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Preliminary spawning and larval rearing of the sea cucumber *Holothuria sanctori* (Delle Chiaje, 1823): A potential aquaculture species

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ABSTRACT

Hatchery production is critical to the successful aquaculture of a species. This study describes the first captive breeding and reports on the embryonic development and early larval stages of the sea cucumber *Holothuria sanctori*, a species native to the Mediterranean and eastern Atlantic. Various induction methods were applied to obtain spawning in adult *H. sanctori* specimens; gametes were obtained from a spontaneous spawning event and from broodstock induced by mechanical shock coupled with algal stimulation. Fertilized eggs were then cultured at two different densities (0.3 egg/ml and 1 egg/ml) and fed two microalgae densities ($5 \times 10^3 - 2 \times 10^4$ cells/ml and $1 \times 10^4 - 4 \times 10^4$ cells/ml) from the microalgae *Nannochloropsis* sp. and *Amphora* sp. and larval growth was recorded. Embryonic and larval development of *H. sanctori* were monitored on a daily basis for one month and auricularia larval growth was registered at days 5, 10, 15, and 20 after fertilization. The embryonic and larval stages were observed up to the late auricularia stage (14 days after fertilization). The best larval growth was observed at the lower egg and algal densities.

1. Introduction

Holothurians are valuable members of the marine environment for their ability to maintain the productivity of the ecosystems by ingesting the organic matter content in the sediment (Yingst, 1976; Moriarty, 1982; Amon and Herndl, 1991; Ramofafia et al., 1997; Uthicke, 1999; Zhou et al., 2006; Tolon et al., 2017a,b), increasing the nutrient solubility and acting on the bioturbation of the substrate through activities of displacement, ingestion, and defecation (Bakus, 1973; Uthicke and Klumpp, 1998; Uthicke, 1999, 2001; Slater and Carton, 2009). Their role contributes to nutrient cycling, to the reduction of stratification of the seafloor sediments and are important for various ecological processes (Bakus, 1973).

They are also important in trade, as many oriental communities are using sea cucumber for food and medicinal purposes (Chen, 2003, 2004; Ferdouse, 2004; Raison, 2008). Usually, the edible part of the animal is its body wall that is processed and served as a dried product commonly named "Trepang" or "Bêche-de-Mer" in the global market. Their high level of protein and low amount of fat makes sea cucumber interesting products for human nutrition. More recently, several studies on sea cucumber have found anticancer (Zou et al., 2003), anti-inflammatory (Collin, 1998), and antioxidant (Althunibat et al., 2009) effects and have reported a number of bioactive compounds (Zou et al., 2003; Zhong et al., 2007; Bordbar et al., 2011). These nutritional and medicinal uses result in a constant increase in the demand and thus of the price. To offset this demand, global catches of sea cucumber increased to 51,000 tons (wet weight) in 2018 compared to 26,000 tons (wet weight) in 2000 (FAO, 2020). The increasing demand is causing the overexploitation of wild species so that many target species have been declared to be heavily exploited and depleted. Global reviews of significant fishery in regions such as the Indian Ocean (Conand, 2008) suggest that more than half of the sea cucumber stocks are overexploited, and population extinction has been reported in some localities (Hasan, 2005).

Some European countries contribute to the Bêche-de-Mer market by exporting their wild catches to the main import destinations mainly located in Asia. For instance, Turkey, the main Mediterranean producer, and Portugal harvest, and export species such as *Holothuria mammata*,

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Holothuria polii, Holothuria sanctori, Holothuria tubulosa, *Holothuria arguinensis*, *Holothuria forskali,* and *H. mammata* (Aydin, 2008; González-Wangüemert et al., 2016). In Spain, several companies are exporting sea cucumbers to China (Gomez, 2011; González-Wangüemert et al., 2016; Siegenthaler et al., 2015). Locally, the sea cucumber *Parastichopus regalis* is consumed in some Spanish regions where it is prized by people and been reported to be the most expensive seafood product reaching 130 EUR/kg fresh weight in the Catalan market (Ramón et al., 2010). The fishery of this species has been extended to France where a 1-ton (wet weight) harvest of *P. regalis* was registered for the year 2018 (FAO, 2020).

