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Spawning, Larval Development, and Juvenile Rearing of White Teatfish *Holothuria fuscogilva* in the Hatchery in the Philippines

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The significant exploitation of the high-value white teatfish, *Holothuria fuscogilva*, has raised global concerns about the species' wild populations. Aquaculture technology development may potentially restore dwindling stocks; however, it remains to be established in the Philippines. This study reported the first successful mass production of *H. fuscogilva* in the Philippines. The broodstock was collected on two occasions from Tubajon, Laguindingan, Misamis Oriental (May and June 2020). All broodstock survived with no evisceration in 30 min (27.8–35.2 ˚C) of transport to the hatchery. Broodstock was induced to spawn using spawning techniques adopted from those of Agudo (2006) with modification in three events (July, August, and December 2020). However, successful spawning only occurred when the temperature was reduced by 7 ˚C (extreme cold shock). *H. fuscogilva* produced 3.4 million eggs in three spawning inductions at > 88% fertilization rate. The eggs hatched 2–3 d post-fertilization. Generally, the embryonic development of *H. fuscogilva* was radial holoblastic, and the larval development had the same pattern as other sea cucumber species. The auricularia stage was observed at Days 4–27, followed by the doliolaria stage at Days 28–32, and pentactula stage at 33–39 d post-fertilization. An average of 1.1% of the fertilized eggs proceeded to the juvenile stage at 40–45 d post-fertilization. Over 4 mo, juveniles grew at 0.03 ± 0.002 g d^{-1}. This is the first documentation of the larval development of *H. fuscogilva* and the first production of the juvenile stage in the Philippines, where 15,000 juveniles are being grown in the hatchery for future broodstock.

Keywords: early development, hatchery, *Holothuria fuscogilva*, larvae, sea cucumber, spawning induction

INTRODUCTION

The white teatfish, *Holothuria fuscogilva* (Cherbonnier 1980), is a tropical sea cucumber (Holothuroidea) species commonly found in seagrass beds, reef slopes, and lagoons at depths of 3–40 m in the Indo-Pacific region (Conand 1981; Reichenbach 1999). The species is one of the largest sea cucumber species, reaching a maximum size of 57 cm and a maximum live weight of 4000 g (Conand 1990; Leopardas *et al.* 2021). *Holothuria fuscogilva* spawns between December and January in New Caledonia and

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from December to March in the Maldives during the northeast monsoon season (Conand 1981: Reichenbach 1999).

Holothuria fuscogilva is commonly exploited for its dried body wall, called "beche-de-mer" or "trepang" (Conand and Byrne 1993; Toral-Granda 2007; Muthiga and Kawaka 2009). Its popularity among commercial sea cucumbers is due to its thick and rigid body wall and consequent lower weight loss with a wet-to-dry conversion ratio of 18.6% (Ngaluafe and Lee 2013). These attributes contribute to high demand in the Asian markets, with an average price of USD 219 kg^{-1} in Hong Kong and USD 154 kg^{-1} in Guangzhou in 2016 (Purcell *et al.* 2018). High fishing pressure of *H. fuscogilva* led to it being listed in the Convention on International Trade in Endangered Species of Wild Fauna and Flora appendix II. It was declared vulnerable by the International Union of the Conservation of Nature (Conand *et al.* 2013).

Nocillado *et al.* (2022) mentioned that sea cucumber aquaculture is a long-term solution for meeting market demand for this highly sought-after species while reducing strain on wild stocks. Aquaculture techniques in several sea cucumber species have been reported – for example, *Holothuria scabra*, *Apostichopus japonicus*, *Stichopus horrens*, *Actinopyga miliaris*, *Isostichopus fuscus*, and *H. fuscogilva* (Agudo 2006; Purcell *et al.* 2012). The standard practice has been to collect mature individuals from the wild and induce them to spawn in tanks by exposing them to thermal shock, adding microalgae to the spawning water, or exposing them to short-term air exposure or combinations of these treatments (Battaglene *et al*. 2002; Agudo 2006; Cheng *et al*. 2021). In the experience of Battaglene *et al.* (2002) and Ramofafia *et al.* (2003), *H. fuscogilva* broodstock from Marau Sound, Guadalcanal, Solomon Islands were induced to spawn with the addition of dried *Schizochytrium* sp. and other algal diets, but larvae failed to develop up to the juvenile stage.

Kiribati was the first (and only) country to produce high-value *H. fuscogilva* juveniles from 1997–2009 consistently, but the technology has been little documented (Friedman and Tekanene 2005; Purcell and Tekanene 2006), and few juveniles were produced (Purcell *et al.* 2009, 2012). Recently, Nocillado *et al.* (2022) reported using neurohormone relaxin-like gonad-stimulating peptide to induce oocyte maturation and spawning of *H. fuscogilva* and documented up to the early juvenile stage.

