

# Small-scale hatcheries and simple technologies for sandfish (*Holothuria scabra*) production

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## Abstract

Sandfish (*Holothuria scabra*) hatchery production is currently being done at various scales across several continents including Australia, Maldives, Vietnam, Pacific island countries, Madagascar and the Philippines. Work in Mindanao in the southern Philippines, through the University of the Philippines Mindanao (UPMin), commenced in 2006. UPMin set up experimental hatcheries, ponds and other facilities by establishing partnerships with two local corporations: Alsons Corporation and JV Ayala Group of Companies. The former facility also has a seawater channel feeding fish ponds, which, through time, has harboured resident populations of sandfish. This channel became a source of broodstock, as well as a 'conditioning area' for sandfish collected from the wild. It also served as the first-stage nursery for juveniles. This paper describes low-cost technology for all stages of culturing *H. scabra* up to production of juveniles  $\geq 10$  g for release, and compares the cost-cutting innovations with those of published protocols. Three local modifications made by the UPMin project team are described here: the use of a seawater channel for broodstock and hapa; mono-algal feeding using *Chaetoceros calcitrans*; and the use of recycled or locally made materials. Broodstock can be kept for weeks in the channel with zero mortality, even without maintenance. In the hapas, juveniles can grow to 5–10 g in 1–2 months at an average survival of 84%. *Chaetoceros calcitrans* was bought from Alsons and scaled up using recycled 250-L PVC barrels. It was used as a feed until the early juvenile stage. These innovations yielded a best performance average of 2.2% survival to 3–5-mm juveniles. This paper attests to the progress and innovations made in sea cucumber research in the Philippines since *H. scabra* production was pilot-tested in the country in 2002.

## Introduction

Sandfish (*Holothuria scabra*) hatchery production is currently being done at various scales across several continents: Australia, Maldives and Vietnam are doing large-scale for commercial production (Bowman 2012; Duy 2012); the Pacific island countries are trialling small-scale production for community-managed sea ranching (Hair et al. 2011); Madagascar uses in-vitro fertilisation to obtain larvae all year round for their partner communities (Eeckhaut et al. 2008); and in the Philippines, seeds are primarily used in

pilot sites for sea ranching and grow-out (Olavides et al. 2011; Juinio-Meñez et al. 2012).

Sandfish production was pilot-tested in the Philippines as early as 2002 (Gamboa and Menez 2003). Work in Mindanao in the southern Philippines, through the University of the Philippines Mindanao (UPMin), commenced in 2006 when UPMin received funding from the Australian Centre for International Agricultural Research (ACIAR). A year later, financial support also came from the Philippine Government through the Department of Science and Technology – Philippine Council for Aquatic and Marine Resource Development. Neither project grant, however, provided any capital outlay, and UPMin did not have a coastal property. Thus began the quest for a research base and a low-cost means of adopting the technology locally.

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The space and facility problems were overcome by establishing partnerships with two local corporations: Alsons Corporation, a large, intensive, commercial milkfish and tilapia industry with aquaculture facilities in Dumoy (Davao City); and JV Ayala Group of Companies, which owns High Ponds Resort, in Toril, Davao City. Alsons' Dumoy facility accommodates one of our two experimental hatcheries. Within the compound is a seawater channel feeding the milkfish and tilapia ponds. This channel became the source as well as the 'conditioning area' for our broodstock, and served as the first-stage nursery for our juveniles. The High Ponds facility of the JV Ayala Group houses our second hatchery and experimental marine pond.

This paper describes a low-cost technology for producing *H. scabra* comprising the following hatchery phases: broodstock collection; broodstock induction (induced spawning); larval rearing and early settlement; nursery, which is divided into first (in the hapa) and second (sand-conditioning) stages; and harvest of  $\geq 10$ -g juveniles for release. It also describes micro-algal culture.

## The seawater channel

In Dumoy, a 300-m-long man-made canal carries sea water from the main pipe to a dyke that feeds all the ponds. Water is pumped five or six times a week, with almost 90% daily exchange efficiency and depths of 2–3 m. Through time, the channel has accumulated a natural sandy–muddy floor with its corresponding flora and fauna, including a population of *H. scabra*. The channel serves two other purposes for our project—as a natural conditioning area for broodstock collected from the wild and as a hapa-nursery system for juveniles.

