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Article

Embryonic development, larval morphology and juvenile growth of the sea cucumber *Athyonidium chilensis* (Holothuroidea: Dendrochirotida)

Desarrollo embrionario, morfología larval y crecimiento de los juveniles del pepino de mar *Athyonidium chilensis* (Holothuroidea: Dendrochirotida)

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Resumen.- Los holotúridos son un valioso recurso para numerosas comunidades costeras en la región Indo-Pacífica. Sin embargo, la explotación no sustentable de muchas especies y el aumento de los precios del producto procesado (bêche-de-mer), han resultado en una disminución de las poblaciones naturales a nivel global. A pesar de que *Athyonidium chilensis* es el holotúrido de mayor importancia económica en la costa Pacífica Sur-este, los estadios de vida tempranos aún no han sido descritos. El desarrollo larval de *A. chilensis* comprendió dos estadios sucesivos, la larva lecitotrófica vitelaria y la larva pentáctula, en aproximadamente 7 días a 13 ± 0,3°C. Una vez asentados, los individuos fueron capaces de alimentarse activamente de microalgas asociadas al sedimento. Treinta y cinco días post-fecundación, los tentáculos bucales y el cuarto par de podios ambulacrales estuvieron completamente desarrollados. Después de 4 meses de cultivo, los juveniles (~1,4 mm de largo) presentaron un gran número de podios en la superficie corporal y algunos comportamientos típicos de individuos adultos fueron observados. La densidad de cultivo tuvo un efecto evidente en el crecimiento y la supervivencia de los juveniles, con las mayores tasas de crecimiento y las más altas supervivencias en el tratamiento con la menor densidad (1 ind cm^{-2}). El presente estudio provee la primera descripción detallada de los estadios de vida tempranos de *A. chilensis*, pero además demuestra que esta especie puede ser cultivada en hatcheries y que el cultivo masivo podría ser eventualmente desarrollado como una medida para mantener una extracción sustentable contribuyendo a la restauración de poblaciones naturales.

Palabras clave: Holotúrido, bêche-de-mer, vitelaria, pentáctula, acuacultura

Abstract.- Holothurians are a valuable resource for coastal communities in the Indo-Pacific region; however, the unsustainable exploitation of the species and the rising wholesale price of the processed product (bêche-de-mer) have resulted in the depletion of wild stocks worldwide. *Athyonidium chilensis* is the most economically important holothuroid in the Southeast Pacific coast; however, the early life-history of the species has not been previously described. *A. chilensis* developed through the lecithotrophic vitellaria and the pentactula larval stages in ~7 days at 13 ± 0.3°C. At settlement, individuals were capable of active feeding on micro-algae associated with the sediment. Thirty-five days post-fertilization, the buccal tentacles and the fourth pair of ambulacral podia were completely developed. After 4 months of cultivation, juvenile (~1.4 mm length) had a substantial number of podia on the body surface and some common adult behaviors were also displayed. An evident effect of the density during the culture of juveniles was observed, with higher growth rates and survival observed in the treatment with the lowest density (1 ind cm^{-2}). This study provides the first descriptions of the early life-history stages of *A. chilensis*, but also shows this sea cucumber can be successfully reared in land-based nursery systems. Culture of this species is feasible and could be potentially developed as an alternative to maintain a sustainable harvest by contributing to the restoration of natural populations.

Key words: Holothuroid, bêche-de-mer, vitellaria, pentactula, aquaculture

Introduction

Sea cucumbers are a valuable resource for coastal communities in the Indo-Pacific region, where they have been exploited unsustainably for decades. The fishing pressure exerted on the species has increased over recent years fueled by the rising wholesale price of the product (bêche-de-mer), resulting in the depletion of wild stocks worldwide (Purcell *et al.* 2002, Hamel *et al.* 2003, Ramofafia *et al.* 2003). The recent use of sea cucumbers in the pharmaceutical industry as antibacterial agents has also increased the demand of the species (see Al-Haj *et al.* 2009), encouraging an improvement of the artificial culture techniques and the acquisition of an adequate knowledge of critical phases in captivity. However, in the last decade, only a few commercial holothurians have been successfully reared to settlement, including some tropical and temperate species (*e.g.*, *Holothuria scabra*, *Holothuria fuscogilva*, *Apostichopus japonicus, Australostichopus mollis)* (reviewed by Ramofafia *et al.* 2003).

