Culture of Tropical Sea Cucumbers for Stock Restoration and Enhancement[1]

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Abstract

Severe overfishing of sea cucumbers has occurred in most countries of the tropical Indo-Pacific. The release of cultured juveniles is being examined at the ICLARM Coastal Aquaculture Centre (CAC) in the Solomon Islands as a means of restoring and enhancing tropical sea cucumber stocks. Sandfish (*Holothuria scabra*) are the tropical species that show the best potential for stock enhancement. Sandfish are of high value, widely distributed and relatively easy to culture in simple systems at a low cost. This paper summarizes information about the culture of *H. scabra* and compares it to that of the temperate species *Stichopus japonicus*. Sandfish live in high nutrient environments at densities of 100s per ha. They have a reproductive peak in September and October, but can be induced to spawn throughout the year. Increases in water temperature and addition of powdered algae are effective ways of inducing spawning. *Chaetoceros muelleri* and *Rhodomonas salina* are two of the better microalgae for feeding the larvae. Sandfish larvae are more robust and easier to rear than those of other tropical species. Larvae metamorphose into juveniles after two weeks at 28°C and settle on diatom conditioned plates. The CAC has produced over 200 000 juveniles from six separate spawnings. Sandfish can be reared on hard substrata until they reach 20 mm in length and are then best transferred to sand substrata. Absolute daily growth rates for juvenile sandfish average 0.5 mm day^{-1} (±0.03 s.e.) and range from 0.2 to 0.8 mm day^{-1}, depending on stocking density, light intensity and addition of powdered algae. Overall, there are good reasons to believe that sandfish can be produced cost-effectively for restocking and stock enhancement. The potential for using cultured juveniles to manage fisheries for sea cucumbers now depends on the development of strategies to optimize the survival of juveniles released into the wild and to evaluate releases on a commercial scale.

Introduction

Tropical sea cucumbers processed into *bêche-de-mer* are a valuable source of income for many coastal communities in the developing nations of the Indo-Pacific. However, increasing demand from China and inadequate management of sea cucumber stocks in many countries have resulted in severe overfishing. It is now apparent that depleted stocks can take decades to recover and that historical "boom" and "bust" cycles will continue unless new ways can be found to manage and restore stocks (Conand and Byrne 1993; Preston 1993; Dalzell et al. 1996; Conand 1997). The release of juvenile sea cucumbers produced in hatcheries is seen as a way of rebuilding wild stocks, a process termed restoration, restocking or reseeding. There is also the potential to increase harvests beyond historical levels through stock enhancement by releasing sufficient cultured juveniles into the wild to reach the carrying capacity of the habitat (Munro and Bell 1997; Battaglene and Bell 1999).

Stock enhancement of temperate sea cucumbers is already practiced successfully in Japan using *Stichopus japonicus*, Selenka (Ito 1995). In China, over 1 000 t of dried *S. japonicus* is produced in marine aquaculture (YSFRI 1991; Ferdouse 1999). To date, there has been no restocking or stock enhancement of tropical sea cucumbers. However, tropical sea cucumbers appear to have the necessary biological attributes for these practices as they feed low on the food chain, are restricted to inshore habitats, and are relatively sedentary and easy to harvest. Research into the feasibility of restocking and stock enhancement of tropical sea cucumbers has been suggested, or is being undertaken, in Ecuador, Philippines, India, Kiribati, Maldives, Marshall Islands and the Solomon Islands (Battaglene and Bell 1999).

Cost-effective production of sea cucumber juveniles is an obvious requirement for successful restoration and stock enhancement programs. This paper provides a brief

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history of the breeding and cultivation of holothurians and the research being done on tropical species at the ICLARM Coastal Aquaculture Centre (CAC) in the Solomon Islands. It focuses on sandfish *Holothuria scabra* Jaeger, 1833, a high-value, widely distributed species and currently the only tropical species that can be mass-produced in hatcheries. The paper also provides information on *S. japonicus*. The emphasis throughout the paper is on the use of cost-effective techniques for the mass-production of juveniles with a view to improving the production, management and conservation of aquatic resources for the well-being of present and future generations of low-income people in developing countries.

