Breeding and rearing of the sea cucumber *Holothuria scabra* in Viet Nam

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Abstract

The aims of this project were to develop large scale breeding and rearing methods for sandfish (*Holothuria scabra*) for commercial culture and/or restocking. Wild collected sea cucumbers were initially difficult to spawn, but after a period in earthen ponds or seabed pens could be induced year-round, using temperature changes, emersion, treatment of water with UV light, and addition of dry phytoplankton. Numerous batches of larvae were reared to settlement and to larger sizes using simple hatchery methods.

Juveniles produced in indoor hatchery tanks (mostly below 3 mm in length) were nursed outdoors to a few grams, or tens of grams, in two or three stages. Nursery methods were tested in different kinds of tanks, earth ponds (sometimes using fine-net bags or hapas), larger bag nets and pens inside ponds, and in the sea using various seabed cages, covers and pens. Both monoculture and polyculture nursery trials (with the shrimp, *Penaeus monodon,* or the babylon snail, *Babylonia areolata*) were carried out.

Nursed juveniles were grown out in ponds, pens and cages. Growth was often rapid, in the range of 1-3 g/day. At best, growth in a pond from 30 to 300 g took only 3 months. Hatchery-produced sandfish from ponds were spawned at less than one year of age, and several batches of their progeny were produced. Big pens (up to 2 000 m^2) were built in marine protected areas and stocked with hatchery-produced sandfish, to test their potential as alternative income sources for local fishermen. Pens proved cheap and effective for broodstock ripening and for ongrowing.

Constraints to commercial culture include low prices paid by dealers, the large area needed for nursery and growout (growth often slows down or stops when stocking densities exceed about 150-300 g/m^2), high variability in survival rate at many stages, predation pressures (including by shrimp), the need to guard pens against theft and problems of pond management. Positive factors include the wide temperature and salinity tolerance of sandfish, ease of containment, good growth in ponds and pens without added feed and the belief that sandfish may help clean the pond floor or seabed of organic wastes from other aquaculture activities.

Coastal population surveys of sandfish have not yet been carried, and only a few small releases of hatchery produced sandfish made. Natural recovery of overfished sea cucumber populations may be delayed at different stages in the life cycle. This needs to be better understood to design and test possible interventions, which might include restocking with hatchery produced juveniles. It is hoped that information here, on growth rates, densities, age at maturity and year-round larval production will be of value in this process.

Keywords: Hatchery, nursery, culture, pond, pen, sandfish

越南糙海参（*Holothuria scabra*）的育苗和养殖

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摘要

该项研究的目的是为了开展大规模养殖糙海参（*Holothuria scabra*）和资源增殖。刚从海里捕捞来的亲参难以诱导产卵，但是在池塘中或是在设于海里的网围中暂养一段时间后，采用水温变化、干露、紫外线照射的海水处理，以及用浮游藻类的干粉诱导等措施都可以使之全年产卵。利用简单的育苗设备就可以得到大批量的幼虫，并培养到大规格幼体。

在室内育苗池内育成的稚参（大部分在3毫米以下）移到室外，经历2-3个阶段，养到几克，甚至几十克。做了不同的室外暂养方法实验，有不同规格的水池、土池（有时用小网目的网袋，或用网围）、大网袋和网围等，设在海里的网箱有的加盖，有的不加盖。还进行了单养，以及与斑节对虾（*Penaeus monodo*），海螺（*Babylonia areolata*）混养的实验。

经过暂养的大规格幼参移到池塘、网围和网箱中养殖。生长速度很快，日生长率约1-3克/日。在池塘中养殖的个体，只用3个月的时间就由30克长到了300克。在池塘中养成的海参，大约一龄可以达到性成熟，并已生产出几批幼参。网围（约2 000 ㎡）被设置在海中避风浪的地方，投放人工苗种，目的是为当地渔民寻找其他生财之道。网围是一种可取的方式，既可用来暂养亲参达到性成熟，又可用于海参养殖。

影响海参养殖的负面因素主要有：中间商的出价太低，暂养和养成需要较大面积（当放养密度超过150-300克/㎡ 时，海参生长速度下降），在不同生长时期造成高死亡率的因素难以确定，捕食动物的危害（包括虾类），以及防范偷盗和其他管理上的问题。有利因素包括该种海参对水温、盐度有较强的抗性，不易逃逸，在不投饲料的情况下可以在池塘和网围中良好生长，并且在养殖过程中，有助于清除池塘或海底的有机废物。

糙海参的资源状况尚未开始调查，仅仅放流过少量人工苗。已被过度开发的海参资源，由于生活史的不同阶段受到干扰，而影响到它的自然恢复。这就需要对可能存在的种种因素有较深入地理解和进行实验，包括放流人工苗种。希望于此介绍的情况，如生长率、密度、性成熟年龄和全年生产幼苗等具有参考价值。

关键词：育苗、暂养、养殖、池塘、网围、糙海参

Introduction

The pioneering breeding work of Dr James in India (James *et al.,* 1994), and subsequent research by Battaglene *et al.* (1999) and Mercier *et al.* (2000) in the Solomon Islands, showed that sandfish, *Holothuria scabra,* is one of the most promising sea cucumber species for aquaculture. It has a short larval phase, relatively high tolerance of changing environmental conditions, and can be processed to produce high-value beche-de-mer (FAO, 1990; Hamel *et al.,* 2001). However, despite anecdotal accounts of commercial-scale sandfish culture (Battaglene, pers. comm.) little is known about the extent to which this species has been farmed, or whether past attempts at growout have been based on wild-caught or hatchery-bred juveniles.

Several authors deal with the spawning and larval rearing of sandfish in the hatchery (James, 1996; Hamel *et al.,* 2001) and early nursery phase until they reach a size of a few grams (Battaglene *et al.,* 1999; Hamel *et al.,* 2001). However, information on subsequent growth rates is scarce, although Shelley (1985) estimated natural growth in Papua New Guinea at about 14 g/month by following cohorts identified from size-frequency measurements and Mercier *et al.* (2000) reported an average size increase of 300 %, from 65 to 197 mm, in 2 months after releasing laboratory reared juveniles in the Solomon Islands. There is also little information on practical systems for producing large quantities of bigger animals. The aims of this project, therefore, were to address these gaps and to develop systems of mass rearing for sandfish to help assess the potential for commercial culture and/or effective restocking of depleted marine areas.

The research described here is a collaborative project between the Viet Nam Ministry of Fisheries and the WorldFish Center (formerly ICLARM). It was done in Khanh Hoa Province, Viet Nam, where there is an important seafood and aquaculture sector. This area was selected because large numbers of shrimp ponds and hatcheries have been built there and many other forms of aquaculture, e.g., cage-culture for spiny lobsters also occur in the area. The existing infrastructure and aquaculture activities assisted the authors to develop and test methods for farming sandfish in shrimp ponds, either as an alternative crop or in polyculture. Shrimp disease problems here, as in many other countries, mean that ponds are often left empty and farmers are interested in new aquaculture species and management systems. The cage and pen-based culture industry also offered opportunities to test grow-out methods directly on the seabed.