Even though the embryonic and larval development of some species of sea cucumbers has been well documented (Cameron & Fankboner 1989, Hamel & Mercier 1996, Battaglene *et al.* 2002, Hamel *et al.* 2003, Laxminarayana 2005, Asha & Muthiah 2002, Asha & Muthiah 2005), most of the species that have been studied belong to the order Aspidochirotida, characterized by an indirect development, *i.e.*, the gastrula develops to an auricularia larva (planktotrophic stage), that transforms into a non-feeding lecithotrophic dololaria (= vitellaria) before settlement at the pentactula stage (Sewell & McEuen 2002). However, direct development, in which the gastrula develops to a lecithotrophic vitellaria larva before settlement (*i.e.*, with no auricularia stage), is the most common form of development in holothuroids and is dominant in 22 of the 25 families (Smiley *et al.* 1991).

Within the family Cucumaridae, *Athyonidium chilensis* represents one of the most common species of echinoderms along the Chilean coast, with a geographic distribution that extends from Ancón, Perú (11º44'5"S), to Punta Gaviota in Chiloé Island, Southern Chile (42º03'55"S). This species inhabits intertidal and subtidal zones, where it can be found in rock pools and crevices, below boulders and also buried in the sand (Pawson 1964, Pawson 1969, Fernández 1998). Although *A. chilensis* is the most commercially important holothurian on the South-East Pacific coast, there is no documented information about the fishery before 1992, nor current records of the magnitude of the artisanal fishery currently operating in Chilean waters (SERNAPESCA 2003, Toral-Granda 2008). The average harvest of the species from 1998 to 2008 was 269 ton per year, with a maximum extraction of 1,510 ton in 2000 (Renbo & Yuan 2004, SERNAPESCA 2008, Toral-Granda 2008).The unsustainable exploitation of this resource due the growing demand from Asian markets forebodes a drastic decline in the wild stocks of *A. chilensis* in the near future. Therefore, studies focused on reproduction, development and feeding behavior in captivity are extremely important in order to understand the life history of the species, determine its possible aquaculture potential and also, make accurate decisions in terms of stock management.

Previous studies have contributed to understand some aspects of the biology, ecology and culture of *Athyonidium chilensis* (Fernández 1998, Guisado *et al.* 1999[1], Moreno 2002, Pérez 2005, González 2006, Fernández 2007, Maltrain 2007, Ruiz *et al.* 2007). However, the early life history of the species has not been completely described and there is a knowledge gap about larval morphology, development, settlement and juvenile growth. The present work was undertaken in order to: 1) characterize for the first time the early life stages of the sea cucumber *A. chilensis* and 2) evaluate the effects of the cultivation density on juvenile growth and survival.

Materials and methods

Broodstock collection and spawning induction

Between February 2005 and December 2006 a total of 112 sea cucumbers were collected from several locations around Valparaíso, Chile. Individuals, which are usually found burrowed in the sand in shallow waters (*i.e.*, 1-5 m deep) were collected by snorkeling or SCUBA, and then transported in insulated containers to the Facultad de Ciencias del Mar y de Recursos Naturales, Universidad de Valparaíso, Valparaíso, Chile. Once at the laboratory, specimens were placed in 1000 L tanks with running sea water at 13 ± 0.3ºC (mean ± SD) for a period of 24-48 h.

Typically, groups of thirty sea cucumbers with body lengths larger than 15 cm (*i.e.*, 250-400 g) were placed in five 67-L tanks (6 specimens per tank) filled with micro-

[1]Guisado C, J Saavedra & A Hernández. 1999. Inducción al desove y desarrollo larvario de *Athyonidium chilensis* (Semper, 1868). Libro de Resúmenes XIX Congreso de Ciencias del Mar, Chile, Impresos Universitaria, Antofagasta, pp 120-121.

filtered seawater (1 µm) and then induced to spawn by combining two different techniques, thermal stimulation (Battaglene *et al.* 2002, Giraspy & Ivy 2005, Laxminarayana 2005) and addition of food *ad libitum* using the micro-alga *Chlorella neustonica* (Pérez 2005).

Larval rearing and juvenile maintenance

When spawning was successful, female gametes were deposited in 3-L glass containers and male gametes were transferred to a 250 ml beaker. In order to carry out fertilization, 2 ml of spermatozoa were added to each 3-L containers obtaining an approximate gamete ratio of 100:1 (sperm-egg).

Once fertilization was verified under the microscope the eggs were transferred into five 67-L tanks (*i.e*., 3 eggs ml^{-1}) filled with micro-filtered and UV sterilized seawater. Fifty percent of the seawater in each tank was daily renewed from the second day of cultivation and a constant temperature was maintained (13 ± 0.3ºC [mean ± SD]). After 3 or 4 days post-fertilization (pf), only larvae swimming in the water column were selected and then transferred into a 1000-L tank (0.5 larva ml^{-1}). Two air stones positioned at the bottom of the tank provided continuous aeration and ensured gentle water circulation.

