

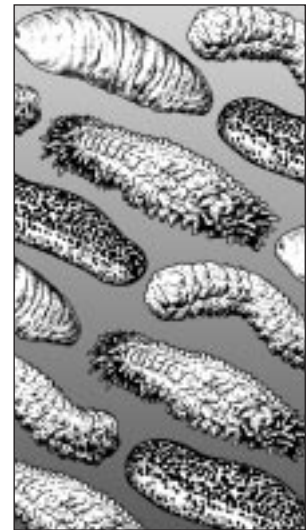


Secretariat of the Pacific Community

# BECHE-DE-MER

Number 14— February 2001

I N F O R M A T I O N B U L L E T I N



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## EDITORIAL

This is the 14th issue of the Bulletin. I would like to take this opportunity to thank all those who have already contributed to it and ask that you take an active role in its improvement, as many of you have already indicated that the Bulletin is useful to you.

Is the current presentation by section, i.e. 1) New Information, 2) Correspondence, 3) Publications, satisfactory? Are there any other new columns that you would like to see included? Your suggestions and comments are necessary for the Bulletin's development.

In the 'New Information' section, the columns on 'In situ spawning observations' and 'Asexual reproduction through fission observations' have received a favourable response with two new articles (p. 26 and p. 23).

Many of the articles in this issue deal with sea cucumber aquaculture, in particular, a few summaries and an article on a new hatchery in Madagascar (p. 2). This issue also includes other articles on the Galapagos (p. 22) and the Singapore market (p. 12).

Previous issues of the Bulletin are now available on the SPC's website at [www.spc.int/coastfish/News/bdm/bdm.htm](http://www.spc.int/coastfish/News/bdm/bdm.htm). The "Echinoderms Newsletter" is also available on the Web at [www.nmnh.si.edu/iz/echinoderm](http://www.nmnh.si.edu/iz/echinoderm).

An echinoderms forum was created after the International Conference in Dunedin. You can subscribe by contacting [sabine.strohr@nrm.se](mailto:sabine.strohr@nrm.se) or by sending a e-mail to [listserv@nrm.se](mailto:listserv@nrm.se) and including on the first line of the message SUBSCRIBE ECHINODERM-L, your surname and first name, but no other text.

Chantal Conand

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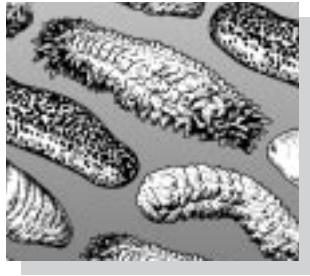
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# new info

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## A sea cucumber hatchery and mariculture project in Tulear, Madagascar

*Michel Jangoux<sup>1,2,3</sup>, Richard Rasolofonirina<sup>1,3</sup>, Devarajen Vaitilingon<sup>1,3</sup>, Jean-Marc Ouin<sup>1,3</sup>, Guy Seghers<sup>1,3</sup>, Edouard Mara<sup>3,4</sup> and Chantal Conand<sup>5</sup>*

This project arose from recent and very alarming reports of widespread overexploitation that may, in the short term, lead to the disappearance of echinoderm resources (particularly sea cucumbers) along the entire west coast of Madagascar (Conand 1998; Conand et al 1997).

Collecting sea cucumbers at low tide or by free diving is a traditional activity in Madagascar (Rasolofonirina and Conand 1998). Trepang production has been ongoing in Madagascar since the beginning of the century and was destined exclusively for export to Indochina.

Beginning in 1990, the market underwent rapid expansion and in 1994 catches reached a peak of some 650 mt of trepang exported to Singapore and Hong Kong. At a price of USD 4000 per tonne ("official" rate), this corresponded to overall earnings of more than USD 2.6 million. In more concrete terms, this means that every 10 kg of good quality sea cucumbers harvested provide between one and two euros directly to fishermen [1 euro ≈ 0.94 USD, 03/01/01].

During the period when the resource was abundant, a fisherman could collect several hundred kilos in a single day, thereby earning between 25 and 50 euros, in a country where the current monthly salary of a labourer is about 40 euros! Since 1995, while demand on the international market has remained high, and has even increased, there has been a significant drop in the quality of Malagasy trepang. This is due to the increasing scarceness of

those species with a high commercial value, the growing harvest of lower commercial value species, and a decrease in the sizes of the specimens collected (Conand 1999). Trepang exports went from 650 mt in 1994 to 320 mt in 1996, which shows a significant decrease in available resources. Since 1996, harvest zones have clearly expanded through the illegal use of diving tanks to collect specimens (Maillaud 1999). This technique not only accelerates the resource's disappearance but is also responsible for a large number of fatal or severely debilitating accidents due to the lack of preparation and training on the part of those involved.