The continuous and increasing demand for Bêche-de-Mer has also led to the commercial exploitation of less economically valuable species, such as *H. sanctori* (Aydin, 2008; González-Wangüemert et al., 2016) which will lead to a certain overfishing scenario if irresponsible catches are not managed and market needs are not sustained by other sources such as aquaculture. Therefore, several studies have investigated sea cucumber aquaculture, including hatchery production, as preliminary steps for the successful aquaculture of such species. Among the species of interest in Europe are *H. arguinensis, H. tubulosa*, *H. mammata*, *H. forskali*, and *H. polii* (Domínguez-Godino et al., 2015; Santos et al., 2016; Domínguez-Godino and González-Wangüemert, 2018; Rakaj et al., 2018, 2019; and Laguerre et al., 2020). However, no hatchery production studies have been performed on *H. sanctori*. *H. sanctori* exists naturally in the Mediterranean Sea and the eastern Atlantic Ocean from the Bay of Biscay and Portugal to the Azores, and the Canary Islands, associated with the rocky substrates (Pérez-Ruzafa, 1984; Navarro et al., 2012). Its susceptibility increases since it was reported in shallow waters of the intertidal puddles (Pérez-Ruzafa, 1984; Navarro et al., 2012) and yet the aquaculture production techniques have not been established. Hence, its production cycle needs to be studied in order to offset the potential market needs and conserve the wild stock. Thus, the present study evaluates the broodstock induction methods and larval development of the sea cucumber *H. sanctori* under controlled rearing conditions.

2. Material and methods

2.1. Broodstock collection and maintenance

Adult specimens of *H. sanctori* (n = 69, mean weight = 106.95 ± 9.63 g) (Fig. 1) were collected from Taliarte (27.9892°N, 15.3753°W; Las Palmas de Gran Canaria, Spain) (Fig. 2) by scuba diving at the end of July 2019. The broodstock were immediately placed in a 20 L container until their transfer to the biofiltration reservoir of the ECOAQUA Institute, University of Las Palmas de Gran Canaria (Las Palmas de Gran Canaria, Spain). The broodstock were maintained in the reservoir, naturally filled with sediments, with flowing seawater at ambient temperature and under natural photoperiod until their transfer to the aquaculture experimental facility for spawning induction and larval production experiments.

2.2. Spawning induction

Spawning induction trials were carried out within the aquaculture experimental facility of the ECOAQUA Institute during August 2019. Prior to the induction procedure, broodstock were washed with fresh seawater to remove algae and sediment and were randomly placed in ($1 \times 0.8 \times 0.35$) m^3 tanks at an approximate density of 375 g/m^2 (7 broodstock/tank) and then subjected to three different spawning induction treatments, each treatment was tested in triplicate.

Treatment (1) Mechanical shock plus Thermal shock, Treatment (2) Mechanical shock plus Algal stimulation, and Treatment (3) Mechanical shock plus Thermal shock plus Algal stimulation.

Fig. 1. *H. sanctori* specimen. Scale bar = 4 cm.

- Mechanical shock consisted in keeping the specimens in dry conditions at the ambient temperature of 25 °C during 90 mins, then submit them to a strong water current by exiting the water from a hose with a narrow opening, which increases the intensity of the water flow during 3 min.
- Thermal stimulation consisted in increasing the water temperature by 6 °C above ambient (from 23 °C to 29 °C), using aquarium heaters, and subject the specimens to this temperature during 90 mins before returning them to seawater at ambient temperature of 23 °C.
- Algal stimulation was performed by adding *Spirulina* sp. to the tanks (0.1 g/L) and maintain the condition during 60 min before placing the specimens back in seawater at ambient temperature of 23 °C.

Once males and females had spawned, eggs were left during one hour in 10 L fertilization buckets (10 egg/ml). The fertilized eggs were then siphoned through a 40 μm sieve and washed with 1 μm filtered fresh seawater to remove excess spermatozoa before being transferred to 10 L buckets. The water containing fertilized eggs was stirred to uniformly distribute the eggs, and three replicate samples (4 ml) were taken to estimate the fertilization rate under the microscope using a counting chamber, before eggs were transferred to the larval tanks.

2.3. Embryonic and larval culture

During the larval rearing trial, larval density and feed rate were tested. The fertilized eggs were stocked at low (0.3 egg/ml) or high (1 egg/ml) densities in 200 L conical bottom larval tanks, filled with 1 μm fresh filtered and UV sterilized seawater, and fed at low (5×10^3 cells/ml to 2×10^4 cells/ml) or high (1×10^4 cells/ml to 4×10^4 cells/ml) microalgae densities when the auricularia stage was reached. Each treatment was tested in triplicate, represented as follows: Treatment (A) - low larval density and low feed rate; Treatment (B) - low larval density and high feed rate; and Treatment (C) - high larval density and high feed

Fig. 2. Sampling location (Map, *Google Maps*, Accessed 20 October 2020).

rate. Tanks were maintained in a hatchery room with windowpanes to be kept under natural photoperiod conditions (13:11 h light-dark). Tanks were provided with aeration, constant water flow (40% daily exchange), and the water temperature was maintained at (23 ± 0.5 °C). The algae tested as a source of feed were *Nannochloropsis* sp. and *Amphora* sp., provided in equal proportions (3 times/day) with concentrations gradually increased from the first day of feeding until the fifteenth day of feeding, when the algal concentration was fixed until the end of the experiment. Microalgae were grown in F/2 medium and using the batch culture method. Salinity and temperature were monitored throughout the entire duration of the experiment.