Embryonic and larval development was documented by taking random samples (30 larvae) every 15-30 min during the first 24 h pf; hereafter, samples were taken every hour until the early vitellaria stage was observed. Samples were photographed and measured using a light microscope (40X magnification) equipped with an ocular micrometer and also analyzed using a scanning electron microscope. After 10 days of cultivation (*i.e.,* when feeding behavior was observed), the larvae were fed daily with the microalga *Tetraselmis suecica* (MLB1094; 1,200 cells ml^{-1}), and after 42 days of cultivation, 0.5 g of Algamac 2000™ were also provided once a week. Once settlement was observed, 145 g of fine sand (> 0.5 mm) was added to the tank in order to increase survival. Hereafter, size was determined at daily or weekly intervals by taking measures of the maximum length (ML in µm, defined as the distance between the mouth and the anus; Hamel & Mercier 1996) of 30 individuals during the first 5 months of cultivation. Growth was evaluated using a polynomial regression. Survival was evaluated at days 1, 32, 60 and 157 by haphazardly placing six 100 cm^2 quadrats on the bottom of the 1000-L tank and estimating the density of individuals (ind cm^2) by counting the juveniles inside each quadrat.

Effects of cultivation density on juvenile growth and survival

Three experimental densities were evaluated in this study. Juveniles obtained in the laboratory (75 days pf; 620 ± 45 µm; N = 180) were placed in 3000 ml glass containers (25 cm diameter and 12 cm of height) at densities of 1, 3 and 5 ind cm^{-2} (*i.e.,* 64, 191 and 318 individuals per container, respectively), with each density being conducted in triplicate. Additionally, and based on preliminary experiments, 3 g of fine sand was used as a substrate. Seawater was renewed daily and a constant temperature was maintained (13 ± 0.3°C). Juveniles were fed on a daily basis with the micro-alga *Tetraselmis suecica* (1,200 cells ml^{-1}), and the supplement Algamac 2000™ was also added once a week. In all three treatments (1, 3 and 5 ind cm^{-2}), the ML of juvenile sea cucumbers were measured at the beginning and at the end of the experimental period (*i.e.,* day 75) using a light microscope at 40X magnification, allowing the estimation of the growth rate (GR) of juveniles as follow, $GR = (ML_1\text{-}ML_0)\ t^{-1}$, where: ML_1 = final maximum length (µm), ML_0 = initial maximum length (µm) and t = experimental period (days). Due the logistic of handling so many soft-bodied juvenile sea cucumbers, we were unable to follow individual traits during experiments. Therefore, we calculated the size for each period by averaging the ML of 20 haphazardly collected juvenile in each of the three replicate containers per treatment. After measurements, all individuals were returned alive to the corresponding containers. One-way analysis of variance (ANOVA) was used to test for differences in GR among the experimental densities examined. Normality and homocedasticity requirements were tested using Lilliefors and Levene tests, respectively, and data did not require transformation to meet ANOVA assumptions.

Survival was quantified twice a month by visually inspecting the total number of juvenile remaining in each of the replicate containers. Differences in survival of juveniles over time, among different experimental densities, and the corresponding interactions were evaluated using a two-way ANOVA. Significant differences were examined using Tukey's HSD multiple comparison tests. Normality and homocedasticity requirements were tested using Lilliefors and Levene tests, respectively. Since our response variable was based upon percentages (*e.g.*, % of juvenile remaining in each replicate container), data were arcsine transformed before analysis.

Results

Spawning and embryonic development

In general, the stress involved with collection and transportation of the specimens was not enough to induce them to spawn; however, the mixture of the two inducing agents (thermal stimulation, *ad libitum* food) seems to be a successful strategy for inducing spawning in wild broodstock. From a total of 112 specimens induced to spawn, only 40% spawned, usually in a 3:1 male-biased proportion. Invariably, males spawned before females, and in both sexes, gametes were released from a single gonopore at the top of the dorsal anterior end. Pre-spawning behaviors were not always clearly evident in both males and females; however, those individuals disturbed prior or during this process, usually failed to spawn.

The chronology of development for *Athyonidium chilensis*, with the corresponding size of the embryos and the photographic illustrations of the stages are presented in Table 1 and Figures 1 and 2. Once fertilization was completed, the estimated mean quantity of eggs in each spawning event was ~ 6 x 10^5. The eggs could be easily seen (360 ± 16 µm) and the fertilization envelope (fe) was observed after 10 min post-fertilization (pf) (Fig. 1A). The first cleavage occurred between 1 to 3 h pf. The second (Fig. 1B), third and fourth cleavages took place between 3 to 6 h pf. During these stages blastomeres (b) of equal size and spherical shape were observed, with a clear separation between them. The animal and vegetal poles continued dividing until the 32-cell stage. At this stage the fertilization envelope was still present (Fig. 1C). After 24-25 h pf, a process of embryo compaction was observed, characterized by a considerable reduction in the space between the blastomeres. After this process, an invagination of the blastoderm (ib) was observed, being the precursor of the blastula stage (Fig. 1D). Rotating movements and constant migration throughout the water column were also observed in the embryos, and after 48 h pf a clearly visible elongation of the blastula indicated the transformation into a gastrula (Fig. 1E). The blastopore was clearly visible in the aboral region (Fig. 2A, B) and a small lateral depression at the equatorial region of the embryo's body began to form, indicating the position of the future vestibule (Fig. 2C). After 4 to 5 d pf the embryo began its transformation into a vitellaria larva (Fig. 1F).

