DOI: 10.1111/are.13487

ORIGINAL ARTICLE

WILEY Aquaculture Research

Spawning and rearing of *Holothuria tubulosa*: A new candidate for aquaculture in the Mediterranean region

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Abstract

Holothuria tubulosa (Gmelin, 1788) has recently shown an increased demand in Asian markets, becoming one of the intensively exploited holothurian species in the Mediterranean Sea. A risk is that over-harvesting is likely affecting both the species' natural stocks and the benthic communities. In this scenario, sea ranching and restocking through aquaculture could assist in mitigating its overexploitation. This study is the first to demonstrate the successful artificial breeding and rearing of *H. tubulosa*, and its consequent potential as a new species for the Mediterranean aquaculture industry. Here we describe the spawning induction, larval development and early juvenile growth in hatchery cultures, aimed at developing a spawning and rearing protocol for this species. The trials were conducted from July to October in both 2014 and 2015. *Holothuria tubulosa* was induced to spawn by testing four different methods. Thermal stimulation plus thermal shock emerged as the most efficient method to obtain active and healthy gametes. Larval development in *H. tubulosa* progressed through five stages, reaching the juvenile stage in 27 days. Two different microalgal feeding regimens were tested for larval breeding. Under the best feeding conditions, 7% of the larvae metamorphosed into settled juveniles, adhering to artificial substrates previously conditioned with benthic biofilm. Our results indicate that *H. tubulosa* shows good performance in hatchery rearing during the larval phases, indicating that this species could be a new candidate for aquaculture in the Mediterranean region, both for production and restocking proposes.

KEYWORDS
Holothuria tubulosa, larval feeding, larval rearing, mariculture, sea cucumber, spawning

1 | INTRODUCTION

Deposit-feeding sea cucumbers are common members of marine benthic communities and important ecosystem engineers that play a key role in sea floor dynamics by processing and bioturbating the sediment (MacTavish, Stenton-Dozey, Vopel & Savage, 2012; Purcell, Conand, Uthicke & Byrne, 2016).

Some species of sea cucumber are also edible and considered a delicacy in many Asian markets, where, in their dried form, they are traded as a luxury seafood called trepang or bêche-de-mer (Purcell, Hair & Mills, 2012; Toral-Granda, Lovatelli & Vasconcellos, 2008). Of the approximately 1,400 species of holothuroids worldwide (Han, Keesing & Liu, 2016; James, 2001; Pawson, 2007), nearly 60 species are commercially exploited (Kinch, Purcell, Uthicke & Friedman,

2008; Purcell, Samyn & Conand, 2012; Purcell, Hair et al., 2012). The high commercial value and the increasing demand has intensified exploitation in the wild, this remaining the only source available for many sea cucumber species (Conand, 2004; Purcell, Lovatelli, Vasconcellos & Ye, 2010; Purcell, Hair et al., 2012; Sicuro & Levine, 2011; Toral-Granda et al., 2008). The resulting massive and unregulated exploitation has led to the collapse of several natural stocks and is likely impacting significantly on vulnerable benthic communities (Purcell et al., 2013).

In this context, aquaculture could be a sustainable alternative to meet the current market demand (Anderson, Flemming, Watson & Lotze, 2011; Bartley & Bell, 2008) by reducing pressure on wild stocks and by providing restocking actions. Furthermore, several sea cucumber species have been shown to have a valuable application in Integrated Multi-Trophic Aquaculture (IMTA) (Paltzat, Pearce, Barnes & McKinley, 2008; Slater & Carton, 2007; Tolon, Emiroglu, Gunay & Ozgul, 2017; Yokoyama, 2013; Yu, Zhou, Yang, Ma & Hu, 2014). Their use in IMTA systems has been found to mitigate the negative effects of intensive aquaculture through waste particulate subtraction and processing (Ahlgren, 1998; Barrington, Chopin & Robinson, 2009; Slater & Carton, 2009; Tolon et al., 2017).

Recent years have witnessed a growing interest in the sea cucumber aquaculture industry on a global scale, as extensively noted and reported by FAO reports and numerous studies (Han et al., 2016; Lovatelli et al., 2004; Purcell, Hair et al., 2012; Toral-Granda et al., 2008). Aquaculture production has increased more than fourfold, rising from 46,000 tonnes in 2004 to 200,000 tonnes in 2014 (FAO, Global Production Statistics 1950–2014). To date, production of holothuroids by aquaculture has been developed mainly for six Indo-Pacific species (Domínguez-Godino, Slater, Hannon & González-Wangüermert, 2015; Purcell, Hair et al., 2012), supplying a portion of the market demand, especially for the Japanese sea cucumber, *Apostichopus japonicus* (Chen, 2004; Han et al., 2016; Lovatelli et al., 2004; Purcell, Hair et al., 2012).

The increasing demand from Asian markets has led to the rapid exploitation of new fisheries elsewhere, such as the Mediterranean area (Antoniadou & Vafidis, 2011; González-Wangüemert, Valente & Aydin, 2015) where the fishing effort for holothuroids is on the increase. In Turkish waters alone, there are approximately 120 active fishing vessels with a harvest that ranges from 720,000 to 1,080,000 sea cucumbers per day (González-Wangüemert, Aydin & Conand, 2014; González-Wangüemert, Valente, Henriques, Domínguez-Godino & Serrão, 2016).

In the Mediterranean Sea, the main target species displaying commercial interest are *Holothuria polii*, *Holothuria tubulosa*, *Holothuria mammata* and *Parastichopus regalis* (González-Wangüemert et al., 2014, 2015, 2016; Purcell, Samyn et al., 2012). These sea cucumbers are considered a good source of nutrients and a seafood suitable for human consumption (Aydin, Hüseyin, Bekir, Yilmaz & Sevim, 2011; Çakly, Cadun, Kisla & Dicer, 2004; Pereira, Valentão, Teixeira & Andrade, 2013; Roggatz et al., 2016; Sicuro et al., 2012).

Holothuria tubulosa, the object of this study, is a Mediterranean species (González-Wangüemert et al., 2016; Valente, Serrão & González-Wangüemert, 2015) generally associated with rich organic bottoms and seagrass beds where it thrives in a bathymetric range from 1 to 100 m (Bulteel, Jangoux & Coulon, 1992; Gustato & Villari, 1979; Massin & Jangoux, 1976; Tortonese, 1965). This sea cucumber displays separate sexes without sexual dimorphism; it presents an external fertilization and has an annual reproductive pattern, spawning mainly during the summer (Despalatović, Grubelić, Šimunović, Antolić & Žuljević, 2004; Kazanidis, Lolas & Vafidis, 2014; Ocaña & Tocino, 2005). *Holothuria tubulosa*, given its trophic behaviour, has been recently proposed as a candidate in the IMTA system by Tolon et al. (2017). The same authors have successfully reared wild specimens under finfish net cages, without supplementary food by exploiting aquaculture waste.

Holothuria tubulosa has become one of the target species actively harvested in Turkey, Greece, Italy, Spain and many other countries, prior to exportation to Eastern Asian markets (Aydin, 2008; Çakly et al., 2004; González-Wangüemert et al., 2014, 2015). Unfortunately, to date, the management measures for a sustainable fishery of this target resource are still scarce or even completely absent along the Mediterranean coasts.

As yet, no artificial reproduction protocols have been developed for Mediterranean species, in Europe the only sea cucumber successfully reproduced in captivity is the Atlantic species *Holothuria arguinensis* (Domínguez-Godino et al., 2015).

In this study, we present details of the different methods for spawning induction, the development of embryos, larvae and juveniles of *H. tubulosa*, aiming at developing a hatchery-based larval-rearing protocol for this Mediterranean species.

2 | MATERIALS AND METHODS

2.1 | Broodstock collection and maintenance

Adult specimens of *H. tubulosa* (880 individuals; mean weight: 247.3 ± 15.7 g; mean ± *SE*) were collected by snorkelling (1–6 m depth) at Torre Astura (central Tyrrhenian Sea, Italy 41°24′29″N, 12°45′51″E) between July and October in 2014 and 2015. After collection, specimens were maintained in 30 L tanks equipped with aerators; a dry ice pack was placed inside the tanks to maintain the temperature below 28°C. No loss of broodstock occurred during transportation. The broodstock were transferred to the Laboratory of Experimental Ecology and Aquaculture (Tor Vergata University, Rome), and were acclimated and maintained indoors in 600 L tanks with filtered seawater at 23–24°C (in closed circulation systems with biological and mechanical depuration). The holding tanks had been previously filled with sediment collected from the sampling site. After 7 days, specimens were fed with additional dry extruded pelleted fish feed (EFICO YM 854 for Gilthead Seabream; Biomar SAS, Nersac, France) at a rate of 5 g per kg of weight broodstock per day. Broodstock were maintained in the holding tanks for 2 weeks prior to spawning induction at a density of approx. 40 specimens per tank.