2.4. Embryonic and larval development

Five main larval stages are described in the literature for different sea cucumber species. These stages include early, mid, and late auricularia, doliolaria, and pentactula. In the present study, embryonic and larval development were monitored microscopically from fertilization of the eggs up to the late auricularia stage. Embryos were sampled by taking 4 ml water samples (n = 3) from the fertilization bucket at hourly intervals to describe the cell divisions and take measurements. Daily water samples were taken from each treatment, for 26 days, to monitor larval development, and register larval size (length and width) (Fig. 3) for 10 larvae per tank.

2.5. Data analysis

Larval size (n = 10) was examined in the different treatments (A, B, and C) on days 5, 10, 15, and 20 after fertilization and was used as an indicator to compare larval growth between treatments. The results were statistically analyzed using SPSS (version 22.0 for Windows; Inc., IBM, Chicago, IL, USA). One-way ANOVA was performed to test the effect of different feed ratios at low stocking density on larval growth in treatments (A) and (B) and to test the effect of different stocking densities at high feed ratio on larval growth in treatments (B) and (C). Data showing significant differences ($P < 0.05$) were analyzed by paired comparisons using Tukey's HSD. The assumption of normality and homogeneity of variances was assessed with Shapiro–Wilk test and Levene's test.

3. Results

3.1. Spawning

During the experiment of the different methods tested for spawning induction, larvae were obtained from a spontaneous spawning of the broodstock and from broodstock that were induced by mechanical shock coupled with algal stimulation. The spontaneous spawning took place at night after the transfer of the broodstock to the induction tanks, and the induced spawning also took place at night after two days of induction stimulation.

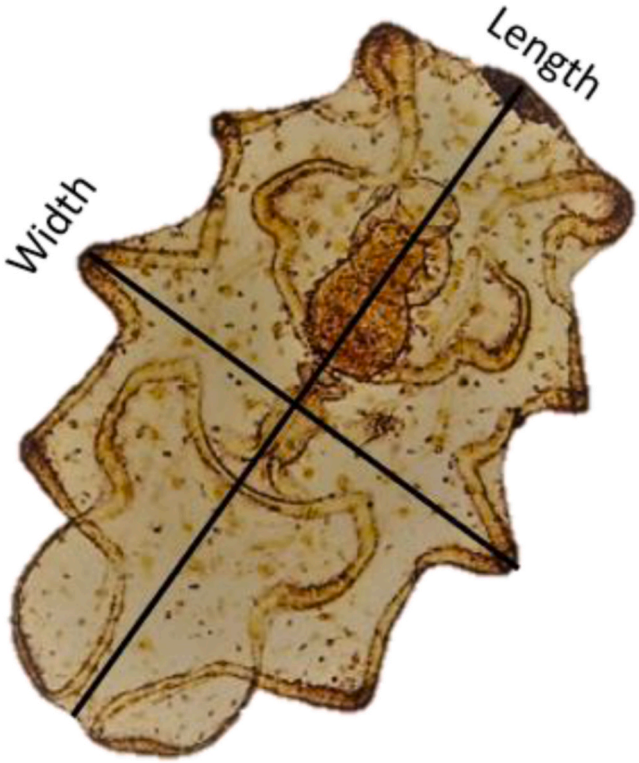

Fig. 3. Size parameters of the auricularia larvae.

No apparent trend was observed among broodstock regarding pre- or during spawning behavior except that the spawned specimens were clinging to the walls of the tanks and close to the water surface. Male and female gametes were expulsed from a single gonopore at the top dorsal anterior surface of the body (Fig. 4). The eggs of females were spherical in shape of orange color and could be discerned with the naked eye, with a mean diameter of 139.47 ± 0.13 μm, while sperm was emitted from males' gonopore as a white steady stream (Fig. 4).

H. sanctori appears to be photosensitive as it has been observed that specimens react quickly to light directed at them and tend to move away from it. It is also noted that it is easier to manipulate the specimens after sunset than during the day.