The situation is such that if no action is taken, the region is headed for disaster in both human and ecological terms. On the human level, the increasing scarcity of a high-value export product can only lead to increased poverty and instability in the village communities on the west coast of Madagascar, which have gradually concentrated their activities around the exploitation of trepang. On the ecological level, sea cucumbers are a vital element for sustaining coastal ecosystems in tropical areas as they are macro-detritivores that consume various organic detritus (e.g. excrement, cadavers, moultings).

In the long term, the project in Tulear should ensure that instruction in sea cucumber resource control and production is provided to specialised staff members. Also, practical training, first by project participants and later by the newly trained staff members, in on-site methods in rearing species of

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commercial interest should be given to those village communities that depend on sea cucumbers.

Meeting this training objective will come about through acquiring skills to routinely produce – through use of simple, reproducible and sufficiently rapid methods – marketable-sized high value sea cucumbers from juveniles produced in hatcheries. Initially, this involves building a hatchery and learning to master the pre-juvenile events of the life cycle of exploited species (i.e. fertilisation, embryonic development, larval growth, metamorphosis and immediately post-metamorphic organogenesis). The next step is to set up a pilot farm at an appropriate site to describe, control and optimise the processes involved in rearing specimens in a natural setting.

The project's two phases (hatchery and grow-out) will be guided by 30 years of research experience in sea cucumber biology acquired by marine biologists at the Universities of Brussels and Mons (Belgium) (Jangoux and Lawrence 1982; Coulon and Jangoux 1993; Gosselin and Jangoux 1996; Grosjean et al. 1998) and the University of La Réunion (France). They have produced a wide range of works on larva farming and biology and on the life cycles, reproductive cycles and eating patterns of adult echinoderms, in particular sea cucumbers.

The work underway is funded by Belgium's University Corporation for Development (Coopération Universitaire pour le Développement – CID) and revolves around a triple partnership involving the Universities of Brussels and Mons and the Madagascar University of Tulear, with the assistance (consultancy) of the University of La Reunion. It began in spring 1999 and is programmed to run four years (1999 – 2003). The project consists of first putting into place and then ensuring the scientific management of a hatchery that is designed to mass produce juvenile *Holothuria scabra* (sea cucumbers) and *Triplaneustes gratilla* (sea urchins) using genitors taken from the wild. Once this type of production has been mastered, the project will begin rearing juveniles of commercially exploitable specimens. Successful completion of the hatchery phase – planned for some time in 2002 – should make it possible to launch a second phase, which will consist of setting up a pilot farm to grow-out juveniles.

The situation is simple. The resource will become scarcer and eventually disappear if the current level of exploitation continues. Only a mariculture activity can save the situation by “doubling” the process of forming wild populations through the production of commercially exploitable specimens on farms (optimising juvenile grow-out). Because these juveniles would be produced in hatcheries, this should bring about a decrease in the pressure on wild populations. Once a simple and easy standard rearing method has been applied, it will be possible to do the same at a variety of coastal sites (aquacultural farms) using specimens supplied by the hatchery, which would maintain a minimum level of pressure on wild populations (whose ecological importance is well known). After these populations have recovered, rearing techniques could be transferred to juveniles collected directly on site (this would obviously imply that the skills to ensure stock management did exist).

The Tulear hatchery (Aqua-Lab) began in April 2000 and is currently operational. Its main section consists of a 120 m<sup>2</sup> air-conditioned building (Fig. 1) containing six rooms for growing seaweed (Fig. 2), rearing larvae (Fig. 3), growing out juveniles (Fig. 4A), caring for genitors (Fig. 4B), microscopic analysis, computer processing (Fig. 5) and servicing aquarium equipment.

Initial fertilisation of the sea cucumbers, which began in May 2000, has led to the production of a limited number of 1-2 cm juveniles. The larvae are fed planktonic algae imported from Europe (species from the genera *Phaeodactylum* and *Chaetoceros*) while the juveniles are fed finely chopped macro-algae from the reef (Fig. 6).