2.2 | Spawning induction and fertilization

Before stimulation, breeders were transferred and maintained for 48 hr in substrate-free aquaria to void their gut contents. The sea cucumbers were then cleaned and washed with filtered seawater to remove sediment and other organisms, and finally transferred to 600 L spawning tanks containing 5 μm filtered and UV-sterilized seawater.

The following methods were performed in parallel on broodstock groups of 18–22 individuals to induce spawning. Each approach described below was replicated in total for 16 trials from July to October with six trials in 2014 and ten in 2015.

- *Mechanical shock* (dry shock): Broodstock were kept dry for approximately 30 min, subjected to a strong water jet, and were transferred back into the spawning tanks (Renbo & Yuan, 2004; Yanagisawa, 1998).
- *Thermal shock*: Water temperature was quickly raised by 3–5°C (from 23°C to 26–28°C), was maintained over 1.5 hr, and returned to the starting temperature (Battaglene, Seymour, Ramofafia & Lane, 2002; Dabbagh, Sedaghat, Rameshi & Kamrani, 2011; Domínguez-Godino et al., 2015).
- *Thermal stimulation*: Water temperature in spawning tanks was raised gradually by 2–3°C using aquarium heaters (from 23°C to 25–26°C) and maintained at the elevated temperature for at least 2 days (this method was drawn up on the basis of hatchery observations).
- *Combined thermal stimulation and thermal shock*: Water temperature in spawning tanks was gradually raised by 2–3°C (from 23°C to 25–26°C) and maintained. On day 2, a thermal shock was performed by quickly raising the water temperature by 3°C and returning it back to 25–26°C. The combination of these two methods was designed to ensure a better control of the spawning time.

When none of the methods induced spawning, the broodstock were maintained in the holding tanks and spawning induction was performed again 2 weeks later; instead, when spawning induction was successful, the broodstock showed signs of pre-spawning movements. Males were immediately identified, as they started spawning first, and were transferred into separate tanks. The remaining specimens manifesting pre-spawning behaviour were assumed to be female and isolated in 20 L spawning buckets until the release of eggs. Water in the buckets was stirred to uniformly distribute the eggs. Five samples (1 ml) were collected to estimate the number of released eggs/female; eggs were counted using a Sedgewick-Rafter cell chamber under a light stereomicroscope. At this stage, the spermatozoa solution (obtained from isolated spawning males) was added at low concentration (*circa* 1–5 $\times$ 10^4 spermatozoa/ml) in the female spawning buckets to minimize polyspermy (Hamel & Mercier, 1996). After the addition of the spermatozoa solution, eggs were continuously monitored. Thirty minutes after fertilization, eggs were removed from the tanks by using a 60 μm bucket sieve. Collected eggs were then washed with filtered and sterilized seawater to remove the residual sperm. Larval rearing was conducted from early August to September in 2014 and repeated in the same period in 2015.

2.3 | Larval and juvenile culture

The fertilized eggs were transferred and maintained in a 10 L round-bottom flask at a density of 1 egg/ml (Battaglene & Bell, 1999; Domínguez-Godino et al., 2015; Pitt, 2001). An air driven pipette was placed into each flask to maintain sufficient aeration and ensure gentle water circulation. During the first day, embryonic development was followed continuously by taking 10 ml water samples (five replicates) every hour, after which samples were taken every 6 hr until the early auricularia stage was reached. From the first occurrence of the early auricularia stage, the larvae were monitored daily to observe development. Larvae were reared indoors using 5 μm filtered and UV-sterilized seawater. Water temperature was maintained at 24°C by conditioning the hatchery room environment. Salinity was monitored daily using a Sucrose Brix Refractometer and was maintained between 36‰ and 37‰. The hatchery photoperiod was designed according to the natural circadian cycle using low brightness, and avoiding direct lighting on cultures. EDTA was dissolved in culture water at low concentrations (2 mg/L) to improve water quality by reducing heavy metal concentrations (Agudo, 2006). Water was partially exchanged (50%) on a daily basis using a 60 μm mesh screened siphon. Dead larval deposits, when they were observed, were removed by gently siphoning the bottom of the flasks with a pipette.

When a fully developed gut was observed (third day following fertilization), larvae were fed daily using a mixture of microalgae *Isochrysis galbana*, *Chaetoceros calcitrans* and *Tetraselmis suecica* in equal proportions (cells/ml). The microalgae were cultured in 2 L plastic bag photo-bioreactors using Guillard's f/2 growth medium (Guillard & Ryther, 1962) at 24°C using a 12:12 hr light–dark photoperiod. Only algae under exponential growth were administered.

Two different feeding regimes were tested for larval rearing:

- High microalgae regime of 20,000–40,000 cells/ml from early auricularia to late auricularia, as recommended in the literature for several sea cucumber species (Agudo, 2006).
- Low microalgae regime of 5,000–10,000 cells/ml from early auricularia to late auricularia.

The experiment was repeated twice with three replicates for each condition. When the larvae reached the doliolaria stage, they were transferred from the 10 L round-bottom flask to 30 L aquaria, each containing 6 PMMA (Plexiglas®) settlement panels (0.20 m $\times$ 0.40 m stacked with a 30 mm gap), covered with a well-developed diatom-biofilm. The settlement panels were prepared in advance by immersing them for 2 weeks in naturally illuminated tanks inoculated with benthic diatoms (*Navicula* spp., *Nitzschia* spp. and *Phaeodactylum tricornutum*), and supplemented with f/2 beta

growth medium to promote biofilm development. Since not all larvae metamorphosed at the same time, microalgae mixture was administered in the 30 L aquaria during the first 3 days to feed the remaining auricularia larvae. To provide sufficient aeration and to ensure water circulation, two air stones were placed on the bottom of each aquarium. Approximately 50% of the water was changed daily using 60 μm mesh siphons. Juveniles were fed by adding ALGAMAC 3050 as supplementary feeding to the benthic diatoms. From the day of their appearance, 0.01 g of ALGAMAC was dissolved in each aquarium daily.

2.4 | Sampling and specimen preparation

Water samples (10 ml, five replicates) were taken daily after the first day to follow larval development, record developmental stages, and register the mean length and width for a minimum of 30 larvae during the planktonic phase. After settlement, the number of juveniles was estimated by counting the juveniles in five subareas, using prepared sampling plates. In the following results we present the mean ± *SE* of this sampling.

Eggs, embryos, larvae and juveniles were fixed in 2% formaldehyde in filtered seawater. The specimens were viewed using a Leica MZ12 stereomicroscope and a Leica DMLB 2000 light microscope (Leica, Wetzlar, Germany).

3 | RESULTS

3.1 | Spawning induction and fertilization

The following outcomes were observed during the trials performed to test various spawning induction methods: (i) *Mechanical shock*: no response was observed applying this method in all 16 trials; (ii) *Thermal shock*: spawning occurred sporadically (i.e. spawning successful in four trials); (iii) *Thermal stimulation*: spawning was successful in 8 out of 16 trials (pre-spawning behaviour was mainly observed after the first day of treatment); and (iv) *Combined thermal stimulation and thermal shock*: spawning was successful in 11 out of 16 trials. Thermal shock, performed on the second day of the thermal stimulation, permitted better control of the spawning event.

Among the different trials, thermal stimulation + thermal shock was selected as the best method to induce spawning in *H. tubulosa*. Pre-spawning behaviour was identified in broodstock. It was observed that individuals sought the standing position, many of them being deployed on tank walls close to the water surface. Breeders clung to the substrate with the posterior part of the body, and extended the anterior end of the body to facilitate the dispersal of gametes from the apical gonopore (Figure 1).

Males spawned before females by releasing a thin and steady stream of sperm from gonopore. Males spawned continuously in the erect position for about 2–2.5 hr with short interruptions. Females began spawning approximately 1–1.5 hr after males. They often raised the anterior body, moving and twisting upward until a short and powerful (4–8 s) squirt of eggs was released from the gonopore. Each female completed spawning with 4–6 distinct squirts/jets with a 5–10 min interval between each squirt. This powerful ejection dispersed the eggs much more widely in respect to the streams of sperm.

