previous results. Nevertheless, it is important to consider that the former review only reports rates during larval growth, not during the combined larval and postlarval phases as in our study. Unfortunately, growth rates during early development are not routinely reported, and to our knowledge, this is the first work on the growth rates of *A. maura* during its early development.

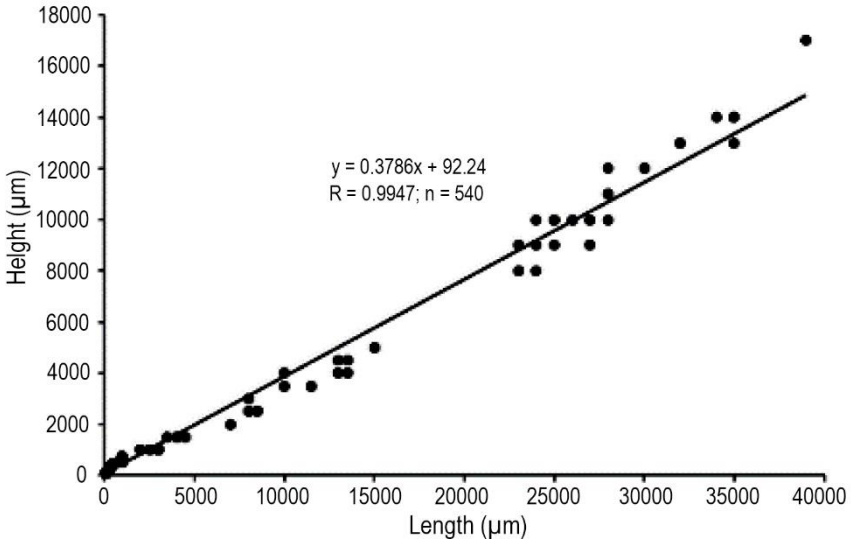

Figure 3. Height and length (µm) relationship in *Atrina maura*. The model equation, correlation coefficient (R), and number of data (n) are shown.

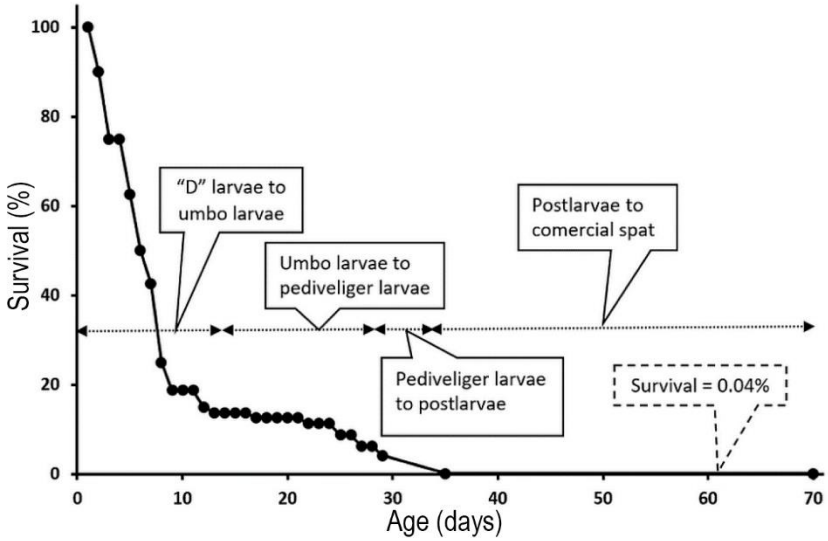

Figure 4. Survival (%) throughout developmental stages in *Atrina maura* during hatchery production.

The first general result of the present study confirms the difficulty of producing pen shells spat in controlled conditions (laboratory or hatchery; Zhou et al. 2020). This problem has been observed for *Atrina pectinata* in Japan (Ohashi et al. 2008), *Pinna nobilis* in Spain, and *A. maura* in Mexico. For this last species, Melguizo-Robles (2011) executed nine production runs, from which only two were successful (22.2%), while Ángel-Dapa et al. (2015) made seven runs, and in all of them, larvae died between 13 and 21 days of culture. In our study, the rate was also low, with 12.5% of success (one of eight). Although there are no published reports of commercial hatchery experiences, managers of several facilities also report low spat production rates, indicating that commercial production of the species is not profitable until now.