History of Sea Cucumber Breeding

The breeding and cultivation of holothuroids dates back about 50 years. By far the most comprehensive information available on breeding sea cucumbers is that for *S. japonicus* (YSFRI 1991; Ito and Kitamura 1997; Yanagisawa 1998). Research on *S. japonicus* in Japan began in the 1930s with the first recorded production of juveniles in 1950. However, commercial production has only occurred in the last ten years with over 2.5 million juveniles being released in 1994. The objective of the Japanese release programs has been to increase the proportion of valuable red and blue/green varieties over the less valuable black variety. China started research on *S. japonicus* in the 1970s and by 1985 had developed less sophisticated but reliable techniques for producing juveniles. According to Ferdouse (1999), mainland China produced 2 375 t of dried sea cucumber of which 1 023 t came from marine aquaculture.

Hatchery culture of *H. scabra* dates back to 1988, when small quantities of juveniles were first produced experimentally in India using the techniques developed in China for *S. japonicus* (James et al. 1988). The emphasis of early research was on the production of *H. scabra* juveniles for farming, although the possibility of stock enhancement was suggested (James et al. 1988, 1994; James 1996). The farming of wild-caught *H. scabra* juveniles in meshed enclosures has been practiced in India and Indonesia for at least two decades (Tiensongrusmee and Pontjoprawiro 1988; Daud et al. 1993; Muliani 1993; James 1996). Despite the initial breeding successes, and for reasons that remain unclear, commercial hatcheries did not develop in India. However, the techniques developed in India have now been applied experimentally in Australia, Indonesia, Maldives and the Solomon Islands (Martoyo et al. 1994; Morgan 1996; Battaglene and Seymour 1998).

Attempts to breed other tropical species have generally met with poor results (Appendix 1).

Broodstock

Broodstock can be collected from the wild and induced to spawn immediately or held in land-based tanks and conditioned in captivity. Sandfish live in high nutrient environments at densities of 100s per ha. Broodstock is generally collected by divers and can be a hazardous occupation in tropical waters inhabited by crocodiles. Tropical sea cucumbers can be difficult to hold in captivity and reduced feeding, weight loss and poor gonad development has been reported frequently. It has been more common for wild-caught animals to be used as broodstock. At least 15 and as many as 200 animals are collected for spawning induction. The number of animals contributing to each hatchery run is an important genetic consideration, particularly if cultured juveniles are to be released in the wild. For this reason, repeated use of the same broodstock should be avoided (Yanagisawa 1998).

Broodstock can be sexed by taking a biopsy needle and aspirating a small piece of gonad. A 1:1 sex ratio of males to females is desirable (Yanagisawa 1998). Maturity in *S. japonica* can be measured from the diameter of the largest oocytes. Unfortunately, maturity in *H. scabra* cannot be gauged from the diameter of the largest oocytes but gonosomatic indices of 5-8% indicate reproductive peaks (Ramofafia, pers. comm.). Rapid changes in water temperature, salinity and pressure should be avoided during transport of broodstock because they can cause evisceration and premature spawning. At the CAC, up to 20 broodstock are transported in individual plastic bags within a single insulated box for periods of up to ten hours by a small airplane.

In Japan, captive broodstock is fed dried brown algae and held at a water temperature 5°C below ambient to synchronize development (Ito 1995; Yanagisawa 1998). *H. scabra* have been conditioned in captivity using a variety of products including soybean powder, rice bran, chicken manure, ground algae and prawn head waste in both India and Indonesia (James 1996; Rosliwati, pers. comm. 1996). In the Solomon Islands, *H. scabra* were held in captivity in 4 000-l fiberglass tanks for over six months.