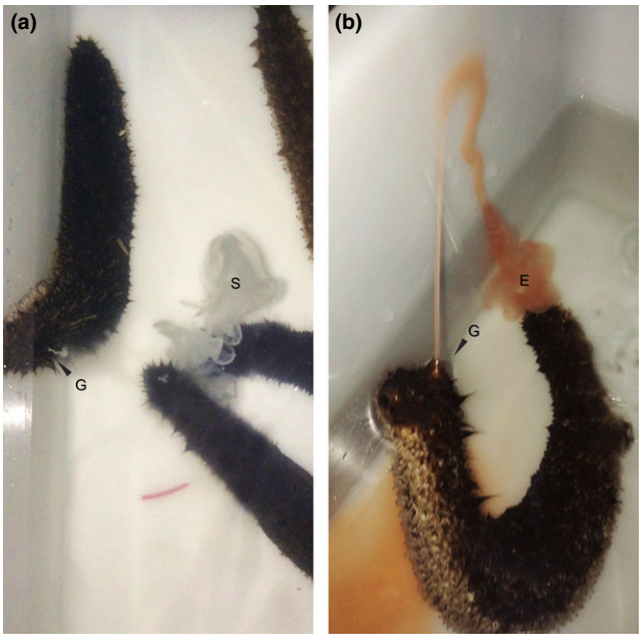

FIGURE 1 Spawning of *Holothuria tubulosa*. (a) Male: gonopore (G), sperm (S). (b) Female: gonopore (G), eggs (E)

Mature eggs of *H. tubulosa* were spherical, visible to the naked eye, with a mean diameter of 151.2 ± 2.1 μm (Figure 2a). The quantity of eggs varied widely among individuals, ranging from 0.4 to 12 million per spawning female (mean 3.48 ± 1.41 million; $n = 12$). During spawning induction, low aeration was provided using air pipettes inside the spawning tanks. Since the eggs were heavier than seawater, they quickly sank to the bottom of the bucket.

3.2 | Embryonic and larval development

The fertilization membrane of mature eggs (mean length 151.2 ± 2.1 μm) was inflated within 1–5 min after the addition of sperm, increasing their dimension to a mean length of 172.1 ± 1.7 μm ($n = 30$ larvae). At this stage, the fertilization envelope became clearly visible (Figure 2b). The first cleavage appeared 110 min after fertilization. Cell division was holoblastic, generating two blastomeres of similar size (Figure 2c). The second and third cleavages were observed at 150 and 195 min, respectively, after fertilization (Figure 2d, e). The morula stage was observed at approximately 6 hr after fertilization and the blastula stage was reached 12 hr after fertilization, exhibiting a typical central blastocoele surrounded by a small and single layer of cells (Figure 2f). At this stage, the embryo started rooting and moving (Figure 2g). At 20 hr, the invagination of the blastula wall began and the first gastrula was observed (mean length 177.1 ± 1.6 μm) (Table 1). In the following

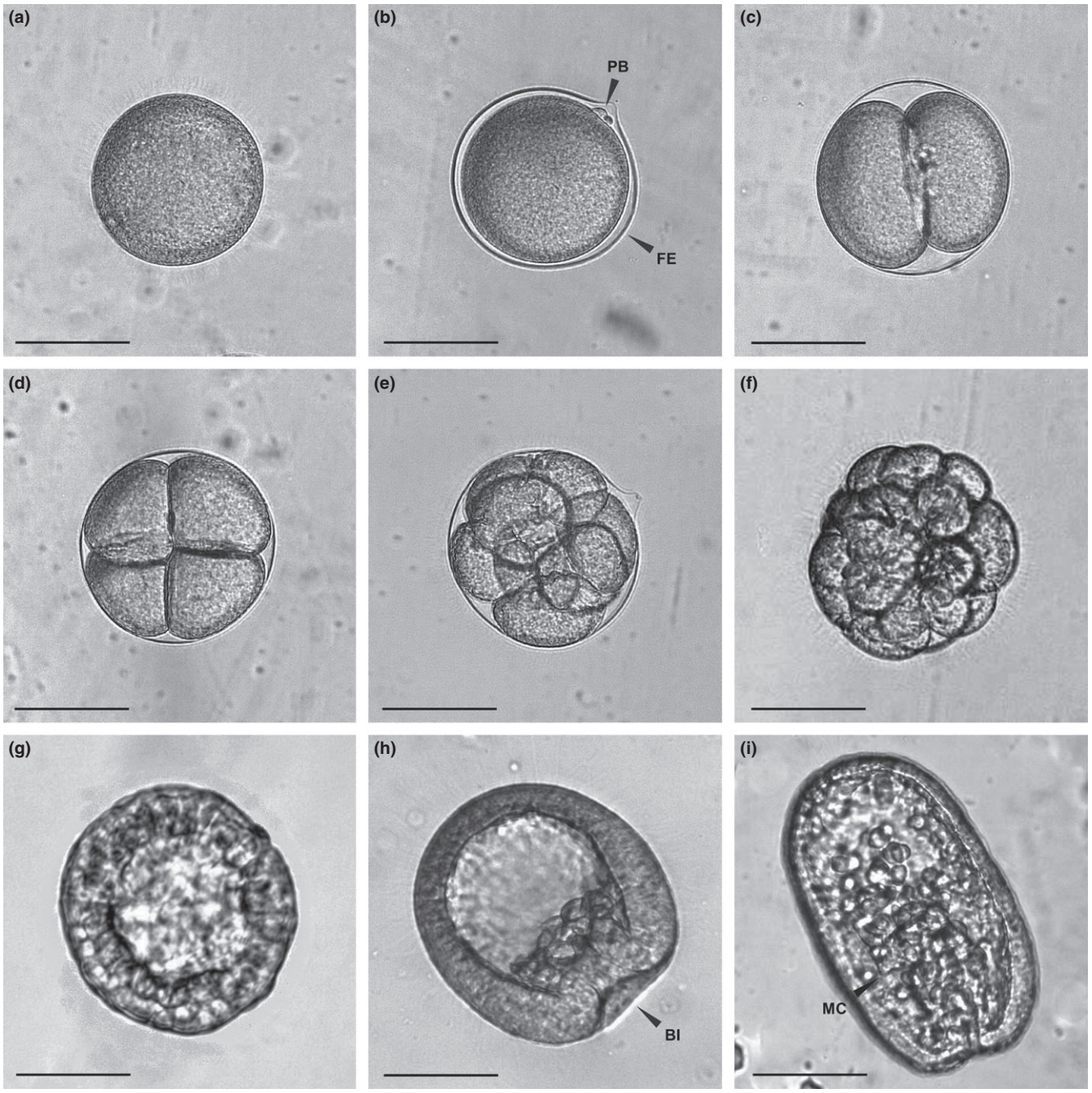

FIGURE 2 Embryonic development of *Holothuria tubulosa* (a) Unfertilized egg, (b) Fertilized egg with a fertilization envelope (FE) and two polar bodies (PB), (c) First cleavage, (d) Second cleavage, (e) Third cleavage, (f) Morula, (g) Rotary blastula, (h) Early gastrula with blastopore (Bl), (i) Mid gastrula;mesenchyme cells (MC), Scale bars = 100 μm

24–30 hr, the gastrula continued to elongate until they reached the early auricularia stage (Figure 3a).

Under the rearing conditions in this study, *H. tubulosa* progressed through five stages of larval development: early auricularia, mid auricularia, late auricularia, doliolaria and pentactula. Early auricularia larvae were completely developed by day 3 post fertilization. The mean length and width of early auricularia larvae were 434.2 ± 5.0 μm and 260.9 ± 3.5 μm, respectively, and the larvae appeared translucent with a functional digestive tract, including a buccal cavity, oesophagus and stomach (Figure 3b). The first feeding instance was observed at this stage. Larvae increased in size, reaching the mid auricularia stage on day 7 post fertilization (Figure 3c). The mean length and width at this stage were 693.2 ± 7.2 μm and 464.1 ± 4.9 μm respectively. Larvae reached the late auricularia stage on day 20 post fertilization (Figure 3d), and had a mean length of 786.7 ± 12.3 μm and width of 532.4 ± 7.2 μm, respectively, these being the maximum dimensions registered during any of the planktonic stages. Larval intestines increased in size throughout the

TABLE 1 Embryonic development and size (mean ± *SE*; *n* = 30) of *Holothuria tubulosa* at 24°C

Stage	Time (min, hr, d)	Size (μm)
Unfertilized egg	–	151.2 ± 2.1
Elevation of the fertilization envelope	0–5 min	172.1 ± 1.7
2-cell division	110 min	–
4-cell division	150 min	–
8-cell division	195 min	–
16-cell division	255 min	–
Morula	360 min	–
Blastula	12 hr	173.0 ± 1.7
Early gastrula	20 hr	177.1 ± 1.6
Late gastrula	2 d	302.7 ± 4.3
Early auricularia	3 d	434.2 ± 5.0
Mid auricularia	7 d	693.2 ± 7.2
Late auricularia	20 d	786.7 ± 12.3
Doliolaria	24 d	423.6 ± 6.6
Pentactula	26 d	431.7 ± 8.0
Juvenile	27 d	512.3 ± 10.1
Juvenile	37 d	783.2 ± 20.7
Juvenile	45 d	1479.9 ± 78.1
Juvenile	90 d	8700 ± 1400

hr, hours; min, minutes; d, day.

development of auricularia larvae. The axohydrocoel made its first appearance during the mid-auricularia stage (Figure 3c), widely expanding during the late auricularia stage (Figure 3d). Late auricularia larvae became non-feeding doliolaria after 24 days post fertilization. At this stage, a rapid metamorphosis from the late auricularia to the doliolaria stage took place in 82% of the larvae. Metamorphosis did not take place in the remaining (18%) of the larvae.