3.2. Embryonic development

Stages of embryonic development are described in Table 1 and Fig. 5. Once the mature eggs were fertilized, the fertilization envelope could be observed (Fig. 5b). At this point, the fertilization rate was found to be high, estimated at 96%. The fertilized egg passed through a holoblastic cell division, generating two blastomeres of similar sizes (first cleavage)

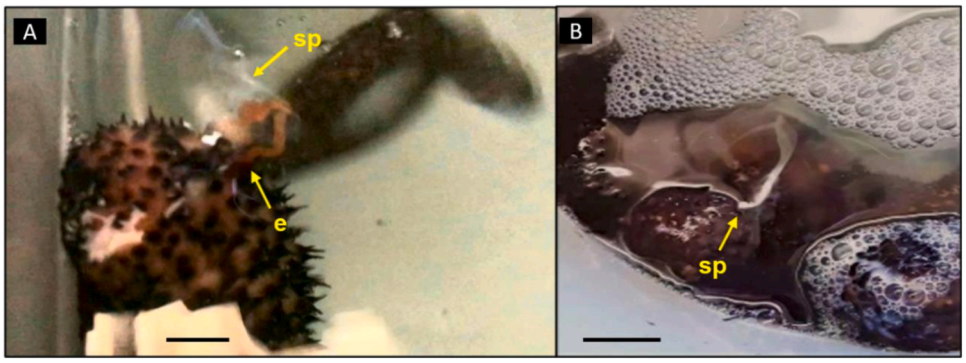

Fig. 4. Spawning behavior of *H. sanctori*. (A) Female gametes (e), Male gametes (sp). (B) Male gametes (sp). Scale bars = 4 cm.

Table 1
Size (mean ± SD; n = 10) and time after fertilization (AF) of *H. sanctori* embryonic stages development.

Embryonic stage	Size (μm)	Time AF (h, min)
Unfertilized egg	139.47 ± 0.13	
Fertilized egg	143.42 ± 0.11	0 min
2 cell egg	145.26 ± 0.32	1 h 30 min
4 cell egg	146.31 ± 0.10	2 h 30 min
8 cell egg	148.42 ± 0.30	3 h 30 min
16 cell egg	221.58 ± 0.23	4 h
32 cell egg	218.42 ± 0.16	4 h 35 min
64 cell egg	212.21 ± 0.31	7 h 20 min
Blastula	153.42 ± 0.21	11 h 10 min
Gastrula	277.11 ± 0.17	57 h 30 min

AF: After fertilization

(Fig. 5b) observed 1 h 30 min after fertilization. The cleavage continued until reaching the blastula stage (Fig. 5h) 11 h 10 min after fertilization, with a clear central blastocoel surrounded by a small single layer of cells. Gastrulation then started 16 h 50 min after fertilization through invagination of the blastula wall forming a blastopore (Fig. 5l) and continued to elongate, reaching full elongation at 57 h 30 min after fertilization (Fig. 5o).

3.3. Larval development

Development of *H. sanctori* larvae was observed throughout the auricularia stages, the larvae did not then metamorphose to later stages and the doliolaria was not observed with its characteristics including the appearance of hyaline spheres and tentacles.

Early auricularia larvae were first observed 78 h after fertilization (Table 2) with a hatching rate of 62%. They were transparent pelagic larvae with a functional digestive tract, for feeding, comprising a clear buccal cavity, esophagus, intestine, and cloaca (Fig. 6). Ciliary bands were observed to be used for feeding and locomotion from the early auricularia (Fig. 6a) and up to the late auricularia (Fig. 6c). The larvae reached the mid auricularia stage at day 6 after fertilization and remained in that stage during 8 days. The axohydrocoel (Fig. 6) emerged during this stage and expanded with elongation of the larvae throughout the auricularia before it stopped to differentiate.

3.4. Larval growth

Treatment (A), appeared to provide the best mean larval lengths and widths of 858.5 ± 97.7 μm and 630.2 ± 84.9 μm, respectively, followed by treatment (B), presenting larvae with mean lengths and widths of 850.8 ± 95.5 μm and 615.6 ± 82.2 μm, respectively, and finally, treatment (C), presenting larvae with mean lengths and widths of 812 ± 107 μm and 573.6 ± 97 μm, respectively. However, there were no significantly different lengths or widths ($P < 0.05$) between

