Hatchery experience and useful lessons from *Isostichopus fuscus* in Ecuador and Mexico

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Abstract

This paper summarises lessons learned from captive breeding of the sea cucumber *Isostichopus fuscus* in land-based installations on the coast of Ecuador and Mexico. This species has been intensively fished in Mexico, along mainland Ecuador and around the Galapagos Islands. Management efforts have traditionally been challenged by local economic and social conditions. Populations of *I. fuscus* have thus been severely depleted over the past decades, generating interest in aquaculture and restocking. Spawning, fertilisation, larval rearing, disease control and juvenile growth have been documented in two privately owned hatcheries. Data from trials conducted in Ecuador over several years indicate that, under optimal conditions, juveniles can be grown to a size of ~8 cm in length in 3.5 months and to commercial size in ~18 months. Preliminary tests have shown that growing juvenile sea cucumbers in shrimp ponds is feasible. In Mexico, successful spawnings were restricted to late summer and autumn/fall months, when cultures of larvae and early juveniles yielded growth rates similar to or greater than those recorded in Ecuador. Grow-out of juveniles in shrimp ponds was impeded in both countries by skin infections, leading to high mortality rates, whereas juveniles placed in cages in the ocean (in Mexico) exhibited reasonable growth rates and better survival (to 90%). Overall, studies demonstrate that, with proper disease control, millions of juvenile *I. fuscus* can be reared in captivity annually, thus providing an alternative to fisheries, or a way to maintain sustainable harvests and eventually contribute to restoration of the natural populations.

Introduction

Isostichopus fuscus (Figure 1) is a deposit-feeding sea cucumber that is mainly found on reefs and sandy bottoms along the western coast of the Americas, from northern Peru to Baja California, Mexico (Castro 1993; Toral-Granda 1996; Sonnenholzner 1997; Gutierrez-Garcia 1999). Like many other commercial sea cucumber species, *I. fuscus* has been widely fished over past decades to meet the growing demand for beche-de-mer in the major Asian markets. As the waters along mainland Ecuador became depleted, the fisheries shifted to the Galapagos Islands in the early 1990s, raising international apprehension over the fate of this unique archipelago, which has been recognised as a national park and marine reserve. Since then, attempts by government at regulating sea cucumber harvests, and banning them in some areas, have met strong opposition from local fishers in Ecuador. In fact, illegal fisheries have always been a concern and still occur along the mainland coast, around the Galapagos Islands and elsewhere in the distribution area of *I. fuscus*. In 1994 the Government of Mexico imposed a total closure because this species was considered locally endangered. However, the closure was not obeyed by

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Figure 1. Adults of *Isostichopus fuscus* photographed (**A**) in situ (Galapagos Islands) and (**B**) in land-based installations (mainland Ecuador) showing the main colour morphs

fishers, leading to a decrease in the biomass, which is now only 2% of the original biomass in some regions (Castro 1995; Aguilar-Ibarra and Martinez-Soberon 2002). Currently, the fishery in Mexico is managed under concessions and stricter activity controls (Toral-Granda 2008).

Official information on the fisheries and actual total catches are difficult to obtain and remain sparse (Salgado-Castro 1993; Castro 1997; Sonnenholzner 1997; Gutierrez-Garcia 1999; Jenkins and Mulliken 1999). Nevertheless, recent data and reports on average capture sizes (Sonnenholzner 1997; Martinez 2001) indicate that *I. fuscus* populations have declined substantially and that natural stocks may irreversibly crash in the near future. Stock recovery has yet to be observed in any region (Toral-Granda 2008).

Despite this situation, a very limited number of studies has been conducted on the reproductive biology, spatial distribution, population structure, growth and survival rate of *I. fuscus* (Herrero-Pérezrul 1994; Fajardo-Leon et al. 1995; Toral-Granda 1996; Sonnenholzner 1997; Herrero-Pérezrul et al. 1999; Hamel et al. 2003; Mercier et al. 2004, 2007; Toral-Granda and Martínez 2007; Becker et al. 2009). Some authors have mentioned that aquaculture and restocking should be investigated as possible solutions to the current crisis (Gutierrez-Garcia 1995, 1999; Fajardo-Leon and Velez-Barajas 1996; Jenkins and Mulliken 1999).

Until recently, aquaculture in Ecuador and Mexico was largely focused on shrimp. The emergence of white spot disease in 1999–2000 has severely affected the industry and resulted in the bankruptcy and closure of numerous farms. Consequently, both countries now have abandoned shrimp farm infrastructures that could very well be put to use for the development of other species, such as sea cucumbers.

This paper summarises efforts made to cultivate *I. fuscus*, including methods of larval development and juvenile growth in land-based nursery systems on the coasts of Ecuador and Mexico. Major findings from Ecuador have been outlined previously (Hamel et al. 2003; Mercier et al. 2004, 2007; Becker et al. 2009), whereas data from Mexico are presented here for the first time.

Results show that aquaculture of this species is feasible and that it could potentially be developed as an alternative to fisheries. In addition, it could be used to maintain sustainable harvests and eventually contribute to the restoration of natural populations. Further research to complement the work presented here is being conducted on the feeding, growth and reproductive biology of this highly prized sea cucumber, which is a dominant feature of the Mexican and Ecuadorian marine ecosystems. In time, hatchery production and restocking of *I. fuscus* might provide part of the solution to the current sea cucumber fishery crisis.

