Breeding and culture of the sea cucumber Holothuria scabra in Vietnam

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The sea cucumber Holothuria scabra (sandfish) has been commercially exploited over a wide tropical and subtropical range for centuries. It can produce a high-value grade of beche-demer if processing is carried out well. In many cases sandfish have made up the most valuable fraction of the total sea cucumber trade from particular producing countries, both in terms of price per kilogram and of total value. However processing weight losses can be as high as 95%, and wet-weight prices to fishermen are typically in the range \$US1-3/kg. Higher prices are generally paid for larger specimens.

Two subspecies are reported from the South Pacific (Conand 1990), with *H. scabra var. versicolor* generally breeding at and reaching a larger size, living in deeper water and having a wider colour range. Sandfish found in Vietnam (at least in Khanh Hoa Province) appear to be closer to *H. scabra* in terms of their small size and size at first maturity. However the colours range from black through dark brown to light beige, often with transverse stripes. They also appear less deeply ridged than many pictured from Oceania.

Sandfish are found in sandy estuarine or lagoon conditions at depths of 2-25m or more, often in patches of high concentration. They are easily overexploited. Fishermen/divers who collect shellfish and sea cucumbers along the Khanh Hoa coast (free-diving or using hookah diving gear) commonly say they that there are now few sandfish to be found where they were once plentiful. However stocks remain in Cam Ranh Bay (about 60km south of Nha Trang) and Van Ninh (a similar distance north), which are still fished.

The species may have potential for commercial aquaculture and for restocking or stock enhancement. Sandfish have been bred on an experimental scale in India, Indonesia, Solomon Islands, Vietnam and the Maldives. There are also accounts of commercial culture based on cage or pond storage and growth of wildcollected animals. Some people in the Cam Ranh and Van Ninh areas ongrow some collected sandfish in shrimp-style ponds or in pens, to benefit from the higher prices paid for larger animals. However it is not known on what scale or how successful these activities are, or whether culture together with shrimp is anywhere practiced successfully.

In Solomon Islands a release of small numbers of hatchery-produced sandfish juveniles of a few grams weight suggested that they are quickly attacked by a range of coral reef fish, but survive better in a mangrove-seagrass environment (Dance et al. in press). Seagrass beds may be the natural habitat of newly settled sandish juveniles (Mercier et al 2000). Seabed culture and releases of sandfish in Vietnam may benefit from the severe depletion of fish stocks.

Sandfish are broadcast spawners with roughly equal numbers of males and females. In some regions of their wide geographical range, sandfish with ripe gonads can be found in most months of the year. This is the case for example in Solomon Islands, at about 9°S. In other regions there seems to be a single spawning season of only a few months duration. This may be more common in higher latitudes (Morgan 2000) with bigger seasonal temperature ranges. Freshly collected sandfish, or animals that have been cultured for many months at low density in ponds, pens or tanks have all been spawned by

different workers. Temperature shocks, drying, water jetting, high concentrations of dry algae and UV irradiation of water are commonly-used induction techniques, with 1-3 million eggs per spawning per female.

Animals bought in Cam Ranh (at about 12° N) were small, typically averaging not more than 150-200g. In the first year after work started they could only be spawned once, in February (after some months of ongrowing) when they averaged 260g. In the second year, after further ongrowing in ponds or seabed pens, broodstock groups of 200 – 600g individual weight have been spawned once or twice every month, from September to July, so far.

Hatchery rearing of eggs and larvae, at densities up to 1/ml, through hatching, auricularia, doliolaria, pentacularia and early juvenile stages, has generally been quite easy, using indoor fibreglass or concrete tanks of 1.7-6.2m. Only partial water changes were made, about 20-30% per day. Incoming seawater was sand filtered, stored briefly in a reservoir tank then and then passed through 1µ filter bags or cartridges. 5ppm EDTA was routinely added after water changes in hatchery tanks. Larvae were fed algae from open outdoor batch cultures: Chaetocerus muelleri and calcitrans, Nanochloropsis occulata, Platymonas sp., Isochrisis galbena, Rhodamonas salina etc., at gradually increasing concentrations. Feeding rate was generally judged by colour since it was difficult to count or otherwise estimate residual algal cell densities.

When auricularia numbers dropped with the start of metamorphosis preconditioned stacks of plastic plates (pvc, polythene or polypropylene) were added to the tanks. Conditioning systems included painting plates with a slurry of dry algae and allowing them to air-dry, immersing in benthic diatom cultures (Navicula etc) or Platymonas cultures for a few days, or immersing in running unfiltered seawater for a few days, with or without fertilization. All the materials and conditioning systems seemed to work reasonably well. Live algae were supplemented after the start of settlement with dry algal preparations, mainly commercially produced Spirulina plus some Schizochytrium (Algamac 2000). Most competent larvae have metamorphosed and settled within 10-20 days of spawning, depending on temperature and feeding.

