

A review on the recent advances in the biology and aquaculture technology of *Holothuria scabra*

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Abstract

The highly valued sea cucumber *Holothuria scabra* is currently listed as endangered (EN) species in the IUCN Red List due to overfishing in most of its native locations, spurring the development of *H. scabra* aquaculture to ensure sustainability of the industry and species in the wild. This review presents a summary on the biology of *Holothuria scabra* and recent advancements of its aquaculture technology. The life cycle, morphology and internal anatomy of *H. scabra* are discussed. Recent findings on the reproductive behavior and mechanism of the population are reviewed together with the possible factors that influence gamete maturation and spawning events. This review also covers the notable recent advances on *H. scabra* aquaculture technology such as the studies on broodstock diet which indicated bacteria and carbonate minerals to be the main diets; the favorable polyculture results of *H. scabra* juveniles with red tilapia and *Kappaphycus striatum*; and the discovery and subsequent patent of *H. scabra* oocyte maturation inducing substance prepared from sea urchin spawns.

Keywords: *Holothuria scabra*, sea cucumber, echinoderm, broodstock, reproductive biology, aquaculture technology

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Introduction

The sea cucumber *Holothuria scabra* Jaeger, commonly known as sandfish or trepang, has become one of the highly valued species in the food fish industry since the 1980's especially in the Asian market. It is currently valued at more than USD1000 per kg dry weight, just a little under the market value of *Apostichopus japonicus*, also dubbed the Japanese sea cucumber. Therefore, it is not surprising that *H. scabra* has become overfished in most of its natural habitats resulting in the species being listed as endangered (EN) under the International Union for Conservation of Nature (IUCN) Red List (Purcell *et al.*, 2014). In Sri Lanka, the unregulated overfishing of sea cucumbers has resulted in the collapse of the industry (Kumara *et al.*, 2005), and fishing activities have recently been restricted to the north, east, and north-western coastal waters (Dissanayake and Stefansson, 2012). Other countries like India (Sakhtivel and Swamy, 1994) and Papua New Guinea have imposed a ban or moratorium on sandfish trading (Purcell *et al.*, 2014; Purdy *et al.*, 2017) in an effort to prevent the impending collapse. The need to enhance the natural stocks of *H. scabra* in its native location has helped to spur the development of sea cucumber aquaculture technology. Fortunately, just like the sea cucumber *Apostichopus japonicus*, its temperate counterpart, *H. scabra* is also one of the more adaptable species for aquaculture production due to its biology and natural habitat (Hamel *et al.*, 2001).

The biology of *H. scabra* had been studied extensively for decades; the earliest mention was in a literature by Jaeger (1833, cited in Hamel *et al.*, 2001). Yet for many years the existing information was not readily accessible to researchers due to the publication and language barriers. In 2001, Hamel *et al.* compiled many of the then existing references into one comprehensive review on *H. scabra*. The 16-year-old literature was a great help in the advancement of *H. scabra* research, either for fundamental studies or for the aquaculture application and technology. A more recent review was compiled and edited by Brown and Eddy (2015) covering the advancement of echinoderm aquaculture in general. This paper aims to present a current summary on the biology of *H. scabra* and the advancement of its aquaculture technology and management in the past sixteen years.

The Biology of Holothuria scabra

Taxonomy

The holothurians can be grouped and differentiated from other echinoderms based on the existence of a calcareous ring located anterior to the oesophagus as well as the buccal tentacles that extend out of the pharynx and serve as feeding apparatus. Unlike other echinoderms, the holothurians also possess an outer skeleton (test) consisting of microscopic ossicles and a well-developed haemal system. Holothurians are further classified into six orders based on the anatomy of the

calcareous ring, the shape and arrangement of the ossicles, the number and form of the buccal tentacles extending out of the mouth, and the presence of respiratory trees. Of the six holothurian orders, only two orders are known to be exploited commercially (Hamel *et al.*, 2001; Slater and Chen, 2015). The minority comes from the order Dendrochirotida, which is associated with 10 buccal tentacles. Only one species, *Cucumaria frondosa*, is commercially exploited within this order mainly for the extraction of its bioactive compound, frondoside A (Takashi *et al.*, 2005; Slater, 2015). Most of the commercial sea cucumbers belong to the order Aspidochirotida, which has 18-30 buccal tentacles.