Methods and results

Spawning and fertilisation

Adult sea cucumbers were routinely collected from nearby coastal areas in Ecuador or Mexico to serve as broodstock. The adults were adapted to captive conditions in large tanks or raceways for a few days or weeks prior to spawning. Various methods of spawning induction were initially tested. However, close monitoring and spawning experiments later

revealed that the species follows a predictable lunar spawning periodicity. Patterns of gamete release were investigated on the coast of Ecuador using several hundred newly collected individuals monitored nearly every month for 4 years. Between 1% and 35% of individuals consistently spawned 1–4 days after the new moon (Figure 2) (Mercier et al. 2007). Most spawnings occurred on the same evening, although some gamete release was often recorded over two to four consecutive evenings. On a spawning night, males typically initiated gamete release around sunset, and females spawned just after the peak male broadcast. The percentage of spawning individuals was higher, and a greater overlap between male and female peak

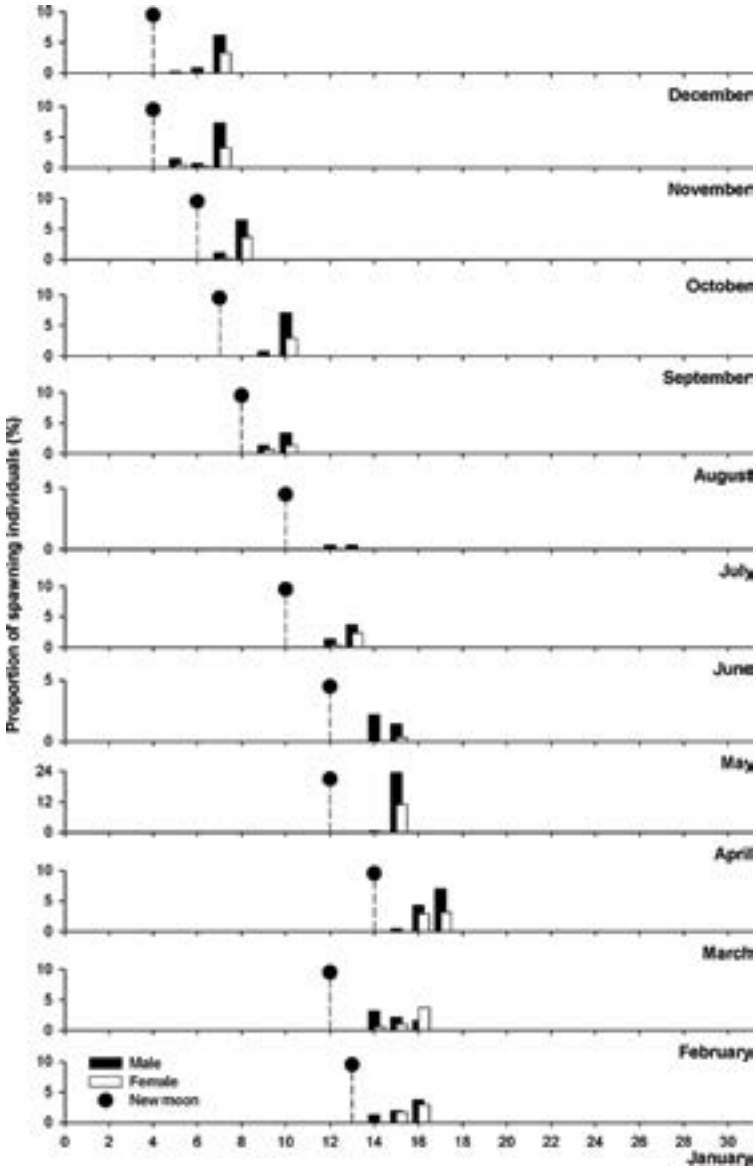

Figure 2. Example of the typical spawning periodicity recorded in captive *Isostichopus fuscus* in Ecuador (from Mercier et al. 2007)

spawning activity was noticed, during clear conditions compared with overcast conditions (Mercier et al. 2007). Preliminary data from Mexico confirmed the same lunar pattern, although individuals mostly started to spawn 2–3 days before the new moon, and continued to do so for 2–4 consecutive days.

In Ecuador, it has thus been possible to obtain male and female gametes on a monthly basis; only a limited number of spawning trials have been unsuccessful, mostly due to poor environmental conditions (e.g. heavy rain). In Mexico, spawning events were recorded solely between June and December of each year, with maximum success in late summer and autumn/fall (August to December).

The broodstock in Ecuador typically consisted of 300–400 adults maintained in large 30-t tanks. Males and females were isolated in buckets as soon as they showed signs of imminent spawning (typical posture with anterior end rising and moving right to left and up and down). Clear morphological distinctions between male and female gonopores at that stage allowed trained personnel to sort them before the actual gamete release. Each female was placed separately in a 300-L spawning tank and maintained there until it had released its oocytes. Once the female had been removed from the tank, dry sperm obtained surgically from three males (sperm extracted from the gonad without adding any sea water until use may be kept at 4 °C for up to 48 hours) was diluted in sea water to allow cell count and prepare the solution required to achieve the desired final concentration of spermatozoa in the tanks. The best fertilisation rates and lowest occurrence of polyspermy were obtained with a concentration of 500–1,000 spermatozoa/mL. Spawning of both males and females occasionally occurred in the broodstock tanks; already fertilised gametes were then transferred to culture vessels. Similar techniques were used in Mexico.

Larval development

After fertilisation, the eggs were rinsed to remove excess sperm. A few hours later, the developing larvae were transferred to the hatchery tanks, where their development was closely monitored (Hamel et al. 2003; Mercier et al. 2004). The routine protocol included daily cleaning of the tanks during the first days, followed by installation of a flow-through system. In Mexico, the flow-through system was used from the very beginning of the culture. The larvae were fed every day using a mix of live microalgae (dominated by *Rhodomonas* and *Dunaliella* in Ecuador, and *Chaetoceros* and *Dunaliella* in Mexico) at a frequency and concentration dictated by the daily observation of digestive tract contents. With improvement of the rearing techniques over the past few years, including the use of running sea water and temperature control (see below), a 30–50% survival rate has regularly been achieved, although the average survivorship remains at 8–30% of juveniles developed from each larval run (Hamel et al. 2003; Mercier et al. 2004).