## The hatcheries

Two small-scale hatchery facilities were constructed, one each in Dumoy and High Ponds. The Dumoy hatchery was a 30-m<sup>2</sup> area located between two big holding tanks for tilapia fingerlings. It was basically a nipa-roofed structure with coco-wood posts. Sea water, electricity and space were all provided free by Alsons. At High Ponds, the 80-m<sup>2</sup> hatchery was roofed with corrugated PVC sheets and walled with chicken wire. In addition, a 6,000-m<sup>2</sup> freshwater earthen pond was converted to marine water for experimental use. The space for the hatchery, use of the pond and other selected amenities at High Ponds were free, but the project paid for electricity.

## Hatchery protocols

### Broodstock collection and conditioning

For each induction, we would use at least 38 sandfish, sized 130–250 g. The broodstock came from either the resident population in the Dumoy channel or our project sites (Davao del Sur and Davao Oriental), located 1 and 5 hours, respectively, from the hatchery. The animals from the sites were purchased through our local People's Organisation groups and were individually packed in oxygen-filled polyethylene bags containing 1 L of sea water (Agudo 2006). The bags were layered inside a styrofoam chest box for transport.

Wild broodstock were brought to Dumoy, where they were acclimatised for 1 hour by allowing the bags to float in a contained area in the water channel. Each animal was then taken out and dropped gently to the bottom. To avoid mixing with the canal residents, the wild broodstock were released at the seaward end of the channel. This conditioning set-up eliminated feeding and maintenance, yet yielded about 99% survival.

We believe that broodstock conditioning played a role in the success of the larval rearing that followed. For example, the collapse of a batch of larvae on one occasion (January 2010) may have been due to short (i.e. 2 days) broodstock conditioning. The successful batches came either from those conditioned longer or from the resident broodstock population.

### Induced spawning

In earlier induction attempts, broodstock were brought up from the channel onto a floating cage, 2 × 2 × 2 m and ~2 cm mesh size, where they were allowed to defecate for at least 24 hours. It was observed, however, that smaller individuals could squeeze out of the mesh holes, often incurring injury or lesions. An improved practice involves selecting healthy broodstock from the channel and holding them in a bare, flat-bottomed tank containing UV-treated sea water provided with mild aeration.

After about 1 day, the gut-empty broodstock were rinsed with UV-treated sea water. They were divided into two spawning groups (Pitt and Duy 2004) for induction comprising the following steps (Figure 1):

- *Desiccation*. The animals were transferred with care into a dry tank or bin and kept there for 20 minutes.

- *Conditioning in ambient water.* The ‘dry’ animals were moved into a 70-L, 45-cm-high bin filled with 10 cm of filtered sea water, and kept there for 15 minutes.
- *Thermal shock.* The water temperature in the tank was raised 3–5 °C above ambient by slowly adding boiled sea water (James et al. 1994). Induction proceeded for 1 hour.
- *Spirulina bath.* Fifteen grams of *Spirulina* were mixed with 1 L of fresh water then blended well and added slowly to the tank. Induction proceeded for 1 hour.
- *Complete water change.* The *Spirulina* was flushed out by using a hose siphon. Ambient sea water was then slowly added up to about 25 cm depth. After several minutes, one or two individuals would stand and exhibit swaying behaviour. These are signs of readiness to spawn—the gonopore on the dorsal surface of the head begins to swell; males release a long thread of milky sperm, while females release yellowish eggs in two to four bursts that usually shoot out of the water.

Gametes were collected using a beaker. Sperm were scooped out of the water and eggs were collected from each spawning individual by following the direction of the swaying female and positioning the beaker accordingly. Since the released eggs usually shoot out of the water, collecting them required some practice. All sperm were mixed together in a 70-L container, and all eggs in a 20-L container.

To estimate the total egg count, the eggs were pooled in 40 L of sea water. Three subsamples of 1 mL each were taken and counted under a microscope. The average of the three counts was computed. The total number of eggs was roughly estimated using equation (1):

$$\text{Total egg count} = \text{average count/mL} \times 40,000 \text{ mL} \quad (1)$$

About 0.5 mL of sperm from the mixture was introduced to the 40-L egg stock. Too much sperm can lead to polyspermy. Two-cell stage could be observed within the next few hours.