Lumasag *et al.* (2017) were able to produce *H. fuscogilva* juveniles in Northern Mindanao, Philippines, by collecting and spawning *H. fuscogilva* broodstock sourced from shallow seagrass and algal flats inside the Capayas Island Marine Protected Area in Lopez Jaena, Misamis Occidental (de Guzman and Quiñones 2021). His study followed the spawning techniques of Agudo (2006) for *H. scabra* because there are still no established hatchery productions and protocols for *H. fuscogilva* aquaculture in the Philippines. The development of *H. fuscogilva* hatchery technology is compounded by the difficulty in collecting viable broodstock, especially *H. fuscogilva* are scarce in shallow areas, and most are found in deeper waters of the Philippines. These pose a challenge in the mass production of *H. fuscogilva* in the hatchery for stock enhancement and sustainability.

To provide baseline information needed for establishing hatchery protocols for *H. fuscogilva* in the Philippines, this study aimed to mass-produce *H. fuscogilva* juveniles. Specifically, the study investigated: [i] broodstock transport techniques, [ii] spawning induction (SI) techniques, [iii] broodstock's capability to repeatedly spawn, and [iv] the embryonic, larval, and juvenile development of *H. fuscogilva*.

MATERIALS AND METHODS

Broodstock Collection and Transport

Adult *H. fuscogilva* were collected by local fishers at Tubajon, Laguindingan, Misamis Oriental (8°37'53.4"N 124°28'05.7"E) in May and June 2020 (Figure 1). A month prior to the first collection, permission was obtained to obtain broodstock and to use compressor diving. Broodstock was collected from 40–60 m depth at 02:00 AM to avoid stressful exposure of the sea cucumber to high temperatures.

In May 2020, broodstock (n = 18) was transported in plastic boxes (110 L capacity) with a water volume of 50 L and provided with continuous aeration. In a complete block design set-up in triplicate (n = 3), three broodstock were placed in each replicate with two treatments of [i] the addition of two pieces of ice to lower the temperature and [ii] without ice to determine the effect on survival of lowering the transport temperature. Broodstock was transported to Mindanao State University Naawan sea cucumber hatchery for 30 min. Each sea cucumber was checked upon arrival for signs of spawning and evisceration. Evisceration is a method of autotomy involving the ejection of internal organs and is used by animals as a defensive strategy. By dividing the overall number of individuals in each treatment by the total number of individuals eviscerated in each treatment, the incidence of spawning and/or evisceration was estimated. The survival rate of the broodstock in the transport box was also computed. Broodstock (n = 18) was also collected in Tubajon in June 2020, and the transport experiment was repeated. During the transport of broodstock, a