Reproduction and Spawning Induction

The reproduction of *H. scabra* has been studied in Australia, India, New Caledonia, Philippines, Papua New Guinea and the Solomon Islands (Krishnan 1968; Harriot 1980; Ong Che and Gomez 1985; Conand 1993; Ramofafia, unpubl.). In general, *H. scabra* have a biannual peak in the gonosomatic index, indicating that there are two main spawning periods each year. However, closer to the equator, a proportion of the population also appears capable of spawning all

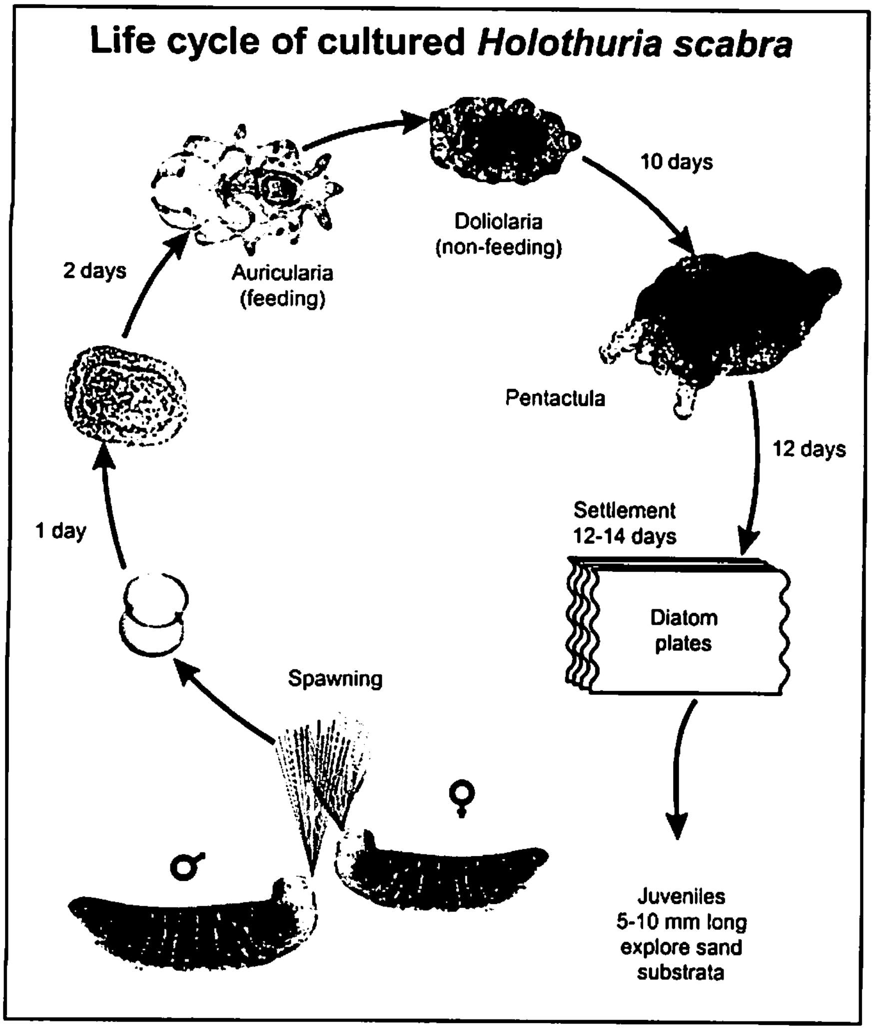

Fig. 1. The 14-day larval cycle of cultured sandfish (**Holothuria scabra*****) at a water temperature of 28°C.***

year round (Ong Che and Gomez 1985; Ramofafia, unpubl.). In the Solomon Islands, the peak reproductive period is in October towards the end of the dry season. Salinity and temperature appear to provide the proximal cues that synchronize and regulate the seasonal reproductive cycle. Contrary to our findings in tanks, other studies have found no evidence for lunar influences (Ong Che and Gomez 1985; Conand 1993). *H. scabra* are dioecious, highly fecund, broadcast spawners.

Thermal stimulation is the most commonly used spawning technique for sea cucumbers, although mature animals will often spawn spontaneously in response to collection and transport stress. Air-drying and the use of water jets stimulate spawning in *S. japonicus* (YSFRI 1991). In our experience, it is not particularly effective for *H. scabra*. Temperatures are typically raised by 3-5°C for one hour to induce spawning using thermal stimulation. Ambient temperatures in the Solomon Islands are around 28±2°C and about 10-50% of mature *H. scabra* will spawn following thermal stimulation, depending on seasonal maturity and lunar periodicity. It is easiest to spawn *H. scabra* in September but the production of ripe eggs is possible all year round. Males are easier to spawn than females (M:F::2.3:1, n=106).