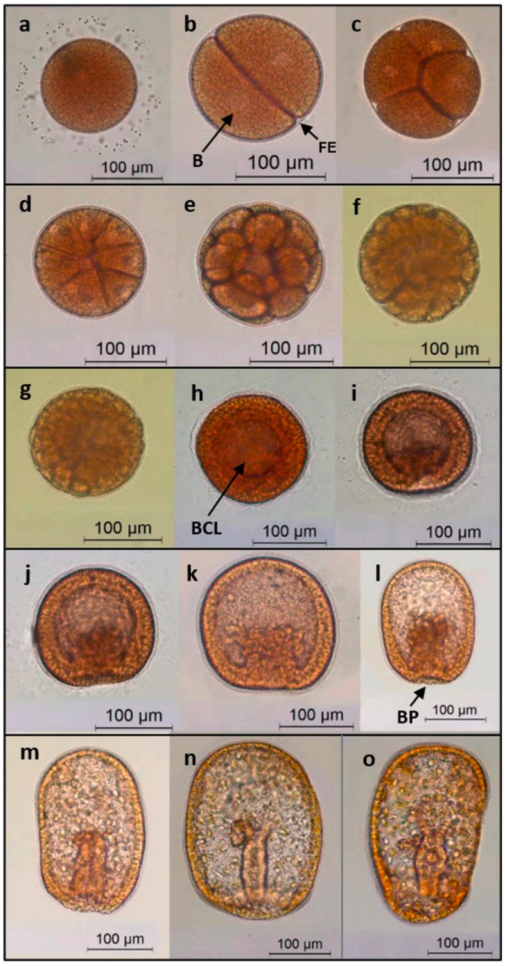

Fig. 5. Embryonic development of *H. sanctori*: (a) Fertilized egg; (b) 2-cell stage with blastomeres (B) and fertilization envelope (FE); (c) 4-cell stage; (d) 8-cell stage; (b) to (h) Cleavage stages; (h) Blastula with central blastocyst (BCL); (i) to (o) Gastrula; (BP) Blastopore.

Table 2
Size (mean ± SD; n = 45), and timing of auricularia larval stages of *H. sanctori* at 23 ± 0.5 °C.

Larval stage	Size (μm)	Time AF (h)
Early auricularia	563.16 ± 125.63	78–150
Mid auricularia	745.6 ± 70.76	150–342
Late auricularia	1013.16 ± 151.46	342–702

AF: After fertilization

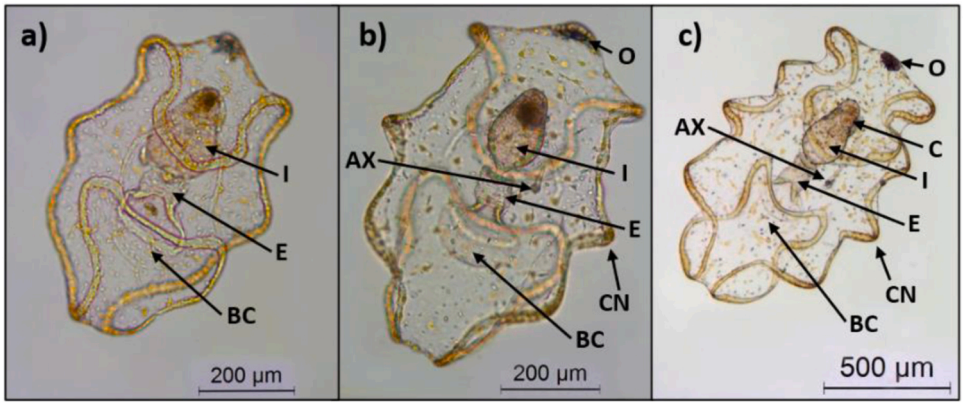

Fig. 6. *H. sanctori* auricularia larval stages: (a) Early auricularia; (b) Mid auricularia; (c) Late auricularia. Arrows indicate to (BC) Buccal cavity, (AX) Axohydrocoel, (E) Esophagus, (I) Intestine, (C) Cloaca, (O) Ossicle, and (CN) Ciliary band.

treatments (A) and (B) (Table 3) or between (B) and (C) (Table 4) throughout the development of the auricularia larvae except for length and width on day 10 after fertilization where the size of the larvae in treatment (A) was significantly higher ($P < 0.05$) than that of treatment (B) (Fig. 7; Fig. 8).

4. Discussion

This study on *H. sanctori* provides a first step in the development of aquaculture production of this commercially valuable species, describing spawning, and embryonic and larval development.

4.1. Spawning

The spontaneous spawning obtained for *H. sanctori* was apparently due to the transportation stress and handling that preceded the broodstock induction procedures. Transportation stress and handling are often sufficient to induce spawning, as demonstrated for *Apostichopus japonicas*, *H. scabra* and *H. fuscogilva* (YSFRI, 1991; Reichenbach, 1999; Agudo, 2006), or with the addition of thermal stress for *H. scabra* and *Actinopyga mauritiana* (Battaglene et al., 2002).