Isostichopus fuscus possesses planktotrophic larvae that need to feed during their pelagic phase and will undergo a series of transformations to reach the juvenile stage (Figures 3–5; Table 1). In most trials, the development, settlement and growth of the juveniles were asynchronous, and different stages/sizes occurred simultaneously in the cultures. Extreme examples were observed in a few tanks where residual auricularia larvae neighboured 4-mm-long juveniles. Table 1 provides developmental kinetics for both countries based on the bulk of the cultures, discarding extreme asynchronies. Figure 6a shows the different sizes of juveniles that may occur in a typical cohort.

Ovulation occurs in the gonadal tubule as the oocytes are released (Figure 3a). Thus, fully mature oocytes (~120 µm in diameter) are expelled directly in the water column at the metaphase-I of meiosis, after the germinal vesicle breakdown. Embryonic development is initiated with the elevation of the fertilisation envelope, roughly 4 minutes after fertilisation. The expulsion of the first polar body occurs ~3 minutes later (Figure 3b). The second polar body follows rapidly within ~2 minutes. The first cleavage is equal, radial and holoblastic, and divides the cell into two equal hemispheric blastomeres (Figure 3c). The second cleavage again occurs along the animal–vegetal axis, yielding more spherical blastomeres. Embryos hatch from the fertilisation envelope as early gastrulae ~10 hours after fertilisation (Figure 3d). These early gastrulae are ciliated and swim; they elongate into full-size gastrulae after ~14 hours (Figure 3e). Auricularia larvae, which constitute the first feeding stage, begin to appear ~24 hours after fertilisation. Growing auriculariae can be observed during the next 2 weeks of culture (Figure 3f; Table 1). At this stage they begin to accumulate hyaline spheres. The oesophagus, sphincter, intestine, cloaca anus are clearly visible. After 16–18 days the auricularia reaches its maximum size of 1.1–1.3 mm; it has left and right somatocoels, as well as an axohydrocoel (Figure 3g) (Hamel et al. 2003; Mercier et al. 2004).

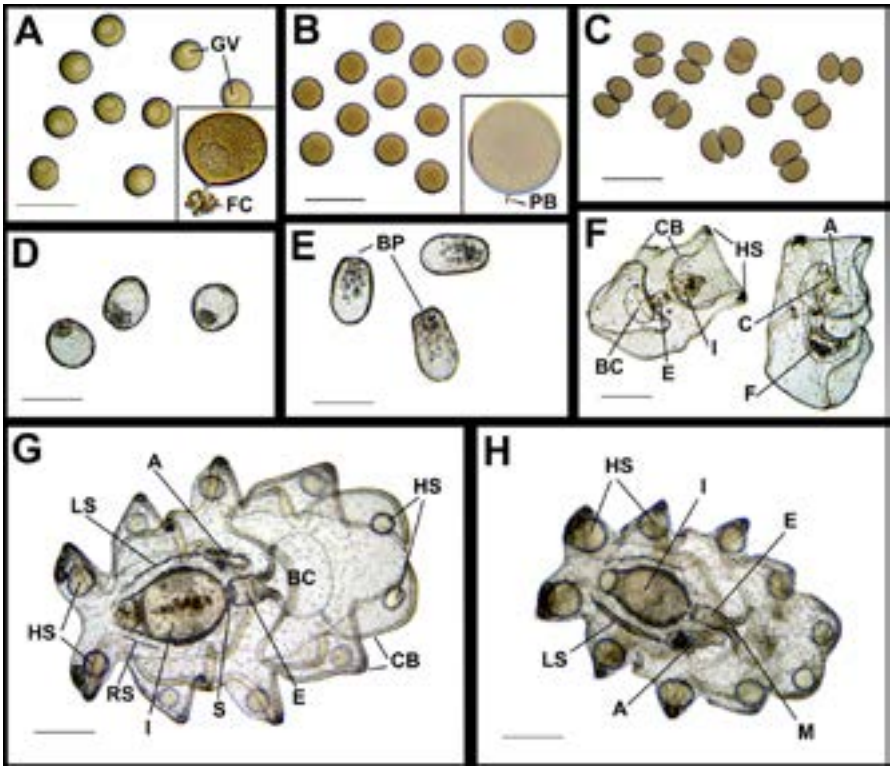

Figure 3. Early development of the sea cucumber *Isostichopus fuscus*; the bars represent 200 µm. **A**: Oocytes collected surgically from a mature gonad; the germinal vesicle (GV) is clearly visible. The insert shows a close-up of an ovulating oocyte with the follicular cells (FC) still attached to it. **B**: Fully mature, newly fertilised eggs with clear germinal vesicle breakdown. The insert shows the expulsion of the two polar bodies (PB). **C**: Two-cell stage. **D**: Newly hatched gastrula. **E**: Elongated gastrula with visible blastopores (BP). **F**: Early auricularia on which the ciliary bands (CB), hyaline spheres (HS), buccal cavity (BC), oesophagus (E), intestine (I), cloaca (C) and anus (A) are identifiable; food items (F) are present in the buccal cavity. **G**: Ventral view of a fully developed auricularia showing the left somatocoel (LS), axohydrocoel (A), hyaline spheres (HS), ciliary bands (CB), buccal cavity (BC), oesophagus (E), sphincter (S), intestine (I) and right somatocoel (RS). **H**: Dorsal view of a metamorphosing auricularia. With a noticeable decrease in size, the buccal cavity disappears and the hyaline spheres (HS) are pulled closer together. The mouth (M), intestine (I), oesophagus (E), left somatocoel (LS) and axohydrocoel (A) are clearly visible.