One potential problem facing the project initially was the reported scarcity of sandfish in the Province. Of the four major bays along the Khanh Hoa coastline (Van Phong, Nha Phu, Nha Trang and Cam Ranh) sandfish were thought to occur only in Cam Ranh. Elsewhere, it was believed that they had been fished to local extinction and that none had been caught in the past decade or so. This turned out to be incorrect. Small-scale commercial landings were still being made in Van Phong, and a few sandfish were found in the Nha Trang area. In Cam Ranh, commercial fishing was still continuing, and some farmers also try to rear small collected sandfish to larger sizes in ponds. A strip of land 300 m wide along the eastern side of this bay, which is closed to fishing by the military, may have helped to keep stocks in better condition there than elsewhere.

Methods and results

Experiments with collected sandfish

Sandfish were bought for broodstock from dealers in Cam Ranh, about 60 km south of Nha Trang. They were small, typically averaging between 150 and 200 g. These individuals could only be spawned once (in February) during the first year. After some months of ongrowing in sandy ponds they had increased their weight to an average of 260 g. Lacking hatchery produced juveniles (or wild sandfish below about 70 g) there were limits to the tank trials that could be done in the first year. However, trials were possible in constructed pens and rented ponds and data were collected from wild sandfish (Pitt *et al.,* 2001).

Very short-term growth trials are rather inaccurate because weight is difficult to ascertain accurately. There was a weight loss of about 4 % on drying adult sandfish for 15 minutes and similar changes due to voiding sand contents over 2-3 days. However, individual short term weight fluctuations, due presumably to changes in the amount of water held inside the body cavity, were often several times larger. These were sometimes caused by conditions during short term storage or transport. Overcrowding in bags or baskets led to swelling by 10-20 % or even more. Sometimes the causes were unknown, perhaps linked with salinity changes.

Sandfish in bare tanks, fed on shrimp pellets or unfed, all lost weight, with animals in the fed tanks losing weight more rapidly than those that were unfed. In tanks with sand, weights were maintained, with little difference among sandfish fed with different diets, i.e., chick feed or wheat flour mixed with shrimp pellets, with algae *Gracilaria* sp., or sea grass. Another interesting observation is that a wide range of finely ground vegetable materials were eaten and defecated, without apparent change of form or colour.

In ponds, sandfish grew at about 1-3 g per day, showing an inverse relation with stocking density. Two attempts to look at different densities and different substrates were cut short by major mortalities caused by heavy rain and stratification. However, there was some indication of a negative density effect and slight advantage of sandy over hard or muddy substrates.

Seabed pens proved useful for holding small numbers of sandfish. Survival was generally very good. Growth rates appeared lower than in ponds, and depended on both location and density.

Spawning and larval rearing

A pen of 800 m^2 was stocked with 392 wild sandfish (average weight of 138 g) in July 2001. These were used regularly as broodstock. After 16 months, the remaining 203 sea cucumbers had to be maintained at higher densities due to relocation of the pens on the farm where the work was done. Hatchery produced second generation broodstock were stored nearby in a 450 m^2 pond.

After further rearing in ponds or pens, broodstock became much easier to spawn. As well as being bigger, they may secrete some chemical to coordinate gonad maturation between themselves, as found for the sea cucumber *Cucumaria frondosa* (Hamel and Mercier, 1999). In New Caledonia, it was also observed that even very large, newly collected sandfish were difficult to spawn. Individuals that had been held in a pond for some months were easier to induce to spawn. Although newly collected wild sandfish were generally used with relative success for spawning in Solomon Islands these had often come from dense lagoon populations.

Groups of 30-45 individuals (average weights 250-415 g) have been spawned on a regular basis since the end of August 2001, and once or twice every month from March 2002 to August 2003. Over a 24-month period, 40 broodstock groups were used and underwent spawning stimulation attempts on 71 occasions. Spawning occurred on 38 of these, producing 275 million eggs. The percentage of successful attempts did not show a clear cycle by time of year (Figure 1), but the average production of eggs (per gram of total spawning group weight) was higher from December to April than at other times (Figure 2). Similarly, although there was no marked difference between the success rate of spawning attempts at different times in the lunar month, there was a higher production of eggs around the new moon than at other times (Figure 3).

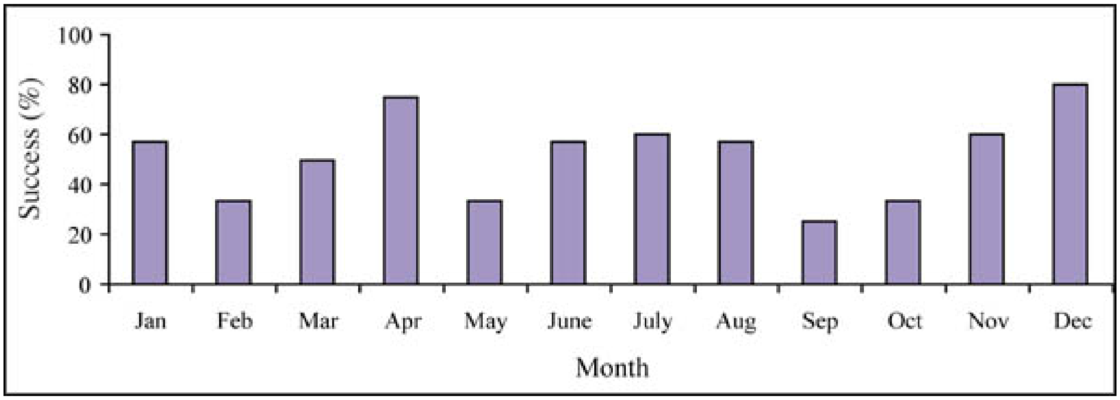

Figure 1. *Percentage of group spawning stimulation attempts which were successful, by month. Groups contained 30-45 adult sandfish.*

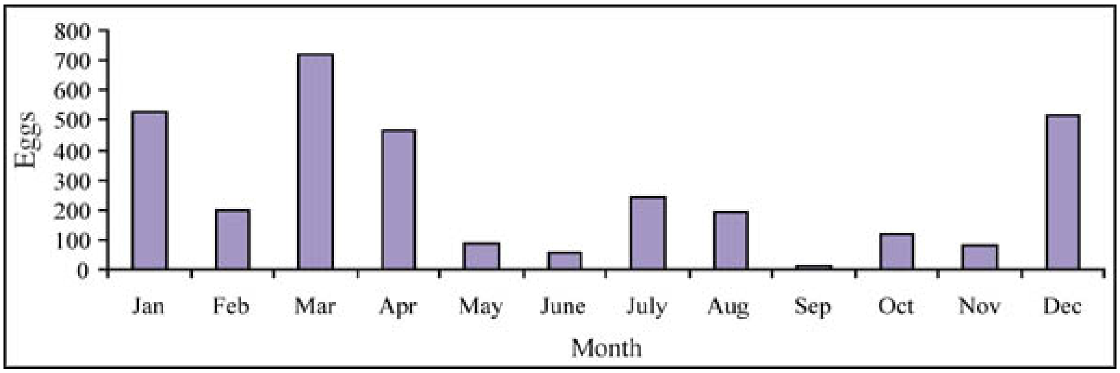

Figure 2. *Monthly average number of eggs per gram of adult sandfish per group of 30-45 adult sea cucumbers.*