Although thermal stimulation has been found to be an effective method for spawning induction of some species of sea cucumbers (Battaglene, 1999; Battaglene et al., 2002; Domínguez-Godino et al., 2015; Domínguez-Godino and González-Wangüemert, 2018; Rakaj et al., 2018), it did not induce *H. sanctori* to spawn in the current study. Instead, *H. sanctori* broodstock showed a response to the mechanical shock coupled with algal stimulation treatment, leading to a release of gametes during the night. This response corroborates with previous findings on some sea cucumber species including *A. japonicas*, *H. scabra*, and *S. horrens* in which mechanical shock and algal stimulation were reported to induce spawning (Renbo and Yuan, 2004; Al-Rashdi et al., 2012; Hu et al., 2013).

The reproduction cycle in sea cucumbers is species-specific and within a species, can be different between geographically separated populations (Kazanidis et al., 2014). The poor response to the different spawning induction treatments may also be due to the fact that the experiments were conducted outside the peak of *H. sanctori* gonad index period during June and July, therefore, future spawning experiments should be conducted during this period (Navarro et al., 2012).

The observed spawning behavior where *H. sanctori* males and

Table 3
Mean (±SD) length and width values (µm) of *H. sanctori* auricularia larvae of treatments (A) and (B) for days 5, 10, 15, and 20, after fertilization (AF).

	Day AF	Treatment (A)	Treatment (B)
Larval length (µm)	5	648 ± 57	649 ± 65
	10	845 ± 44[a]	800 ± 64[b]
	15	907 ± 45	920 ± 37
	20	911 ± 62	903 ± 71
Larval width (µm)	5	447 ± 46	441 ± 64
	10	604 ± 43[a]	563 ± 60[b]
	15	651 ± 51	657 ± 43
	20	719 ± 77	682 ± 63

Different superscripts in the same row indicate significant differences ($P < 0.05$). Treatment (A): 0,3 egg/ml and algal density $5 \times 10^3 - 2 \times 10^4$ cells/ml. Treatment (B): 0,3 egg/ml and algal density $1 \times 10^4 - 4 \times 10^4$ cells/ml.

Table 4
Mean (±SD) length and width values (µm) of *H. sanctori* auricularia larvae of treatments (B) and (C) for days 5, 10, 15, and 20, after fertilization (AF).

	Day AF	Treatment (B)	Treatment (C)
Larval length (µm)	5	649 ± 65	632 ± 86
	10	800 ± 64	785 ± 56
	15	920 ± 37	867 ± 26
	20	903 ± 71	877 ± 88
Larval width (µm)	5	441 ± 64	438 ± 40
	10	563 ± 60	576 ± 70
	15	657 ± 43	580 ± 46
	20	682 ± 63	603 ± 72

Treatment (B): 0,3 egg/ml and algal density $1 \times 10^4 - 4 \times 10^4$ cells/ml. Treatment (C): 1 egg/ml and algal density $1 \times 10^4 - 4 \times 10^4$ cells/ml.

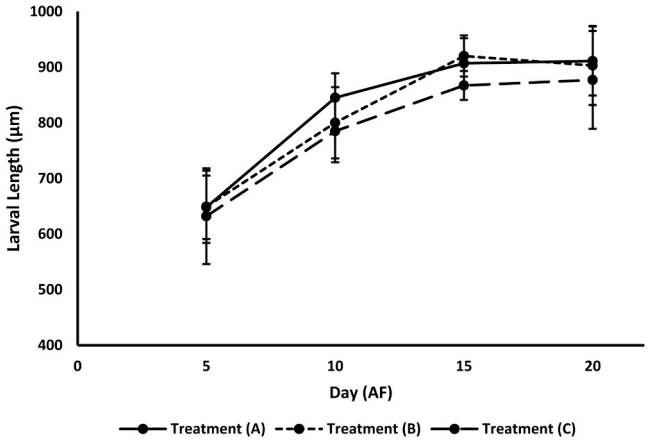

Fig. 7. Mean (±SD) larval length for treatment (A) with initial stocking density 0.3 egg/ml and algal density $5 \times 10^3 - 2 \times 10^4$ cells/ml, treatment (B) with initial stocking density 0.3 egg/ml and algal density $1 \times 10^4 - 4 \times 10^4$ cells/ml, and treatment (C) with initial stocking density 1 egg/ml and algal density $1 \times 10^4 - 4 \times 10^4$ cells/ml.