In the following hours, many auriculariae initiate the transformation that will lead to the doliolaria stage (Figure 3h). During this process, the larvae shrink to nearly 50% of their initial size, the buccal ciliated cavity disappears and the hyaline spheres are pressed closer together (Figure 4a). The doliolaria stage is reached ~19–24 days after fertilisation (Figure 4b; Table 1) as the larvae stop feeding and the cilia are aligned in five distinct crowns along their cylindrical body. At this time, the movement of the primary tentacles can be observed through the translucent body wall. The somatocoel is also visible. A few days later, the doliolaria transforms into an early pentactula possessing five buccal tentacles (Figure 4c). At this stage, the larvae remain close to the substrate, successively going through swimming

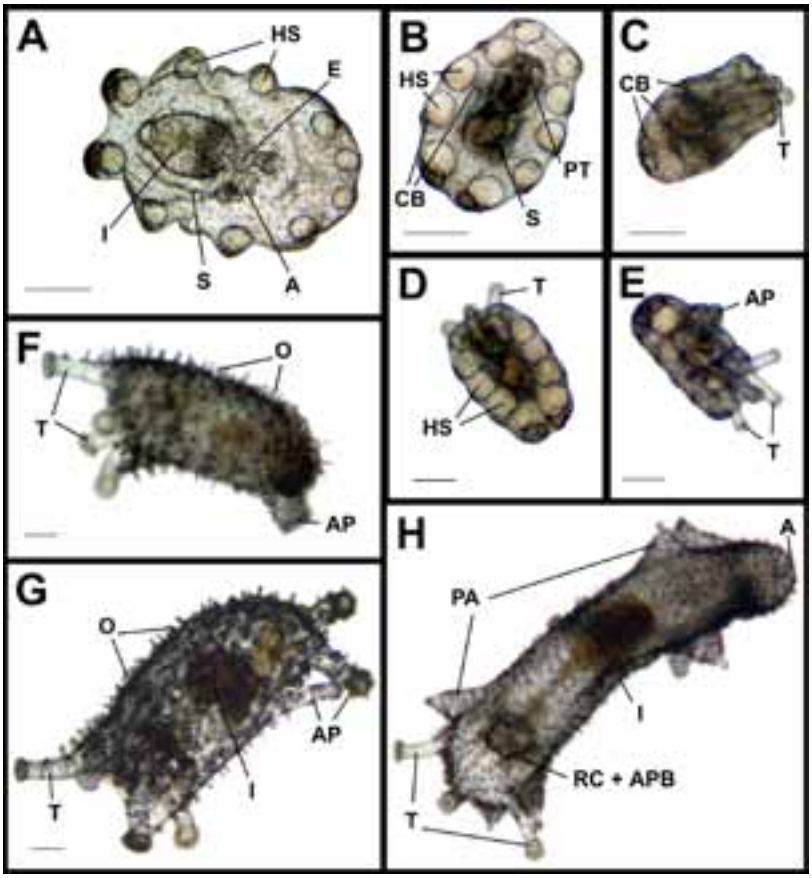

Figure 4. Late development of the sea cucumber *Isostichopus fuscus*; the bars represent 200 µm. **A**: Late metamorphosing auricularia, showing the hyaline spheres (HS), oesophagus (E), intestine (I), somatocoel (S) and axohydrocoel (A). **B**: Fully developed doliolaria with hyaline spheres (HS), primary tentacles (PT), ciliary bands (CB) and somatocoel (S). **C**: Early pentactula with five tentacles (T) and the still-visible ciliary bands (CB). **D**: Dorsal view of newly settled pentactula with tentacles (T) and hyaline spheres (HS). **E**: Ventral view of newly settled pentactula showing the first ambulacral podia (AP) and the five buccal tentacles (T). **F**: Early juvenile, measuring 1.5 mm in length, with tentacles (T), ambulacral podia (AP) and ossicles (O). The hyaline spheres have disappeared. **G**: A 2-mm-long juvenile with five tentacles (T) and three pairs of ambulacral podia (AP). The intestine (I) and ossicles (O) are visible. **H**: A 3-mm-long juvenile showing the tentacles (T), papillae (PA), intestine (I), anus (A) and ring canal and aquapharyngeal bulb (RC + APB)

and settling phases. Definitive settlement, with the complete loss of cilia, completion of metamorphosis and emergence of the two first ambulacral podia, occurs about 22–27 days post-fertilisation in Ecuador and 17–20 days post-fertilisation in Mexico (Table 1; Figure 4d, e). Further details on the development are available (Hamel et al. 2003; Mercier et al. 2004). Hatcheries in Ecuador use corrugated sheets of Plexiglas covered with a rich biofilm to provide settlement substrates and food to settled larvae and

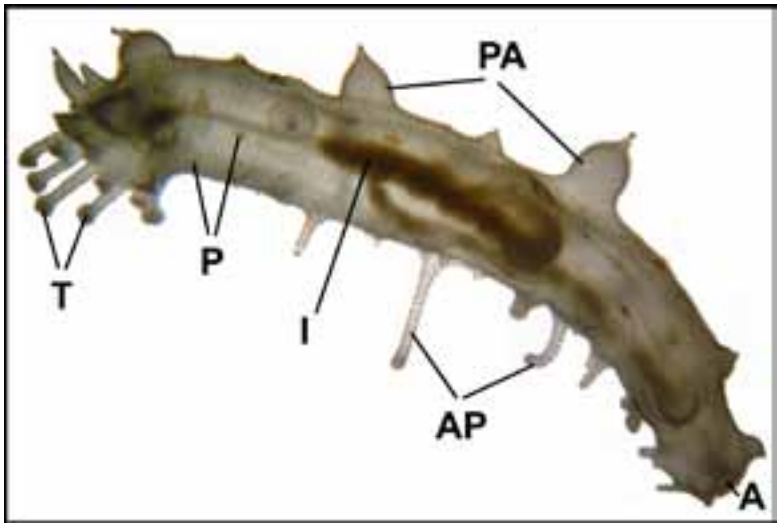

Figure 5. Juvenile sea cucumber *Isostichopus fuscus* measuring 15 mm in length and showing the tentacles (T), early body wall pigments (P), intestine (I), ambulacral podia (AP), anus (A) and papillae (PA)

early juveniles. In Mexico, conditioned, multilayered sheets of locally made fabric mesh are used as settlement substrata and during the early growth phase of juveniles.