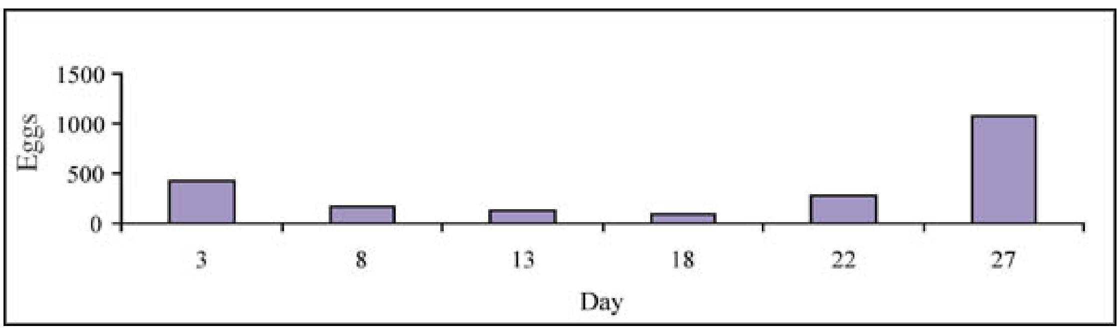

Figure 3. *Average number of eggs per gram of adult sea cucumber per group of 30-45 individuals, by day considering the lunar cycle. (Lunar day 3 refers to the central day of the 5-day period 1-5, day 8 to the period 6-10, and soon, except for 18 which refers to the 4-day period 16-19).*

After transportation (using battery aerators or oxygen-filled bags), broodstock were usually left for a few days in a shallow bare 1 m^3 fibreglass spawning tank. During this time, they voided sand from their digestive tracts. They were sometimes fed with dried grounded *Sargassum* powder. Broodstock were returned to their pen or pond a few weeks later, generally after 2 or 3 spawning stimulation attempts. Anew group of broodstock was collected.

On the morning of a spawning attempt, broodstock were subjected to a range of stresses. These usually included complete or partial drying in shade for 30-45 minutes. This was followed by a slow flow of sun-warmed water at about 5 °C above ambient, often passed through a UV unit. Sometimes ice was used before or instead of drying, to give a cold shock of about 5 °C below ambient before the hot shock. Occasionally, instead of being left dry, the sandfish were subjected to water jetting, or left standing in a couple of centimetres of water. If they were not already spawning at midday, powdered algae (*Spirulina*) were often then added to the water in the tank (30 g in 300-500 litres). If successful, spawning would usually occur between 1300 and 1600 hours and only rarely at night. Sometimes excess spawning males were removed from the spawning tank, but females were not disturbed. Contrary to what was usual in the Solomon Islands, female sandfish often stopped spawning if moved, and failed to start again.

Fertilization occurred inside the spawning tank. Eggs were siphoned out of the tank into a 50 mm mesh bag and washed in filtered seawater to remove excess sperm and powdered algae. Aliquots from stirred buckets were counted and the eggs were stocked in hatchery tanks. Hatching, larval rearing, settlement and early juvenile rearing were usually all carried out in the same indoor fibreglass or concrete tanks of 1.7-6 m^3. Hatchery seawater was sand filtered, stored briefly in a reservoir tank and then passed through (nominal) 1 mm filter bags or cartridges. Partial water changes of about 15-30 % per day were made using a 100 mm mesh outlet screen. EDTA was routinely added after water changes at a rate of about 5 ppm of the added water.

Larvae were fed with algae from open outdoor batch cultures. *Chaetoceros muelleri* and *C. calcitrans, Nanochloropsis occulata, Platymonas* sp., *Isochrisis galbena* and *Rhodomonas salina* were used as available. The algae were given twice daily, at gradually increasing concentrations. Feeding rate was judged by the colour of the water in the rearing tank, since it was difficult to count residual algal cell densities. Tank floors were sometimes cleaned by siphoning after hatching and during early larval rearing.

At the start of metamorphosis, preconditioned stacks of plastic plates (PVC, polythene or polypropylene) were added to the tanks. Several conditioning systems were tried. These included painting plates with dry algae and allowing the plates to air-dry, immersing them in benthic diatom cultures (*Navicula,* etc.) or in *Platymonas* cultures for a few days, or immersing them in running unfiltered seawater for a few days, with or without the addition of chemical fertilizers. All the materials and conditioning systems worked quite well and gave good settlement results on at least some occasions. Conditioning in running seawater was simple, requiring only that plates be put into a conditioning tank 5-7 days after spawning, while painting with *Spirulina* (1-2 g dry algae/m^2) had the advantage that unwanted organisms would not be introduced.

After settlement, live algae were supplemented with algal powder, mainly *Spirulina,* sometimes with added *Schizochytrium* (Algamac, 2000). Depending on temperature, most larvae metamorphosed and settled (or died) within 10-20 days of spawning. Pentactulae and juveniles were usually left indoors (in the same tanks) for a further 2 to 10 weeks, depending on the availability of outdoor tanks for first nursery. Many had by then become juveniles of 1-2 mm in length, although both much larger and many smaller individuals could usually be found, as growth rates were very variable.

Despite filtration, copepods were able to multiply in the hatchery tanks. Heavy infestations destroyed good batches of settled juveniles within a few days, whether due to direct attack or to repeated collisions causing skin damage. On plates with a thick coat of benthic algae, the juvenile sandfish seemed to be somewhat protected. Treatment with the organophosphate insecticide Dipterex (trichlorofon) at 1-3 ppm for 1-3 hours, followed by rapid dilution to about half or one-third the concentration was usually effective in killing swimming stages of the copepods, but not the eggs. Over the years, treatment rates to kill most copepods had to be increased. It is not clear whether this was due to development of resistance, chance infestation with less sensitive species, or a drop in strength of the insecticide as sold.

Juveniles were transferred outdoors by moving the plate stacks on which they had settled, by siphoning the floor (into a bowl or onto a sieve), or by draining and hosing down the tank walls and floor. Juveniles could be separated from dirt by their rapid attachment to clean plastic surfaces, or by catching and washing on a 250 mm mesh screen. Potassium chloride detachment was not used.

Numbers at transfer were estimated either by direct total counts on plates or in bowls, or by counting random samples of known area. Very small juveniles were sometimes re-suspended in a known volume and aliquots counted under a microscope. Overall, survival in the indoor hatchery tank was very variable, but rarely more than 1-2 % from egg to transfer outdoors. Much higher rates of survival through the larval period, and during settlement, were sometimes found in small-scale trials (in tanks, buckets or jars), but over shorter periods.