Doliolaria larvae of *H. tubulosa* displayed the characteristic barrel shape with five ciliary bands (Figure 4a). At this stage, the larvae became smaller than in the previous stages, with a mean length and width of 423.6 ± 6.6 μm and 267.3 ± 3.9 μm respectively. During this stage, the digestive tract disappeared, and in the late doliolaria stage, tentacles became visible (Figure 4b) and larvae started swimming close to the substrate. Hyaline spheres appeared in late auricularia (Figure 3d) and remained visible during the doliolaria stage. At 26 days post fertilization, the first pentactula larvae were observed and became translucent with five anterior tentacles (Figure 4c) and podia. The mean length and width of pentactula larvae were 431.7 ± 8.0 μm and 298.6 ± 4.9 μm respectively. Juveniles appeared at 27–30 days post fertilization. At 37 days post fertilization, juveniles measured a mean size of 783.2 ± 20.7 μm, at 45 days juveniles reached the size of 1479.9 ± 78.1 μm, and their bodies were covered with ossicles of varying shapes (Figure 4d). At 90 days post fertilization, juveniles reached the mean size of 8.7 ± 1.4 mm (Figure 5). Juvenile growth of *H. tubulosa* was variable and some individuals become considerably larger than others.

3.3 | Feeding protocols

Varying the feed rate yielded differences in larval growth, development and survival. Food administered at a high concentration (20,000–40,000 cells/ml) prevented the metamorphosis of larvae, which never progressed past the mid auricularia stage. At the mid-auricularia stage, larvae degenerated and displayed: (i) a spherical shape; (ii) a drastic reduction in the digestive tract; (iii) the disappearance of cilia and ectodermic protrusions; and (iv) larvae sunk to the bottom of the flask, exhibited benthic behaviours, and maintained this form for over 20 days (Figure 6). Food administered at a low concentration (5,000–10,000 cells/ml) resulted in high numbers of metamorphosed larvae and a high number of embryos reaching the juvenile stages.

In this study, 98% of deposited eggs of *H. tubulosa* were mature. We observed a high proportion (96% on average) of successfully fertilized embryos that reached first cleavage, whereas 72% developed into the early auricularia stage. Under the low feeding regime, 51% of larvae reached the late auricularia stage, and 42% metamorphosed into doliolaria. We estimate that, finally, 7% of fertilized eggs metamorphosed into juveniles at 30 days post fertilization and 4% reached the 45th day becoming visible to the naked eye (Figure 7). The survival rate at 90 days post fertilization was estimated to be 1.4%, at this stage. Finally juveniles were transferred into larger aquaria. Considering that some juveniles may have escaped the count by adhering to the internal surface of plate supports instead of to the panels, the survival rate of juveniles reported here may be a slight underestimate.

4 | DISCUSSION

Research involving spawning induction is essential for controlling sea cucumber reproductive processes. Many authors have suggested that physical and mechanical treatments are effective methods for inducing spawning in both tropical and temperate sea cucumbers (Battaglene et al., 2002; Costelloe, 1985; Ramofafia, Byrne & Battaglene, 2003). However, results vary and the responses are species-specific (Abdel Razek, Abdel Rahman, Moussa, Mena & El-Gamal, 2012; Agudo, 2006; Domínguez-Godino et al., 2015; Hu et al., 2010; Kumara, Jayanatha, Pushpakumara, Bandara & Dissanayake, 2013; Zacarías-Soto, Olvera-Novoa, Pensamiento-Villarauz & Sánchez-Tapia, 2013). On the basis of the literature data, we tested out, on *H. tubulosa*, two common methods utilized to induce spawning in sea cucumbers. The mechanical shock treatment, successfully employed in species such as *A. japonicus*, *Bohadschia marmorata*, *Holothuria scabra* and *Stichopus horrens* (Al Rashdi, Eeckhaut & Claereboudt, 2012; Hu et al., 2013; Laxminarayana, 2005; Renbo & Yuan, 2004) proved unsuccessful in *H. tubulosa* broodstock, this remaining unresponsive during the trials. The thermal shock treatment, another effective method widely used to induce spawning in species such as *Actinopyga mauritiana*, *Australostichopus mollis*, *H. arguinensis*, *Holothuria atra*, *Holothuria fuscogilva*, *S. horrens* and *H. scabra* (Battaglene et al., 2002; Domínguez-Godino et al., 2015;

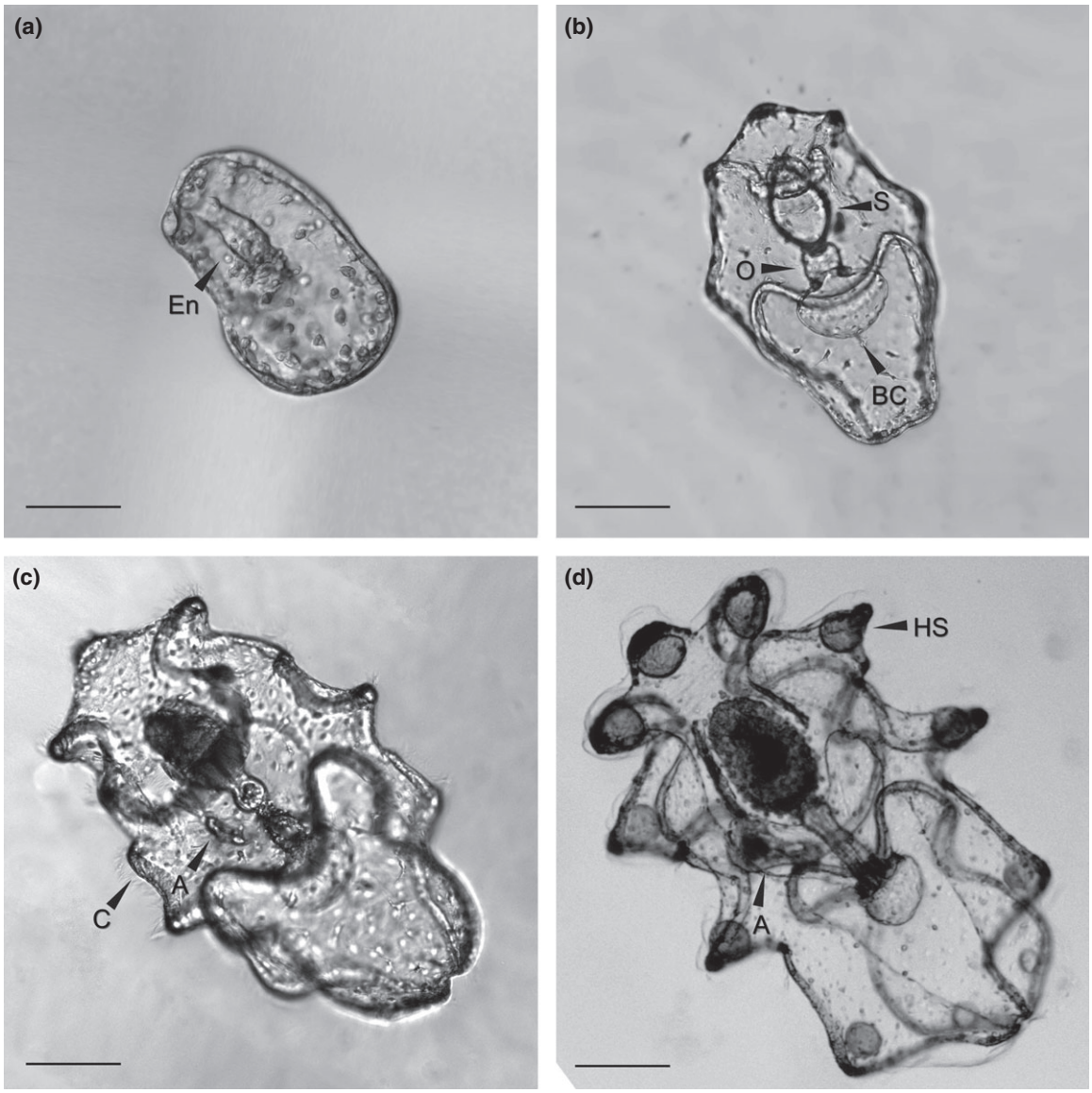

FIGURE 3 Larval development of *Holothuria tubulosa*. (a) Late gastrula: enterocoeles are forming (En). (b) Early auricularia with buccal cavity (BC), oesophagus (O), stomach (S). (c) Mid-auricularia: cilia (C), axohydrocoel (A) are forming. (d) Late auricularia with an axohydrocoel (A) and hyaline spheres (HS). Scale bars = 100 μm

Hu et al., 2013; Laxminarayana, 2005; Morgan, 2009; Ramofafia et al., 2003) proved to be only occasionally effective for *H. tubulosa*.