Juvenile growth

Although the first settled juveniles can be observed as early as day 17–22, a majority of juveniles measuring 1.0–1.5 mm in length are generally found in the tanks after 21–28 days of culture (Figure 4f; Table 1). They reach ~2–3 mm only a few days later (Figure 4g, h), and 5 mm after ~40–48 days. At this stage, the juveniles start to accumulate some reddish-brown pigments. In 8-mm-long juveniles, the tips of the tentacles become ramified. After 50–65 days of culture, the juveniles are 15–18 mm long and 4 mm wide (Figure 5). They possess several papillae and an elongated intestine that already exhibits strong peristaltic movements. The body wall becomes more opaque as the ossicle density and the tegument thickness increase. When the juveniles reach ~20 mm in length, the whitish colouration that characterises the early stages of life is gradually replaced by a brownish tinge typical of adults (Figure 6a). After 72–85 days of culture, the juveniles are ~35 mm long and 10 mm wide (Hamel et al. 2003; Mercier et al. 2004).

The typical growth of *I. fuscus* larvae and juveniles in Ecuador is shown in Figure 7. The average growth of larvae and juveniles follows the second-order polynomial calculation (equation (1)):

$$f(x) = 1658 - 321(x) + 11(x^2) \quad (1)$$

where $f(x)$ is the size in µm and x is the time in days ($r^2 = 0.99$)

The latest cultures in Ecuador have yielded significantly faster growth rates, with juveniles measuring 11 mm after 28 days, 31 mm after 56 days and 56 mm after 77 days. Growth rates are slightly slower in Mexico (Table 1).

Grow-out experiments

In Ecuador the juveniles are usually transferred to larger 18-m^2 pre-conditioned flow-through tanks, with or without conditioned plates (the same as those used for larval settlement), when they reach 0.5–1.0 mm in length. Mexico makes similar use of settlement plates for juvenile growth in flow-through tanks. After about 72 days, some of the juveniles have reached sizes up to 34 mm (Figure 7; Table 1). The maximum size of *I. fuscus* grown in aquaculture facilities is ~240 mm in length or ~490 g (Figure 6b).

Juvenile *I. fuscus* were also successfully reared in shrimp ponds in Ecuador. A preliminary experiment was conducted early in the study to determine if small sea cucumbers (~100–150 g) collected from the wild would grow in ponds in different locations. Enclosures

Table 1. Development of *Isostichopus fuscus*, from fertilisation to 35-mm-long juvenile

STAGE	TIME Ecuador	TIME Mexico
Fertilisation	0	0
Elevation of the fertilisation envelope	4 minutes	5 minutes
Expulsion of the first polar body	7 minutes	10–15 minutes
Expulsion of the second polar body	9 minutes	16–20 minutes
2-cell	52 minutes	21 minutes
4-cell	70 minutes	30–40 minutes
8-cell	95 minutes	64 minutes
16-cell	124 minutes	71 minutes
32-cell	140 minutes	80–90 minutes
Blastula	3 hours	2.5–3.0 hours
Early gastrula	6 hours	6 hours
Hatching	10 hours	9 hours
Late gastrula (elongation)	14 hours	11 hours
Early auricularia	1–2 days	20–25 hours
Auricularia	3–15 days	3–10 days
Late auricularia (early metamorphosis)	16–18 days	11–17 days
Doliolaria	19–24 days	13–18 days
Early pentactula	21–26 days	14–19 days
Settlement (metamorphosis completed)	22–27 days	17–20 days
Juvenile, 1 mm	28 days*	21 days
Juvenile, 2 mm	30 days	30 days
Juvenile, 3 mm	32 days	40 days
Juvenile, 4 mm	38 days	45 days
Juvenile, 5 mm	40 days	48 days
Juvenile, 8 mm	44 days	55 days
Juvenile, 10 mm	47 days	61 days
Juvenile, 15 mm	51 days	65 days
Juvenile, 20 mm	56 days	70 days
Juvenile, 25 mm	63 days	74 days
Juvenile, 30 mm	69 days	78 days
Juvenile, 35 mm	72 days	85–90 days

* For the juvenile stages, the time indicated corresponds to the first noteworthy observations of a particular size in the tanks.

of 1 m^2 were used to facilitate recapture. These sea cucumbers grew an average of 17 g/week and exhibited a 98% survival rate, suggesting that shrimp ponds along the coast can provide a good environment to grow *I. fuscus* juveniles to adult size in a reasonable time frame. However, juveniles grown in tanks and shrimp ponds may both develop skin diseases that can cause massive mortality, especially during months with warmer temperatures and heavy rains (see below).

In Mexico in 2009, an experimental shrimp pond was used for the grow-out of hatchery-reared juveniles (starting with 2-month-old seeds of 3–5 mm and 4–7 mg). During the monitoring phase they grew from an average of 1.07 to 42.07 g in 3 months, but survival was low due to outbreaks of skin disease. Using mesh cages at sea (1.8 × 1.8 × 1.8 m) stocked with 3,000 seeds resulted in a more conservative growth rate (from 2.66 to 26.92 g in 3 months) but greater survival (40–90%). The presence of sponges and other fouling organisms on the mesh (500–1,000 µm), and clogging from accumulated sediments, might have prevented the entry of fresh deposits serving as food to the juveniles. The presence of crabs in the cages was also noted, which might have caused stress (slowing growth) and possibly mortalities.