Table 1. Chronology of development and approximate sizes (mean ± SD; N = 30) of *Athyonidium chilensis* under laboratory conditions (13 ± 0.3 °C). Time of development is presented in hours (h) and days (d) / Cronología del desarrollo y tallas aproximadas (promedio ± DE; N = 30) de *Athyonidium chilensis* bajo condiciones experimentales (13 ± 0,3 °C). Tiempo de desarrollo es presentado en horas (h) y días (d)

Stage	Time (h, d)	Size (µm)
Fertilized oocyte	0 min	360 ± 16
2-cell	1-3 h	342 ± 14
4-cell	3-5 h	367 ± 72
8-cell	5-6 h	358 ± 14
16-cell	6-8 h	400 ± 25
32-cell	8-9 h	442 ± 14
Blastula	24-25 h	325 ± 25
Gastrula	48-49 h	483 ± 38
Vitellaria	4-5 d	433 ± 37
Pentactula	7 d	629 ± 36
Early juvenile	21 d	689 ± 64
Juvenile	35 d	684 ± 11

Larval development

The vitellaria larva of *Athyonidium chilensis* (433 ± 37 µm) was characterized as a free swimming lecithotrophic larva. The vestibule (v) was clearly visible and the five primary buccal tentacles (pt) were observed inside it (Fig. 1F). After 7 days pf these tentacles began to protrude from the vestibule; a process that was the precursor to the pentactula stage (Fig. 1G). The pentactula (629 ± 36 µm) was characterized by a progressive decrease in the swimming capacity presumably due to the loss of the body cilia. Buccal tentacles (bt) were completely protruded and were used for feeding and locomotion. At this stage, the anal pore was also observed in the aboral region of the body. After the settlement of the pentactula (21 days) individuals were capable of active feeding on micro-algae associated with the sediment, using their buccal tentacles to collect and ingest the food.

Juvenile growth and survival

At 21 days pf (689 ± 64 µm ML) the early juvenile possesses, in addition to the buccal tentacles, a pair of ambulacral podia (ap) (Fig. 2 D, E, F). Thirty-five days pf the anus (a) and the fourth pair of ambulacral podia appeared, and juveniles with green pigmentation towards the posterior portion of the body were observed (Fig. 1H). During this period, a survival of ~18% from the total viable larvae was recorded (Fig. 3A). After 4 months, a substantial number of completely developed podia and the characteristic green pigmentation on the body wall were observed. Juvenile *Athyonidium chilensis* (~1,400 µm ML; Fig. 1I) began to display some common adult