In wild reproduction, temperature increase is probably an indispensable condition for the induction of gonadal maturation in sea cucumbers (Guzman, Guevara & Hernandez, 2003; Muthiga, Kawaka & Ndirangu, 2009; Tehranifard & Uryan, 2006). In artificial reproduction, a slow and progressive increase in temperature is successfully applied to induce gonadal maturation in captivity maintained broodstock of *A. japonicus*. This method allows Chinese farmers to obtain healthy gametes even outside the reproductive season (Xilin, 2004; Xiyin, Guanghui, Qiang, Lian & Benxue, 2004).

The two methods below both include a slow increase in temperature and were applied to induce spawning in *H. tubulosa* broodstock. Thermal stimulation treatment successfully induced gamete release 1–4 days after stimulation began. Hence, we hypothesize that this treatment may induce the gonadal maturation, which culminates in a spawning event. Lastly, the combined thermal stimulation and thermal shock method resulted in the best spawning performance for *H. tubulosa*, offering more control of the spawning time respect to thermal stimulation method alone.

During spawning, *H. tubulosa*, as well as many other holothuroid species, raise the anterior end of the body off the substrate to ensure the effective dispersion of gametes (McEuen, 1988). Males spawn first, releasing sperm that potentially stimulates and synchronizes the females to release eggs in short and powerful squirts to facilitate dispersion as observed by Battaglene et al. (2002).

The larval cycle *H. tubulosa* was consistent with that of holothurians reared previously; however, the duration of the larval stages differs from other species (Agudo, 2006; Asha & Muthiah, 2002; Battaglene et al., 2002; Domínguez-Godino et al., 2015). In

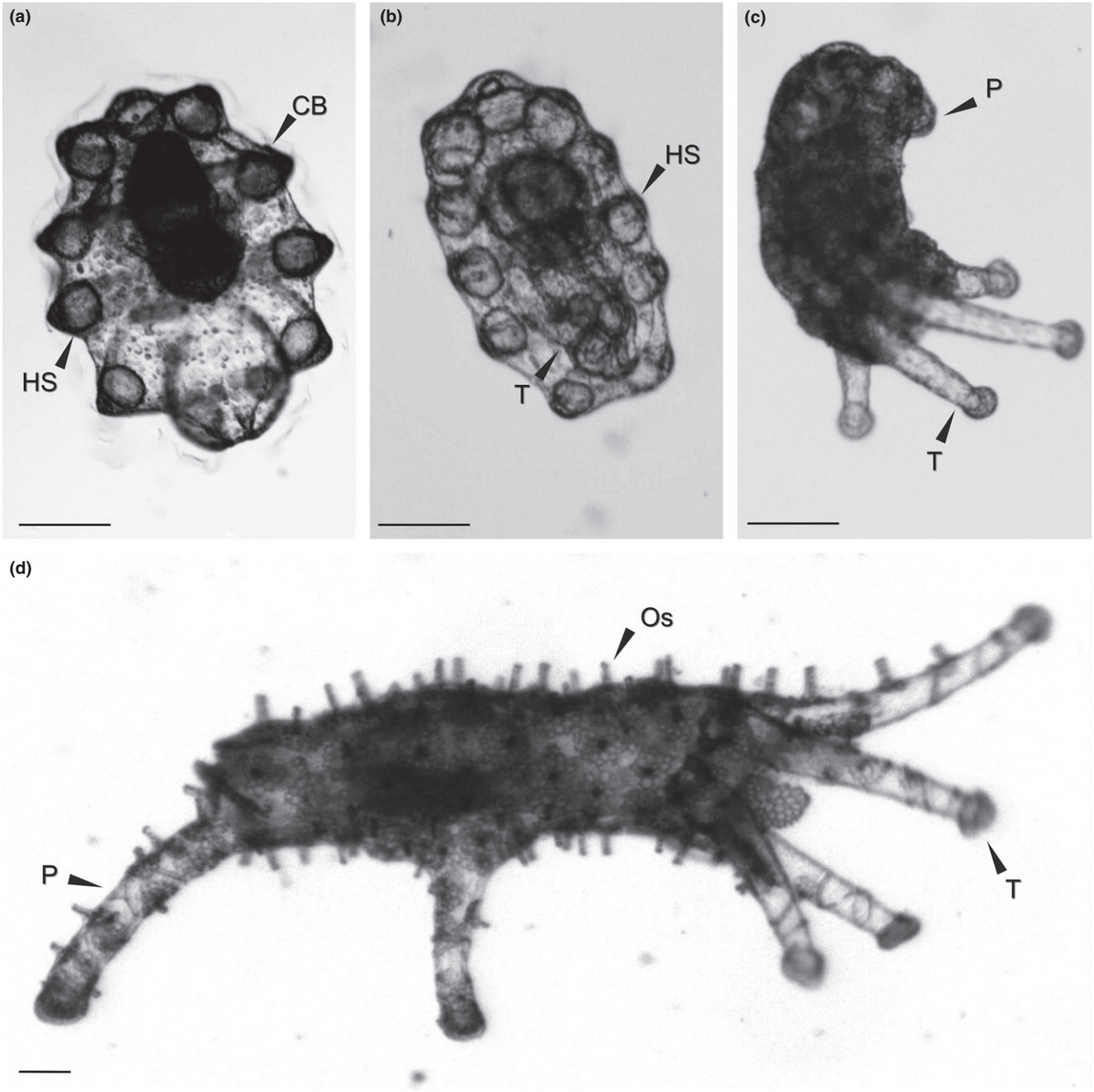

FIGURE 4 Metamorphosis of *Holothuria tubulosa* from doliolaria to juvenile. (a) Early doliolaria, cilia bends (CB) and hyaline spheres (HS). (b) Late doliolaria with tentacles (T). (c) Pentactula with tentacles (T) and podia (P). (d) 45 days juvenile: podia (P), ossicle (Os) and tentacles (T). Scale bars = 100 μm

H. tubulosa, while development from the first divisions to the early auricularia stage took nearly the same length of time as observed in other holothurians, the time period for *H. tubulosa* to reach the doliolaria stage (24 days) was approximately 10 days longer than that observed in many other reared sea cucumbers, including *S. horrens* (Hu et al., 2013), *B. marmorata* and *H. atra* (Laxminarayana, 2005), *H. scabra* (Agudo, 2006), *H. Arguinensis* (Domínguez-Godino et al., 2015) and *A. japonicus* (Renbo & Yuan, 2004). In this regard Hoegh-Guldberg and Pearse (1995) stated that temperature and other factors such as food availability might influence developmental timing in marine invertebrate larvae. Changes in temperature have been observed to result in differences in the larval timing of sea cucumber species such as *Cucumaria frondosa* and *Holothuria spinifera* (Asha, 2004; Hamel & Mercier, 1996).

The larval-rearing protocol developed in this study was compared with those published for other species of sea cucumber. Many authors suggest that water quality management and disease control are key factors for successful breeding (Agudo, 2006; Asha &

FIGURE 5 Juvenile of *Holothuria tubulosa* at 90 days post fertilization. Scale in cm

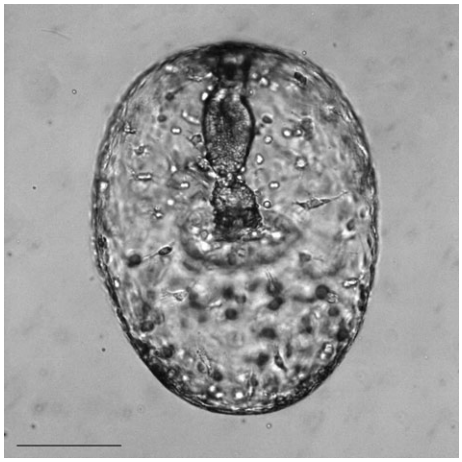

FIGURE 6 Degenerative larvae of *Holothuria tubulosa* fed at elevated concentrations of mixed algae. Scale bar = 100 μm

Muthiah, 2005; Battaglene & Bell, 1999; Battaglene et al., 2002; James, Gandhi, Palaniswamy & Rodrigo, 1994; Pitt & Duy, 2005; Stenton-Dozey & Heath, 2009). According to our observations, the feeding regime is also a relevant factor supporting the success of culturing during the larval stage (Archer, 1996; Asha, 2004; Morgan, 2001). While previous reports involving other species recommend a microalgal concentration of 20,000–40,000 cells/ml (Agudo, 2006; Asha & Muthiah, 2005; Ramofafia et al., 2003), we observed optimal growth and development in *H. tubulosa* larvae with a lower algal concentration (5,000–10,000 cells/ml). Our results indicate that high algal concentration leads to larval degeneration and loss, highlighting overfeeding as a bottleneck for juvenile production in this species. Low rations in microalgae feeding have been reported to be optimal for another species the temperate sea cucumber *A. mollis*, which like *H. tubulosa*, show a similarly slow timing in larval development (Heath, Stenton-Dozey & Jeffs, 2015; Morgan, 2008, 2009; Stenton-Dozey & Heath, 2009). Thorson (1950) suggested that poor food conditions during the larval stages could cause slow growth and prolong larval life in marine invertebrates. However, little is known about the life history strategies and the influence of feeding behaviour on the duration of development in sea cucumbers. Larval duration in *H. tubulosa* could be related to its feeding behaviour, which seems to be adapted for low feeding concentrations.