Within the Aspidochirotida, the commercially exploited sea cucumber comes only from two families, Stichopodidae and Holothuriidae. In terms of aquaculture production, there is only one species from each family currently produced in aquaculture hatcheries namely the temperate species, *Apostichopus japonicus*, and the warm water species, *Holothuria (Metriatyla) scabra* reported by Jaeger (1833). The taxonomic classification of *H. scabra* is as follows (Massin *et al.*, 2009):

Kingdom	: Animalia
Phylum	: Echinodermata
Class	: Holothuroidea
Order	: Aspidochirotida
Family	: Holothuriidae
Genus	: <i>Holothuria</i>
Species	: <i>scabra</i>

Natural Habitat

In his review, Hamel *et al.* (2001) explained observations from various researchers concerning the habitat preference of *H. scabra*. In its natural habitat across the region, adult *H. scabra* is observed to prefer calm coastal areas with sandy muddy substrate instead of coral reefs. They are also observed to be able to tolerate low salinities, and are sometimes even found near estuaries. A study by Mercier *et al.* (1999a, b; 2000a) showed the ability of *H. scabra* to tolerate as low as 20 p.s.u water salinity by burrowing into the sediment. The juveniles are also observed to prefer medium-sized grains (0.4 mm) as substrate and areas where seagrasses are plentiful, especially of the species *Thalassia hemprichii* and *Enhalus acoroides*.

The sandfish *H. scabra* can be found across various regions in the Indo-Pacific which encompasses the latitude range of 30° N to 30° S (Massin, 1999; Massin *et al.*, 2009; Hamel *et al.*, 2001). Native populations have been studied in India, Sri Lanka, New Caledonia, Solomon Islands, Ecuador, Vietnam, Indonesia, the Philippines, Australia, Madagascar, and China (Hamel *et al.*, 2001; Dissanayake and Stefansson, 2012). Extensive aquaculture and farming systems of *H. scabra* have been growing for the past 7 years and are currently on-going in Madagascar, Philippines, Vietnam and Fiji which mainly supply the commercial demand (Hair *et al.*, 2012; Purdy *et al.*, 2017).

Anatomy

The morphology and internal anatomy of *Holothuria scabra* had been covered extensively by various researchers, most notably Bai (1971, 1978, 1980, and 1994), Conand (1989 cited in Hamel *et al.*, 2001), James (1989), and Baskar (1994). The species is usually identified by its smooth, elongated tubular body along the oral-aboral axis, being gray or black at the convex dorsal side, and always white and flat at the ventral side. The dorsal body is sometimes peppered with speckles of black, yellow and/or white patterns. The white ventral side is usually peppered with black or brown dots which are the tube feet. Located ventrally at one end of the tubular body is the oval mouth encircled by 20 tentacles (Conand, 1989 cited in Hamel *et al.*, 2001). At the opposite end of the body and located somewhat dorsally is the anus or cloaca which rhythmically pumps water in and out of the body. The creeping movement of *H. scabra* is regulated by five pairs of muscular lines along the body length (Bai, 1980).

The internal anatomy or viscera of *H. scabra* consists of two tubular branches of gonads with one end connected to the gonopore, the digestive organs which end with the anus, and respiratory trees. *H. scabra* is able to eviscerate most to all of its digestive and respiratory organs as a method of self-preservation. These organs will fully regenerate in about one to two months (Cannon and Silver, 1987; Bai, 1994). When the animals are agitated

during handling, the incidence of evisceration is more common among *H. scabra* compared to other species (Conand, 1989) cited in Hamel *et al.* 2001).

Bai (1980) revealed that the digestive tract of *H. scabra*, comprising the oesophagus, stomach, small and large intestines, is supported by a mesentery. The digestive tract ends into a wide cloacal chamber that terminates at the anus (Slater and Chen, 2015). There is currently no detailed study on the physiology of digestion and nutritional requirements of *H. scabra*.