Complete or partial collapses in the first few days of larval rearing were not uncommon, sometimes with the appearance of mucus strings to which the larvae became attached. Trial use of antibiotics (erythromycin) gave mixed results; positive in jar trials, but negative in tanks. There may be improvements to be made by controlling spermatozoa levels at fertilization, better egg handling, more frequent chlorination of hatchery water supply pipes, different levels of water exchange, use of separate incubation, larval rearing and settlement tanks or earlier transfer outdoors. Tanks were not usually cleaned at all after settlement to avoid loss of juveniles.

Outdoor batch algae cultures were not always reliable. *Chaetoceros muelleri* (or other *Chaetoceros* spp.) and *Rhodomonas salina,* perhaps the best algae for larval culture (Battaglene, 1999), were often in short supply or unavailable when needed. Sometimes the ‘weed’ species, *Nanochloropsis occulata* and/or *Platymonas* sp., formed the main food source.

Complementary research on larval rearing of sandfish being undertaken by students at the Research Institute for Aquaculture No. 3 is also contributing to knowledge about hatchery methods. A summary of this research is included here with the permission of the people involved. Do Thanh Tam examined the effects of transferring larvae to clean tanks prior to introduction of settlement plates. Eight indoor tanks were stocked with 0.7 larvae/ml in 200 litres of water (140 000 larvae/tank), after 2 days incubation in a separate hatching tank. Half the tanks underwent high water changes (40 % every day) and half low water changes (20 % every second day). On day-9 after spawning and before settlement had started, larvae from half of the tanks were transferred to clean tanks containing settlement plates which had been painted with a *Spirulina* slurry (1.5-2 g/m^2 dry matter) and air-dried. Larvae from half of the tanks were not transferred but *Spirulina-painted* plates were put into the rearing tanks (which is the more usual procedure). Twenty days after spawning, estimates of juvenile numbers were made by counting randomly selected sample areas of the plates and tank surfaces. These varied from 3 600-19 500 per tank. Two weeks later all remaining juveniles were counted for stocking in first nursery tanks. Numbers had dropped by about 43 % in this period. There appeared to be no advantage in transfer before settlement, but high water exchange was better than low. The best tanks had over 10 % survival from newly-hatched larvae to juveniles, and produced more than 70 000 juveniles/m^3.

Le Thi Thu Huong investigated the effects of salinity on survival and growth. Larvae were stocked two days after spawning had occurred in normal seawater at the rate of 0.54 larvae/ml in 5 litre buckets indoors, at salinities from 15-40 (3 replicates of 6 salinities). Adjustment of salinities took several hours, at 2 units per hour. Buckets had light aeration and near-opaque covers. Larvae were fed live algae, usually 50 ml *Chaetoceros* sp. daily, and 20 % of the water was changed every second day. At metamorphosis, *Spirulina-painted* plates were inserted in the buckets, and feeding was supplemented with dry *Spirulina* and Algamac. Twenty days after spawning, juveniles on the plates and bucket surfaces were counted. All larvae died within 3 days at a salinity of 15, but metamorphosis and settlement were achieved in salinities from 20-40, with survival rates of recently-hatched larvae to juveniles at up to 44 % (at a salinity of 35).

Le Thi Thuy tested the effects of different feeding regimes, including dried algae. Larvae were raised in 5 litre buckets, stocked and managed as above, using 5 feeds with 3 replicates. Some buckets received live algae, usually 50 ml/ day of *Chaetoceros* sp. or 10 ml/day of the stronger *Nanochloropsis occulata* culture. The other buckets received 5 mg/day of the dry feeds; Frippak micro-encapsulated shrimp larval diet (unfortunately old and in poor condition), *Spirulina* powder, and a mixture of 1 part *Spirulina* and 1 part Algamac 2000. Average survival rates to the juvenile stage for the different feeding regimes were *Chaetoceros* 24.8 %, mixture 12.7 %, *Spirulina* 4.9 %, Frippak 3.8 % and *Nanochloropis* 0.36 %.

As eggs were abundant, nursery and grow-out facilities were more of a production bottleneck than low hatchery survival. Often, because all the nursery tanks were full, juveniles were kept in hatchery tanks long past the time when they were ready for transfer. Their growth slowed and numbers gradually dwindled. The 30 m^3 hatchery could supply far more 1 -2 mm juveniles than the 25 0 m^2 of nursery tanks could support. It is estimated (Pitt and Duy, 2003) that a hatchery of this size in the tropics should be able to supply over 1 000 m^2 of nursery tanks (or hapas and bag nets in ponds), 1.5 ha of nursery ponds and 12 ha of grow-out ponds or pens, to produce 100 tonnes live weight of sandfish per year.

First nursery in tanks

Outdoor first nursery tanks were of 0.5-1 m depth and 0.6-16 m^2 floor area. Inner surfaces were fibreglass, flexible PVC-cloth liner or concrete. They were usually bare, without any added sand. Stocking rates were generally 500-1 500/m^2. Different tank pre-conditioning methods, additional substrate, shade levels, flow rates, water treatments and feeding systems were investigated, some in controlled trials.

Tanks were generally pre-conditioned for a few days using flowing unfiltered water. Dry algae or fine grade shrimp starter feed were usually fed to tanks at a rate of not more than 0.5 g/m^3, twice a day. This was occasionally supplemented by live phytoplankton when available. All tank materials were satisfactory but very small tanks (about 300 litres) did not give good results. It appeared that water should be at least 60 cm deep. Square fibreglass tanks of about 1.6 m^2 surface area and 60 cm depth were useful for experiments. Bigger PVC-lined pools of 10 m^2 and 70 cm depth, or concrete tanks of 6 m^2 or more and 1 m deep, were better for production.

Quite heavy shading seemed to be helpful in the first weeks of first nursery; later it could be reduced. Unfiltered seawater gave better results than filtered sea water, and high flow rates were better than low rates. There was no clear advantage in putting in additional surfaces such as seagrass (*Enhalus* sp.), additional plate stacks or roofing tiles. Small juveniles appeared to avoid the brighter and more open parts of tanks until they attained adult skin colouration. After 1-2 months, yields from first nursery tanks were typically about 100-300 juveniles/m^2 of a wide size range, from very small (less than 0.1 g) up to 2 g and more. On occasion, as many as 500 juveniles/m^2 were obtained with survival up to 50 %. Quite often, however, the survival was much lower and for unknown reasons.

First nursery in ponds

Small juveniles were only transferred directly into a pond once as they disappeared without trace. Total mortality also occurred when small juveniles were placed in a hapa (a 2x2x1 m bag made of 450 mm netting) installed on a raft in a sheltered bay and stocked with post-hatchery individuals of 1-3 mm.

However, the use of hapas inside ponds has given some very good results. Juveniles were transferred either out of water on their 40x50 cm settlement plates separated by damp *Gracilaria* in a styrofoam box, or without plates in oxygen-inflated bags of seawater, with transfer times of 1-2 hours. Newly-installed hapas were stocked (without conditioning), small quantities of *Gracilaria* were added and a few lengths of coconut leaf put inside for shade. Juveniles were not fed and the netting was rarely cleaned.