In most cases, larval cultures of *A. maura* collapse during the umbo larval stage. Attempts have been made to find causes and solutions to this problem by studying various aspects. For example, larvae transitioning from D-veliger larva to umbo larva of this species had high resistance to pathogens, particularly the *Vibrio alginolyticus* strain (Luna-González et al. 2002). Mortality during this phase was observed to occur in both diploid and triploid organisms (Robles-Mungaray 2004). Maeda-Martínez (2008) was one of the first to report

Table 3. Accumulated and relative survival (%) for the developmental stages of the pen shell *Atrina maura* during hatchery production.

Stages	Age (days)	Accumulated survival (%)	Relative survival (%)
D-larvae - umbo larvae	1 to 15	13.750	13.7
Umbo larvae - pediveliger	15 to 29	4.167	26.5
Pediveliger - postlarvae (disoconch)	29 to 35	0.050	1.2
Postlarvae (disoconch) - comercial postlarvae	35 to 70	0.042	84.0

Table 4 Main statistics for the five growth non-linear models adjusted with shell height and length data related to the age in *Atrina maura*. SEC: sum squared error, MEC: mean squared error, RMSE: root mean squared error, AIC: Akaike correction criterion, and AICC: Akaike corrected correction criterion. Significant values of each estimator of prediction error models are indicated in bold.

Statistics	Gompertz	Beta	Exponential	Logistic	Asintotic regression
Shell height					
SEC	169576355.1	181202126.5	169798677.8	3604248374.2	169703791.2
MEC	315784.646	337434.128	315610.925	6711821.926	316021.958
RMSE	561.947	580.891	**561.793**	2590.718	562.158
AIC	6842.912	6878.719	**6841.620**	8493.457	6843.318
AICC	6842.912	6878.719	**6841.620**	8493.457	6843.318
Shell length					
SEC	817425758.	817554438.8	1008537242.3	24875474779.8	926799545.7
MEC	1522208.11	1522447.74	1874604.54	46323044.28	1725883.69
RMSE	**1233.778**	1233.875	1369.162	6806.103	1313.729
AIC	**7692.255**	7692.340	7803.707	9536.613	7760.067
AICC	**7692.255**	7692.340	7803.707	9536.613	7760.067

that part of the larvae floated and died, whereas Goldchain-Goldin (2010) found that floating larvae could be swimming again and that aeration is not related to larval buoyancy. Robles-Rocha (2010) determined that the floating larvae have significantly more lipids than the swimming ones and that there was no perceptible difference in the morphology of the two of them.

Nevertheless, in a finer study of larval morphology, Chacón-Ojeda (2010) found that successful cultures were related to rounder larvae that had changed between height and length; if the larva was taller than long, then it was longer than tall or *vice versa*, and this was called morphometric transition. Melguizo-Robles (2011) mentions that the larval and postlarval culture of *A. maura* differs from other bivalves since larger volumes of water are required to guarantee survival. Finally, Ángel-Dapa et al. (2015) suggest that spawners should be collected in January (low temperature), at 5-8 m depth and during ebb tides to have a higher condition index, higher percentages of mature gonads, resulting in better spawning and larval culture results. Despite all this knowledge, the problem persists, evidence that more studies are necessary to find an integral solution. Some hatchery managers are trying to produce *A. maura* spat by performing several runs and retrieving non-floating larvae to be cultured in large volumes of water. If they succeed, they will sell the spat at three times its original price (Chávez-Villalba et al. 2022). In our study, larvae buoyancy was observed 72 h after spawning, while in *A. pectinata*, this peculiarity was observed after 48 h (Guo et al. 1987); in both species, buoyancy disappeared when larvae entered the pediveliger stage.