At present, chemicals or hormones to induce spawning of sea cucumbers are not available (Marayuma 1980, 1986; Yanagisawa 1998). Stripping eggs from mature females is generally ineffective but sperm dissected from mature male gonads is viable. Storage of sperm at 5°C can extend its viability. Adding blended gonads dissected from mature broodstock may be another effective spawning stimulant. A Japanese scientist working in Kiribati has recently discovered that dried alga is also a good spawning induction agent for *H. fuscogilva*. The results of this research are currently being prepared for publication (Sato, pers. comm. 1998). On the basis of this finding we have induced spawning in *H. scabra, H. fuscogilva, Actinopyga mauritiana* and *A. miliaris* using a commercially available powdered alga, *Schizochytrium* sp., Algamac-2000 (Bio-Marine, Hawthorne, California). It works well and Algamac often induces spawning when thermal stimulation fails. Broodstock are held in 1 000 l of static seawater to which 100 g of blended Algamac is added for one hour. Animals are transferred to clean ambient seawater before spawning. A combination of thermal stimulation and addition of dried algae is recommended. Live phytoplankton do not appear to be good spawning stimulants despite the fact that phytoplankton blooms may stimulate spawning in some temperate species (Hamel and Mercier 1996b).

Rolling and agitated movements often precede spawning in *H. scabra*. Spontaneous spawning of captive broodstock in tanks occurs between 15:00 and 18:00 h, with male sea cucumbers spawning before and for longer than females. Males typically lift half their bodies off the substratum and sway, releasing sperm continuously for up to three hours. Females spawn in a series of short powerful bursts. At the CAC, spawning males are removed when the sperm density reaches 10 000-100 000 ml^{-1} because high sperm densities can cause increased deformities in holothurian larvae (YSFRI 1991; Ito

1995; Hamel and Mercier 1996a). In China, instead of removing males to control sperm density, female *S. japonicus* are placed in egg-boxes (YSFRI 1991). *H. scabra* eggs are kept in suspension by moderate aeration and siphoned from the spawning tank, rinsed in clean seawater for 15 min to remove excess sperm, and incubated for 24 h in 250 l tanks with conical bottoms at 28±2°C. Some species, notably *Actinopyga* spp., have heavier eggs that require stronger aeration during incubation. Most holothuroids are highly fecund. For example, thermally stimulated *H. scabra* spawned on average 1.9 million fertilized eggs per female.

Spawning has been achieved in *H. scabra, H. fuscogilva, H. atra, A. mauritiana,* and *A. miliaris,* and there have been few difficulties in the collection and incubation of fertilized eggs. Some species, e.g., *H. fuscogilva,* have short spawning seasons making them more difficult to study.

Larval Rearing

The larval cycle and the development of commercially important tropical holothuroids is shown in Fig. 1. Egg development is rapid (24 h), and the larval cycle is relatively short at 14 days. In many ways, the larval development of *H. scabra* is very similar to that of *S. japonicus* (Battaglene and Bell 1999). The auricularia is the larval feeding stage. Provided adequate nutrition is given, the larvae become non-feeding doliolaria for a short transition stage before settling as pentactulae. *H. scabra* larvae have been reared at the CAC in 250-750 l fiberglass tanks at densities of 0.1 and 3.9 larvae ml^{-1}. In general, there is a negative relationship between stocking density and survival to settlement. Optimal stocking densities in static incubation tanks appear to be relatively low at around 0.5 larvae/ml^{-1}. Survival to settlement ranges from <1 to 35%. Mortality is greatest at first feeding and settlement (Fig. 2). The rearing water is exchanged by sieving out the larvae every second day until the larvae reaches the doliolaria stage when a 200% daily exchange of flow-through seawater using an internal screen to retain larvae is initiated. Water temperatures fluctuate from 26 to 29°C during rearing trials. Preliminary experiments suggest light intensity should be kept at around 400 lux on the water surface.