The hatchery has been set up at the site of the University of Tulear's Fisheries and Marine Sciences Laboratory. A saltwater pumping station,



Figure 1. Aqua-Lab Hatchery





Figure 2. Planktonic algae cultivation area



Figure 3. Larvae rearing area



Figure 4a.  
Juvenile grow-out area

whose reservoir fills up at high tide and whose water pours into a 30 m<sup>3</sup> settling basin (underground basin), was installed. The water stored in this basin is tapped on demand. It is then filtered (filters with decreasing mesh size down to 1mm) and sterilised by repeated applications of UV before being used in the larvae rearing tanks. This facility has proven satisfactory for rearing sea cucumber larvae but has not yet reached its full potential with regards to sea urchin larvae.

If hatchery production meets expectations, the partners plan to use additional funding from the Belgian Corporation to set up a pilot sea cucumber grow-out farm about 20 km south of the hatchery, at a site which the University of Tulear leases from the Madagascar Ministry of Fisheries. This site, which borders a mangrove, is very satisfactory and corresponds to the ecological requirements of the study species, *H. scabra*. It also possess a natural upwelling of fresh water which would obviously facilitate installation of the permanent research and housing buildings.

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Figure 4b. Genitor care area



Figure 5.  
Microscopic analysis and computer processing room



Figure 6.  
Preparing seaweed to serve as feed for *Holothuria scabra*

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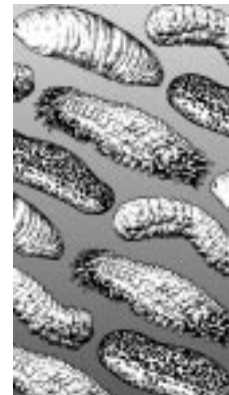
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# The effect of food availability on early growth, development and survival of the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea)

Andrew David Morgan<sup>1</sup>

## Abstract

In the aquaculture of marine invertebrates the interaction between the physical environment and the availability of food may affect the quality of larvae in mass cultures. Larvae from fertilised eggs obtained from the sea cucumber *Holothuria scabra*, induced to spawn in captivity, were placed in 3 l, aerated, concave aquarium bowls and the quality of larvae and their survival, growth and development in response to the rearing environment was observed. At concentrations of 1 and 2 x 10<sup>4</sup> cells/ml of the alga *Isochrysis galbana*, growth and development of larvae increased substantially. At concentrations of 4 and 8 x 10<sup>4</sup> cells/ml, and in the absence of algae, larval survival was less and growth and development was inhibited. Larval quality was compromised by high concentrations of algae. An increase in the concentration of algae may affect levels of pH and un-ionised ammonia, which can affect the development and survival of larvae. It is important to structure feeding regimes so that concentrations of algae do not compromise the rearing environment.

## Introduction

Since Conand (1990; 1993) and Conand and Byrne (1993) assessed sea cucumber fishery resources and the effects of their exploitation, a number of projects have been developed to assess the potential of sea cucumbers for aquaculture. Aspects of the development of holothurian larvae have been described for the sea cucumbers *Cucumaria elongata*, *Stichopus californicus*, *Psolus chitonoides*, *Psolidium bullatum*, *Actinopyga echinties*, *Holothuria leucospilota*, and *H. pardalis* (Chia and Buchanan 1969; Maruyama 1980; Smiley 1986; McEuen and Chia 1991; Mashanov and Dolmatov 2000). Most aspidochirote holothurians follow the larval cycle of pre-auricularia, early, mid- and late auricularia and subsequent metamorphosis to the doliolaria (non-feeding) stage before settlement. The length of the larval cycle differs between species, but the development of *Holothuria scabra* larvae is typical of holothurian ontogenesis, reaching initial settlement after approximately 10 to twelve days in optimal culture conditions (James et al. 1994; Battaglione et al. 1999).

Species of algae that have been fed to larvae of the sea cucumbers *A. echinites*, *H. gresia*, *P. californicus*, and *S. californicus* include *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Dunaliella salina*, *D. tertiolecta* and *Pavlova lutheri* (Burke et al. 1986; Smiley 1986; Balsler et al. 1993; Dautov 1997). Battaglione et al. (1999) fed *H. scabra* larvae with the algae *Chaetoceros muelleri*, *C. calcitrans*, *P. salina*, *Rhodomonas salina* and *Tetraselmis chuii*. Ito (1995),

James et al. (1994) and Ramofafia et al. (1995) indicated that algal concentrations of 2 to 3 x 10<sup>4</sup> cells/ml were optimal for larval ontogenesis of *S. japonicus*, *H. scabra* and *H. atra* respectively, during development in large culture vessels (>100 l). Archer (1996) found that the ingestion rate of larvae of *S. mollis* was reduced when *P. tricornutum* and *D. tertiolecta* was given at concentrations exceeding 6 x 10<sup>3</sup> cells/ml. In the continued presence of high concentrations of algae, larvae stopped feeding.