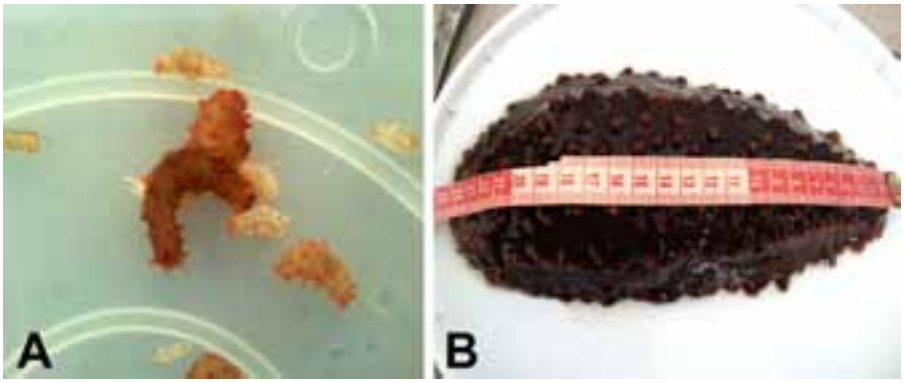

Figure 6. **A**: Juveniles of different sizes, ranging 3–25 mm in length, obtained in the same cohort. **B**: Maximum size of *I. fuscus* obtained through aquaculture (~24 cm long)

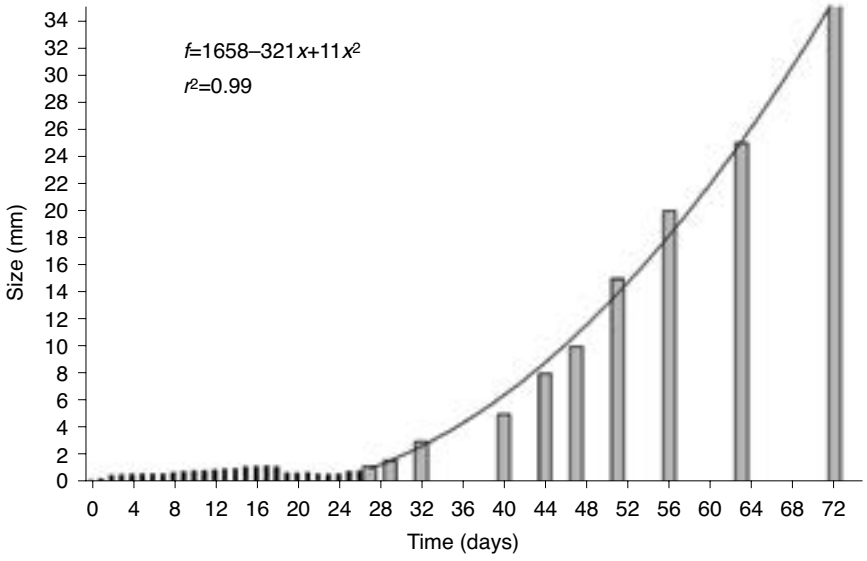

Figure 7. Average growth of larvae (black bars) and juveniles (grey bars) of the sea cucumber *Isostichopus fuscus* in Ecuador

Diseases and other problems

Parasites of the digestive tract in larvae

The most common problem observed during the culture of *I. fuscus* was the development of a disease in the digestive system of early larvae (Figure 8) (Becker et al. 2009). Following the appearance of opaque cells around the digestive tract, the second visible symptom was contraction of the intestine and stomach. In the worst cases, the digestive tract completely shrivelled up and disappeared. Once it became visible, the condition was usually fatal to the larvae.

Upon close examination of the affected larvae under the microscope, the disease was determined to be caused by protozoan parasites (Figure 8a, b). During the first stage of the disease, the parasites can be seen entering through the body wall and the digestive tract, probably inducing the observed contraction. Later in the development of the disease, the

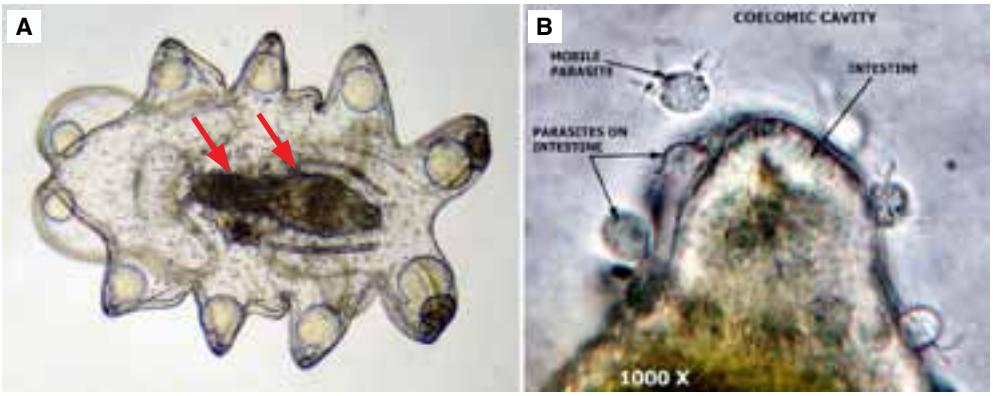

Figure 8. Micrographs of diseased *Isostichopus fuscus*. **A**: Auricularia larva (length 1.2 mm) with digestive tract invaded by parasites (arrows). **B**: Close-up view of the intestine with parasites (parasite diameter 12 µm)

parasites become larger and are present everywhere around the intestine, both inside and outside. The parasites that penetrate the intestine appear to feed on the intestinal contents or tissues, slowly making it shrink, sometimes rupturing the intestinal wall and typically causing the death of the larva within 1–3 days (Becker et al. 2009).