This study, to the best of our knowledge, is the first to successfully induce *H. tubulosa* to spawn and to rear larvae until the juvenile stage using an established hatchery protocol. Considering the species' important ecological role, its high commercial value and its potential use in IMTA systems, we assume that the development of effective breeding and rearing techniques for *H. tubulosa* would be extremely valuable to the Mediterranean aquaculture industry.

5 | CONCLUSION

We have successfully established a method for the hatchery production of *H. tubulosa* juveniles. This method could constitute the basis for future research aimed at evaluating juvenile rearing for direct consumption or for use in stock enhancement programmes. Our preliminary trials regarding *H. tubulosa* reproduction show that this species should be considered as a potential candidate for future marine aquaculture assays. The ability to induce spawning in adults, and the successful rearing of larvae to juvenile stages under hatchery

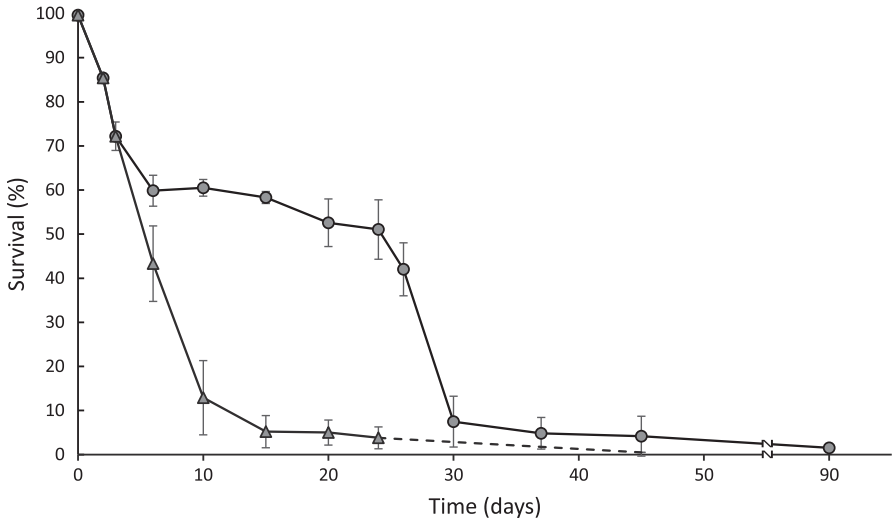

FIGURE 7 Survival of larval stages and juveniles of *Holothuria tubulosa* during 90 days of monitoring in two feeding conditions. High concentration (triangles) 20,000–40,000 cells/ml; Low concentration (circles) 5,000–10,000 cells/ml (mean ± *SE*; *n* = 6) and dotted line representing the degenerative larval stage

conditions, could both support the development of a diversified aquaculture sector in the Mediterranean region.

To build upon the results of this study, further research on optimum larval feeding regimes will be crucial both for effective hatchery production and for clarifying differences in larval ecology between sea cucumber species. In addition, the rearing response in multi-trophic integrated aquaculture should be investigated to assess this species potential for large-scale production.

ACKNOWLEDGMENTS

This research was supported with the contribution of "Piscicoltura del Golfo di Gaeta (P2G)". Special thanks to "Ufficio Tecnico Territoriale Armamenti Terrestri Nettuno" for their help during broodstock collection.

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REFERENCES

Abdel Razek, F. A., Abdel Rahman, S. H., Moussa, R. M., Mena, M. H., & El-Gamal, M. M. (2012). Captive spawning of *Holothuria arenicola* (Semper 1868) from Egyptian Mediterranean coast. *Asian Journal of Biological Sciences*, *5*, 425–431.

Agudo, N. (2006). *Sandfish hatchery techniques*. Nouméa, New Caledonia: Australian Centre for International Agricultura Research, Secretariat of the Pacific Community and the World Fish Center.

Ahlgren, M. O. (1998). Consumption and assimilation of salmon net pen fouling debris by the red sea cucumber *Parastichopus californicus*: Implications for polyculture. *Journal of the World Aquaculture Society*, *29*(2), 133–139.

Al Rashdi, K. M., Eeckhaut, I., & Claereboudt, M. R. (2012). *A manual on hatchery of sea cucumber Holothuria scabra in the Sultanate of Oman*. Muscat, Sultanate of Oman: Ministry of Agriculture and Fisheries Wealth, Aquaculture Centre.

Anderson, S. C., Flemming, J. M., Watson, R., & Lotze, H. K. (2011). Serial exploitation of global sea cucumber fisheries. *Fish and Fisheries*, *12*(3), 317–339.

Antoniadou, C., & Vafidis, D. (2011). Population structure of the traditionally exploited holothurian *Holothuria tubulosa* in the south Aegean Sea. *Cahiers de Biologie Marine*, *52*, 171–175.

Archer, J. E. (1996). *Aspects of the reproductive and larval biology and ecology, of the temperate holothurian Stichopus mollis (Hutton)*. Doctoral dissertation, University of Auckland.

Asha, P. S. (2004). Effect of feed concentrations on larval growth, survival and development of *Holothuria* (Theelothuria) *spinifera* Thee1. *Journal of the Marine Biological Association of India*, *46*(1), 80–86.

Asha, P. S., & Muthiah, P. (2002). Spawning and larval rearing of sea cucumber: *Holothuria* (Theelothuria) *spinifera* Theel. *SPC Beche-de-mer Information Bulletin*, *16*, 11–15.

Asha, P. S., & Muthiah, P. (2005). Effects of temperature, salinity and pH on larval growth, survival and development of the sea cucumber *Holothuria spinifera* Theel. *Aquaculture*, *250*(3), 823–829.

Aydin, M. (2008). The commercial sea cucumber fishery in Turkey. *SPC Beche-de-mer Information Bulletin*, *28*, 40–41.

Aydin, M., Hüseyin, S., Bekir, T., Yilmaz, E., & Sevim, K. (2011). Proximate composition and fatty acid profile of three different fresh and dried commercial sea cucumbers from Turkey. *International Journal of Food Science and Technology*, *46*, 500–508.

Barrington, K., Chopin, T., & Robinson, S. (2009). *Integrated multi-trophic aquaculture (IMTA) in marine temperate waters*. Integrated mariculture: a global review. FAO Fisheries and Aquaculture Technical Paper No. 529 (pp. 7–46), Rome.

Bartley, D. M., & Bell, J. D. (2008). Restocking, stock enhancement, and sea ranching: Arenas of progress. *Reviews in Fisheries Science*, *16*, 357–365.

Battaglene, S. C., & Bell, J. D. (1999). Potential of the tropical Indo-Pacific sea cucumber, *Holothuria scabra*, for stock enhancement. In B. R. Howell, E. Moksness & T. Svasand (Eds.), *Proceedings of the First International Symposium on Stock Enhancement and Sea Ranching* (pp. 478–490). Oxford: Fishing News Books, Blackwell Science.

Battaglene, S. C., Seymour, J. E., Ramofafia, C., & Lane, I. (2002). Spawning induction of three tropical sea cucumbers *Holothuria scabra*, *Holothuria fuscogilva* and *Actinopyga mauritiana*. *Aquaculture*, *207*, 29–47. https://doi.org/10.1016/S0044-8486(01)00725-6

Bulteel, P., Jangoux, M., & Coulon, P. (1992). Biometry, bathymetric distribution, and reproductive cycle of the holothuroid *Holothuria tubulosa* (Echinodermata) from Mediterranean Sea grass beds. *Marine Ecology*, *13*, 53–62.