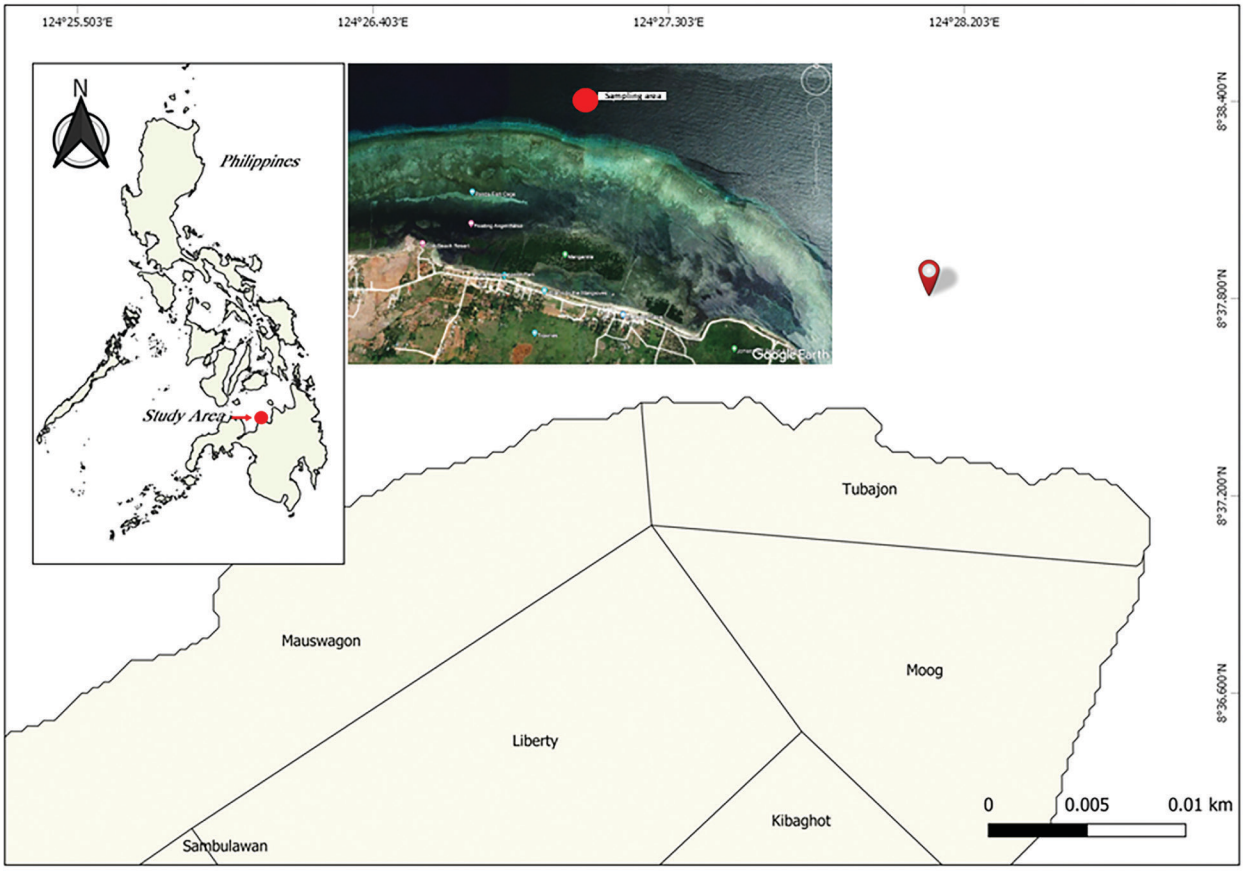

Figure 1. Map of Tubajon, Laguindingan Misamis Oriental showing the sites used for the collection of *Holothuria fuscogilva* broodstock.

refractometer (Atago, Japan) and an alcohol-filled thermometer were used to measure the salinity and temperature, respectively.

Broodstock Conditioning

After thoroughly examining the broodstock, they were placed in a 1000-L fiberglass tank for acclimation. After 24 h, the broodstock was weighed prior to putting it in tanks to monitor condition during holding and later spawning. Afterward, the broodstock was transferred to a 10-ton broodstock conditioning tank (dimensions: 4.6 m x 1.2 m x 1.2 m) with a white sand substrate (grain size: < 5 mm; substrate thickness: 1.27 cm). Tanks were filled with filtered seawater (7 tons) and provided with continuous gentle aeration. At most, 10 individuals (ind.) were put in each tank, and approximately one-third of the water was exchanged weekly. Broodstock was fed 20 L of *Navicula* sp. (750,000 cells mL^{-1}) every morning and remained in the conditioning tanks for 1 mo before SI began.

Spawning Induction (SI) Techniques

SI activities were conducted during the new moon phases of July 2020 (SI1), August 2020 (SI2), and December 2020 (SI3). Six SI techniques (treatments) were simultaneously conducted that were adopted from those of Agudo (2006) with extreme modifications, as shown in Table 1: [i] desiccation, [ii] thermal shock, [iii] food shock, [iv] salinity shock, [v] a combination of i–v, and [vi] extreme cold shock. In all SI activities, except for T5, each treatment was carried out three times at intervals of 30 min. Extreme modifications were made due to unsuccessful SIs observed when following the protocol of Agudo (2006).

The study's experimental tank consisted of 18 120-L plastic boxes with a floor size of 0.18 m^2 filled with 80 L UV-filtered saltwater and aerated continuously. Except for T1, no substrates were added to the tank to eliminate additional factors that could have influenced the results. A total of 36 broodstock were used without sex identification. All broodstock were randomly selected in every SI activity. In every SI activity, each treatment had three replicates with two broodstock per replicate/experimental tank. Temperature and salinity were monitored in each treatment. Once spawning was observed, broodstock was transferred in plastic boxes (120 L) with an ambient water temperature of 28 ˚C and salinity of 30 ppt, served

Table 1. Description of *Holothuria fuscogilva* spawning induction treatments.

Spawning induction technique	Description
[T1] Desiccation	Broodstock was moved into a rectangular (1 m x 0.5 m) container with sand exposed to air for 30 min
[T2] Thermal shock	Adjustment of ambient water temperature by ± 3–5 ˚C for 1 h, either by adding ice to decrease the temperature or using a digital quartz heating rod to increase the temperature.
[T3] Food shock	Addition of 50 g of dried Spirulina powder to a tank containing broodstock, for 1 h.
[T4] Salinity shock	Addition of freshwater to lower the water salinity by 5 ppt for 1 h.
[T5] Combination of T1–T4	Sequential application of desiccation (30 min), thermal shock (± 3–5 ˚C for 1 h), food shock (1 h), and salinity shock (1 h), respectively.
[T6] Extreme cold shock	Abruptly lowering the water temperature by 7 ºC by releasing cool water into the tank for 1 h

as spawning tanks with gentle aeration. Aeration was moderately increased to keep eggs in suspension and fertilized by the male gametes. Estimates of the number of eggs released and determination of the fertilization rate were made by adopting the methods suggested by Agudo (2006). The egg diameter was measured using a micrometer eyepiece placed in the lens of a microscope.

Additionally, the male and female-spawned broodstock were noted and marked for recall during the subsequent experiment. Once the SI activity was done, all 36 broodstock were returned to the broodstock conditioning tanks for recovery. The same set of 36 broodstock was randomly used for SI2 and SI3, following the same SI techniques to repeatedly determine the broodstock's capability to spawn in the hatchery.