**Figure 1.** Induction of *Holothuria scabra*. Upper row: desiccation (left), thermal shock (centre), *Spirulina* bath (right); lower row: spawning male (left) and female (right)

Regardless of the length of conditioning of the broodstock in the water channel, the thermal-*Spirulina* shock (Agudo 2006) proved effective in 9 of our 10 inductions. A summary of the hatchery performance from those successful inductions is shown in Table 1. The single instance when the thermal-*Spirulina* shock did not work was with broodstock that came from the project site and were induced the next day.

Our spawning induction trials were carried out randomly (during any month) based on the available free tanks in the hatcheries. The success of induction alone did not follow any lunar phase, as was also observed by Pitt and Duy (2004). In all nine trials, the males spawned first, which was in keeping with the reports of other authors (Agudo 2006; Duy 2010; Giraspy and Ivy 2010; Pitt et al. 2001).

## Larval rearing

### Larval density

We used a low density of 0.3 fertilised eggs/mL, calculated as (equation (2)):

$$\text{Number of larvae per tank} = \text{desired volume of water in tank} \times 0.3 \text{ fertilised eggs/mL} \quad (2)$$

The volume of fertilised eggs to be used (equation (3)) was:

$$\text{Volume of fertilised eggs} = \frac{0.3 \text{ eggs/mL} \times \text{total volume of fertilised eggs}}{\text{Average count/mL in the 40 L concentrate}} \quad (3)$$

### Rearing tanks

Our 250-L conical-bottom larval rearing tanks were made of marine plywood and custom built to fit the hatchery area. The larval tanks were prepared for stocking by washing them well with chlorine, rinsing with fresh water and then air-drying. Next, each tank was half-filled with 1- $\mu\text{m}$  filtered and UV-treated sea water. The fertilised eggs were then poured in gently and water was brought to the desired volume. Moderate aeration was applied on the first day of rearing.

Rearing tanks were completely covered during the larval stages using thin white cloth overlain with black cellophane bags. This kept the larvae in darkness and also prevented chironomid (bloodworm) infestation.

A week after the appearance of pentactula, the black cellophane was removed to allow light to penetrate the tank and encourage moderate algal growth for the juveniles. This differs from Agudo's (2006) protocol, where the tank is covered for only the first 2 days of larval rearing. We noted that algal growth was better on tank walls that were not very smooth.

### Water monitoring and larval sampling

Temperature and salinity were monitored daily. Temperatures varied in the range 26–29°C, while salinity was 30–34 ppt. The density of larvae was estimated by counting in a known volume of test tube or glass tubing viewed against the light. Developmental stages of larvae were monitored under the microscope.

### Water treatment and water change

Sea water for the larval tanks went through three filtrations: UV light, 10- $\mu\text{m}$  and 1- $\mu\text{m}$  tube filters, and a 1- $\mu\text{m}$  bag filter. Thirty per cent of the water volume was changed daily. To chelate heavy metal residues, we added ethylenediaminetetraacetic acid (EDTA) at 5 g/m<sup>3</sup> per total volume of water changed. When larvae were no longer present in the column, EDTA treatment was halted and water change was done every other day until all juveniles were ready for the hapas. By 2010 we ceased chelating with EDTA and yet our survival rates were improving. During the June and October 2010 batches, the UV light in the Dumoy hatchery broke. We proceeded with the rearing using filtered sea water only. Since survival of juveniles was among the highest in these batches, it appears that UV light and EDTA can be eliminated.

### Larval food and feeding regime

Feeding the larvae started on day 2. Our protocol used just one species, *Chaetoceros calcitrans*, throughout—the regime is shown in Table 2. *Chaetoceros* spp. are some of the best algae for larval rearing (Battaglione 1999). We initially adopted a once-a-day feeding regime with *C. calcitrans* until Mr Duy (Vietnam) suggested splitting the ration at 9 am and 3 pm. This strategy improved the survival rate of our June and October 2010 batches (Table 1). Feeding was thereafter done twice a day until the juveniles were moved out into the hapas. In his seed production manual, Duy (2010) prefers a mixture of algae for optimal growth, and recommends the single species only when there is not enough algal supply. We found single feeding with *Chaetoceros*