Larvae that survive beyond the umbo phase confront a second critical point during the metamorphosis. This stage was characterized by the lowest relative survival of the entire culture cycle (1.19%), like values previously reported for the species (Robles-Mungaray et al. 1996, Melguizo-Robles 2011) and for *A. pectinata* (Ohashi et al. 2008). These last authors are the only ones who comment on the many difficulties encountered when producing this species of pen shell, as larvae and juveniles die by suffocation.

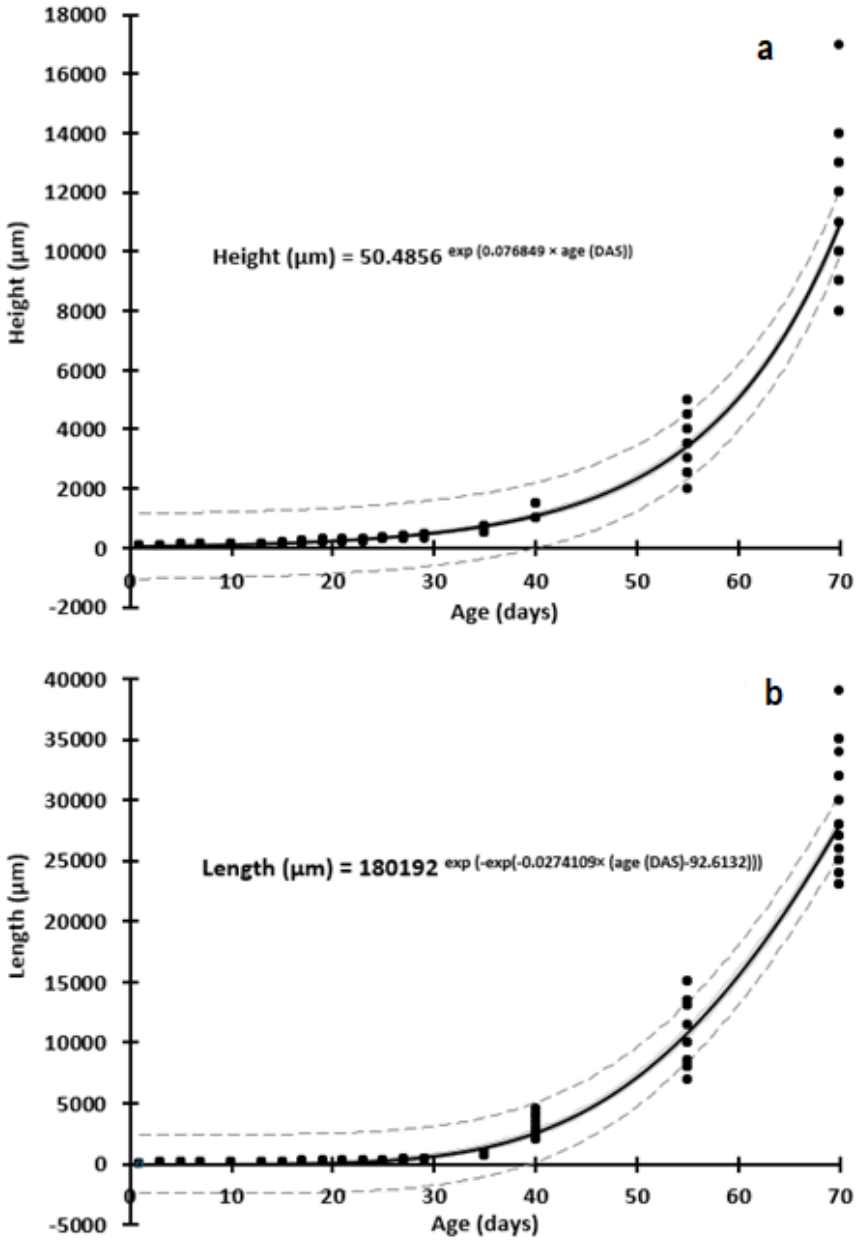

Figure 5. Growth curves of a) shell height and b) length and their relationship with age (days after spawning) in *Atrina maura*. Observed data (•), curves concerning the corresponding models (solid line), and 95% confidence limits (scattered lines) are indicated.