Embryonic and Larval Development

Once spawning occurred, gametes were released and fertilized with sperm. At least 1 h after fertilization, eggs were siphoned from the spawning tank into a 50–80-µm sieve placed in a bowl. Eggs were washed with gentle flow running seawater (30 ppt) and stocked in 500 L fiberglass conical larval tanks at 0.2 eggs mL^{-1} provided with continuous gentle aeration. In the present study, embryonic and larval development was monitored microscopically from fertilization of the eggs up to the pentactula stage. Embryos were sampled by taking 1-mL water samples from the larval tanks in three aliquots. Embryos were placed in the Sedgewick-Rafter chamber and then mounted in the compound microscope to describe the cell divisions, perform larval counts, and measure the length at 5–30 min intervals for 24 h. The hatching rate was determined based on the mean of at least three aliquots of early auricularia larvae multiplied by the tank's total volume over the estimated number of fertilized eggs. After 24 h, monitoring was done daily until the early juvenile stage was observed. In this study, the larval rearing of *H. fuscogilva* adopted the techniques for larval rearing of *H. scabra* of Agudo (2006). Features of the different larval developmental stages *(i.e.* auricularia, doliolaria, pentactula) were observed based on the description by James *et al.* (1994), James (1999), Ramofafia *et al.* (2003), Agudo (2006), and Nocillado *et al.* (2022). In every 1 mL, at least 10 larvae were monitored for their morphological development. The longest portion of the body was measured to get the length of the larvae. Finally, the estimate of the density of each larval stage (early-, mid-, late auricularia, doliolaria, and pentactula) was determined based on the mean of at least three aliquots of larvae multiplied by the total volume of the tank over the total estimated number of fertilized eggs.

Larvae (early to late auricularia) were fed with *Chaetoceros calcitrans* at 10,000–20,000 cells mL^{-1} twice daily (09:00 AM and 03:00 PM). Feeding was paused in doliolaria stage. Settlement plates are commonly used in *H. scabra* rearing but were not added to tanks in this study. Upon reaching the pentactula stage, larvae were fed a combination of *Chaetoceros calcitrans* and *Navicula* sp. at 20,000–40,000 cells mL^{-1} once a day (09:00 AM).

Water exchange was done at 20% every other day. It was paused when doliolaria was observed to reduce the environmental stress due to changes in the external condition during the metamorphosis to the pentactula stage. Water exchange was resumed in the pentactula to the juvenile stages (30–50%) every other day. Water was moderately aerated continuously throughout the study. Daily measurements of temperature, salinity, and dissolved oxygen using Winkler's method were taken in each culture tank throughout the larval and juvenile rearing period.

Juvenile Growth and Development

This study used all the *H. fuscogilva* juveniles produced from the extreme cold shock SI technique. This study established a tank in two replicates for each successful SI activity (SI1, SI2, and SI3). One-month-old post-settled juveniles (n = 1,000) were placed in a 10-ton concrete tank with a floor size of 8 m^2. A total of 6,000 juveniles were used in this study. Stocking density was set to 125 individuals m^{-2} to avoid overcrowding. Juveniles were fed with *Navicula* sp. at a rate of 20,000–40,000 cells mL^{-1} once a day (09:00 AM), and feeding rates were increased

by 50% every month based on the feeding response of the juveniles (*e.g.* the amount of uneaten feed in tanks). About 40–50% of water exchange was done daily, and fecal wastes and uneaten feed were siphoned from the tank to maintain good water quality.

Juvenile growth was monitored monthly for 4 mo by randomly and carefully siphoning at least 50 juveniles to a white tray with water and a ruler to be photo-documented and weighed using a digital weighing scale (Camry, EK 8150 with ±1 g precision). Prior to weighing, water in the tray was completely siphoned out, and juveniles were held for about 2 min to allow the release of water from their respiratory trees. The juvenile-specific growth rate (SGR, % d^{-1}) and daily growth rate (DGR, g d^{-1}) for the whole sampling period were calculated as follows:

$$SGR = \frac{[\ln (W_t/W_o)]}{t} \times 100 \quad (1)$$

where W_0 is the initial weight at $t = 0$, and W_t is the weight at t cultivation days.

$$DGR = \frac{(W2 - W1)}{d} \quad (2)$$

where *W1* and *W2* are the initial and final weights at the beginning and end of the period, respectively, and *d* is the number of days between sampling periods.

Observations on morphological changes such as coloration and the presence of papillae as nipple-like protrusions in the dorsal part were noted.

Data Analysis

One-way analysis of variance (ANOVA) was used to test the differences in the density of larvae and growth (*i.e.* weight) of juveniles among treatments of the larval and juvenile experiments at a 5% significance level. Data were log-transformed when the assumption of homogeneity of variance was not met. Tukey's test at a 5% level of significance was used as ANOVA's *post hoc* test. Mean, standard error, and ranges were also used as descriptive statistics. Excel 2010 (Microsoft) with the add-in Real-Statistics feature was used for statistical testing of the computations.

RESULTS

Transport Techniques

The broodstock collected in Laguindingan was bigger in May with a mean weight of 4.3 kg than in June with a weight of 3.1 kg. All broodstock in both treatments [(i) with ice (27.8 ˚C) and (ii) without ice (35.2 ˚C)] had a 0 % evisceration rate and 100 % survival rate in 30-min travel time.

Spawning Techniques

Only T6 (extreme cold shock) successfully induced the spawning of *H. fuscogilva* in this study. Pre-spawning behavior included rolling and twisting in the spawning tanks. The males *H. fuscogilva* spawned before the females by raising the anterior end of their bodies perpendicularly and often swayed from side to side, releasing a steady stream of sperm from a single gonopore at the top of the anterior end (Figure 2A). Males, erect or lying down, spawned continuously for several minutes or hours, even when they were disturbed. Females started spawning about 1 h after the first male by raising their bodies, similar to males. They formed a bulge in the anterior part of their body and released pale yellow eggs in short, powerful bursts, which lasted for 20–30 s (Figure 2B). Immediately after spawning, the females returned to a horizontal position. Unlike the males, females were not observed to spawn without erecting.