**Table 1.** Summary of combined production performance of the Dumoy and High Ponds hatcheries

Days after moon phase Batch date	Total broodstock source, conditioning	Count of spawners		Minutes after the first spawner <sup>a</sup>		Total fertilised eggs (millions)	EDTA <sup>b</sup>	Initial fertilised eggs in tanks	Count and % survival of 3-mm juveniles	Remarks
		M	F	M	F					
1 day after F.Q. 15 October 2010	38, Dumoy channel residents	12	14	40	55	2.4	No	240,000	5,400 (2.2%)	Hapa by day 38
4 days after F.Q. 23 June 2010	38, Dumoy channel residents	9	10	35	53	2.8	No	360,000	6,400 (1.8%)	Hapa by day 38
2 days after F.Q. 23 February 2010	38, Dumoy channel residents	4	5	20	0	1.28	No	180,000	300 (0.2%)	Collapsed by day 34
5 days after L.Q. 11 February 2010	42, Dumoy channel residents	12	14	27	1	1.98	No	300,000	4,425 (1.5%)	Hapa by day 38
6 days after L.Q. 13 January 2010 (High Ponds only)	40 from wild; conditioned in Dumoy channel for 2 days	10	21	30	11	2.6	No	180,000	0	Collapsed by day 6; short (2-day) conditioning period
2 days after F.M. 4 December 2009	47 from wild; conditioned in Dumoy channel for 4 days	12	20	29	15	1.9	Yes	400,000	539 (0.13%)	Collapsed by day 43; heavily infested with bloodworms
6 days after F.Q. 6 October 2009	46 from wild; conditioned in Dumoy channel for 3 days	14	23	25	51	6.8	Yes	300,000	71 (0.02%)	Collapsed by day 39; heavily infested with bloodworms
6 days after F.M. 13 July 2009	40 from wild; conditioned in Dumoy channel for 6 days	12	13	30	12	2.7	No	300,000	0	Collapsed by day 20; heavily infested with bloodworms
4 days after L.Q. 6 May 2009	43 from wild; conditioned in Dumoy channel for more than 1 week	15	16	36	12	4.3	No	300,000	2,500 (0.83%)	Hapa by day 38

<sup>a</sup> Always a male

<sup>b</sup> EDTA = ethylenediaminetetraacetic acid

F.Q. = first quarter; L.Q. = last quarter; F.M. = full moon

was sufficient, simple and cost-effective. Our best performance was 2.2% survival from fertilised eggs to 3-mm juvenile stage.

### Settlement plates

Settlement plates were made of corrugated polyethylene roof materials that were cut into pieces about 350 × 200 mm. The long sides of each piece were tied together midway with a nylon string to assume a partial fold that could be stacked randomly at the bottom of the tank.

Each piece was washed with detergent and chlorine and air-dried, making sure to keep off insects that might lay eggs on the plates. The rough side was painted with a thin coat of *Spirulina* paste prepared by diluting the powder with just enough water to create a paste-like consistency (Duy 2010). The plates were added once doliolaria were observed, and were stacked randomly in the tanks up to 50% of the water column. The water was changed 3 hours after adding the plates or until all the *Spirulina* bubbles were eliminated. Strong aeration was provided.

### Rearing problems

Infestation of chironomids and copepods, both in the tanks and on settlement plates, was the main cause of low survival or population crashes in our hatcheries. In four of the five crashes, the white cloth tank covers were removed at settlement stage. These tanks showed severe chironomid infestation. We decided to keep the cover on until harvest time at ≥3 mm, and this improved the survival rate. Chironomids at the High Ponds site were more difficult to control, probably due to the shaded location of the hatchery and the presence of more trees in the immediate surroundings. The hatchery in Dumoy, on the other hand, is sandwiched between two concrete structures (Figure 2).

We found that copepods can survive even in UV-treated and filtered seawater systems, as was also reported by Pitt and Duy (2004). They can destroy good batches of settled juveniles within a few days. To address this, proper aseptic procedures were observed, such as chlorination of water pipes and tanks before and after each batch, and covering the

**Table 2.** Daily feeding concentration of *Chaetoceros calcitrans* used for *Holothuria scabra* production in this protocol

Day from fertilisation	Larval stage	<i>Chaetoceros</i> cells/mL	Aeration
2	Early auricularia	20,000	gentle
4	Mid auricularia	20,000–25,000	moderate
6	Mid and late auricularia	25,000–30,000	moderate
8	Late auricularia	30,000–40,000	moderate
10 (till transfer to hapa)	Doliolaria (till 0.5-mm juvenile)	30,000–40,000	strong (once plates are added)



**Figure 2.** The hatchery at High Ponds, Toril (left) is shaded by trees on one side and at the back, while that in Alsons, Dumoy (right), is more exposed to sunlight.

tanks immediately after water change. Our staff were told to be mindful of being carriers of contaminants. For example, they had to rinse their hands before doing tank water changes, especially if they had come directly from monitoring the hapas in the channel. Agudo (2006) recommends thorough rinsing of all rearing materials with freshwater before and after use, and storing them in containers with chlorinated water.