At the CAC, feeding of *H. scabra* larvae commences on the second day after hatching and up to four species of micro-algae are used, starting at 20 000 cells ml^{-1} and gradually increasing to 40 000 cells ml^{-1} after 14 days. The different species of algae are fed on an equal dry cell weight basis with *Chaetoceros muelleri (gracilis)* as the standard. Experiments have been conducted in replicated 500 ml glass flasks to determine the best algae to feed *H. scabra* and *H. fuscogilva* larvae. Larvae survive and grow best on diets of *Rhodomonas salina* (a red alga with a large cell size, 8-12 mm) and *Chaetoceros muelleri* (diatom, 5-8 mm excluding spines), and to a lesser extent *Chaetoceros calcitrans* (diatom, 3-6 mm). Larvae fail to metamorphose if fed on *Isochrysis galbana* or *Tetraselmis chuii.* The proportion of competent larvae reared on *R. salina* is higher at algal cell densities of 10 000 ml^{-1}, than at 3 000 and 7 000 ml^{-1}. Feeding equal parts *R. salina* and *C. muelleri* is more effective than feeding *R. salina* alone. If facilities for growing algae are limited then the best all-round choice appears to be *Chaetoceros muelleri.* Not only is it one of the better algae for sea cucumbers but it is also tolerant of high temperatures and relatively easy to grow. In the future, it may be possible to avoid live feeds altogether as commercial diets and filtration systems improve (Yanagisawa 1998).

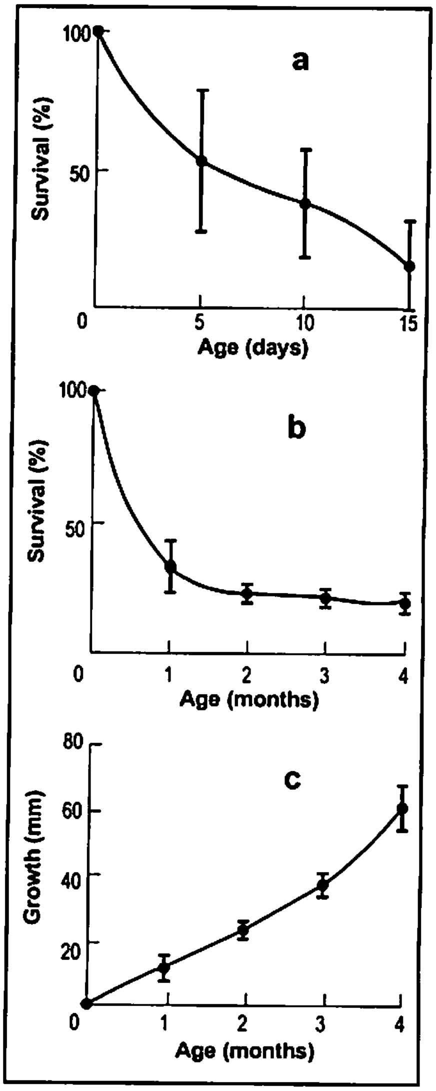

Fig. 2. Generalized survival and growth data for cultured* Holothuria scabra*: a - larval survival to settlement; b - juvenile survival after settlement; c - juvenile growth after settlement. (Data from 26 trials. Bars are s.e.)

There is considerable scope for improving the survival of *H. scabra* larvae and, as techniques are refined, survival to settlement will hopefully reach the 80-90% currently achieved with *S. japonicus* (Ito and Kitamura 1997; Yanagisawa 1998). The progress with other species has not been as good. On two occasions, *H. fuscogilva* larvae were reared to the late auricularia stage before the majority of larvae died. Similar mortalities have occurred with *Actinopyga* spp. at settlement.

The lack of lipid sphere reserves in the late auricularia suggests that poor nutrition might have been a contributing factor to mortalities. Auricularia appear to require the accumulation of lipid reserves in order to metamorphose and settle (Dautov and Kashenko 1995). The distinct hyaline or lipid spheres first visible in late stage *H. scabra* auricularia appear to be good indicators of larval competency. Alternatively, the recent successful production of juvenile *H. fuscogilva* in Kiribati was achieved using *C. muelleri* and antibiotics (Sato, pers. comm., 1998). This suggests *H. fuscogilva* may be more susceptible to bacteria than *H. scabra*.