Çakly, S., Cadun, A., Kisla, D., & Dicer, T. (2004). Determination of quality characteristics of *Holothuria tubulosa* (Gmelin, 1788) in Turkish sea (Aegean region) depending on sun drying process step used in Turkey. *International Journal of Food Science & Technology*, *13*, 3–16.

Chen, J. (2004). Present status and prospects of sea cucumber industry in China. In A. Lovatelli, C. Conand, S. W. Purcell, S. Uthicke, J. F. Hamel & A. Mercier (Eds.), *Advances in sea cucumber aquaculture and management*. FAO Fisheries Technical Paper No. 463 (pp. 25–38). Rome.

Conand, C. (2004). Present status of world sea cucumber resources and utilization: An international overview. In A. Lovatelli, C. Conand, S. W. Purcell, S. Uthicke, J. F. Hamel & A. Mercier (Eds.), *Advances in sea cucumber aquaculture and management*. FAO Fisheries Technical Paper No. 463 (pp.13–24). Rome.

Costelloe, J. (1985). The annual reproductive cycle of the holothurian *Aslia lefevrei* (Dendrochirota: Echinodermata). *Marine Biology*, *88*, 155–165.

Dabbagh, A. R., Sedaghat, M. R., Rameshi, H., & Kamrani, E. (2011). Breeding and larval rearing of the sea cucumber *Holothuria leucospilota* Brandt (*Holothuria vegabunda* Selenka) from the northern Persian Gulf, Iran. *SPC Beche-de-mer Information Bulletin*, *31*, 35–38.

Despalatović, M., Grubelić, I., Šimunović, A., Antolić, B., & Žuljević, A. (2004). Reproductive biology of the holothurian *Holothuria tubulosa* (Echinodermata) in the Adriatic Sea. *Journal of the Marine Biological Association of the U.K.*, *84*, 409–414.

Domínguez-Godino, J. A., Slater, M. J., Hannon, C., & González-Wangüermert, M. (2015). A new species for sea cucumber ranching and aquaculture: Breeding and rearing of *Holothuria arguinensis*. *Aquaculture*, *438*, 122–128.

FAO, Global Production Statistics 1950–2014. Retrieved from http://www.fao.org/fishery/statistics/global-aquaculture-production/query/en.

Gmelin, J. F. (1788). *Systema naturae: per Regna tria naturae, secundum classes, ordines, genera, species, cum characteribus, differentiis, synonomis, locis*. Beer, vol. *1*(6).

González-Wangüemert, M., Aydin, M., & Conand, C. (2014). Assessment of sea cucumber populations from the Aegean Sea (Turkey): First insights to sustainable management of new fisheries. *Ocean & Coastal Management*, *92*, 87–94.

González-Wangüemert, M., Valente, S., & Aydin, M. (2015). Effects of fishery protection on biometry and genetic structure of two target sea cucumber species from the Mediterranean Sea. *Hydrobiologia*, *743*, 65–74. https://doi.org/10.1007/s10750-014-2006-2

González-Wangüemert, M., Valente, S., Henriques, F., Domínguez-Godino, J., & Serrão, E. (2016). Setting preliminary biometric baselines for new target sea cucumbers species of the NE Atlantic and Mediterranean fisheries. *Fisheries Research*, *179*, 57–66.

Guillard, R. R. L., & Ryther, J. H. (1962). Studies of marine planktonic diatoms: I. *Cyclotella nana* Hustedt and *Detonula convervacea* (Cleve). *Gran Canadian Journal of Microbiology*, *8*, 229–239.

Gustato, G., & Villari, A. (1979). On the ecology and species frequency of the genus *Holothuria* in the Gulf of Naples. In M. Jangoux (Ed.), *Echinoderms* (pp. 187). Rotterdam: Present and Past.

Guzman, H. M., Guevara, C. A., & Hernandez, I. C. (2003). Reproductive cycle of two commercial species of sea cucumber (Echinodermata: Holothuroidea) from Caribbean Panama. *Marine Biology*, *142*, 271–279.

Hamel, J. F., & Mercier, A. (1996). Early development, settlement, growth, and spatial distribution of the sea cucumber *Cucumaria frondosa* (Echinodermata: Holothuroidea). *Canadian Journal of Fisheries and Aquatic Sciences*, *53*, 253–271.

Han, Q., Keesing, J. K., & Liu, D. (2016). A review of sea cucumber aquaculture, ranching, and stock enhancement in China. *Reviews in Fisheries Science & Aquaculture*, *24*, 326–341. https://doi.org/10.1080/23308249.2016.1193472

Heath, P., Stenton-Dozey, J., & Jeffs, A. G. (2015). Sea cucumber aquaculture in New Zealand. In N. Brown, & S. Eddy (Eds.), *Aquaculture of echinoderms* (pp. 353–360). New York: John Wiley & Sons.

Hoegh-Guldberg, O. V. E., & Pearse, J. S. (1995). Temperature, food availability, and the development of marine invertebrate larvae. *American Zoologist*, *35*(4), 415–425.

Hu, C., Li, H., Xia, J., Zhang, L., Luo, P., Fan, S., … Wen, J. (2013). Spawning, larval development and juvenile growth of the sea cucumber *Stichopus horrens*. *Aquaculture*, *404*, 47–54.

Hu, C., Xu, Y., Wen, J., Zhang, L., Fan, S., & Su, T. (2010). Larval development and juvenile growth of the sea cucumber *Stichopus* sp. (Curry fish). *Aquaculture*, *300*, 73–79. https://doi.org/10.1016/j.aquaculture.2009.09.033

James, D. B. (2001). Twenty sea cucumbers from seas around India. *Naga, The ICLARM Quarterly*, *24*, 4–8.

James, D., Gandhi, A., Palaniswamy, N., & Rodrigo, J. X. (1994). Hatchery techniques and culture of the sea-cucumber *Holothuria scabra*. *CMFRI Special Publication*, *57*, 1–40.

Kazanidis, G., Lolas, A., & Vafidis, D. (2014). Reproductive cycle of the traditionally exploited sea cucumber *Holothuria tubulosa* (Holothuroidea: Aspidochirotida) in Pagasitikos Gulf western Aegean Sea Greece. *Turkish Journal of Zoology*, *38*, 306–315.

Kinch, J., Purcell, S., Uthicke, S., & Friedman, K. (2008). Population status, fisheries and trade of sea cucumbers in the Western Central Pacific. In V. Toral-Granda, A. Lovatelli & M. Vasconcellos (Eds.), *Sea cucumbers: A global review of fisheries and trade*. FAO Fisheries and Aquaculture Technical Paper No. 516 (pp. 7–55). Rome.

Kumara, P. A., Jayanatha, J., Pushpakumara, J., Bandara, W., & Dissanayake, D. C. T. (2013). Artificial breeding and larval rearing of three tropical sea cucumber species *Holothuria scabra*, *Pseudocolochirus violaceus* and *Colochirus quadrangularis* in Sri Lanka. *SPC Beche-de-mer Information Bulletin*, *33*, 30–37.

Laxminarayana, A. (2005). Induced spawning and larval rearing of the sea cucumbers *Bohadschia marmorata* and *Holothuria atra* in Mauritius. *SPC Beche-de-mer Information Bulletin*, *22*, 48–52.

Lovatelli, A., Conand, C., Purcell, S., Uthicke, S., Hamel, J. F., & Mercier, A. (2004). *Advances in sea cucumber aquaculture and management*. FAO Fisheries Technical Paper No. 463 pp. 440. Rome.

MacTavish, T., Stenton-Dozey, J., Vopel, K., & Savage, C. (2012). Deposit-feeding sea cucumbers enhance mineralization and nutrient cycling in organically-enriched coastal sediments. *PLoS ONE*, *7*(11), e50031.

Massin, C., & Jangoux, M. (1976). Observations écologiques sur *Holothuria tubulosa*, *Holothuria polii* et *Holothuria forskali* (Echinodermata, Holothuroidea) et comportement alimentaire de *H. tubulosa*. *Cahiers de Biologie Marine*, *17*, 45–59.

McEuen, F. (1988). Spawning behaviors of northeast Pacific sea cucumbers (Holothuroidea: Echinodermata). *Marine Biology*, *98*, 565–585. https://doi.org/10.1007/BF00391548

Morgan, A. D. (2001). The effect of food availability on early growth, development and survival of the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea). *SPC Beche-de-mer Information Bulletin*, *14*, 6–12.

Morgan, A. D. (2008). The effect of food availability on phenotypic plasticity in the larvae of the temperate sea cucumber *Australostichopus mollis*. *Journal of Experimental Marine Biology and Ecology*, *363*, 89–95.