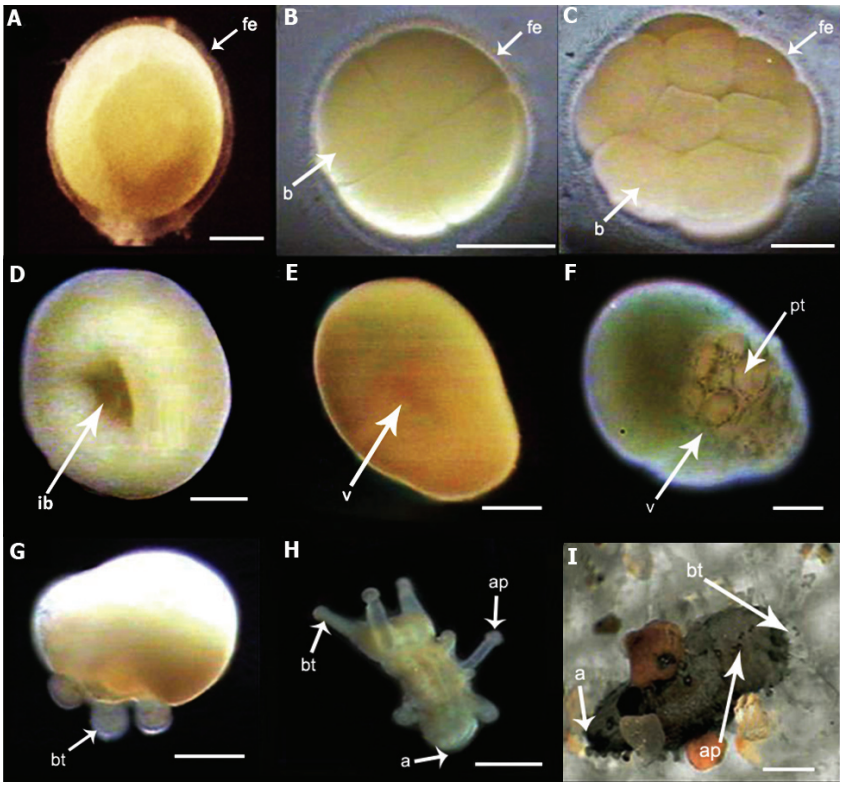

Figure 1. Light microscopy of the embryonic and larval development of *Athyonidium chilensis*. A. Fertilized oocyte with fertilization envelope (fe) completely elevated. Scale bar = 120 µm. B. 4-cell stage. (b) blastomere. Scale bar = 180 µm. C. 32-cell stage. Scale bar = 150 µm. D. Blastula. (ib) invaginating blastoderm. Scale bar = 100 µm. E. Gastrula. (v) vestibule. Scale bar = 100 µm. F. Vitellaria. (pt) primary tentacles. Scale bar = 150 µm. G. Pentactula. (bt) buccal tentacles. Scale bar = 200 µm. H. Juvenile 35 days post-fertilization. (a) anus, (bt) buccal tentacles, (ap) ambulacral podia. Scale bar = 230 µm. I. Juvenile 120 days post-fertilization. Scale bar = 275 µm / Microscopía óptica del desarrollo embrionario y larval de *Athyonidium chilensis*. A. Oocito fertilizado con la membrana de fecundación (fe) completamente elevada. Escala = 120 µm. B. Estadio 4 células. (b) blastómero. Escala = 180 µm. C. Estadio 32 células. Escala = 150 µm. D. Blástula. (ib) invaginación del blastodermo. Escala = 100 µm. E. Gástrula. (v) vestíbulo. Escala = 100 µm. F. Vitelaria. (pt) tentáculos primarios. Escala = 150 µm. G. Pentáctula. (bt) tentáculos bucales. Escala = 200 µm. H. Juvenil 35 días post-fecundación. (a) ano, (bt) tentáculos bucales, (ap) podios ambulacrales. Escala = 230 µm. I. Juvenil 120 días post-fecundación. Escala = 275 µm