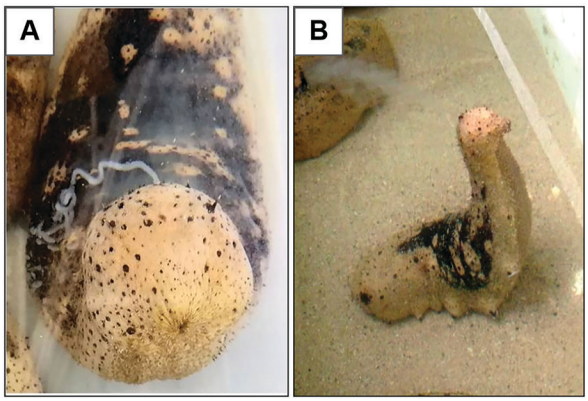

Figure 2. The spawning activity of male (A) and female (B) *Holothuria fuscogilva*.

A total of 3.4 million eggs from five *H. fuscogilva* females were produced in three successful SI activities. The highest fecundity was approximately 2.4 million eggs released in SI1, followed by SI3 with 0.6 million eggs, whereas SI2 produced 0.3 million eggs. Egg diameter ranged from 228.6–230.0 µm. The fertilization rate was highest in SI3 (99.0%), where two females and two males (2F:2M) spawned, followed by SI2 with 98.9% (1F:1M) and SI1 with 88.7% (2F:1M). Of the spawning individuals, the average female weight was 3.2 kg, and the males weight was 2.9 kg. The hatching rate (%) was calculated on the appearance of auricularia larvae approximately 48 h after fertilization. Despite having the lowest fecundity, the highest hatching rate of 98.9% was observed in SI2, followed by SI3 and SI1 with 18.4 and 17.1%, respectively.

Embryonic and Larval Development

The embryonic and larval development of *H. fuscogilva* was documented using the production of *H. fuscogilva*. The fertilized eggs were observed from 2–5 min post-fertilization (Figure 3A). The first cleavage occurred 1 h 15 min post-fertilization (Figure 3B). The four-cell division was documented at 1 h and 52 min post-fertilization (Figure 3C), whereas multi-cell divisions were observed from 2 h 25 min up to 4 h 17 min (Figures 3D-G). The multi-cell stage was marked transitioning to the blastula stage at 6 h 4 min (Figures 3H–I) and finally developed to the blastula stage at 10 h 47 min post-fertilization (Figure 3J). The blastula stage then transitioned to the gastrula at 13 h and reached the fully developed gastrula at 24 h 42 min (Figure 3K). Generally, the embryonic development of *H. fuscogilva* was radial holoblastic.

The early auricularia stage (mn length 794.1 ± 50.9 µm) was observed at 2–3 d post-fertilization (survival 44.8%) (Figure 5). This stage had a visible mouth, esophagus, and stomach (Figure 4A). The mid-auricularia stage (mn length 1,244.2 ± 13.4 µm) was observed at 4–12 d

Figure 3. The embryonic development of *Holothuria fuscogilva*. The fertilized egg at 2–5 min (A), first cleavage at 1 h 15 min (B), multi-cellular division at 2–4 h (C–G), transition to blastula stage at 6 h 4 min (H–I), fully developed blastula at 10 h 47 min (J), gastrula at 24 h 42 min (K), and newly hatched auricularia at 2–3 d (L). Scale bar = 100 µm.