Morgan, A. D. (2009). Assessment of egg and larval quality during hatchery production of the temperate sea cucumber, *Australostichopus mollis* (Levin). *Journal of the World Aquaculture Society*, *40*(5), 629–642.

Muthiga, N. A., Kawaka, J. A., & Ndirangu, S. (2009). The timing and reproductive output of the commercial sea cucumber *Holothuria scabra* on the Kenyan coast. *Estuarine, Coastal and Shelf Science*, *84*(3), 353–360.

Ocaña, A., & Tocino, L. S. (2005). Spawning of *Holothuria tubulosa* (Holothurioidea, Echinodermata) in the Alboran Sea. *Zoologica Baetica*, *16*, 147–150.

Paltzat, D. L., Pearce, C. M., Barnes, P. A., & McKinley, R. S. (2008). Growth and production of California sea cucumbers (*Parastichopus californicus* Stimpson) co-cultured with suspended Pacific oysters (*Crassostrea gigas* Thunberg). *Aquaculture*, *275*(1), 124–137.

Pawson, D. L. (2007). Phylum echinodermata. *Zootaxa*, *1668*, 749–764.

Pereira, D. M., Valentão, P., Teixeira, N., & Andrade, P. B. (2013). Amino acids, fatty acids and sterols profile of some marine organisms from Portuguese waters. *Food Chemistry*, *141*(3), 2412–2417.

Pitt, R. (2001). Review of sandfish breeding and rearing methods. *Beche-de-mer Information Bulletin*, *14*, 14–21.

Pitt, R., & Duy, N. D. Q. (2005). Breeding and rearing of the sea cucumber *Holothuria scabra* in Viet Nam. In A. Lovatelli, C. Conand, S. W. Purcell, S. Uthicke, J. F. Hamel & A. Mercier (Eds.), *Advances in sea cucumber aquaculture and management*. FAO Fisheries Technical Paper No. 463 (pp. 333–346). Rome.

Purcell, S. W., Conand, C., Uthicke, S., & Byrne, M. (2016). Ecological roles of exploited sea cucumbers. *Oceanography and Marine Biology: An Annual Review*, *54*, 367–386.

Purcell, S. W., Hair, C. A., & Mills, D. J. (2012). Sea cucumber culture, farming and sea ranching in the tropics: Progress, problems and opportunities. *Aquaculture*, *368*, 68–81. https://doi.org/10.1016/j.aquaculture.2012.08.053

Purcell, S. W., Lovatelli, A., Vasconcellos, M., & Ye, Y. (2010). *Managing sea cucumber fisheries with an ecosystem approach*. FAO Fisheries and Aquaculture Technical Paper No. 520 pp. 171. Rome.

Purcell, S. W., Mercier, A., Conand, C., Hamel, J. F., Toral-Granda, M. V., Lovatelli, A., & Uthicke, S. (2013). Sea cucumber fisheries: Global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries*, *14*(1), 34–59.

Purcell, S. W., Samyn, Y., & Conand, C. (2012). *Commercially important sea cucumbers of the world*. FAO Species Catalogue for Fishery Purposes No. 6. Rome.

Ramofafia, C., Byrne, M., & Battaglene, S. (2003). Development of three commercial sea cucumbers *Holothuria scabra*, *Holothuria fuscogilva* and *Actinopyga mauritiana*: Larval structure and growth. *Marine & Freshwater Research*, *54*, 657–667. https://doi.org/10.1071/MF02145

Renbo, W., & Yuan, C. (2004). Breeding and culture of the sea cucumber *Apostichopus japonicus*. In A. Lovatelli, C. Conand, S. W. Purcell, S. Uthicke, J. F. Hamel & A. Mercier (Eds.), *Advances in sea cucumber*

aquaculture and management. FAO Fisheries Technical Paper No. 463 (pp. 277–286). Rome.

Roggatz, C. C., González-Wangüemert, M., Pereira, H., Rodrigues, M. J., da Silva, M. M., Barreira, L., … Custódio, L. (2016). First report of the nutritional profile and antioxidant potential of *Holothuria arguinensis*, a new resource for aquaculture in Europe. *Natural Product Research*, *30*(18), 2034–2040.

Sicuro, B., & Levine, J. (2011). Sea cucumber in the Mediterranean: A potential species for aquaculture in the Mediterranean. *Reviews in Fisheries Science*, *19*, 299–304.

Sicuro, B., Piccinno, M., Gai, F., Abete, M. C., Danieli, A., Dapra, F., … Vilella, S. (2012). Food quality and safety of Mediterranean sea cucumbers *Holothuria tubulosa* and *Holothuria polii* in southern Adriatic Sea. *Asian Journal of Animal and Veterinary Advances*, *7*, 851–859.

Slater, M. J., & Carton, A. G. (2007). Survivorship and growth of the sea cucumber *Australostichopus* (*Stichopus*) *mollis* (Hutton 1872) in polyculture trials with green-lipped mussel farms. *Aquaculture*, *272*(1), 389–398.

Slater, M. J., & Carton, A. G. (2009). Effect of sea cucumber (*Australostichopus mollis*) grazing on coastal sediments impacted by mussel farm deposition. *Marine Pollution Bulletin*, *58*(8), 1123–1129.

Stenton-Dozey, J., & Heath, P. (2009). A first for New Zealand: Culturing our endemic sea cucumber for overseas markets. *Water & Atmosphere*, *17*, 20–21.

Tehranifard, A., & Uryan, S. (2006). Reproductive cycle of *Stichopus hermanni* from Kish Island, Iran. *SPC Beche-de-mer Information Bulletin*, *24*, 22–27.

Thorson, G. (1950). Reproductive and larval ecology of marine bottom invertebrates. *Biological Reviews*, *25*(1), 1–45.

Tolon, M. T., Emiroglu, D., Gunay, D., & Ozgul, A. (2017). Sea cucumber (*Holothuria tubulosa* Gmelin, 1790) culture under marine fish net cages for potential use in integrated multi-trophic aquaculture (IMTA). *Indian Journal of Geo Marine Sciences*, *46*, 749–756.

Toral-Granda, V., Lovatelli, A., & Vasconcellos, M. (2008). *Sea cucumbers: A global review of fisheries and trade*. FAO Fisheries and Aquaculture Technical Paper No. 516 pp. 331. Rome.

Tortonese, E. (1965). *Echinodermata*, *Fauna d'Italia*, *IV*. Calderini (Ed.) pp. 422. Bologna.

Valente, S., Serrão, E., & González-Wangüemert, M. (2015). West vs East Mediterranean Sea: Origin and genetic differentiation of the sea cucumber *Holothuria polii*. *Marine Ecology*, *36*, 485–495.

Xilin, S. (2004). The progress and prospects of studies on artificial propagation and culture of the sea cucumber, *Apostichopus japonicus*. In A. Lovatelli, C. Conand, S. Purcell, S. Uthicke, J. F. Hamel & A. Mercier (Eds.), *Advances in sea cucumber aquaculture and management*. FAO Fisheries Technical Paper No. 463 (pp. 273–276). Rome.

Xiyin, L., Guanghui, Z., Qiang, Z., Lian, W., & Benxue, G. (2004). Studies on hatchery techniques of the sea cucumber, *Apostichopus japonicus*. In A. Lovatelli, C. Conand, S. Purcell, S. Uthicke, J. F. Hamel & A. Mercier (Eds.), *Advances in sea cucumber aquaculture and management*. FAO Fisheries Technical Paper No. 463. (pp 87–296). Rome.

Yanagisawa, T. (1998). Aspects of the biology and culture of the sea cucumber. In S. De Silva (Ed.), *Tropical mariculture* (pp. 291–308). London: Academic Press.

Yokoyama, H. (2013). Growth and food source of the sea cucumber *Apostichopus japonicus* cultured below fish cages—potential for integrated multi-trophic aquaculture. *Aquaculture*, *372*, 28–38.

Yu, Z., Zhou, Y., Yang, H., Ma, Y. E., & Hu, C. (2014). Survival, growth, food availability and assimilation efficiency of the sea cucumber *Apostichopus japonicus* bottom-cultured under a fish farm in southern China. *Aquaculture*, *426*, 238–248.

Zacarías-Soto, M., Olvera-Novoa, M. A., Pensamiento-Villarauz, S., & Sánchez-Tapia, I. (2013). Spawning and larval development of the four-sided sea cucumber, *Isostichopus badionotus* (Selenka 1867), under controlled conditions. *Journal of the World Aquaculture Society*, *44*(5), 694–705.

How to cite this article: Rakaj A, Fianchini A, Boncagni P, Lovatelli A, Scardi M, Cataudella S. Spawning and rearing of *Holothuria tubulosa*: A new candidate for aquaculture in the Mediterranean region. *Aquac Res*. 2017;00:1–12. https://doi.org/10.1111/are.13487