The initial five trials of these first nursery batches in hapas (carried out without any tank comparison) gave extremely good results. The best survival was 97 % and the average was 56 %. This was better than had been achieved in tanks. Growth too was good. Nursed juveniles of 1 g mean weight were produced after an average of 41 days.

Two trials were run to compare first nursery in hapas with small fibreglass tanks of 1.7 m^2 floor area and 60 cm depth. Small larvae (mostly of length 1-2 mm) were taken from the same hatchery tank on the same or following days. However, in the first trial mean survivals were 31 % for 4 tanks compared with only 26 % for 3 hapas. In the second trial, survival was very low: 19 % for 4 tanks and 13 % for 3 hapas. Mean sizes after 6 weeks were 0.5 to 0.8 g, with no clear difference between rearing systems.

Part of the problem with the latter two trials was that ponds were being used for multiple batches of sandfish of different sizes at the same time, in bag-nets, pens and the main body of the pond. Therefore, they had not been dried for many months and appeared very eutrophic. Consequently, the hapa meshes clogged and the hapa floors became muddy with low dissolved oxygen levels. Another drawback was the short life of the mesh used when exposed to sunlight. Nevertheless, the use of hapas in shrimp ponds is considered to have many benefits as a method to get around the considerable cost of pumping water to onshore tanks to provide the conditions needed for first nursery.

Blocks of small independently manageable ponds could be very valuable for nursery work at all stages. Ideally, they should have sandy floors and be in a location where salinity can be kept above 20 in the wet season. An aeration system would also be useful to keep pond floors from becoming anaerobic and break up any stratification caused by rain.

Second nursery

Juveniles from the first nursery bare tank system were usually sorted by size and stocked in outdoor tanks for a second nursery period. These tanks were supplied with a thin layer (3-5 mm) of fine sand on the floor. They were generally unshaded. Numerous experiments were run to look at the effects of flow rate, substrate type, density, diet, shading, water filtration and co-culture with shrimp during the second nursery stage.

Complementary research on feeding during the first nursery phase has also been done by Mai Van Dinh (unpublished data). This experiment used 15 (lightly shaded) outdoor tanks of 0.57 m^2 with sand on the floors and a water flow of about one exchange per day, in which 30 juveniles of about 0.77 g average weight were stocked per tank and fed 0.5 g/day of different dry diets (5 diets, 3 replicates). They were weighed every 2 weeks. Fine shrimp starter food supported faster growth than (a) the same shrimp food mixed with *Spirulina* and powdered seaweed; (b) 'five-powder' (consisting of finely-ground corn, rice, soybeans, black and green beans); (c) chicken manure; or (d) those left unfed (Figure 4).

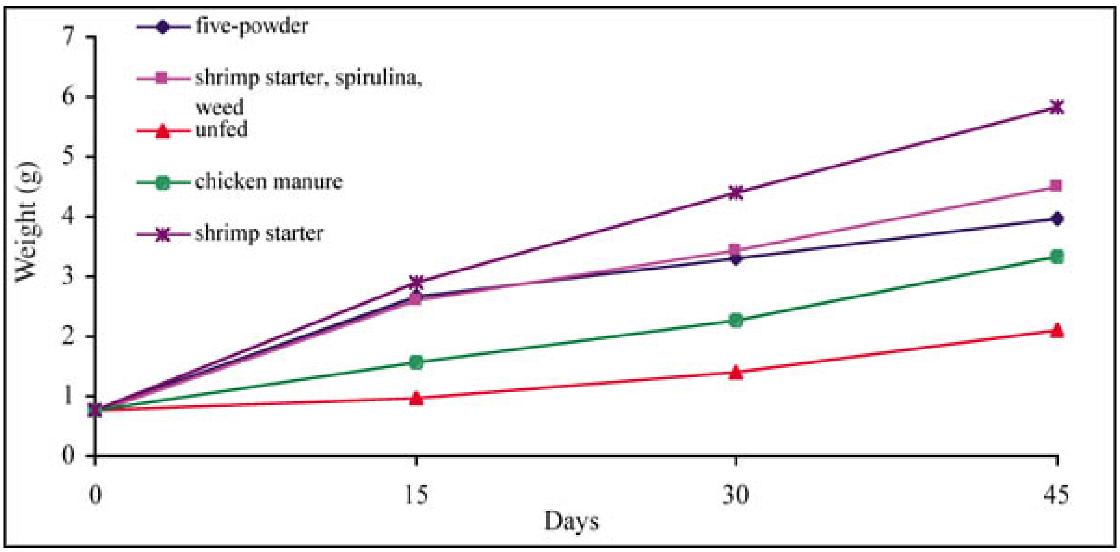

Figure 4. *Growth (average weight) of juvenile sandfish fed with different dry diets during the second nursery stage.*

A factorial trial in 16 outdoor tanks (1.7 m^2) was designed to assess the contributions of supplied food, development of food organisms inside the tank based on photosynthesis and incoming suspended particles as food sources. It compared growth with added food (0.6 g/m^2 twice daily of fine shrimp starter feed) or without feeding with flows of filtered or unfiltered water (exchanging about half the tank volume per day) and with opaque or clear covers on the tanks. Tanks were stocked with 35 juveniles each (average weight 2.1 g), which were weighed every 2 weeks. It turned out that both feeding and light were necessary for good growth (Figure 5). After 6 weeks of these treatments, all tanks were given food, unfiltered water and light for another 4 weeks and growth recovered in most of the tanks that had been held back.

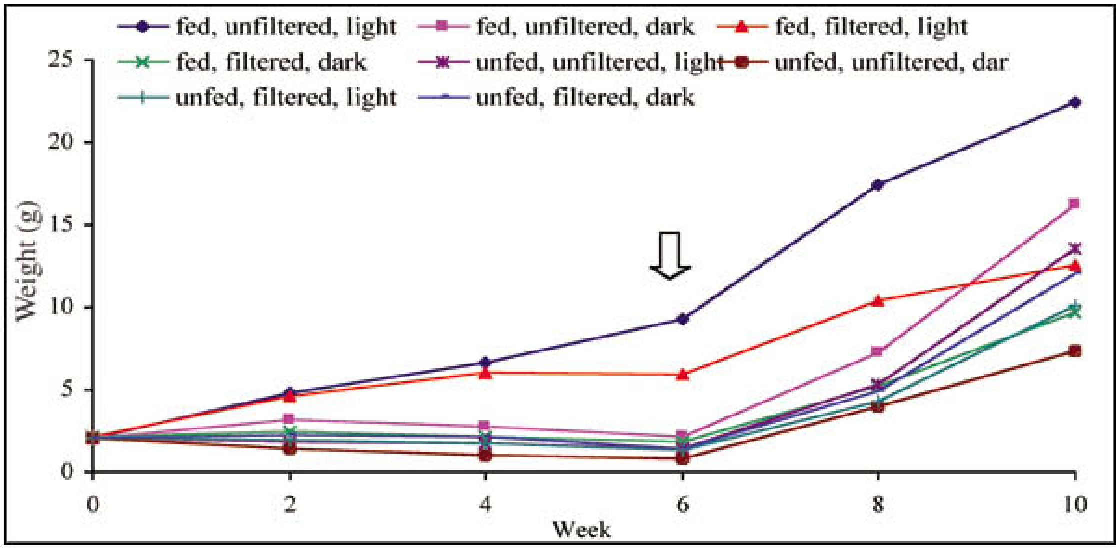

Figure 5. *Growth of juvenile sandfish under different rearing combinations of fed (shrimp starter) or unfed, unfiltered or filtered water, light or dark. After six weeks, all tanks were given optimal conditions (arrow); i.e., fed, unfiltered water and light.*