In the present study I used *H. scabra* larvae produced in captivity from induction of spawning (Morgan 2000) to investigate the effect of different concentrations of the alga *I. galbana* on larval quality. The quality of larvae was determined by observation of symmetry of shape, complexion of the dermis and development of the larval arms.

## Materials and methods

### Culture vessels

Experiments were conducted in 3 l concave aquarium bowls at 27°C with a 16:8 LD cycle using UV-sterilised seawater filtered to 0.2 mm, to eliminate most bacteria, for use in larval rearing experiments. Concentrations of the alga *Isochrysis galbana* at 0, 1, 2, 4 and 8 x 10<sup>4</sup> cells/ml were assigned to bowls randomly with three replicates per treatment. Larvae were obtained by inducing a number of *H. scabra* to spawn in captivity (Morgan 2000). The fertilised eggs were washed of excess sperm and allowed to develop in a 250 l flow-through hatch tank before

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use in the experiment. The experiment was terminated after 11 days as under optimal nutritional regimes, larvae metamorphose into non-feeding doliolaria around this time. Consequently, it was not necessary to assess the quality of larvae in response to the culture environment after this time.

### Maintenance

Every day 50% of the seawater was siphoned out of each 3 l concave aquarium bowl using a plastic tube with a 100 mm mesh screen glued to one end. The remaining seawater and larvae were drained into a clean bowl containing 1.5 l of 0.2 mm filtered UV-sterilised seawater. The amount of algae was adjusted by taking three 10 ml samples from each bowl and counting the algal density with a high speed particle counter (model FN Coulter Counter;  $\pm 250$  cells/ml).

### Growth and survival

Larvae were stocked in the 3 l aquarium bowls at a density of 1 larva/2 ml of filtered sea water. Growth and survival measurements were made preceding the transfer of larvae and remaining seawater to a clean bowl. Every second day, larval survival was measured by taking five 20 ml sub-samples from each bowl and counting the number of surviving larvae using a stereo-dissecting microscope. On alternate days 30 to 50 larvae were sampled from each bowl and total growth in length measured with a compound microscope and micrometer eyepiece. Percentage survival was adjusted for the removal of larvae.

### Development and larval quality

Larval stage was recorded by observation of the hydrocoel, left and right somatocoel and lateral folds or 'arms' (Smiley 1986; Pedrotti 1995; Dautov and Kaisheko 1995) using a compound microscope with a micrometer eyepiece ( $n = 10$  larvae/bowl). The hydrocoel is situated to the left of the oesophagus (Fig. 1) and enlarges throughout development. The elaboration of the hydrocoel refers to the thickening

and subsequent development of five nodules late in the larval cycle.

Early auricularia larvae had a simple convoluting ciliated band with undeveloped lateral arms, a 'globular' hydrocoel, and very little development of the left somatocoel and no right somatocoel. The mid-auricularia stage was recorded as occurring when the four lateral folds could be seen developing, the left somatocoel extended more than half way down the gut and the hydrocoel was elongated. Late auricularia occurred when lateral folds exhibited overt folding and the hydrocoel had elaborated, the left somatocoel extended to the rear of the stomach and the right somatocoel was clearly visible. The appearance of the dermis and symmetry of shape were also recorded.

### Data analysis

A one-way analysis of variance (ANOVA) was used to obtain differences in the mean growth rate of larvae for each concentration of algae from days two to 10. A one-way analysis of variance (ANOVA) was used to obtain differences in the mean survival of larvae for each concentration of algae at the end of the experiment. Differences between treatments were tested for using least square means (LS means) and the Bonferroni correction for multiple