The respiratory system of *H. scabra* is an intricate network of tubules or vessels formed from the cloacal wall in one common main stem that branches to the left and right into many finer branches. Two or three shorter branches may also form out of the base of the common stem. This network of tubules is the respiratory tree that terminates into the perivisceral coelom and are always covered in coelomic fluid (Bai, 1980; Slater and Chen, 2015). The cloaca also plays an important role in the respiratory system by drawing sea water in and out of the cloacal chamber and respiratory trees with its rhythmical pumping (Bai, 1980). Gas exchange occurs through the thin tubular wall into the coelomic fluid that is circulated around the body through the water vascular and haemal system. However, gas exchange also occurs throughout the integument and tube feet which explains the ability of *H. scabra* to eviscerate all its internal organs

including the respiratory trees when necessary.

Body wall composition

The integument of *H. scabra* is composed mainly of collagenous proteins and connective tissues. This body wall or dermis is the main body part desired as food of which characteristics and texture determines the value of the sea cucumber as a commodity. The Holothuriidae possess a thin epidermis composed of microscopic calcareous ossicles. Getting rid of this part is one of the most labor intensive and time consuming activities in the traditional processing of sea cucumbers.

The studies on the composition of *H. scabra* body wall are few, covering only the gross composition and done mostly on dried specimens. They generally agreed that *H. scabra* contain around 30-60% (DW) crude protein and around 2% (DW) of fat (Springhall and Dingle, 1967; Ozer *et al.*, 2004; Ibrahim *et al.*, 2015). Springhall and Dingle (1967) also showed the body wall containing calcium, sodium, potassium, and phosphorus in small amounts. A study conducted by Yahyavi *et al.* (2012) showed higher content (>36%) of polyunsaturated fatty acids (PUFA) in the lipid profile of *H. scabra* body wall compared to the monounsaturated fatty acids (MUFA) at 25%. In comparison, the much studied composition of *A. japonicus* body wall also showed protein as the highest component of the body wall. According

to Saito *et al.* (2002) the main protein component of the body wall is the collagen fibrils containing glycine, glutamic acid, proline, and aspartic acid. The structure of these collagen fibrils is what gives the body wall its texture, an important component for the use of sea cucumber in the culinary field. The content of carbohydrates and lipids in the body wall of *A. japonicus* is found to be low. Most of the lipids are contained in the gonads, being the reproductive organs, especially when they are mature. Saito *et al.* (2002) also found thiamine, riboflavin, carotenoid, and niacin in *A. japonicus* body wall as well as various minerals and trace elements.

A number of studies had been done on the extraction of various bioactive compounds with pharmacological properties from various sea cucumber species, including *H. scabra* (Slater, 2015). A recent study by Assawasuparek *et al.* (2016) on *H. scabra*, showed that the body wall extract contains scabraside D, a sulfated triterpene glycoside, found to inhibit tumor growth in mice. Other bioactive compounds found in *H. scabra* extract are echinoside A (Collin and Adrian, 2010), saponins (Yibmantasiri *et al.*, 2012) and chondroitin sulfates (Takashi *et al.*, 2005) that show active antioxidant and antimicrobial activities.

Reproductive biology

The *H. scabra* has separate sexes but exhibit monomorphism. It is a broadcast spawner, known for biannual

seasonal mass spawning in the natural habitat. The peak spawning seasons across various populations seemed to fall in October to January and March to July (Hamel *et al.*, 2001). Nevertheless, some studies also observed that certain small populations of *H. scabra* spawn all year long. These studies give rise to the possibility that these *H. scabra* populations may have an asynchronous gonadal development that enables them to breed all year long (Purwati, 2006; Slater and Chen, 2015). The most notable study that supports this notion is by Purwati (2006) which investigated the tubules and gonadal indices of Indonesian *H. scabra* populations from three vastly different regions. The study showed that during each sampling occasion the researchers would always find animals with fecund gonadal stage, with after-spawned gonads, and without visible gonads.