Culture with *Penaeus monodon* shrimp post-larvae (80/m^3) often produced improved growth for the sandfish without appearing to harm the shrimp. However, there were also cases of mass losses of the sandfish, particularly with high densities of larger, underfed shrimp. First, some flat or bitten sandfish were seen then, within a few days, all the sandfish would die or vanish. There was no doubt, both from tanks and from aquarium observations, that under some circumstances *P. monodon* shrimp will prey on juvenile sandfish. A release of hatchery-bred megalops larvae of swimming crabs (*Portunus pelagicus*) into a second nursery sandfish tank resulted in similar patterns of damage. The crabs developed well, but survival of sandfish was low. In aquaria, small rabbitfish (*Siganus* sp.), under consideration as tank cleaners also appeared to cause the death of sandfish juveniles.

Juveniles from harvested hapas in ponds were transferred to larger bag-nets inside ponds. These were 4 x 4 m or 6 x 6 m square cages of 1.2 m height, made from a cheap 1 mm mesh. They gave good protection against potential predators (shrimp, fish and crabs) and dissolved oxygen levels on the bag-net floor were often higher than on the pond bottom. Survival and growth could be good; in one case 96.5 % of 1.2 g juveniles reached an average weight of 13.6 g in 2 months, at a high density of 460 g/m^2.

Later nursery and ongrowing in pens and cages at sea

A trial was conducted together with the Hon Mun Marine Protected Area Management Project on the largest island (Hon Tre) of Nha Trang Bay. Two sites were chosen, after discussion with local fishermen as being apparently suitable for sandfish. At each site a nearby resident agreed to observe and guard the pens. The first site (Bich Dam) was inside a very sheltered bay in water of 1.5 - 2.5 m depth, with sandy-silt substrate, a little coral or coral rubble and some seagrass. The second site (Vung Ngan) was more exposed to the south, in water of 4-5 m depth, on a substrate of sand with some rocks and coral sand. Although big seas were rare here (with fishing boat moorings and lobster cages in the immediate vicinity), there were often quite noticeable water movements, even close to the seabed.

Four open pens were constructed at each of the two sites by building fences standing 80 cm high off the seabed. The fence bottom was buried in the substrate and pegged down, with sandbags arranged around the edge on top of the pegs. Each pen enclosed an area of 46 m^2. Some pens were stocked more than once.

Even in the more sheltered location, survival of small juveniles (2.3 or 6.3 g average) was poor. Two batches disappeared within a month, while only 18 % of a third batch reached 200 g average after 9 months. Most small juveniles disappeared within the first few weeks. Wounded, bitten or flat (eviscerated) juveniles were found, similar in appearance to those from tanks containing shrimp or swimming crabs. Survival of bigger juveniles (18 or 32 g) was fair, with 70-82 % averaging 140-220 g after 6-8 months. In the more exposed location, survival after 3 months again appeared to depend on release size. Recovery was zero for the juveniles stocked at 4.9 g average weight, 3 % for those stocked at 11.4 g, 11 % at 30.4 g and 50 % for those stocked at 50.6 g. However, after about 4 months none of the sandfish were found, due perhaps to wave action or to theft.

Seabed cages used for culturing babylon snails were tried for rearing small (nursed) sandfish juveniles. Rigid cages consisted of 2mx2mx30cm steel frames covered on all six faces with 1 mm or 3 mm plastic mesh. Hinged doors in the top surfaces allowed access. The floors lay directly on the seabed and sand was added to cover the netting. Each cage was stocked with 50-200 juveniles. Survival after 2 months was above 50 % for juveniles of average initial weight 1.5-6.3 g, even in a cage with babylon snails. There was good growth to 8.7-31.6 g, depending on initial size and density. However, for the smallest (0.5 g) juveniles only 24 % were recovered. Babylon snail farmers commonly use cages or pens with netting walls rising above high water. Cage floors are usually covered with sand, while pen walls are buried and pegged down in the seabed. The smallest mesh is usually about 4 mm knot to knot. Sandfish were supplied to several farmers, with apparently good results (although clear data were never easy to retrieve). In two cages at one site, juveniles of 5 g appeared to reach 44 and 70 g in five weeks. At another site, 2 g juveniles reached 16-20 g, and 4 g juveniles reached 33 g after one month.

Seven different attempts at grow-out during the nursery phase directly on the seabed were carried out at two sites. On three occasions, sheets of 2 mm netting were laid directly on the seabed, with the edges buried and pegged down (like clam-culture parks). In two other attempts, similar 2 mm netting was formed into shallow boxes (open below but covered on top), with walls about 35 cm high. The walls of these boxes were also partly buried and pegged down, and the roof was held off the sand with numerous small foam floats. Two long netting bags were also made and laid on the seabed. The floor of these bags was partly covered with sand and in one case the roof had small foam floats attached.

Reasonable numbers of sandfish were retrieved in only two out of seven of these trials. From one 11.7 m^2 box-shaped net, 47 %of 250 juveniles released at 3.3 g were collected after 3 months, averaging only 12.2 g. From a 14.1 m^2 bag, 39 % of 210 juveniles released at 3.1 g were collected after a similar period, averaging 11.2 g. From other attempts, only a few or no sandfish were found. These systems might be made to work reliably if there was easy access to suitable sheltered sites with firm substrate (sand or silt) and nets were cleaned at least weekly. However, use of these bags would then probably be costly in diver time. The use of bag-nets and pens in ponds, or co-culture in existing babylon snail cages seem be more promising methods.

Two pilot-scale pens were built in marine protected areas along the Khanh Hoa coast to test sandfish farming as an alternative income source for local fishermen. The first was in collaboration with the International Marinelife Alliance. They had recently established a locally managed Marine Protected Area (MPA) at Ran Trao, a patch of silty sand, seagrass, coral rubble and coral reef in Van Phong Bay some 60 km north of Nha Trang. The protected area (0.5 km^2) was a couple of kilometres offshore from the same village (Xuan Tu) where the sea cucumber project's broodstock pens and experimental ponds were located.

The pen consisted of a fence of 5 mm mesh 145 m long and 1 m high built in an approximate circle in water of 5-6 m depth. Steel posts supported the net at 2 m intervals. The foot of the net was buried 10-20 cm and fixed down with bamboo pegs every 50 cm. Long narrow sandbags were laid around the foot of the net on top of the pegs. It enclosed an area (1 600 m^2) of silty sand with some short seagrass. Although partly protected by a submerged fringing reef, it was exposed to the southeast.