## Nursery

### *First phase: $\geq 3$ -mm juveniles into hapas*

In our experience, juveniles do not grow uniformly in the tanks. This is still a difficulty in our optimisation of a low-cost technology, although significant improvement in the rate of survival to 3 mm was obtained when the feeding regime was split into two, tanks were fully covered to prevent chironomid infestation, and rough inner surface tanks and rough settlement plates were used.

By day 38, a good number of juveniles had reached  $\geq 3$  mm in size. This group was harvested and moved into the hapas. Harvesting involved using a fine, soft watercolor paintbrush to detach the bigger juveniles

from the plates. After harvesting, those plates still with smaller juveniles were dropped back into the tanks without repainting with *Spirulina*. A thin film of algae could regrow on the plates overnight. With this thinning-out process, juveniles were harvested at least three times within a 3–7-day interval.

The hapas, measuring  $1 \times 2 \times 1$  m with a mesh size  $\sim 1$  mm, were made of the same material as that used by local pond operators. Because the water level in the channel changes regularly, floating hapas were designed (Figure 3). The four surface corners of the hapa were tied to bamboo poles fixed to paddle wheel buoys; and the bottom corners and the middle floor were fastened with weights to keep the floor submerged in the water all the time. The hapas were conditioned for 3–5 days to allow a substantial mat of biofilm to grow. Then 400 juveniles ( $\geq 3$  mm length) were transferred into them (Figure 4). The algal mat served as natural food for the growing juveniles and eliminated the manual task of feeding them. In the channel, growth of biofilm is fast and periodic thinning was done by gently scrubbing the outer sides of the hapa. In Vietnam the hapas are tied to bamboo poles that are fixed on the substrate of the pond. The



**Figure 3.** The seawater channel at Alsons, Dumoy (left), and the floating hapas (right)

**Table 3.** Survival of juveniles in the hapas inside the seawater channel at Dumoy

Batch / date	Total count of 3–5-mm juveniles	Total hapas (@ 400 juveniles per hapa)	Mean wet weight (g) after 30 days	Count and % survival after 30 days
15 October 2010	5,400	14	5.45	3,051 (56.5%)
23 June 2010	6,400	16	2.5	2,717 (42%)
11 February 2010	4,000	10	3.5	3,822 (96%)
6 May 2009	2,000	5	2.1	1,453 (73%)





**Figure 4.** Harvesting  $\geq 3$ -mm juveniles from a plate (left); releasing the juveniles onto a hapa (centre); 1-month-old juveniles in the hapa (right). A synaptid (foreground) and seahare egg case (tip of caliper) are seen with the juveniles.

water level in their ponds follows the natural high- and low-tide cycle, but growth of biofilm is not as thick as that in the Dumoy water channel.

A summary of the survival rates of juveniles in the hapas is shown in Table 3. Our best record was 96%. Low survival in June and October 2010 occurred when juveniles were kept in hapas longer than 35 days. As in the tanks, juveniles in the hapas do not grow uniformly, and harvesting was also done two to three times. The first harvest was conducted at 30–35 days, and juveniles  $\geq 2.0$  g were thinned out. One to two more batches of late shooters would catch up at 2–3-week intervals. Longer time in the hapa and frequent handling seemed unfavourable for the juveniles. This staggered harvesting can be a natural pacing for grow-out or sea-ranching releases. It should be well managed in order to avoid a glut at the nursery and ensure continuous release and, consequently, continuous harvest.

Predators in ocean nurseries include crabs and carnivorous fishes (Dance et al. 2003; Lavitra et al. 2009). Surprisingly, they were not a problem in the water channel. Although synaptids and *Dollabella* (Figure 4) were common invaders, we noted no threats to the growing juveniles. The weights in the bottom corners of the hapas have to be checked regularly—without them the floor rises up to the surface and could expose the juveniles to more direct heat from the sun and warmer water temperature.