Male and female gonads comprise filamentous tubules that are elongated, branched, and with variable sizes and colors depending on the maturity state of the gonads. According to Bai (1980) and James (1989), the female gonads are said to be thick, brown tubules with visible eggs that appear as white dots when ripe. However, Conand (1993) described ripe female gonads as translucent and the mature female gonadal tubules are shorter, heavier, and bigger in diameter than those of the males. Mature female tubules have been observed to contain vitellogenic oocytes, while mature male tubules contain only spermatozoa (Tuwo, 1999). Purwati

(2006) showed that the tubular development is synchronous across the gonads and consists of only a single tuft for each spawning cycle. Tubules are reabsorbed after spawning, thus signifying a resting stage in which the gonads are not visible. The study showed that it may take as long as 12 months for the developing tubules to reach fecund stage.

Both studies by Conand (1993) and Purwati (2006) showed that the gonadal index (GI) for both males and females indicated large variations across the same population at the same sampling occasion. This seemed to confirm the asynchronous gonadal development model in *H. scabra* populations. However, Purwati (2006) did find that the GI values of *H. scabra* population in Indonesian waters peaked at two different periods in a year, specifically at the end of each monsoon season, indicating a bi-annual spawning season. This finding was in agreement with the *H. scabra* population study in India by Khrisnaswamy and Krishnan (1967), in Australia by Harriot (1980), in New Caledonia by Conand (1989, 1990, 1993), in Sri Lanka by Moiyadeen (1994), and in Solomon Islands by Battaglione (1999b, c). Several environmental factors were proposed by various studies as to be the cue for gamete maturation and spawning event. These factors include the fluctuating water temperature during the transitional period of monsoon seasons (Conand, 1993; Battaglione, 1999c), change in photoperiod (Morgan, 2000),

lunar influence (Mercier, 1999c, 2000b), and the change in water salinity (Khrisnaswamy and Krishnan, 1967; Battaglène, 1999a, 2000).

The heterogeneity of gonadal development across the population makes the duration of gametogenesis stages difficult to be concluded. However, most studies agree that *H. scabra* gametogenesis include immature and mature gonadal stages, followed by spawning, after-spawning or spent, and resting stages where tubules are reabsorbed (Ong Che and Gomez, 1985; Conand, 1993; Tuwo, 1999; Morgan, 2000, Purwati, 2006). The development and maturity of gonadal tubules seemed to be determined by the protein and lipid storage in the intestines (Khrisnaswamy and Krishnan, 1967). Conversely, a study by Morgan (1999) seemed to indicate that the opposite may also be true. The growth and metabolism in food-deprived *H. scabra* actually utilize the nutrients from the gonads and gametes.

Life cycle

Various researchers have conducted studies on the life cycle development of *H. scabra*. The collected results from these studies corroborate each other in general except on few details. In the published article, Hamel *et al.* (2001) compiled and summarized the two major sources of information on *H. scabra* life cycle studies conducted in India (James *et al.*, 1988, 1994; James and James 1993) and studies conducted

in Solomon Islands (Battaglène, 1999a, c; Battaglène and Bell, 1999; Battaglène *et al.*, 1999). The development of *H. scabra* can be summarized in stages as shown in Fig. 1.

There are variations in the findings of *H. scabra* oocyte diameter and time frame of blastula formation reported by different researchers in the past. Oocyte diameter was claimed to be in various sizes ranging from 70 μm (Ong Che and Gomez, 1985) to 210 μm (Conand, 1993). The varying results may be caused by the non-uniformity in the research protocols across the studies (Hamel *et al.*, 2001). Blastula formation was reported to be completed in 40 minutes (Battaglène, 1999a, c; Battaglène and Bell, 1999) to 3 hours (James *et al.*, 1988, 1994). Personal observation of the embryonic stage of *H. scabra* seemed to confirm the latter.

Larval stage

The size of fertilized *H. scabra* eggs are observed to be around 100-200 μm (Slater and Chen, 2015). Fertilization usually occurs immediately upon contact with the sperms.

In captive breeding, polyspermy is usually a problem and must be avoided by controlling the sperm concentration in the water. This is done by removing the spawned males from the spawning tank once the desired sperm concentration is achieved. Polyspermy is also avoided by washing the fertilized eggs gently before stocking them in the hatching tanks.