The Ran Trao pen was stocked in July 2002 with 1 458 hatchery-produced sandfish from various pond nursery experiments (ca. 0.93/m^2), averaging 97 g. Growth was somewhat disappointing with an average weight reaching 180 g by April 2003. There was then a partial harvest, which only fetched about US$ 0.84/kg. By late September 2003, the net fence was in poor condition, having fallen down, torn or lifted in many places. The remaining sandfish were not harvested but left in the sea for local restocking. Sandfish remained quite plentiful within the pen (which still seemed to be a substantial barrier to migration) and a samples averaged 335 g.

The second pilot-scale pen was in the same sheltered bay (Bich Dam) as the earlier pen nursery experiments, in the area of the Hon Mun MPA and maintained in collaboration with their staff. It was made from a strip of 8 mm netting 60 cm high and nearly 200 m long. It was laid in a rectangle in water 1.5-2.5 m deep, supported on bamboo posts every 2.5 m. The substrate was mainly silty sand with some broken coral. The foot of the net was buried about 5-15 cm, held down with bamboo pegs but without sandbags. It enclosed an area of about 2 000 m^2 and was stocked in March 2003 with 1 460 (ca. 0.73/m^2) hatchery-bred sandfish nursed in ponds, averaging 84 g. By August these sandfish averaged 221 g, a growth rate of 1.05 g/day.

Later nursery and ongrowing in ponds

Sandfish juveniles of various sizes were transferred for further nursery and ongrowing into earth ponds, or pens inside ponds. Efforts were made to rid the ponds of crabs and shrimp (by drying or spreading insecticide-laced bait) and fish (using saponin) before stocking. Conditions inside the ponds were often tested by releasing 50-100 juveniles in a small pen (9-25 m^2) built inside the pond before the main stocking. Survival in a pen inside a pond was often better than in the main body of the pond, although growth slowed down sooner. This led to the use offences built inside the pond along the base of the banks.

In one early success, juveniles of 30 g stocked at a density of 1/m^2 exceeded 300 g in just 3 months without any losses. These hatchery-bred sandfish were later spawned at less than one year of age and several batches of second generation juveniles have been reared.

In the example shown below, juveniles of 2.3, 5.8 and 11.6 g average weight were stocked in six experimental (coral and coral sand substrate) ponds at about 1/m^2 (Figure 6). After 113 days, the small juveniles averaged 119 and 148 g, the middle-sized ones 166 and 156 g and the large ones 200 and 182 g.

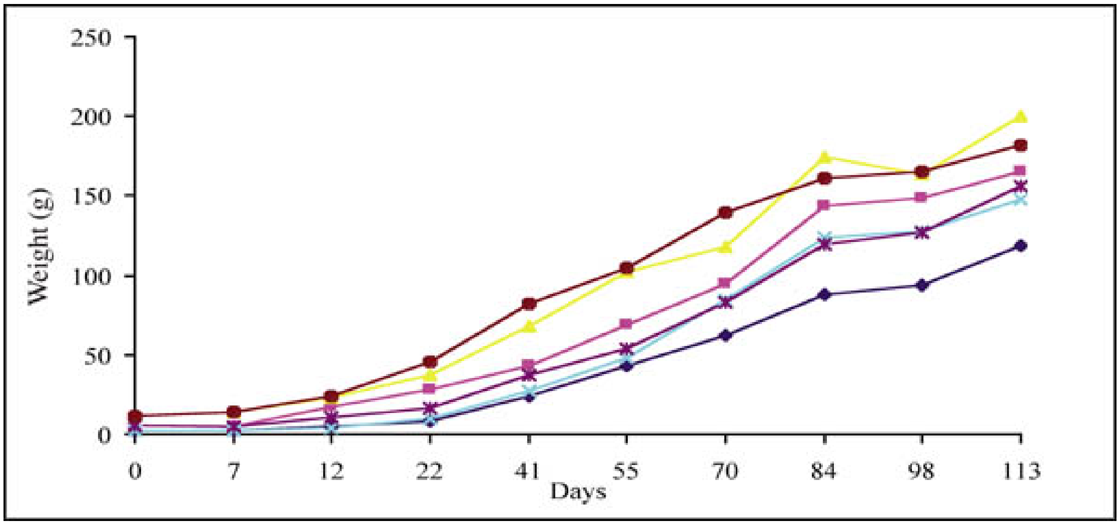

Figure 6. *Average weights (g) of sandfish stocked in ponds as juveniles for a period of 113 days.*

On a few occasions, sandfish in ponds became sick, with white lesions on the dorsal surface. *Vibrio* bacteria (tentatively identified as *V. salmonicida*) were isolated from these lesions, but it is not known what the primary cause was; possibly attack by crabs, which were never possible to exclude completely from the ponds and pens. Sometimes the diseased individuals recovered spontaneously in ponds or recovered after being moved to a tank with clean seawater.

Battaglene (1999) observed that growth of juveniles in tanks slowed down or stopped when sandfish density reached about 200-225 g/m^2. This was generally confirmed, although in ponds the limit could be a little higher and on the seabed it was often lower. Growth also often stopped at an average weight of about 250-350 g, whether the density was high or not. It is not yet known whether this is due to energy being put into gonad development and spawning, to depletion of a limiting nutrient in the substrate or to some other cause.

Co-culture with shrimp in ponds

With help from the Support to Brackish Water and Marine Aquaculture (SUMA) program of DANIDA, the scientists of the Research Institute for Aquaculture No. 3 (RIA3) carried out a series of experiments in earth ponds and small scale containers to look at the feasibility of co-culture of sandfish with *Penaeus monodon* shrimp (Thu, 2003). In the first year, wild collected sandfish were used, however, after artificial breeding became routine, these experiments used hatchery produced juveniles.

There was reasonable survival to harvest of both shrimp and sandfish in two out of four co-culture ponds on the first attempt, and one of two co-culture ponds on the second. Apparently, reduced levels of total organic matter and hydrogen sulphide were found in ponds with sandfish and shrimp, as opposed to with shrimp alone, and growth rates of shrimp were improved. In buckets, sandfish juveniles survived at salinities of 15 (95 % after one month), at 10 (92 % after 3 weeks) and at 40 (83 % after one month). At a salinity of 45, all were dead after one week. Sandfish juveniles in buckets tolerated the use of the chemicals dolomite, zeolite and lime (used to manage water quality in shrimp ponds) at below 30 ppm.

Conclusions

Prospects for commercial culture of sandfish

In trying to answer the question ‘can the development of hatchery methods of sandfish lead to commercial-scale farming in Viet Nam’ there are a number of positive and negative factors that need to be considered. The positive factors are:

- Sandfish are easy to contain inside simple, low submerged fences.
- Sandfish do not need feeding in pens or ponds at medium densities (1.5-3 tonnes/ha).
- A second crop may make low density shrimp culture more profitable.
- Sandfish might improve benthic conditions and water quality in shrimp ponds.
- Sandfish are likely to thrive on detritus around fish, lobster or babylon snail cages.