The parasites have never been observed in the larvae before hatching. However, the condition develops rapidly shortly thereafter, suggesting that the causal agents are present in the surrounding environment, and that they enter the larvae at the first opportunity. They seem to remain inactive until the larvae start to feed. Afterwards, they can be seen to develop in different areas of the mouth and, most commonly, the digestive tract (stomach and intestine). A form with thin appendixes can be found attached all over the larvae, but the amoeboid form is mostly observed around the digestive organs; it has the ability to move in and out of what appears to be a trophosoite form (Becker et al. 2009).

We have tried different methods of collecting the gametes to establish whether the parasites were coming from the sea water itself or from the spawning adults. It has proven impossible to develop a culture without the presence of the parasites at one stage or another, even when using artificial sea water from the onset. It would seem that the parasites are either present around the gametes and/or develop spontaneously in the culture (possibly from aerosols).

Close monitoring of the early larval stages allows detection of the first occurrence of the parasites, and enables control of the disease through adjustments of environmental parameters. If the disease is not contained in its earliest phase, the whole culture usually crashes. This problem is especially prevalent during the hottest and rainiest months of the cycle in both countries. Decreasing the temperature to ~24–26 °C and increasing aeration in the cultures mitigates proliferation of this parasite; however, even lower temperatures may slow or interrupt the development of the larvae.

Disease of the body wall (skin) in juveniles and larger individuals

In Ecuador and Mexico, grow-out trials in shrimp ponds or large tanks have so far yielded mixed results, with significant mortality due to a disease affecting the body wall (Figure 9). This condition may cause degeneration, evisceration and eventually death. Some promising treatments were devised in Mexico with daily usage of antibiotics for several weeks, but cured animals could develop the disease again later on. The best way to prevent and cure this condition in Mexico is currently to grow *I. fuscus* directly in the ocean or transfer any affected individual to the field as soon as the skin disease is detected.

Problems related to quality of food and water

Due to variable and often poor environmental conditions along the coast where the water was being pumped, a very complete filtration system, including UV treatment, had to be installed to provide the best possible water quality throughout the trials. The conventional treatment used for prawn culture was not dependable enough to grow sea cucumber larvae with optimum success, especially *I. fuscus*, which requires

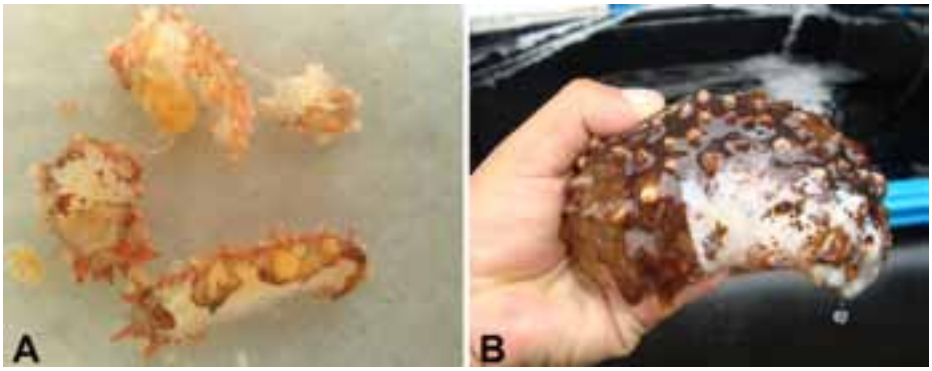

Figure 9. **A**: Juveniles and **B**: adult individuals of *Isostichopus fuscus* affected by body wall (skin) disease; individuals are ~2–3 cm long in A and ~20 cm in B.

high-quality oceanic water. Strict sanitary measures were adopted in the handling of gametes and larvae to maximise survival rates and minimise incidence of infections and diseases. Bacterial counts were routinely made from water samples to monitor the efficiency of the sanitary and filtration procedures.

Bacterial contamination of algal cultures was another common problem that had to be overcome. Growing larvae need large quantities of healthy live algae to develop steadily, especially during the auricularia stage. Inability to provide a healthy mix of algae can significantly delay growth and metamorphosis for extended periods. Thus, it has proven crucial to develop a system of algae production that is reliable and efficient.

As the size of the cultures grew from a few tens of thousands to over 2–3 million larvae per month, rearing conditions had to be maintained and eventually improved to avoid mass mortalities.

Outlook

After 10 years of research and development:

- A good portion of the effort has been placed on adapting shrimp farm equipment and larval rearing conditions to fit the needs of *I. fuscus*.
- The species has been found to follow a predictable lunar spawning cycle, which facilitates the collection of mature gametes (oocytes and spermatozoa).
- A larval rearing protocol has been developed using flow-through systems, an optimal micro-algae diet, water quality management and disease control.
- In successful trials, survival rates from fertilised egg to settlement varied from 2–13% in Mexico to ≥30% in Ecuador.
- Based on the best growth rates, juveniles can reach 8 cm (~25–27 g) in 110 days in shrimp ponds (Ecuador) and 90 days in cages (Mexico), with survival rates of up to 90%.
- While grow-out of sea cucumbers in tanks and shrimp ponds appears to be promising under optimal conditions, cage culture (sea farming) might be a more reliable and simple option because it is free of disease.

Future goals

Future research aims to:

- improve the diets and conditioning of adults to spawn when maintained in tanks to avoid having to continuously collect broodstock from the wild
- finetune hatchery and larval rearing protocols to maximise (scale-up) commercial mass-production
- optimise the control of larval and juvenile parasitic infestations and infections
- experiment with grow-out techniques to determine the best diet, substrates and location to grow the sea cucumbers to commercial size
- determine the commercial and ecological prospects for hatchery-produced *I. fuscus* (e.g. marketing, restocking)
- explore the possibility of culture away from shrimp habitat / installations.