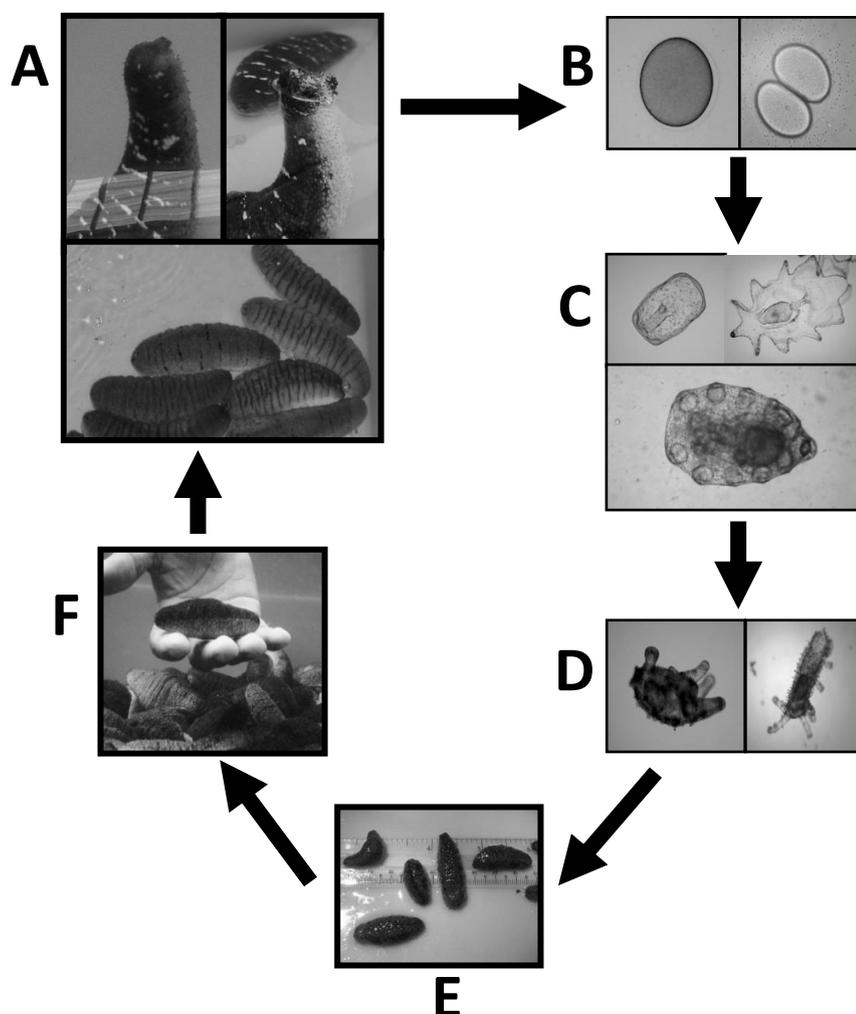


Figure 1: A. Mature broodstock and spawning activity. B. Fertilized egg and cell division (48 hours). C. Planktonic stage (10-14 days) consists of three different stages (Top left clockwise: Gastrula, Auricularia larvae, Doliolaria). D. Benthic stage consists of two different stages (left-right: Pentactula, early juvenile). E. Juvenile at 1 g. F. Juvenile at 10 g (4 months). (Pictures are from personal collection.)

Under the optimal condition of 27°C, fertilized eggs developed into blastula in 3-5 hours. Eggs hatch in 24-48 hours as gastrula, the non-feeding planktonic larvae stage, which then develop into auricularia larvae (Hamel *et al.*, 2001).

Auricularia larvae are the feeding planktonic larvae that generally develop within 36-48 hours after fertilization. Auricularia feeds on microalgae and its

larval development can be divided into the early (300-400 µm length), middle (400-700 µm length), and late auricularia (700-1000 µm length). The three stages of auricularia are differentiated through body length and width, feed type, size and quantity, and an increasingly defined body structure. The late auricularia has pronounced arms and hyaline spheres. The hyaline

spheres are postulated to be a lipid-based energy storage that is crucial for the survival of the next larval stage (Battaglione, 1999a, c). In an optimum rearing condition, the auricularia larvae develop for 10-14 days before transforming into the doliolaria larvae.