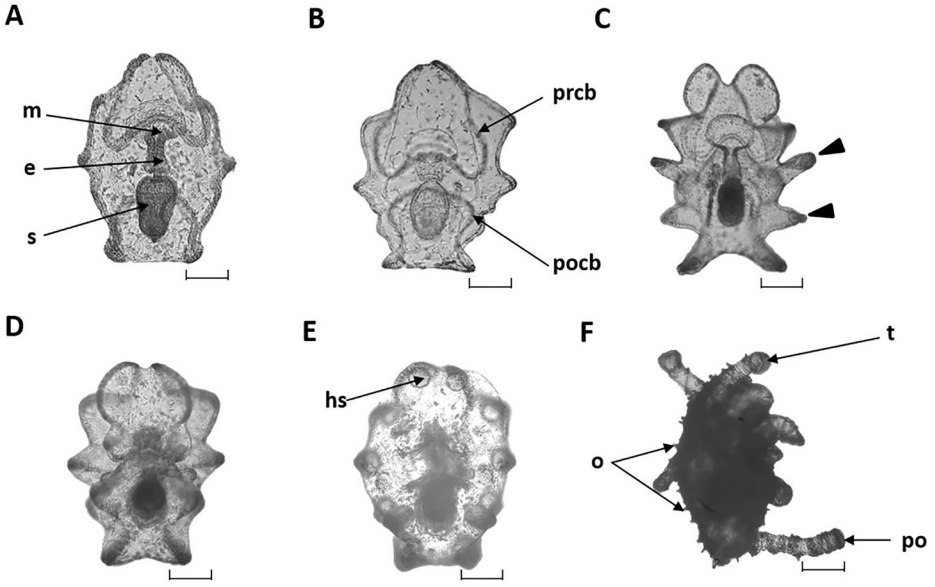

Figure 4. The larval stages of the white teatfish *Holothuria fuscogilva*. Early auricularia at 2–3 d post fertilization (dpf) (A), mid-auricularia at 4–12 dpf (B), late auricularia at 13–16 dpf (C), late auricularia transitioning to the doliolaria stage (D), doliolaria at 17–28 dpf (E), and pentactula at 29–33 dpf (F). Legend: [m] mouth, [e] esophagus, [s] stomach, [prcb] preoral ciliated band, [pocb] postoral ciliated band, [hs] hyaline spheres, [o] ossicles, [t] tentacles, and [po] podia. Scale bar = 100 µm.

post-fertilization (survival 39.2%) (Figure 5). This stage had preoral and postoral ciliated bands, which are used by the larvae for feeding and locomotion (Figure 4B). The late auricularia stage (mn length = 1,362.7 ± 68.6 µm) was observed at 13–16 d post-fertilization (survival 28.9%) (Figure 5). This stage had lateral processes and hyaline spheres (Figure 4C). A significant change in the size and appearance of the larvae signaled the beginning of the metamorphosis from the auricularia stage into the doliolaria stage. The lateral processes vanished along the esophagus and mouth at the doliolaria stage (mn length = 931.4±35.3 µm) with 19.3% survival (Figure 5), leaving just the stomach and hyaline spheres intact and close to one another. The doliolaria were distinguished by their barrel-like shape, five transverse cilia rings, and lack of feeding (Figure 4E). The transitional process included resorption of the preoral and anal lobes and lateral approaches, as well as fragmentation of the ciliated band into ciliary rings. These ciliary rings grew in place of the lateral processes and were supported by raised epithelial ridges. Because of morphogenetic changes in the gut and coelom, the advanced doliolaria stage was no longer translucent. The hyaline spheres were absorbed during this stage. The first pentactula (mn length = 1,803.9±103.8 µm) was observed at 29–33 d post-fertilization with 9.7% survival (Figure 5). This stage had five tentacles and ossicles (Figure 4F).

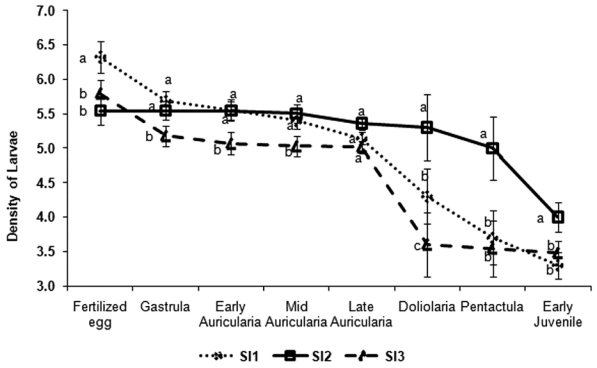

Figure 5. The density of larvae from larval to juvenile stages of *Holothuria fuscogilva* in three successful spawning inductions: SI1 (dotted line), SI2 (solid line), and SI3 (broken line). The density of larvae was log-transformed to log 10. The error bar represents the standard error of the experiments in three replications.

Juvenile Development and Growth

In this study, only 1.1% of the fertilized eggs survived the early juvenile stage. A total of 15,000 *H. fuscogilva* early juveniles were observed after 40–45 d, with SI2 having the highest survival of 2.9% (10,000 ind.), followed by SI3 of 0.5% (3,000 ind.) and SI1 of 0.1% (2,000 ind.) (Figure 5).

The early juvenile stage of *H. fuscogilva* had a distinct yellowish body and dark patches all over (Figure 6). Papillae were visible as nipple-like protrusions on the dorsal surface. Well-developed tentacles and ossicles could be seen, whereas the ring canal and aqua-pharyngeal bulb were not visible.

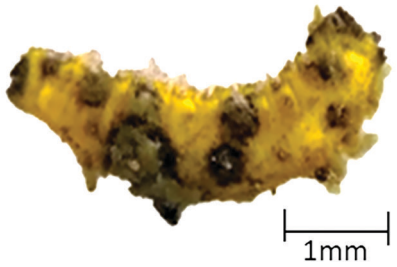

Figure 6. *Holothuria fuscogilva* juvenile at 45 d post-fertilization.

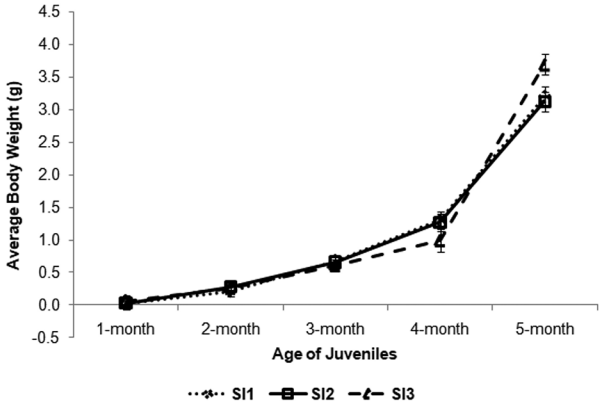

Figure 7. Monthly average body weight (g) of *Holothuria fuscogilva* juveniles during 4-mo hatchery rearing with no significant difference ($p > 0.05$): SI1 (dotted line), SI2 (solid line), and SI3 (broken line).