#### *Second phase: sand-conditioning of $\geq 2.0$ -g juveniles*

Juveniles  $\geq 2.0$  g were conditioned in the substrate before they were released for grow-out or sea

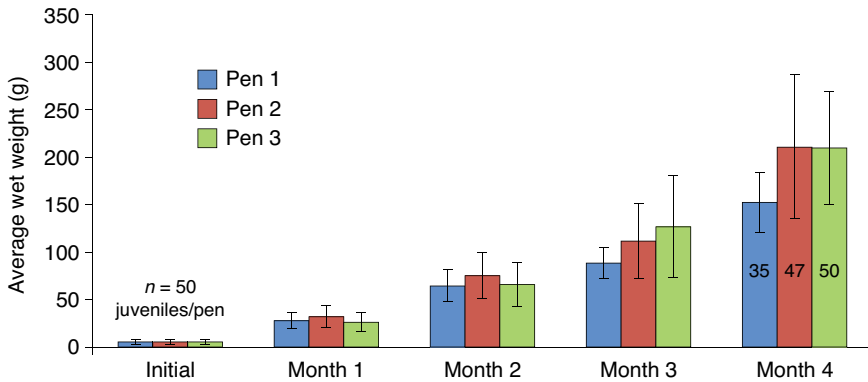
ranching. We conducted several pond experiments using various sized juveniles from the hapas. In one of the trials, three pens ( $3 \times 5 \times 0.4$  m each, made of PVC screen, with a mesh size of 15 mm) were laid out in one portion of the pond. To each pen, 50 juveniles (3–13 g) were introduced. After the first month, the average wet weight was 21.3 g (i.e. growth rate of 0.77 g/day) (Figure 5). After 4 months, the survival rate was 70–100%, with an average wet weight of 191 g. In another experiment involving smaller juveniles, survival after a month was 58–80% and average growth rate was 0.42 g/day. While growth and survival of juveniles varied, the marine pond proved, at the very least, to be a reliable juvenile sand-conditioning area.

In Vietnam *H. scabra* are commercially grown to  $\geq 500$  g in marine ponds. The ponds are converted shrimp ponds and are irrigated by natural rise and fall of the tides. In the Philippines there are also many abandoned shrimp farms, and their potential for sea cucumber grow-out is recommended for further investigation.

#### **Harvest of $\geq 10$ -g juveniles for release**

We recommend  $\geq 10$  g for release size, as the bigger the juvenile, the greater the chances of survival, especially in sea ranches. Juveniles were packed in groups of five in oxygen-filled polyethylene bags containing 1 L of sea water, and transported to the release site in the same way that broodstock are transported. On site, the bags were allowed to float on the water for about 30 minutes to acclimatise the juveniles to the ambient temperature (Figure 6).





**Figure 5.** Growth of juveniles in pens within a marine pond in High Ponds. Vertical bars represent standard error while numbers inside bars in month 4 represent the surviving individuals.



**Figure 6.** Transporting the juveniles to site of release—community partners at work

### ***Chaetoceros* culture**

A significant reduction in production cost in terms of labour and raw materials was achieved by using a single-species feed, *Chaetoceros calcitrans*, for the larvae. This regime was adopted from practices in Vietnam. Every week, 1 L of stock culture was brought in by Alsons from their algal laboratory in another city. Using the formulation of Agudo (2006), the stock was scaled up to 10 L inside an air-conditioned algal room (Figure 7; refer also to appendix). Sea water for culture passed through UV-sterilisation, microfiltration and chlorination–dechlorination. These 10-L stocks in turn became the seed for outdoor upscaling in 250-L recycled PVC drums. Another suspected source of chironomids was the scaled-up