Doliolaria is the non-feeding planktonic larval stage. At this stage the larvae are looking for a suitable substrate to settle on. This stage is one of the bottlenecks in sea cucumber hatcheries with high mortality rate as doliolaria are known to float around and never settle when a suitable substrate is not found. They appear as black dots in the water to the naked eyes and can be found floating near the surface and tank walls. They are also reported to be phototactic (Battaglione, 1999a, c). In a suitable environment, doliolaria is known to settle within 24-48 hours, however, they have been observed to delay settlement up to 96 hours when there is no suitable substratum (Mercier *et al.*, 2000a). A study by Mercier *et al.* (2000a) showed strong preference by doliolaria larvae to settle on the blades of the *Thalassia hemprichii* and *Enhalus acoroides* seagrass species as compared to other substrates such as artificial leaves, crushed corals, or other plant species. Upon finding the preferred substratum for settlement, the planktonic doliolaria larvae would transform into the benthic pentactula larvae.

The pentactula larva is the feeding benthic stage, possessing tentacles that are used in grabbing food and feeding.

At this stage, the pentactula larva will continue its development by body elongation. Further development would show the larva moving about and its movement can be traced from the "trail" of feces it leaves behind. This larval stage usually continues for 15-18 days in which the pentactula larvae gradually transform into a juvenile stage (James *et al.*, 1994).

Juveniles

The juvenile stage can be recognized by the disappearance of the tentacles under the mouth hood and the further definition of tubular and elongated body shape bearing the varietal coloration and specific patterns of the adults. Reports vary, but approximately 30-45 days after fertilization, the 1 cm long juvenile sea cucumbers emerge (James and James, 1993; James *et al.*, 1994; James, 1996).

The development of juvenile stage to adult stage is known to require the introduction of sandy substrates. Based on personal observation, the juveniles' body size increased sharply after the introduction of sandy substrates. Studies by Mercier *et al.* (1999a, b) found that juveniles prefer muddy sandy substrates especially sand with an average grain size of 0.4 mm. A later study showed juveniles to remain among the seagrass up to 10 cm body size before migrating out into open sandy areas (Mercier *et al.*, 2000a). This study and several others (James *et al.*, 1994; Uthicke and Benzie, 1998 cited in Hamel *et al.*, 2001; Uthicke and

Benzie, 1999) seemed to indicate a downward migration behavior of *H. scabra* in the wild. More studies are needed in order to confirm this hypothesis.

Reports on the growth rate of juveniles vary and many of the studies are conducted in a laboratory setting. Two studies conducted in the field showed a big difference in the growth rates of juvenile *H. scabra*. Mercier *et al.* (2000b) stated a growth rate of 10-15 cm/month from an initial size of 65 mm while Manikandan (2000) observed a growth rate of only 1.4 cm/month from an initial size of 15 mm. It is thus speculated that the growth rate increases with the size of the animal.

Adults

The subadult *H. scabra* can be differentiated from juveniles through their behavior as well as the body weight and size. In the wild, it is common to find adults of up to 2 kg body weight and 30 cm long, although these are getting rare due to overexploitation. Sexually mature adults, however, are difficult to determine just from body weight and size because *H. scabra* as small as 200-250 g can spawn successfully and be as fecund as those double in body weight (Harriot, 1980). The size of first sexual maturity has been studied but results vary greatly. Studies by Shelley (1981) and Lokani (1995) observed that *H. scabra* becomes sexually mature at around 140 mm body length. However, Baskar and James (1995) observed that

H. scabra first spawning occurred when the animals were about 200-230 mm body length. Yet other study by Harriot (1980) recorded first sexual maturity of *H. scabra* at 184 g of body weight instead of length. The inconsistent reports on first sexual maturity may be due to the non-uniform methods of defining first sexual maturity across the different studies.

The life span of *H. scabra* is unconfirmed but James *et al.* (1994) and the Marine Products Exports Development Authority (MPEDA) (1989) suggested that it could be more than 10 years. Fecundity and reproductive viability of adult sea cucumber throughout its lifespan is yet to be studied in detail.