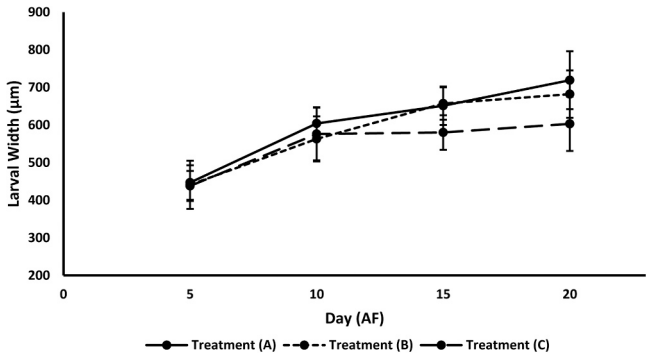

Fig. 8. Mean (±SD) larval width for treatment (A) with initial stocking density 0.3 egg/ml and algal density $5 \times 10^3 - 2 \times 10^4$ cells/ml, treatment (B) with initial stocking density 0.3 egg/ml and algal density $1 \times 10^4 - 4 \times 10^4$ cells/ml, and treatment (C) with initial stocking density 1 egg/ml and algal density $1 \times 10^4 - 4 \times 10^4$ cells/ml.

females broodstock remained just below the surface of the tank, releasing sperms and eggs by the gonopore, was similar to those reported in other sea cucumber species such as *H. arguinensis* and *H. forskali* (McEuen, 1988; Domínguez-Godino et al., 2015; Laguerre et al., 2020).

4.2. Embryonic and larval development

The observed embryonic development and larval stages of *H. sanctori* in the current study were similar to those reported for several other sea cucumber species including *A. japonicus*, *H. arguinensis*, *H. forskali, H. leucospilota, H. mammata*, *H. polii*, *H. scabra*, *H. tubulosa,* and *Stichopus horrens* while they differed in size and timing values of the embryonic and larval stages between the current study and other studies (Yan et al., 2012; Hu et al., 2013; Soliman et al., 2013; Domínguez-Godino et al., 2015; Domínguez-Godino and González-Wangüemert, 2018; Huang et al., 2018; Rakaj et al., 2018, 2019; Laguerre et al., 2020).

H. sanctori fertilized eggs had a medium size of 143 µm compared to higher values reported for *H. polii*, *H. forskali*, *H. tubulosa*, and *H. mammata* of approximate sizes; 247, 189, 172, and 167 µm, respectively (Domínguez-Godino and González-Wangüemert, 2018; Rakaj et al., 2018, 2019; Laguerre et al., 2020), and lower values reported for *Isostichopus fuscus* and *A. mauritiana* of approximate sizes; 120 and 110 µm, respectively (Ramofafia et al., 2003; Mercier et al., 2004). The early auricularia size of 563 µm exceeded the one reported for *H. arguinensis*, *H. tubulosa*, *H. mammata*, *H. polii*, and *H. forskali* with recorded values of 500, 430, 350, 300, and 300 µm, respectively (Domínguez-Godino et al., 2015; Domínguez-Godino and González-Wangüemert, 2018; Rakaj et al., 2018, 2019; Laguerre et al., 2020).

The timing of the emergence of auricularia larvae in *H. sanctori* (3 days 6 h) was close to that observed in *H. polii* and *H. tubulosa* (3 days) (Rakaj et al., 2018, 2019), where the breeding temperature of *H. polii* and *H. tubulosa* was 24 °C while for *H. sanctori* in the present study, was 23 °C. larval rearing temperature may explain these results in part as water temperature plays an important role in larval development and breeding time (Asha and Muthiah, 2005). As for the doliolaria stage that could be reached in some species such as *H. polii* and *H. tubulosa* at 8, and 24 days, respectively, after fertilization, it could not be observed up to 30 days after fertilization in *H. sanctori*. The hyaline spheres that usually emerge in the late auricularia and thus can be clearly seen in the doliolaria as a feature, were not observed as their development did not take place. This is probably linked to the microalgae used in the feeding regimes; *Nannochloropsis* sp. and *Amphora* sp., as the feeding regime and feed suitability are crucial for larval growth, and development and have consequences on duration, shape, and survival of larvae (Morgan, 2001; Knauer, 2011; Laguerre, 2020). Three different microalgae: *Chaetoceros* sp., *Tetraselmis* sp., and *Isochrysis* sp., are commonly used in larval feeding protocols for different sea cucumber species (Ramofafia et al., 1995; Purcell et al., 2012; Domínguez-Godino et al., 2015; Domínguez-Godino and González-Wangüemert, 2018; Rakaj et al., 2018, 2019; Laguerre et al., 2020) where it was planned to use these microalgae on larval production of *H. sanctori* in the current study, but there were some limitations in obtaining them before the start of the experiment. A recent study on *Parastichopus tremulus* indicated that the suitability of larval feed could affect larval metamorphosis to later stages, as the larval feed used in that study was algal paste, and the results were consistent with those in the current study. Larvae have not metamorphosed beyond the late auricularia (Schagerström et al., 2021). Hence, the results obtained in this study regarding the development limitation of larvae will rather be linked to the suitability of the algae and not the availability, as the provided feeding amounts were similar to the ones provided for sea cucumber species in other studies (James et al., 1994; Asha and Muthiah, 2002; Asha, 2004; Laxminarayana, 2005; Rakaj et al., 2018) and did not exceed 40×10^4 cells/ml. The amount provided took into consideration that high microalgae concentrations have been proved to reduce the ingestion rate and inhibit larval growth and development of Holothurian larvae (Morgan, 2001).