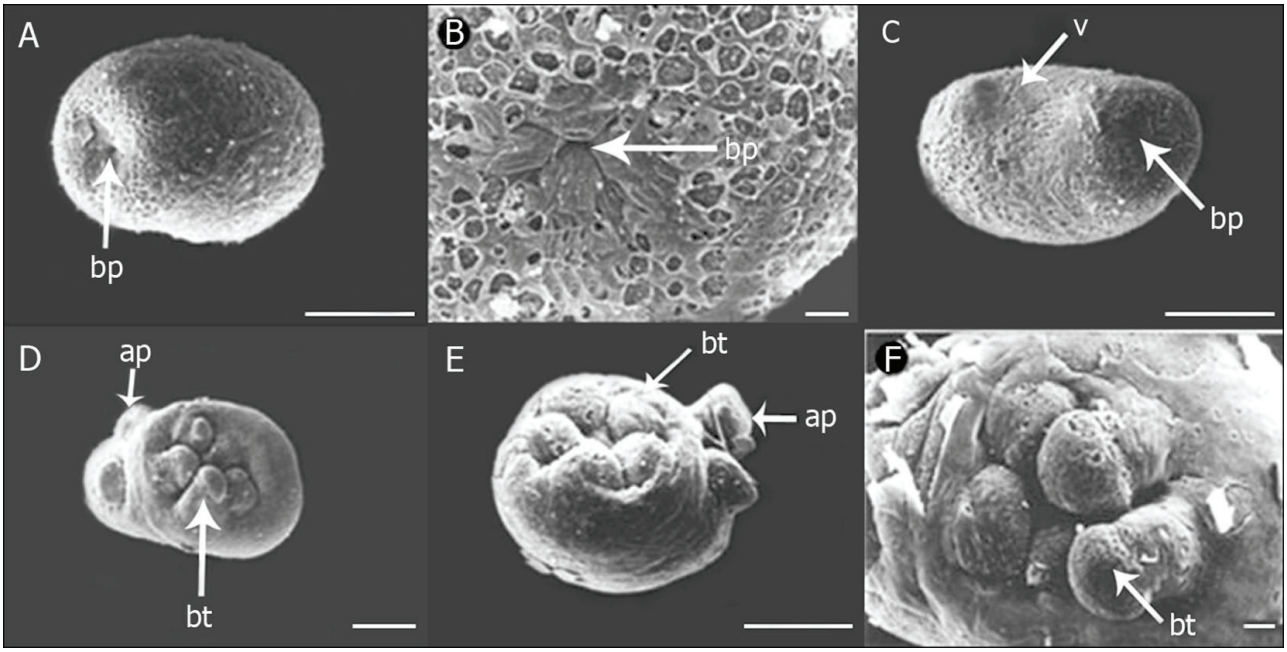

Figure 2. Scanning electron microscopy of the larval development of *Athyonidium chilensis*. A-B. Gastrula with blastopore (bp) in the aboral region. C. Gastrula with the future vestibule (v) forming. D-E. Early juvenile. (bt) buccal tentacles, (ap) ambulacral podia. F. Buccal tentacles in detail. Scale bar (A, C, D, E) = 100 µm. Scale bar (B) = 10 µm. Scale bar (F) = 50 µm / Microscopía electrónica del desarrollo larval de *Athyonidium chilensis*. A-B. Gástrula con blastoporo (bp) en la zona aboral. C. Gástrula con el futuro vestíbulo (v) en formación. D-E. Juvenil temprano, donde (bt) tentáculos bucales, (ap) podios ambulacrales. F. Detalle de los tentáculos bucales. Escala (A, C, D, E) = 100 µm. Escala (B) = 10 µm. Escala (F) = 50 µm

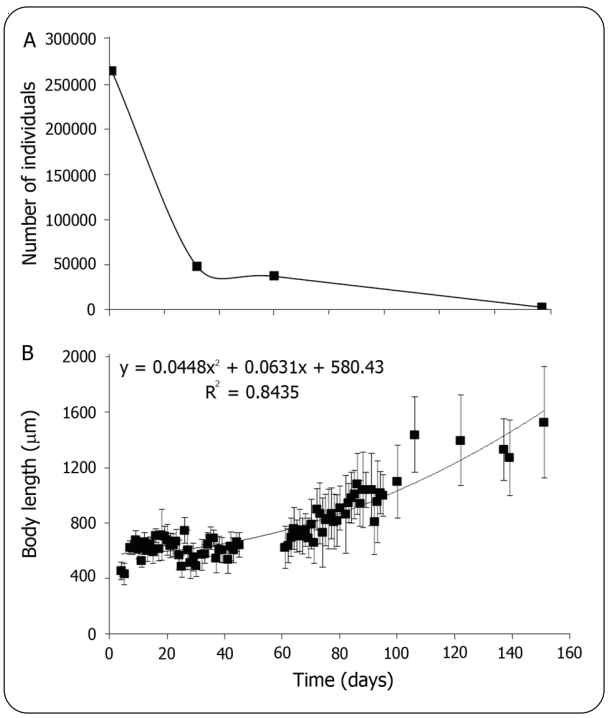

Figure 3. A. Survival. B. Size of *Athyonidium chilensis* during the first five months of cultivation. Vertical bars represent SD / A. Supervivencia. B. Talla de *Athyonidium chilensis* durante los primeros cinco meses de cultivo. Barras verticales representan DE

behaviors such as burying and anal pumping movements. Individuals significantly increased in size overtime (R^2 = 0.8435, $F_{(1, 78)} = 253$, $P < 0.001$; Fig. 3B). After 160 days of cultivation juveniles attained a size of ~1500 µm ML and survival decreased to 1.08% (Fig. 3A, B). During the following months the survival decreased to 0.24% and a maximum size of ~1,900 µm ML was recorded in the few remaining juveniles after approximately 8 months pf.

Effects of cultivation density on juvenile growth and survival

After 75 days, the GR (µm d^{-1}) of juvenile *Athyonidium chilensis* reared in laboratory conditions did not varied significantly among the three cultivation densities tested (one-way ANOVA, $F_{(2, 6)} = 1.038$, $P = 0.410$). Although there was a trend of higher GR in the treatment with the lowest density (1 ind cm^{-2}; 1,647 ± 152 µm ML), this value was not significantly different from the values obtained in the other treatments (1,523 ± 152 µm ML and 1,487 ± 114 µm ML for treatments with 3 and 5 ind cm^{-2}, respectively) (Fig. 4).

The survival of juvenile *Athyonidium chilensis* decreased significantly in all density treatments over time

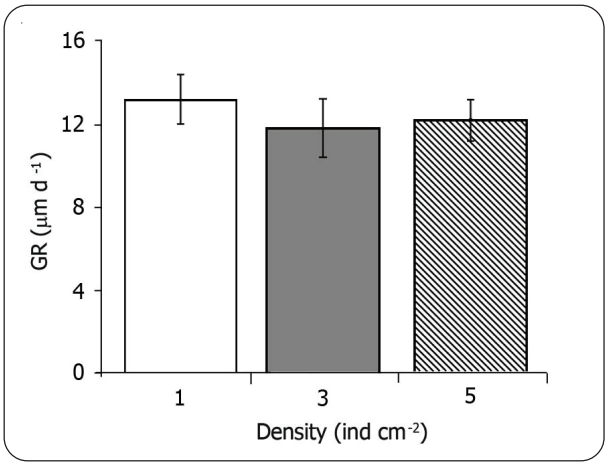

Figure 4. Growth rate (GR) of juvenile *Athyonidium chilensis* reared in laboratory under three experimental densities (1, 3 and 5 ind cm^{-2}). Vertical bars represent SD / Tasa de crecimiento (GR) de juveniles *Athyonidium chilensis* mantenidos en laboratorio bajo tres densidades experimentales (1, 3 y 5 ind cm^{-2}). Barras verticales representan DE