Advancement in the Aquaculture Technology and Management of H. scabra

On broodstock nutrition

The study on the effect of sea cucumber farming on sediment by Plotieau *et al.* (2013) showed a great reduction of bacterial population, reduced fine grain (<250 μm), big reduction in the concentration of photosynthetic bacteria, and reduced carbonate proportion (aragonite, calcite, magnesian calcite) in the sediment, but not much change was found in the total organic matter. It could be inferred from this study that adult sandfish live mainly on bacteria rather than organic matter. The study also showed that sandfish is able to digest the inorganic materials of the sediments. This observation was

confirmed by Schneider *et al.* (2013) and Collard *et al.* (2014) who showed that the dissolution of CaCO_3 into seawater in the ocean is greatly helped by sea cucumbers which secrete digestive acids to dissolve the minerals in their gut. Besides the mineral content in the substrate, a study by Shi *et al.* (2015) also showed that the size of sand particles have a cumulative effect on feed nutrient absorption and growth rate of the sea cucumber.

Another possible diet formulation for intensive land-based culture of *H. scabra* may be gleaned from studies conducted on *Apostichopus japonicus*, a species of temperate sea cucumber commercially farmed for consumption. One of the related studies was performed by Yuan *et al.* (2006) using feed formulated with 75% dried bivalve feces and 25% powdered algae. The study found a conflict between using dried material (feces and algae) which had been naturally stripped of any bacteria population and using fresh material which may still contain beneficial bacteria. The dried feed enabled easy feed preparation and storage, but the powdered algae or dried feces alone was shown to be an unsuitable diet for growth. Fermented powdered algae, however, was shown to perform better as a food source for sea cucumbers. This study seemed to affirm the hypothesis of Plotieau *et al.* (2013) that bacteria may comprise the majority portion of what sea cucumbers actually feed on.

In relation to broodstock feed, a study by Asha and Muthiah (2007) on juvenile growth showed the researchers coming up with their own induction-stimulating feed formulation. This feed was composed of rice bran, soya powder, and *Sargassum* spp. powder at 4:1:2 ratio. The feed was given at 5% body weight daily. In the area of feed supplement, a preliminary study by Ko *et al.* (2009) had shown that the addition of α -tocopherol or Vitamin E had a positive impact on weight gain, feed conversion rate and survival of juvenile *A. japonicus*. The optimum dietary level of α -tocopherol is found to be between 23.1-44 mg/kg diet. More studies are needed to conclude the effect of dietary vitamin E in the whole body lipid and protein contents of sea cucumbers, particularly *H. scabra*. Such studies may also be able to answer whether vitamin E supplementation in sea cucumber diet can improve its reproductive ability.

On captive broodstock management and farming

The poor sustainability of broodstock viability in captivity is well documented (Morgan, 2000; Mercier and Hamel, 2013). The use of captive-bred or F1 broodstock in sea cucumber hatchery so far was only implied in Pitt and Duy (2004) and Agudo (2006). Generally, in captivity, broodstock would start losing weight and decreasing in reproductive productivity after a few cycles. The population would also see an increasing mortality

due to diseases. Attempts had been made for inland polyculture of sea cucumber with other aquatic species but most studies focused on juvenile *H. scabra* rather than broodstock management. The co-culture of juvenile *H. scabra* with juvenile blue shrimp (*Litopenaeus stylirostris*) showed no positive results (Purcell *et al.*, 2006), and was even found to be inviable since the survival of *H. scabra* was observed to be very low (Bell *et al.*, 2007). Mills *et al.* (2012) studied the polyculture of *H. scabra* with *Penaeus monodon* in Vietnam which gave disastrous results due to sea cucumber predation by the prawns. A more promising result was found in the polyculture of juvenile sea cucumber with red tilapia (Sithisak, Pongtippatee, and Withyachumnarnkul, 2013) in 30 ppt seawater. Sea cucumbers were found to grow faster in polyculture ponds than in monoculture ponds.

Beltran-Gutierrez (2012) and Beltran-Gutierrez *et al.* (2016) investigated the polyculture of sea cucumber with red seaweed, *Kappaphycus striatum*, which produced acceptable results since no negative effects were observed on sea cucumber growth albeit without a positive effect as well. Another study by Namukose *et al.* (2016) demonstrated an integrated mariculture system of *H. scabra* with seaweed *Eucheuma denticulatum* which could enhance the growth rate of the seaweed as well as reduce the organic matter in the sediments at medium *H. scabra* stocking densities.