Metamorphosis and settlement are critical stages in the development and culture of sea cucumber larvae (Smiley et al. 1991; Yanagisawa 1998). High survival is dependent on the larvae being competent to metamorphose and then responding to settlement cues. Settlement cues include certain bacteria and diatoms, although the exact chemicals involved are unknown (Yanagisawa 1998). Settlement preferences of *H. scabra* larvae are the subject of an ecological study currently being conducted in the Solomon Islands that suggests that they settle on seagrass leaves in the wild (Mercier et al. b, in review). Cultured *H. scabra* pentactulae settle on tank surfaces, mesh screens and fiberglass plates, conditioned in seawater for 4 to 10 days. Conditioning provides a bacterial film and fine coating of epiphytes including diatoms that stimulate metamorphosis and settlement. Plates are conditioned in outdoor tanks under direct sunlight. When adding plates to larval rearing tanks care is needed to avoid contaminating the tanks with copepods and protozoa which also graze on the biological film. James et al. (1994) recommend coating plates in an algal extract of *Sargassum* sp. In the Solomon Islands, Algamac is added from day 10 at 0.5 g 500 l^{-1} as a potential settlement cue and source of food for newly settled pentactulae.

The settlement of *S. japonicus* juveniles is undertaken on vinyl chloride, polycarbonate jagged plates coated with benthic diatoms, using similar technology to that developed for sea urchins and abalone (Ito 1995; Yanagisawa 1998). The species, density and size of diatoms are important factors in obtaining good settlement and there is a linear relationship between settlement and diatom concentration. Some 50% of *S. japonicus* larvae metamorphose and settle on surfaces with 200 000 *Navicula* sp. cells cm^{-2}. Production of suitable periphitic diatom-coated plates takes up to 5 months and involves media enrichment, control of light intensity, regular washing and elimination of copepods using pesticides (Ito 1995; Ito and Kitamura 1997). The use of polycarbonate plates can be expensive and time consuming on a large-scale and requires specialized washing equipment. Almost any conditioned surface will attract settlement provided it is not toxic to larvae. For example, in China, silk cloth, polyethylene sheets, tiles and stones have been used to settle *S. japonicus* (YSFRI 1991).

Detachment and Grading

Newly settled juveniles attach firmly to settlement surfaces and can be difficult to detach. There are at least three reasons why it is necessary to detach sea cucumbers (YSFRI 1991; Ito 1995; Hatanaka 1996; James 1996). First, juveniles need to be detached from settlement surfaces when moved from indoor hatchery tanks to outdoor land-based nursery tanks. Second, survival and growth of juvenile sea cucumbers is improved when they are transferred regularly to new tanks. Third, there is a large degree of variation in the growth of cultured sea cucumbers and detachment facilitates grading and counting. Harvest and grading can be undertaken using physical methods such as siphons, water jets and fine brushes (Ito 1995; James 1996). However, these methods are time consuming, labor intensive and potentially harmful to the delicate podia of juveniles. Alternatively, detachment and grading of juvenile *S. japonicus* and *H. scabra* can be done using potassium chloride (0.5-1% w/w KCl) in association with water jets and selective mesh screens (Ito 1995; Yanagisawa 1996; Battaglene and Seymour 1998). The use of KCl does not harm juvenile sea cucumbers but does effectively kill some tropical copepods.

Juvenile Growout

There are currently two main methods used for commercial rearing of juvenile holothurians: plate-culture and, for want of a better term, bottom-culture. Plate-culture was developed in Japan for *S. japonicus* and is similar to that used in the culture of abalone and sea urchins (Uki 1989; YSFRI 1991; Ito 1995; Hagen 1996; Yanagisawa 1998). Juvenile *S. japonicus* are reared in land-based tanks on sets of plates held inside fine-mesh bags. New plates are added as the natural benthic diatoms are depleted. The larval tanks are usually rectangular raceways, constructed originally in concrete but increasingly in fiberglass, and are often >10 m^3 in size. Most hatcheries supplement the diet of juveniles with artificial feeds and juveniles can be weaned off benthic diatoms and fed powdered brown algae (Livic, Riken Vitamin Co. Ltd., Tokyo) (Yanagisawa 1998). Bottom-culture was developed in China for *S. japonicus* and later adapted for *H. scabra*. It involves a short plate or polyethylene collector phase, followed by extensive culture in concrete tanks (YSFRI 1991; James et al. 1994). Newly settled *H. scabra* juveniles feed on the bacterial and diatom film that naturally occurs in tanks conditioned with running seawater. In India, this natural film is supplemented with an extract of *Sargassum* sp. When the juveniles reach 10-20 mm in length, they are transferred to fine sand substrata and fed powdered alga *Ulva lactuca* (James et al. 1994; James 1996).