In 4 mo of rearing in the hatchery, there was no significant difference between the monthly growth performance of *H. fuscogilva* juveniles from the three SIs of extreme cold shock technique (one-way ANOVA, $p > 0.05$) (Figure 7). After a 4-mo rearing in the hatchery, the average body weight of 5-mo-old *H. fuscogilva* juveniles was 3.3 ± 0.13 g with a specific growth rate of 3.6 % d^{-1} and a daily growth rate of 0.03 ± 0.002 g d^{-1} (Figure 7). The water temperature, pH, salinity, and dissolved oxygen during the rearing of the juveniles in the hatchery were 27.0 ± 1.0 ˚C, 8.5 ± 0.4, 31.0 ±1.0 ppt, and 6.9 ± 0.3 mg L^{-1}, respectively.

DISCUSSION

Transport Techniques

In this study, all *H. fuscogilva* broodstock survived 30 min of transport without evisceration and mortality, with and without ice at 27.8 and 35.2 ˚C, respectively. This species is relatively hardy, and the authors found that they fared well over a short transport time. Somewhere between 30 min and 5 h, *H. fuscogilva* has been reported to start eviscerating, a sign of stress (Battaglene *et al.* 2002). In other sea cucumber species, such as *Holothuria scabra,* evisceration was observed in 5 h holding time. Tuwo *et al.* (2019) suggested that evisceration could be triggered by the presence of dead and decaying sandfish in the transport bag. Future studies should determine the safe transportation duration limit for this *H. fuscogilva* species.

Spawning Techniques

In this study, male and female spawning activity of *H. fuscogilva* was the same as reported previously (*e.g.* Battaglene *et al.* 2002; Ramofafia *et al.* 2003), where pre-spawning behavior included rolling and twisting in the spawning tanks and the males spawned before the females. Sexual maturity of *H. fuscogilva* occurs at 1.5 kg (Reichenbach 1999). Broodstock for this study was at least 1.8 kg and was collected in May and June when *H. fuscogilva* gonads are mature in the Philippines (Leopardas *et al.* 2021).

Holothuria fuscogilva in New Caledonia was found to spawn during the warm season (October–March) (Conand 1993) and during the northeast monsoon in the Maldives in December and March (Reichenbach 1999). In other research on *H. fuscogilva*, thermal stress induced spawning in males but not females (Battaglene *et al.* 2002), whereas both sexes spawned after adding *Algamac* and *Schizochytrium* sp. while increasing the temperature (Battaglene *et al.* 2002; Ramofafia *et al.* 2003). Thermal stimulation is a common method of inducing spawning in many invertebrates (Loosanoff and Davis 1963) and is the most often cited stimulant for induced spawning in sea cucumbers (Smiley *et al.* 1991; Yanagisawa 1998; Morgan 2000). However, thermal stimulation proved less effective with *H. fuscogilva* (Battaglene *et al.* 2002), and lowering water temperature by 3–5 ˚C did not induce spawning in this study. Instead, a decrease of 7 ˚C proved to be the only effective spawning treatment, suggesting that *H. fuscogilva* needs an extreme cold shock to stimulate spawning. *Holothuria fuscogilva* lives in deep water, with much lower temperatures than in shallow areas; thus, more extensive temperature changes from the ambient temperature may be required to trigger spawning.

Furthermore, in this study, using the SI technique of extreme cold shock, 1 individual *H. fuscogilva* produced 0.7 million eggs, higher than 0.2 million from the report of Battaglene *et al.* (2002). It also showed that the same female broodstock laid every SI activity in this study. The highest hatching rate obtained by SI2 partly proved that broodstock could repeatedly be spawned monthly. As Battaglene *et al.* (2002) mentioned, the female broodstock of *H. fuscogilva* was highly fecund and capable of multiple spawning. It is also reported in the study of Leopardas *et al.* (2021) that *H. fuscogilva* in Laguindingan marked a variation in tubule length, an indication that this species follows the tubule recruitment model, wherein tubules are progressively recruited from the base of the anterior gonad that eventually develops to become the fecund tubules (Sewell *et al.* 1997). This also highlights the capability of *H. fuscogilva* broodstock to be held in the hatchery for multiple spawning, an answer to the difficulty of broodstock collection in the wild.

Embryonic and Larval Development

In this study, the eggs were larger than reported by Ramofafia *et al.* (2003) and Nocillado *et al.* (2022) (approx. 230 μm compared to 151.7 μm and > 140 μm, respectively). The mean length of early, mid, and late auricularia in this study was considerably bigger than that reported by Ramofafia *et al.* (2003) and Lumasag *et al.* (2017). The density of the larvae decreased with each larval stage, with the metamorphosis to the doliolaria stage as the steepest decline. In this study, survival was observed from 28.98% in the late auricularia stage to 19.30% in the doliolaria stage. In different sea cucumber species, high mortality is also observed during its metamorphosis to doliolaria stage. It can be shown in the study by Huang *et al.* (2018) on *H. leucospilota* and the study by Ivy and Giraspy (2006) on *H. scabra* having a high mortality rate of 49.1 and about 30%, respectively, observed when the late auricularia stage metamorphosed to the doliolaria stage.