Although the polyculture of *A. japonicus* with bivalves such as abalone (Kang *et al.*, 2003) has been successful in China (Mercier and Hamel, 2013), it is worth noting that, to date, no study could be found with regards to the polyculture of tropical abalone with *H. scabra*.

Effect of environmental factors on reproductive biology

A study by Collard *et al.* (2014) noted that in a short period of less than 12 days, the ocean acidification in which the sea water pH dropped to around 7.4-7.7, caused acidosis of the coelomic fluids (CF) in *H. scabra*. However, the acidosis of CF in the short-term did not seem to affect the respiration process and ammonia secretion. Further studies need to be carried out to determine whether long term CF acidosis will eventually affect the reproductive ability and viability in a negative manner.

A study by Battaglione *et al.* (2002) on wild caught broodstock presented an overview into the spawning methods and peak periods of *H. scabra*, *Holothuria fuscogilva* and *Actinopyga mauritiana*. This study showed that *H. scabra* could be induced to spawn throughout the year as compared to other species in the study but the percentage of successfully spawned broodstock was the greatest only in August to November with September being the most productive month. Even so, the percentage of successfully spawned broodstock in the month of

September amounted to only 35%. Various environmental factors are suspected to act as or have influence on the spawning cues for *H. scabra* in the wild. The environmental factors include the change in temperature, photoperiod and its intensity, the lunar cycle which influences tidal fluctuations, and the availability of certain types of food. The change in temperature or thermal stimulation is quite a common cue for spawning events of invertebrates, and this is also the case with *H. scabra* (Morgan, 2000). Battaglione *et al.* (2002) also reported that spontaneous spawning occurred following capture and transport stress on the broodstock. Concerning captive broodstock, there have been reports of successful spawning following physical and thermal stimulation on *H. scabra* held in tanks (Morgan, 2000).

On broodstock sex differentiation, sex hormones and oocyte maturation

One of the difficulties in breeding *H. scabra* is in determining the sex of the broodstock since *H. scabra* is a monomorphic species. Sex can only be determined through a biopsy of the gonads, dissection of the animals, and spawning (Hamel *et al.*, 2001). The difficulty of sex identification combined with the difficulty in determining gamete maturity make it necessary to obtain a sufficiently large population of wild stock for each spawning attempt (Mercier and Hamel, 2013).

Assisted fertilization of extracted sea cucumber oocytes has not been successful due to the unique two maturation steps in which the first oocyte maturation phase is stopped at first meiotic phase specifically at prophase-I stage (Leonet *et al.*, 2009). Oocytes would only mature when the meiotic phase resumes just before spawning presumably due to the activation of a gonad-stimulating substance (GSS), which would then stimulate the production of a maturation-inducing substance (MIS) such as 1-methyl-adenine (1-Me-A) in sea stars (Yamashita *et al.*, 2000). In the case of the sea cucumber, the MIS is an unknown endocrine substance. However, Leonet *et al.* (2009) claimed to have successfully discovered a complex substance, termed as the maturation-inducing fractions (MIF), which could be prepared from sea urchin (*Tripneustes gratilla*) spawns, that allowed the maturation of 28-90% of extracted *H. scabra* oocytes. An international patent had been filed for this finding which indeed was a significant breakthrough.

Currently, there is a scarcity of studies on the putative sex chromosomes of *H. scabra* or even the general chromosome number of the species. Chromosome studies had been performed on other echinoderm species which generally showed a range of 36 to 46 diploid chromosome numbers (Colombera and Venier, 1976, 1980). A study by Okumura *et al.* (2009) showed a diploid number of 44 for *A. japonicus*

which is the same as the other two species that belong to different families. This suggests that chromosome number may similar or vary only slightly among animals within the class Holothuroidea.

There is also no detailed study on the sex-related hormones, sex-related chemical cues, or maturity-related chemical cues of *H. scabra*. Such studies may be fundamental in the development of a field test-kit to ease sex identification of *H. scabra* broodstocks. In contrast, various such studies had been carried out on *A. japonicus*. A study by Katow and Katow (2014) has resulted in the development of a detection kit for mature *A. japonicus* females by measuring their gonad-stimulating substance-like (GSSL) peptide immunochromatography.

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