Despite careful filtration copepods were able to enter and breed in the hatchery tanks. Heavy infestations destroyed good batches of settled juveniles within a few days, perhaps because repeated collisions caused skin damage. Treatment with the organophosphate insecticide Dipterex (trichlorofon) at 1-2ppm for 1-2 hours (followed by rapid dilution to about half or one-third) was usually effective in killing swimming stages, but not eggs.

After settlement pentacularia were usually left indoors for 2-6 weeks, depending on the availability of outdoor tanks for first nursery. Many had by then become juveniles of 1-2mm in length, although much larger and smaller animals could usually be found because growth rate is very variable. Juveniles were transferred outdoors either by moving the plate stacks on which they had settled, by siphoning the floor onto a seive or by draining and hosing down the tank walls and floor. Stocking rates outdoors for the first nursery stage were generally 500-2000/m².

A wide range of first nursery conditions were assessed, usually in bare tanks from 0.6-16m² surface area, of 0.5-1m. in depth and made from fibreglass, flexible pvc-cloth liner or concrete. Different tank preconditioning methods, additional substrate, shade levels, flow rates, water treatments and feeding systems were all looked at, some in controlled trials.

Results have not yet been fully analyzed but they have broadly agreed with the findings of Battaglene et al (1999). It appeared that water should be at least 60 cm deep. Shading seemed to be helpful, at least in the first weeks of first nursery. Unfiltered seawater gave better results than filtered, and high flow rates better than low. There was no clear advantage in putting in additional surfaces such as sea grass (*Enhalus* sp.), additional plate stacks or roofing tiles, although small juveniles appeared to avoid the brighter and more open parts of tanks.

All tank materials were satisfactory on at least some occasions but very small tanks (about 3001) did not give good results. Most consistent were square fibreglass tanks of about 1.8m² surface area and 60cm depth, perhaps because it was easy provide one or more water changes per day. However they are too small for large-scale production. Good first nursery rearing runs were sometimes achieved in bigger tanks; PVC-lined pools of 10m² area and 70cm depth or concrete tanks of 6 or 16m² and 1m depth. Tanks were generally preconditioned for a few days using flowing unfiltered water. Dry algae were usually fed to tanks at a rate of about 1g/m³ twice a day. This was sometimes supplemented by live phytoplankton when available.

There is still a high degree of hit-ormiss uncertainty about the success of transfer outdoors and first nursery rearing, with some tanks and batches performing well and others poorly. Disappearance of nearly all transferred juveniles within a few days of transfer was not uncommon. Yields from first nursery tanks, after 1-2 months, were typically about 200-300 juveniles/m², of a wide size range, from very small (less than 0.1g) up to 2g or more. On occasion as many as 500 juveniles/m² were obtained, with survival up to 50%.

The juveniles were then usually sorted by size and stocked in tanks with fine sand on the floor, for a further period of nursery. Dry algae feeding was then usually partly replaced by shrimp postlarva starter food, which is cheaper. At this stage culture with shrimp postlarvae (at about 80/m³) often showed improved growth for the sandfish without appearing to harm the shrimp. However, with shrimp of about the same size as or bigger than the sandfish in the same tank there were some mass losses of the sandfish.

Sandfish juveniles of various sizes have been transferred for further further nursery and/or ongrowing, into earth ponds, seabed pens or seabed cages. Again the results have been mixed. In examples of successful tranfers to ponds, juveniles of 1.6g average took $1\frac{1}{2}$ or 2 months (in different ponds) to reach 60g average, 5.5g juveniles took 2¹/₂ months to reach 130g, 28g animals took 11/2 months to average 96g and animals of 30g (at low stocking density) took only 3 months to reach 300g. In all cases growth slowed down or stopped when sandfish density reached about 200-250g/m². Closed seabed cages used for farming babylon snails (Babylonia areolata) are being tried as nursery facilities for sandfish (with or without the snails), and open seabed pens are also being used for nursery and growout trials.

A single attempt at transferring 1-2mm juveniles to a pond failed completely; none were ever found again. Juveniles of a few grams also disappeared from ponds shortly after stocking on some occasions. Currently attempts are made to exclude fish and crabs where juveniles are stocked, at least while the sandfish are small. In some ponds many sandfish 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 was the primary cause of the lesions. Sometimes these animals recovered spontaneously in ponds or after being moved to a tank with clean seawater.