Doliolaria is the non-feeding, highly motile barrel-shaped larval stage, demersal, and started to occupy the lower part of the rearing tanks (Asha and Muthiah 2002; Ramofafia *et al.* 2003). In this study, no feeding was made during doliolaria stage. The good hyaline sphere development in the larvae supports the metamorphosis of late auricularia to doliolaria larvae, which also probably aided doliolaria's survival and preceded the mass production of juveniles. The hyaline spheres play a role in larval buoyancy (Dautov 1997) and serve as a nutritional source for the larvae during metamorphosis (Dautov 1997; Duy *et al.* 2016; Ramofafia *et al.* 2003). Neutral lipids in the hyaline spheres remained per pentactula, which provided a good amount of "buffer" for metabolic costs until the juvenile has a fully functioning digestive system (*i.e.* through the peri-metamorphic period) (Peters-Didier and Sewell 2019). Thus, giving suitable food to the larvae is necessary to ensure high survival for further research.

In the present study, the larval development of *H. fuscogilva* appears to have taken longer than reported in previous studies. The mid- and late-auricularia stages were observed at 12 and 16-d post-fertilization, respectively, whereas it only took 5 and 12-d post-fertilization, respectively, in the study of Ramofafia *et al.* (2003). The low survival rate and development delay probably have been attributed to the low temperature (26.62 °C) and salinity (30.98 ppt) recorded in the present study. Reports on other sea cucumber species indicated ideal temperatures and salinity. According to Seeruttun *et al.* (2008), *H. atra* growth was greater at 28 °C, whereas Asha and Muthiah (2005) reported that the temperature of 28–32 °C and a salinity of 35 ppt was optimal for the normal growth and development of the *H. spinifera* larvae and lower salinities led to larval deformities and disintegration. Furthermore, James *et al.* (1994), Battaglene (1999), Chen and Chian (1990), and Ramofafia *et al.* (1995) have reported that water temperatures between 27 and 30.8 °C as the optimum for the larvae of tropical sea cucumbers *H. scabra*, *A. echinites*, and *H. atra.* Similarly, Huang *et al.* (2018) reported that a temperature of 29−33 °C and a salinity of 27−30 ppt was found to be best for the *H. leucospilota* larvae. Fluctuation of temperature significantly affects the growth and survival of sea cucumbers not only of the class Holothuroidea (Asha and Muthiah 2005) but also of the genus *Apostichopus* among others (Dong *et al.* 2007; Li and Li 2010). In the study of Nocillado *et al.* (2022), the *H. fuscogilva* larvae were maintained at a temperature of 28 to 29.5 ˚C and the salinity range was 34–35 ppt, and the larvae developed to the juvenile stage. Even though our study had a 1–3 °C lower temperature than the optimum temp of other species, SI2 still produced juveniles with a 2.9% survival rate. The temperature fluctuations might not cause mortalities but might result in a relative decline in the developmental dynamics of the sea cucumber (Hamel and Mercier 1995). Further investigation is highly recommended by looking at the ideal and optimum conditions (*e.g.* temperature, salinity, pH, *etc.*) needed for the *H. fuscogilva* larvae development and production.

Juvenile Growth and Development

Holothuria fuscogilva juveniles were observed at 40–45 d in this study, considerably later than the 27 d reported by Lumasag *et al.* (2017). In contrast, *H. fuscogilva* juvenile survival rate was higher, 1.12 %, compared to the 0.45 % survival recorded by Lumasag *et al.* (2017). However, the current study recorded much lower growth rates for tank-reared juveniles than those in the wild, as reported by Lumasag *et al.* (2017) (SGR of 3.6% d^{-1} compared to 16.6% d^{-1}). Slow growth in the hatchery may have been due to inadequate food quality and quantity [see Ito (1995) and Morgan (2001)]. However, this indicates that juveniles readily grow once they are in their natural habitat and that restocking and survival of hatchery-produced juveniles in the wild presents a promising potential.

CONCLUSION

Holothuria fuscogilva in the Philippines has been shown to spawn in the hatchery when the temperature was decreased by 7 °C. Some countries have succeeded in closing the life cycle of *H. fuscogilva*. However, this is the first published description of the spawning and development of larvae and juveniles. The aquaculture techniques for *H. fuscogilva* in this study demonstrates the species' potential to be bred repeatedly in the hatchery in the Philippines and may help to overcome the problems associated with gathering wild broodstock.

This study's baseline information also helped establish hatchery protocols for *H. fuscogilva* in the Philippines. It is recommended that further research be conducted on broodstock transport over more extended periods and the development of simplified technology for administering extreme cold shock for SI (*e.g.* use of ice to reduce water temperature by 7 °C). In addition, research designed to optimize microalgal diets and determine physicochemical parameters needed for larval and juvenile development are necessary to refine the protocol for the *H. fuscogilva* hatchery in the Philippines. These will address the challenge in the mass production of *H. fuscogilva* for stock enhancement and sustainability.

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