(two-way ANOVA, $F_{(5, 36)} = 50.204$, $P < 0.001$). Significant differences in survival were also detected among treatments (two-way ANOVA, $F_{(2, 36)} = 5.101$, $P = 0.011$; Fig. 5), with no interaction effect between the time and treatment (two-way ANOVA, $F_{(10, 36)} = 1.131$, $P = 0.368$). Overall, the survival of juveniles in the treatment with 1 ind cm^{-2} was not significantly different to the survival in the treatment with 3 ind cm^2 (Tukey's test, $P = 0.378$); but it was significantly different to the lower values obtained in the treatment with 5 ind cm^{-2} (Tukey's test, $P = 0.008$). No significant differences were detected between the treatments with the larger densities (Tukey's test, P = 0.174) (Fig. 5).

Discussion

Liberation of gametes in *Athyonidium chilensis* was only successfully achieved after thermal stimulation and addition of food. In contrast to previous studies (*e.g.*, Battaglene *et al.* 2002), spontaneous spawning was never observed after the transportation of individuals, or during conditioning periods in laboratory. However, increase of the water temperature proved to be a reliable method for obtaining gametes in this species, a technique also used to induce spawning in a range of tropical and temperate sea cucumbers, including *Holothuria scabra, Holothuria fuscogilva*, *Actinopyga mauritiana* and *Australostichopus mollis* (Battaglene *et al.* 1999, Mercier *et al*. 2000, Battaglene *et al.* 2002, Ramofafia *et al.* 2003, Morgan 2009).

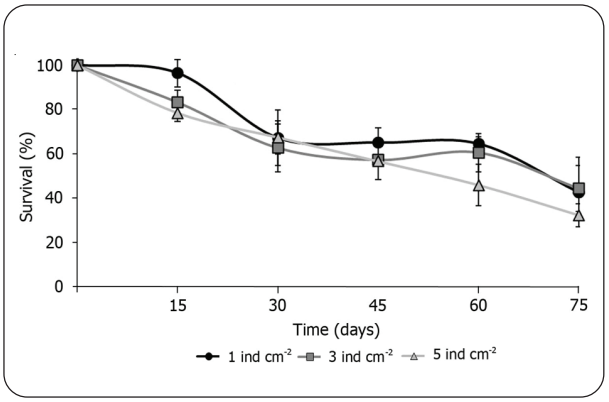

Figure 5. Survival of juvenile *Athyonidium chilensis* reared in laboratory under three experimental densities. Black circles: 1 ind cm^{-2}, dark-grey squares: 3 ind cm^{-2} and light-grey triangles: 5 ind cm^{-2}. Vertical bars represent SD / Supervivencia de juveniles *Athyonidium chilensis* mantenidos en laboratorio bajo tres densidades experimentales. Círculos negros: 1 ind cm^{-2}. Cuadrados grises: 3 ind cm^{-2}. Triángulos grises: 5 ind cm^{-2}. Barras verticales representan DE

After spawning induction, males consistently spawned before the females. This same reproductive behavior has been previously described by several authors in a vast range of sea cucumbers regardless the induction method used, *e.g.,* Costelloe (1988) in *Aslia lefevrei*; McEuen (1988) in *Psolus chitonoides*, *Psolidium bullatum* and *Cucumaria miniata*; Asha & Muthiah (2002) in *Holothuria spinifera*; Battaglene *et al.* (2002) in *H. scabra*, *H. fuscogilva* and *A. mauritiana*; Laxminarayana (2005) in *Bohadschia marmorata*; Renbo & Yuan (2004) and Fujiwara *et al*. (2010) in *Apostichopus japonicus.* Indeed, some authors have suggested that the sperm, or something associated with it, is a proximal signal for synchronizing female to spawn (see Battaglene *et al*. 2002). Therefore, in order to successfully carry out fertilization in laboratory is essential to maintain a total control over the broodstocks, because spontaneous spawning could cause polyspermia, a common problem with *in vitro* fertilizations and a lethal condition for marine invertebrates.