Survival rates to 1-2mm were usually only a few percent, but as eggs were plentiful this was not the main bottleneck to production. Hundreds of thousands of settled 1-2mm juveniles were produced (and tens of thousands have been reared to larger sizes). Hatchery survival might be improved with more reliable algae culture systems. Rhodamonas salina, perhaps the best species for larval culture (Battaglene 1999) was rarely available at the right time and Chaetocerus was often in short supply. Sometimes the 'weed' species Nanochloropsis and/or Platymonas formed the main food source.

Production has been limited mainly by the lack of nursery and ongrowing space. The 35m³ hatchery could supply several times more 1-2mm juveniles than the total of 200m² of nursery tanks available could absorb. The nursery facilities in turn could feed tens of hectares of nursery and grow-out ponds or pens.

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Figure 1 and 2 (above/below). Palm oil fruit bunches are harvested from oil palm trees, Elaeis guineensis. Photo courtesy of MPOB.

microorganism. This process almost doubled the protein content of raw PKM, from about 17% to 32% crude protein. Since T. koningii is a cellulolytic fungus, the reducing sugar content of the fermented PKM was also higher compared to raw PKM. However, when the fermented biomass was incorporated into tilapia diets, a marked reduction in fish growth was observed. We believe that despite the higher protein and digestibility of the fermented PKM, mycotoxins might have been released during the fermentation process. Further studies are being planned to use mycotoxin adsorbers to alleviate these problems in the use of fermented PKM.

Another way to increase the protein content of PKM is to extract the protein using chemical and physical processes. Isolating proteins from PKM will essentially eliminate the problems of low nutrient digestibilities. Despite the high costs of such processes, we are currently conducting some initial studies to see if the protein isolate is of high enough nutritive value for high value marine fish.

Amino acid supplementation

Some studies have reported that amino acid supplementation can improve the growth of fish fed plant-based diets. PKM is low in sulfur amino acids and probably lysine, which are essential amino acids necessary for optimal fish growth. A feeding trial conducted with hybrid catfish showed that up to 20% raw PKM could be incorporated into



catfish diets without any negative effects on growth performance. However, at 40% PKM, growth was significantly depressed and this was not alleviated with the addition of 1.2% dietary L-methionine. One possible reason could be that methionine may not be the first limiting essential amino acid in the PKM-based diets. Further studies involving the use of other essential amino acid and combinations thereof are currently being planned.

Utilization of feed enzymes

The low digestibility of PKM is commonly attributed to the high levels of non-starch polysaccharides (NSP) found in the cell wall materials. These anti-nutritional factors impair the digestibility and utilization of nutrients present in PKM either by direct encapsulation of the nutrients or by increasing the viscosity of the intestinal content thereby reducing the rate of hydrolysis and absorption of nutrients in the diet. The use of proteolytic, fibrolytic or carbohydrate-degrading enzymes to PKM-based diets have great potential in releasing unavailable nutrients and energy.

Studies have shown that tilapia fed PKM pretreated with commercial feed enzymes consistently show better growth and feed utilization efficiency compared to fish fed similar levels of raw

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PKM. Up to 30% enzyme-treated PKM could be incorporated into red tilapia diets without significantly depressing growth (Ng et al., unpublished data). However, direct inclusion of exogenous enzymes in diets for tilapia have so far not been successful. Research is currently being carried out in our laboratory to further optimize the use of feed enzymes in PKM-based diets, varying parameters such as the type, levels and application method (direct, pretreatment, post-extrusion coating).

Conclusion

Initial studies on the use of PKM in tilapia and catfish diets have generated encouraging results with fish growing well on dietary levels as high as 20%. Studies with grass carp were even more encouraging in terms of higher levels of raw PKM being used in their diets (Ng and Teoh, unpublished data). It is anticipated that with further research on enhancing the nutritive value of PKM, this low cost locally available oilseed meal can be used as a viable partial substitute for many of the imported feed ingredients resulting in savings in feed costs for the local fish farmers.

Culture of sandfish

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Without these facilities many animals were kept in tanks at high density (where they grew only slowly), long past the time when they were ready for transfer.

The area of bare tanks for first nursery needs to be about 15-30 times the area of the hatchery. The area for nursery in tanks with sand and ponds has to be 10-20 times bigger again, depending on the size of juveniles required. A basic hatchery and fairly simple culturing methods should be able to produce enough seed for at least pilot-scale commercial aquaculture and/ or restocking trials as long as there is sufficient tank and pond space for the nursery stages.

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