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References

Aguilar-Ibarra A. and Martinez-Soberon G. 2002. Economic reasons, ecological actions and social consequences in the Mexican sea cucumber fishery. SPC Beche-de-mer Information Bulletin 17, 23–26.

Becker P., Eeckhaut I., Ycaza R.H., Mercier A. and Hamel J.-F. 2009. Protozoan disease in larval culture of the edible sea cucumber *Isostichopus fuscus*. Pp. 571–573 in 'Echinoderms', ed. by L.G. Harris, S.A. Bottger, C.W. Walker and M.P. Lesser. CRC Press, London.

Castro L. 1993. The fisheries of the sea cucumbers *Isostichopus fuscus* and *Parastichopus parvimensis* in Baja California, Mexico. Proceedings of the 8th International Echinoderm Conference, Dijon, France, p. 504.

Castro L. 1995. Management options of the commercial dive fisheries for the sea cucumbers in Baja California, Mexico. SPC Beche-de-mer Information Bulletin 7, 20.

Castro L. 1997. Review of recent developments in the Baja California, Mexico, *Isostichopus fuscus*, *Holothuria impatiens* and *Parastichopus parvimensis* fisheries. SPC Beche-de-mer Information Bulletin 9, 26–27.

Fajardo-Leon M. and Velez-Barajas A. 1996. Pesqueneria de pepino de mar. In 'Estudia del potencial pesquero y acuicola de Baja California Sur', ed. by M. Casas-Valdez and G. Ponce Diaz, Vol. 2, 151–165. Mexican Ministry of Fisheries and the Environment (SEMARNAP) and Centre of Research and Advanced Studies (CICIMAR), La Paz.

Fajardo-Leon M.,Velez-Barajas A., Marso-Rojas J.A., Singh-Cabanillas J. and Michel-Guerrero E. 1995. Population structure and reproductive cycle of the sea cucumber *Isostichopus fuscus* (Echinodermata: Holothuroidea) in Santa Rosalia, Southern Baja California, from September 1992 to September 1993. National Fisheries Institute, Ministry of Fisheries.

Gutierrez-Garcia A. 1995. Feasability of an on-growing system for culturing the sea cucumber *Isostichopus fuscus* in the sea of Cortez, Mexico. Institute of Aquaculture: University of Stirling.

Gutierrez-Garcia A. 1999. Potential culture of sea cucumber in Mexico. SPC Beche-de-mer Information Bulletin 11, 26–29.

Hamel J.-F., Ycaza R. and Mercier A. 2003. Larval development and juvenile growth of the Galapagos sea cucumber *Isostichopus fuscus*. SPC Beche-de-mer Information Bulletin 18, 3–8.

Herrero-Perezrul M.D. 1994. Comparative study of reproduction of *Isostichopus fuscus* Ludwig, 1875 and *Neothyone gibbosa* Deichman, 1941 (Echinodermata: Holothuroidea) at La Paz Bay. MSc thesis, Centre of Research and Advanced Studies (CICIMAR), National Polytechnic Institute, Mexico.

Herrero-Pérezrul M.D., Reyes Bonilla H., Garcia-Dominguez F. and Cintra-Buenrostro C.E. 1999. Reproduction and growth of *Isostichopus fuscus* (Echinodermata: Holothuroidea) in the southern Gulf of California, Mexico. Marine Biology 135, 521–532.

Jenkins M. and Mulliken T.A. 1999. Evolution of exploitation in the Galapagos Islands: Ecuador's sea cucumber trade. Traffic Bulletin 17.

Martinez P.C. 2001. The Galapagos sea cucumber fishery: risk or an opportunity for conservation? SPC Beche-de-mer Information Bulletin 14, 22–23.

Mercier A., Ycaza R.H. and Hamel J.-F. 2004. Aquaculture of the Galapagos sea cucumber, *Isostichopus fuscus*. In 'Advances in sea cucumber aquaculture and management', ed. by A. Lovatelli, C. Conand, S. Purcell, S. Uthicke, J.-F. Hamel and A. Mercier. FAO Fisheries Technical Paper No. 463, 347–358. Food and Agriculture Organization of the United Nations: Rome.

Mercier A., Ycaza R.H. and Hamel J.-F. 2007. Long-term study of gamete release in a broadcast-spawning holothurian: predictable lunar and diel periodicities. Marine Ecology Progress Series 329, 179–189.

Salgado-Castro L.R. 1993. Sea cucumber fisheries of the Pacific coast (*Parastichopus parvimensis*) and *P. californicus* and *Isostichopus fuscus*, from the Gulf of California. National Fisheries Institute and Ministry of Fisheries.

Sonnenholzner J. 1997. A brief survey of commercial sea cucumber *Isostichopus fuscus* (Ludwig, 1875) of the Galapagos Islands, Ecuador. SPC Beche-de-mer Information Bulletin 9, 12–15.

Toral-Granda M.V. and Martínez P.C. 2007. Reproductive biology and population structure of the sea cucumber *Isostichopus fuscus* (Ludwig, 1875) (Holothuroidea) in Caamaño, Galápagos Islands, Ecuador. Marine Biology 151, 2091–2098.

Toral-Granda V. 1996. Biologia reproductiva del pepino de mar *Isostichopus fuscus* en la Isla Caamaño, Santa Cruz, Galápagos. Honours thesis, Universidad del Azuay, Cuenca, Ecuador.

Toral-Granda V. 2008. Population status, fisheries and trade of sea cucumbers in Latin America and the Caribbean. In 'Sea cucumbers: a global review of fisheries and trade', ed. by V. Toral-Granda, A. Lovatelli and M. Vasconcellos. FAO Fisheries and Aquaculture Technical Paper No. 516, 211–229. Food and Agriculture Organization of the United Nations: Rome.