The negative factors are:

- The current local wet (whole-body) price is only US$ 0.70-2/kg.
- The processing weight loss is 90-95 %.

- Sandfish in central Viet Nam do not appear to reach the large sizes (over 1 kg) of those in the South Pacific.
- Culture is density-limited; nursery and growout need large areas.
- Sandfish are vulnerable to water stratification, low salinities and anoxic conditions in ponds.
- Sandfish can be easily stolen, particularly from large submerged pens.
- Predators of juveniles include crabs, shrimp, some snails and fish.

Farmers have shown some interest in growing hatchery produced sandfish. Juveniles have been supplied to both pond and pen farmers usually at around 2 g average weight, but few if any appear to have yet reared significant quantities to sell, however, clear feedback from farmers has always been difficult to obtain. The low price paid by some local processors (under US$ 1/kg wet weight) has discouraged major efforts to overcome the technical problems of pond management. Successful shrimp culture, albeit a high-risk enterprise, remains the primary goal of most pond operators. The situation might be improved with better processing and marketing, perhaps on a cooperative basis. At a guaranteed live-weight price of US$ 2/kg or more there would certainly be more interest.

A longer term goal would be development of profitable systems of pond polyculture. Potential advantages are the more efficient use of feeds, a reduced load on the environment and a lower risk to farmers than intensive shrimp culture. Components might include shrimp at low densities, filter feeding bivalves, seaweed, fish or gastropods. Sandfish could fit well into such systems.

Prospects for restocking depleted populations of sea cucumbers with hatchery reared juveniles

At present, hatchery breeding methods only exist for a very small number of the commercial tropical sea cucumber species and large juveniles remain expensive to produce. It is far from obvious that the release of hatchery juveniles will be either useful or cost effective in restoring overfished sea cucumber populations. A few points to consider are outlined below.

If populations do not recover after fishing has been controlled, or recover only slowly, effective interventions will depend on the stage(s) in the life cycle where the recruitment bottlenecks occur. For example, if the remaining adults are too sparsely distributed for fertilization to occur, concentrating spawners in a few suitable sites and/or bringing in adults from nearby areas might be cheaper and easier than a hatchery breeding program.

Restocking without effective protection is likely to be futile. Where some stocks still remain, testing the effects of the two measures (protection and release) may not be easy or cheap (although genetic profiling could prove useful), requiring good pre- and post-intervention surveys at multiple sites.

It is not known to what extent planktonic sea cucumber larvae control their own dispersal by tide and current. While released, juveniles might survive, grow and spawn in a particular location. The long term effect of a restocking program may be limited if their larvae cannot not reach and settle in suitable nursery areas. The geographical scale on which release tests need to be done is also not clear, since it is not known how far larvae travel.

Juveniles of most species are rarely found and their nursery requirements are little known.

Despite the patchiness of data, a comparative study of the histories of different sea cucumber fisheries worldwide might be useful, in particular looking at recovery times between fishing pulses and the effectiveness of traditional and modern management methods.

It is hoped that some of the findings of this project, as well as contributing to practical large scale breeding, may also add to the knowledge needed for management of sandfish populations. In particular it has been shown that:

- Sandfish are capable of rapid growth (1-3 g/day) under good conditions.
- They can reach maturity at less than one year of age.
- They are partial spawners and can be spawned year round under tropical conditions. It is likely that a female will be able to spawn several times in a year in nature, producing 1-2 million eggs or more each time. Female sandfish can therefore probably produce of the order of 10^7 eggs in an undisturbed lifetime.
- Some shrimp, crabs and perhaps rabbitfish can damage and kill sandfish juveniles.
- Adults, and even larvae, can survive salinities as low as 20.

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References

Battaglene, S.C. 1999. Culture of tropical sea cucumbers for the purposes of stock restoration and enhancement, p. 11-25. *In: The Conservation of Sea Cucumbers in Malaysia: Their Taxonomy, Ecology and Trade.* Baine, M. (Ed.). Proceedings of an international conference 25 February 1999, Kuala Lumpur, Malaysia, Heriot-Watt University.

Battaglene, S.C., Seymour, J.E. & Ramofafia, C. 1999. Survival and growth of cultured juvenile sea cucumbers, *Holothuria scabra. Aquaculture,* 178:293-322.

FAO. 1990. *The fishery resources of Pacific island countries. Part 2: Holothurians,* by C. Conand. Food and Agriculture Organization of the United Nations, Rome, Italy. Fisheries Technical Paper No. 272.2. 143 pp.

Chinh, N. 1996. Study on the technological process for artificial reproduction and commercial growout of scallop (*Chlamys nobilis* Reeve 1852) and sea cucumber (*Holothuria scabra* Jaeger 1883 & *Actinopyga echinites* Jaeger 1883). *In: Science & technology studies, 1991-1995.* Program KNO4, Centre for Technical Information and Fisheries Economics, Hanoi. p. 91-105.

Hamel, J.-F. & Mercier, A. 1999. Mucus as a mediator of gametogenic synchrony in the sea cucumber *Cucumaria frondosa* (Holothuroidea: Echinodermata). *Journal of the Marine Biological Association United Kingdom,* 79:121-129.

Hamel, J.-F., Conand, C., Pawson, D.L & Mercier, A. 2001. The sea cucumber *Holothuria scabra* (Holothuroidea: Echinodermata): Its biology and exploitation as beche-de-mer. *Advances in Marine Biology,* 41:129-223.

James, D.B., Gandhi, A.D., Palaniswamy, N. & Rodrigo, J. X. 1994. *Hatchery and culture of the sea-cucumber Holothuria scabra.* Central Marine Fisheries Research Institute Special Publication No. 57.

James, D.B. 1996. Culture of sea-cucumber. *Bulletin Central Marine Fisheries Research Institute,* 48:120-126.

Mercier, A., Battaglene, S.C. & Hamel, J.-F. 2000. Periodic movement, recruitment and size-related distribution of the sea cucumber *Holothuria scabra* in Solomon Islands. *Hydrobiologia,* 440:81-100.

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

Pitt, R., Thu, N.T.X., Minh, M.D. & Phuc, H.N. 2001. Preliminary sandfish growth trials in tanks, ponds and pens in Vietnam. *SPC Beche-de-mer Information Bulletin,* 15:17-27.

Pitt, R. & Duy, N.D.Q. 2003. To produce 100 tons of sandfish. *SPC Beche-de-mer Information Bulletin,* 18:15-17.

Shelley, C.C. 1985. Growth *of Actinopyga echinites* and *Holothuria scabra* (Holothurioidea: Echinodermata) and their fisheries potential (as beche-de-mer) in Papua New Guinea. *Proceedings of the Fifth International Coral Reef Congress,* 5:297-302.

Thu, N.T.X. 2003. *Shrimp culture in combination with sea-cucumbers (Holothuria scabra) to improve the environment in ponds.* Project report. Ministry of Fisheries Research Institute for Aquaculture No. 3, FSPS Programme, SUMA component. 43pp.