The average diameter of the newly released eggs of *Athyonidium chilensis* was 360 ± 16 µm. This size corresponds to the second of four modes of egg-size frequency distributions of holothurians defined by Sewell & Young (1997). The second mode is characterized by an egg-diameter around 421 µm (range: 150-950 µm) and is typical in species with lecithotrophic or brooding development. Some examples include other dendrochirotes species such as *Aslia lefevrei*: (*i.e.,* 400-650 µm; Costelloe 1988) and *Cucumaria frondosa* (*i.e.,* 900 µm; Hamel & Mercier 1996).

Embryos of *Athyonidium chilensis* in laboratory conditions remained buoyant and constantly swimming until the vitellaria stage (4-5 d post fertilization). The timing to reach this stage was similar compared to other species as *Aslia lefevrei* (7 d; Costelloe 1988), *Psolus chitonoides* and *Psolidium bullatum* (3-4 d, respectively; McEuen & Chia 1991) and *Cucumaria frondosa* (8 d; Hamel & Mercier 1996). As in those species, the vitellaria of *A. chilensis* was also preceded by a reduction in the larval length. The mean size recorded in this study for the lecithotrophic vitellaria larvae of *A. chilensis* was of 433 ± 37 µm, similar to the sizes recorded in *A. lefevrei* (*i.e.,* 500-650 µm; Costelloe 1988) and *P. bullatum* (*i.e.,* 475-560 µm; McEuen & Chia 1991), but much smaller to the values recorded by Hamel & Mercier (1996) in *Cucumaria frondosa* (*i.e.,* 1.55 mm).

Juvenile *Athyonidium chilensis* obtained after 35 days pf were capable of actively using their crown of tentacles for feeding, and their four pairs of ambulacral podia for locomotion and attachment to the substrate. During the first 240 days of cultivation in laboratory, juvenile *A. chilensis* reached an average length of 1.9 mm and grew approximately at a rate of 6 µm d^{-1} since settlement. Contrasting growth patterns have been recorded in other holothuroids in laboratory and field, being largely influenced by environmental conditions, such as temperature, food supply, and the degree of environmental disturbance. Costelloe (1988) recorded for *Aslia lefevrei* and approximate growth rate of 35 µm d^{-1}, reaching a size of 3.5 mm after 6-8 weeks. Similarly, Hamel & Mercier (1996) recorded for *Cucumaria frondosa* a growth rate of around 26 µm d^{-1} in laboratory, with individuals reaching 10 mm during the first year of cultivation. Faster growth rates have also been recorded in some aspidochirotes species, for example, juvenile *Isostichopus fuscus* grew at a rate of 0.5-1 mm d^{-1}, reaching a length of 3.5 cm after only 72 days (Hamel *et al.* 2003), with similar patterns also being recorded in juveniles *Holothuria scabra* (0.5 mm d^{-1}; Battaglene *et al.* 1999) and *Stichopus* sp. (1 mm d^{-1}; Hu *et al*. 2010).

As in others sea cucumber reared in land-based nursery systems, *Athyonidium chilensis* showed low survival during the first 30 days of cultivation. High mortalities were also recorded at hatch and during the first 20 days post-metamorphosis in *Holothuria spinifera* (Asha & Muthiah 2002), *C. frondosa* (Hamel & Mercier 1996, Hamel *et al.* 2003), *H. scabra* (Ramofafia *et al.* 2003)

and *Australostichopus mollis* (Morgan 2009), among others. Few procedures have been proposed to avoid high mortalities in early stages of holothurians during cultivation, but one of the most important is the maintenance of an appropriate density of individuals. Past experience has shown that high densities of individuals increases mortality and reduces the availability of space and food, causing malnutrition, slow growth and high variability of size (Xiyin *et al.* 2004, Agudo 2006). Although our results suggests that at low density (1 ind cm^{-2}) juveniles grew similarly to those conspecifics at higher densities (3 and 5 ind cm^{-2}), they had overall better survival, agreeing with the findings of Battaglene *et al.* (1999) and Xilin (2004), whose observed the highest survival in the low density treatments (*i.e.,* 1.7 ind cm^{-2} and 1 ind cm^{-2} for *H. scabra* and *Apostichopus japonicus*, respectively).

In summary**,** although *Athyonidium chilensis* as well as other holothurian species, undergoes critical phases in captivity, *e.g.,* fertilization, larval development and settlement, this species showed a rapid embryonic and larval development and settlement. Juvenile growth and survival was affected by the cultivation density, factor that could affect the food availability on cultured conditions. This study provide the first observations on larval development and juvenile growth of *A. chilensis* in the SE Pacific coast, showing that this sea cucumber can be successfully reared in land-based nursery systems and that the culture of this species can potentially be developed as an alternative to maintain sustainable harvest and eventually contribute to the restoration of natural populations. Further research should target to improve the spawning induction methods and principally, the feeding and growth of juveniles sea-cucumbers in captivity.

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