Over the past two years, CAC has modified the bottom-culture technique and developed simple, cost-effective methods for mass-rearing of *H. scabra* juveniles (Battaglene et al., in press). There is a critical period in the culture of newly settled juveniles (<5 mm) during which high mortality occurs. After one month, survival averages 34.4% (Fig. 2) if juveniles are reared on plates but is lower and more variable if they are detached and reared on hard substrata. On plates, juveniles grow to a mean size of 13 mm in length (range 3 to 41 mm) after one month (Fig. 2). Experiments indicate that it is better to delay transfer to sand until the juveniles reach a size of 20 mm and 1 g. Survival of *H. scabra* >20 mm was high: mortality was <4% over 2 months and restricted to tanks stocked at high density or with reduced light. Experiments in which light was reduced by shading indicate that diatoms and epiphytic algae are important sources of food for cultured juveniles up to 50 mm in length. However, addition of powdered algae does not improve growth or survival except at high densities. Growth of juveniles >20 mm is highly variable and density dependent: coefficients of variation average 25.8% (±2.2 s.e). Absolute daily growth rates for juveniles ranged from a mean of 0.2-0.8 mm day^{-1}, with an overall average of 0.5 mm day^{-1} (±0.03 s.e.) over two months. This is equivalent to a weight range of 0.1-0.4 g day^{-1} and an average of 0.2 g day^{-1} (±0.02 s.e.). Growth in *H. scabra* slowed when densities reached approximately 225 g m^{-2}. This is equivalent to six 40 g individuals m^{-2}. Juveniles that were stunted as a result of being held at high densities grew at the same rate as other juveniles once the stocking density was reduced.

It is now evident that newly settled *H. scabra* juveniles can be reared in tanks using simple technology, little or no added feeds and at a low cost. As a result, there should be no major impediment to the production of juveniles for stock enhancement programs, provided juveniles can be released successfully into the wild at sizes of <60 mm and 20 g. The three months it takes to produce juvenile *H. scabra* of this size and the ease of culturing them, compares favorably with other tropical marine invertebrate species under active consideration for stock enhancement (Battaglene et al., in press).

The large number of juveniles that are required for stock enhancement and the possibility that they may need to be 60 mm in length at release, has prompted an investigation into the possibility of growing sandfish in shrimp farming ponds. Tank experiments show that *Penaeus monodon* can cohabit with sandfish juveniles, although there are some indications that *H. scabra* may be detrimental to prawns at high densities. Two 30 000 l concrete shrimp nursery ponds were stocked with small *H. scabra* juveniles. After 20 weeks, survival in the ponds averaged 93% and juveniles grew at 0.7 g day^{-1} at low density and 0.3 g day^{-1} at high density. Experimental releases of juveniles into larger shrimp ponds will be done in 1999.

Alternatively, if larger cultured juveniles are required for restocking and stock enhancement it may be possible to grow them using the farming methods already developed in India and Indonesia. Farming of *H. scabra* is commonly undertaken in rectangular meshed cages. These pens are typically anchored to the mud by wooden poles and the bottom of the cage covered in sediment to 50 mm. According to James et al. (1994), cages need to be cleaned regularly and replenished with new mud in order to obtain good survival and growth. Farmed juveniles are fed various waste products, including chicken manure and the bottom sludge from prawn farming ponds.