4.3. Larval growth

Results of the larval lengths indicated that, for most of the days observed, treatment (A) with low stocking density and low feeding ratio enabled better growth followed by treatment (B) with low stocking density and high feeding ratio for the mid and late auricularia larval stages in comparison with treatment (C) with high stocking density and high feeding ratio. The same trend was observed for larval widths as treatment (A) was the one that revealed wider larvae than the ones observed in treatments (B) and (C). This outcome was reinforced when the statistical analysis showed that the lengths and widths of the larvae in treatment (A) on the tenth day after fertilization were significantly higher ($P < 0.05$) than in treatment (B). This may indicate that the feeding regimen could influence the larval growth of *H. sanctori*. A low concentration of microalgae ($5 \times 10^3 - 2 \times 10^4$ cells/ml) administered to *H. tubulosa* larvae was also found to provide better growth and development than a higher concentration ($2 \times 10^4 - 4 \times 10^4$ cells/ml) regimes (Rakaj et al., 2018). Accordingly, the advantage of the larval growth in treatment (A) ($5 \times 10^3 - 2 \times 10^4$ cells/ml) in the current study could be explained based on findings that suggest better growth under lower feed concentrations. However, these results refute the hypotheses of some studies performed on other species such as *A. japonicus, H. spinifera, H. scabra,* and *H. atra* that propose higher feeding regimes of $2 \times 10^4 - 4 \times 10^4$ cells/ml to optimize larval growth and development (James et al., 1994; Asha and Muthiah, 2002; Asha, 2004; Laxminarayana, 2005). Further, the higher growth trend of larvae in treatment (B) with lower stocking density than larvae in treatment (C) with a higher stocking density makes stocking density a factor to be considered when cultivating *H. sanctori*. The better performance of larvae with low stocking density corroborates a previous study on *H. scabra* suggesting that the best larval density is in the range of 0.3–0.7 larvae/ml (James, 1996), but it is also contrary to work protocols used in hatchery production for other species (*H. mammata, H. arguinensis,* and *H. tubulosa*), where the larval stocking density is reported to be 1 larva/ml (Domínguez-Godino et al., 2015; Domínguez-Godino and González-Wangüemert, 2018; Rakaj et al., 2018).

Future studies are to be performed using microalgae commonly used in larval feeding protocols for different sea cucumber species to determine their suitability for larval growth and development of *H. sanctori* and to estimate the adequate stocking density to possibly develop a complete larval rearing protocol adapted to the requirements of this sea cucumber species.

5. Conclusions

The present study was able to shed light on the spawning induction and larval production of the sea cucumber *H. sanctori* for the first time and achieved considerable progress clarifying the embryonic and larval development of such species. This description of the embryonic and larval development allowed to obtain preliminary results that are very encouraging for the domestication of this species and bring a way to the diversification of future production. The study also highlighted that an improper feeding regime may inhibit the development of larvae, while algal and stocking density can affect their growth. For *H. sanctori*, in the present study, reduced algal and stocking density enabled better larval growth during the auricularia larval stages. Additional experiments will be required to monitor the development and survival of *H. sanctori* using other microalgal sources, in order to estimate their nutritional efficiency and to optimize the larval production protocols. Further research is needed to improve larval survival, to optimize rearing techniques and to close the production cycle of *H. sanctori* to develop new, innovative, integrated and profitable aquaculture.

CRediT authorship contribution statement

Mohammad Magdy: Writing – original draft, Formal analysis, Investigation, Visualization. **Francisco Otero-Ferrer**: Conceptualization, Methodology, Editing. **Gercende Courtois de Viçose**: Conceptualization, Methodology, Investigation, Writing – review and editing, Supervision, Project administration. All authors read and approved the final manuscript.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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