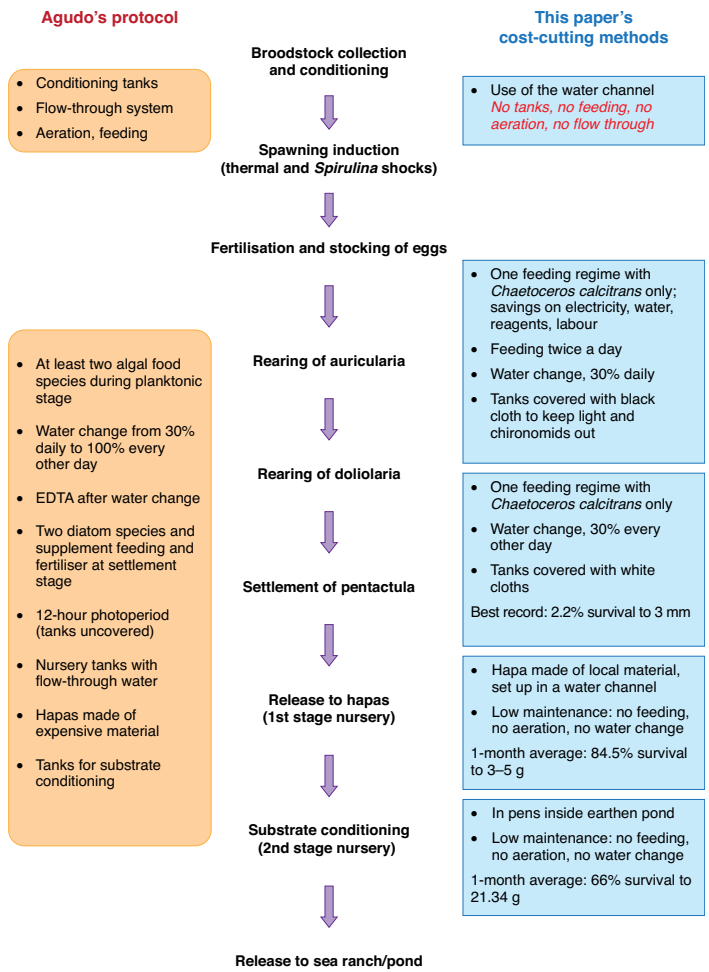
*Chaetoceros* cultures. It was necessary to bring the drums out in the open for exposure to sunlight, but they were tightly covered with thin, white cloth to prevent chironomid infestation.

### **Conclusions**

The cost-cutting innovations in this paper are compared with those of Agudo’s (2006) protocol (Figure 8). Protocols to produce sandfish are already established, and *H. scabra* has been found to grow in various systems (James et al. 1994; Battaglione et al. 1999; Gamboa et al. 2004; Pitt and Duy 2004; Agudo 2006; Duy 2010). Three local modifications made by the Mindanao project team have been described here: mono-algal feeding using *Chaetoceros calcitrans*; the



**Figure 7.** The algal room (left); some *Chaetoceros calcitrans* jugs inside (centre); outdoor upscaling making use of recycled glucose syrup barrels (right)



**Figure 8.** Comparison of cost-cutting innovations in this paper with those of Agudo's (2006) protocol

use of a seawater channel for broodstock conditioning and hapa nursery; and use of recycled or locally made materials. These modifications were made in the context of partnership: the mono-algal feeding was adopted from the system employed by Mr Duy in RIA3, Nha Trang, Vietnam; and the water channel, marine pond and hatchery spaces were provided by two private partners, Alsons Corporation and the JV Ayala Group of companies. This paper attests to the progress and innovations made in sea cucumber research in the Philippines since *H. scabra* production was pilot-tested in Bolinao in 2002 (Gamboa and Juinio-Menez 2003).

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## Appendix. Modified *Chaetoceros* upscaling formulation

The technology in this paper does not maintain an algal culture. Instead, a 4 L stock of *Chaetoceros* is purchased from Alsons every other week and scaled up outdoors in the hatchery using recycled polyethylene barrels. The formulation we modified is described here and is not intended for maintaining pure stock cultures.

### Chlorination–dechlorination

12.5% chlorine strength stock solution. Usage: 0.2 mL (stock) per litre of culture volume (Agudo 2006). Although chlorine content is not indicated in sodium hypochlorite powder, for calculation purposes we estimated the strength to be around 70%, based on references on the internet about sodium hypochlorite available in the Asian market.

250 g thiosulphate in 1-L solution to make stock solution. Usage: 0.2 mL (stock) per litre of culture volume (Agudo 2006).

### *Chaetoceros* upscaling formulation (modified from Agudo 2006)

1. Fertiliser—Manusol (30:10:10): for 10-L jug culture, 0.25 g is needed; for 200-L culture, 5 g is needed
2. Silicate—sodium metasilicate: for 10-L jug culture, 0.375 g is needed; for 200-L culture, 7.5 g is needed.