Predators, Parasites and Disease

There is little published data on parasites and diseases of cultured holothuroids. James et al. (1994) suggested that copepods and ciliates are the main predators of auricularia. Copepods also compete with juveniles for food and can be removed with regular chemical treatments, e.g., Trichlorophos 0.5-1 ppm for 8 h used in Japan and Dipterx 2 ppm for 2 h in India (James et al. 1994; Ito 1995; Yanagisawa 1996; Ito and Kitamura 1997). In the Solomon Islands, direct predation by copepods has not been observed but they have caused mortality by rapidly multiplying and depleting food resources in tanks receiving unfiltered water, especially in those containing newly settled juveniles. Routine filtering of seawater with 5 mm nominal filters and regular transfer of juveniles to clean tanks can prevent copepod infestations. Regular transfer also allows counting and grading and avoids the use of pesticides.

Restoration and Stock Enhancement

Now that there are good reasons to believe that juvenile *H. scabra* can be mass-produced cost-effectively, it is important to determine whether released juveniles can survive and grow to a harvest size in a reasonable time frame. For this it is necessary to know more about the life history of juvenile sandfish. ICLARM is collaborating with two Canadian scientists to study this (Mercier et al. a and b, in review). The objectives are to identify the nursery habitats of juvenile sandfish, including any changes in habitat with size and variation in recruitment among and within years, as well as other factors regulating the abundance of juveniles, e.g., predation. On the completion of hatchery research in 1999, ICLARM's research program will focus on the development of optimal release strategies for juveniles with field experiments involving the release of cultured juveniles. Once optimal release strategies have been determined, ICLARM will undertake commercial-scale releases to establish the economic feasibility of restoring and enhancing sea cucumber stocks.

In evaluating the potential for restocking tropical sea cucumbers, three additional pieces of research will be undertaken. First, there will be an assessment of environmental impacts. Second, ICLARM will assist other research organizations to identify the genetic structure of sea cucumber stocks and ensure adequate genetic diversity of released juveniles. To date, very little is known about genetic variation within species of tropical sea cucumbers. Indeed, for some species, the taxonomy is still being resolved. Third, ICLARM will try to develop appropriate methods for marking cultured juveniles so that they can be distinguished from wild stock at harvest. Without this technology, it will be difficult to assess the effectiveness of large-scale restocking experiments (Munro and Bell 1997; Battaglene and Bell 1999).

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Appendix 1. A partial list of holothurian species that have been the subject of culture attempts. Species in bold type have been successfully cultured through to settlement.

Species	Temperate/Tropical	Country	Reference
Phaeodactylum tricornutum	unknown	Russia	YSFRI (1991)
***Platymonas* spp.**	unknown	Russia	YSFRI (1991)
Stichopus japonicus	temperate	Japan	Yanagisawa (1998)
Stichopus japonicus	temperate	China	YSFRI (1991)
Stichopus horrens	tropical	Hawaii	SPC # 7 page 25
Cucumaria frondosa	temperate	Canada	Hamel and Mercier (1996a)
Cucumaria elongata	temperate		Chia and Buchanan (1969)
Psolus chitonoides			McEuen and Chia (1991)
Psolidium bullatum			McEuen and Chia (1991)
Parastichopus californicus	temperate	Canada	Cameron and Fankboner (1989)
Eupentacta fraudatrix		Russia	Dolmatov and Yushin (1993)
Holothuria scabra	tropical	India	James et al. (1988)
Holothuria scabra	tropical	Indonesia	Martoyo et al. (1994)
Holothuria scabra	tropical	Australia	Morgan (1996)
Holothuria scabra	tropical	Solomon Is.	This paper
Holothuria scabra	tropical	Maldives	Oceanworld
Holothuria atra	tropical	Solomon Is.	Ramofafia et al. (1995)
Holothuria fuscogilva	tropical	Kiribati	Sato, pers. comm.
Holothuria fuscogilva	tropical	Solomon Is.	This paper
Holothuria nobilis	tropical	Guam	Preston (1990)
Actinopyga echinites	tropical	Taiwan	Chen and Chian (1990)
Actinopyga mauritiana	tropical	Guam	Preston (1990)
Actinopyga mauritiana	tropical	Solomon Is.	This paper
Actinopyga milieris	tropical	Solomon Is.	This paper

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