Bivalve hatcheries generally do not report these issues. However, there is a study on the pearl oyster *P. sterna* indicating that like our study, the transition from D-larvae to umbo larvae and metamorphosis are the critical phases during spat production (Hoyos-Chairez et al. 2020). Nevertheless, it should be noted that there are other phases for *A. maura* production where no inconveniences have been reported, such as broodstock conditioning, spawning, the transition from umbo larvae to pediveliger, and the overall nursing stage.

The morphometric transition observed by Chacón-Ojeda (2010) and related to a successful culture of *A. maura* was also detected in our study. We noticed during the larval development of the species that the relative extent of shell height (SH) *vs*. shell length (SL) seems to shift periodically over several days. For example, for D-veliger larvae, SL is greater than SH; at the end of the umbo larvae phase, SH is greater than SL, and during the transition from the umbo to pediveliger, SL progressively increases again and reaches greater

values than SH. After metamorphosis, the SL of postlarvae is always greater than the SH. These changes in shell relative proportions had not been previously reported for any species within the Pinnidae or other species of bivalves. In terms of larvae size, the D-veliger larvae of *A. maura* (65 µm SH, 75 µm SL) are similar to that of *Atrina tuberculosa* (67-70 µm SL) (Niebla-Larreta 2006). In contrast, all larval stages are smaller than in *A. pectinata* (Ohashi et al. 2008). For example, *A. maura* showed 400 µm SH, 414 µm SL for pediveligers, and 567 µm SH, 875 µm SL for early postlarvae, while sizes in *A. pectinata* were 520 µm SH, 545 µm SL and 600 µm SH, 900 µm SL, respectively for each stage (Guo et al. 1987). It was possible to observe a coincidence of greater SL than SH for pediveliger of *A. pectinata* respect *A. maura*, but for postlarvae occurred, the opposite. The results of Guo et al. (1987) and our results indicate that pediveliger larvae in these species do not present eyespot but a prominent foot.

Modeling

Most published studies on growth patterns in juveniles and adults of *A. maura* from field cultivation have used growth rate or a single mathematical model (generally Von Bertalanffy or exponential) as the criterion for analysis and comparison (Leal-Soto et al. 2011, Góngora-Gómez et al. 2016). Only Aragón-Noriega (2013) reported a multi-model approach for field cultivation from juveniles to adults of *A. maura* and selected the Gompertz model according to the lowest value of AIC. Nevertheless, in the former study, the values of the logistic and Schnute models were very close to the value of the Gompertz model. This result coincides with the model selected in the present study for shell length using the same selection criteria. In the case of the relationship between age (DAS) and SH, the best fit was an exponential equation, but only with a difference of 1.292 less than the Gompertz model, which was the second most suitable. The Gompertz and exponential growth equations belong to the non-asymptotic and potential form, with small increments early in life and larger increments as the organism grows old. This form of growth occurs in *A. maura* considering both the larval-postlarval and juvenile-adult stages, so the Gompertz model is appropriate for this species and possibly other pen-shell species.

CONCLUSIONS

The spat production protocol for pen shells differs from that generally used for most bivalve species (Guo et al. 1987, Ohashi et al. 2008) since it is necessary to select swimming larvae that require double or triple the amount of water to minimize mortality. Here we present a complete production cycle with detailed descriptions from D-veliger larvae to commercial spat stages. This information could progress the production of *A. maura* in the hatchery and serve as a basis for the spat cultivation of other pen shell species. We confirmed that the most difficult phase to overcome is that of the umbo larvae, but once it is surmounted, there are great possibilities of reaching the commercial spat stage. Metamorphosis is another critical stage, but it does not necessarily imply the production collapse but a phase with high mortality where alternatives should continue to be examined to reduce it. It is necessary to find solutions or alternatives to these problems as hatchery-produced spat seems to be the only viable option for pen shell commercial supply since spat collection in the field is not guaranteed.

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