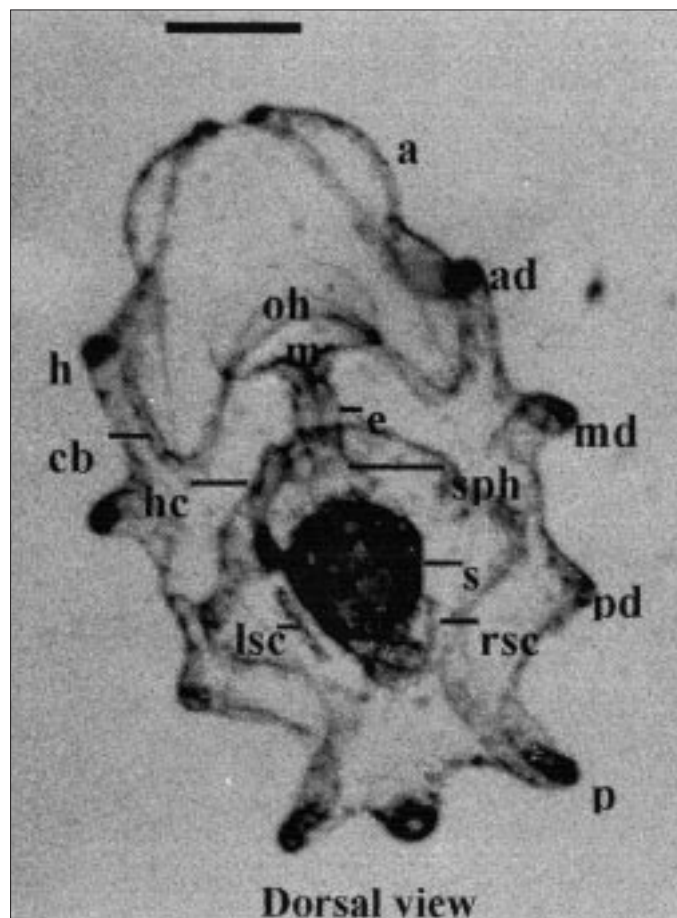


Figure 1

Photograph of *Holothuria scabra* larvae late in development, indicating features used to identify larval stage (scale bar indicates 200  $\mu$ m). a, anterior fold. ad, anteriodorsal fold. md, mid-dorsal fold. pd, posteriodorsal fold. p, posterior fold. rsc, right somatocoel. lsc, left somatocoel. hc, hydrocoel. m, mouth; s, stomach. cb, ciliary band. h, hyaline sphere. sph, sphincter muscle. oh, oral hood.

comparisons (5% significance level divided by the number of multiple comparisons;  $n = 10$ ). Differences in the rate of development between early, mid- and late auricularia were graphed.

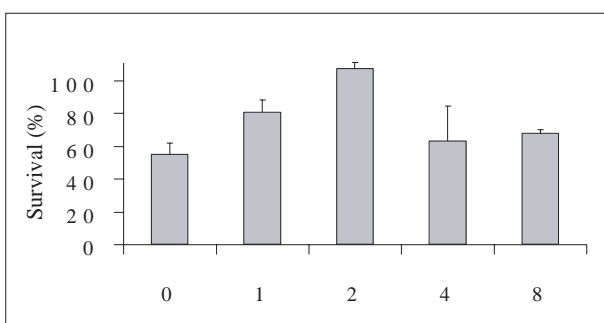
## Results

### Survival

Survival of larvae at  $2 \times 10^4$  cells/ml differed from all other concentrations of algae except  $1 \times 10^4$  cells/ml ( $p < 0.005$ ; Table 1). Few larvae died at  $2 \times 10^4$  cells/ml while  $55 \pm 6.7$  percent of larvae remained at  $0 \times 10^4$  cells/ml at the end of the experiment (Figure 2). Other concentrations of 1, 4 and  $8 \times 10^4$  cells/ml had  $81 \pm 7$ ,  $63 \pm 21$  and  $68 \pm 2.2$  % of larvae left respectively, on day eleven (Figure 2).

**Table 1.** Analysis of variance (ANOVA) results for numbers of *Holothuria scabra* larvae surviving after 11 days and p-values for Least squares means at 0, 1, 2, 4, and  $8 \times 10^4$  cells/ml of the algae *Isochrysis galbana* ( $p < 0.005$ ).

Source	DF	SS	MS	F value	Pr>F
Model	5	136	27	5	0.0164
Error	9	47	5		
Corrected total	14	183			
LS Means	1	2	4	8	
0	0.0379	0.0016	0.4932	0.4065	
1		0.062	0.118	0.1652	
2			0.0042	0.005	
4				0.8551	
8					



**Figure 2.**

Mean number ( $n = 3$ ) of *Holothuria scabra* larvae surviving after eleven days at concentrations of 0, 1, 2, 4, and  $8 \times 10^4$  cells/ml of the algae *Isochrysis galbana* (mean  $\pm$ S.E.).

### Growth

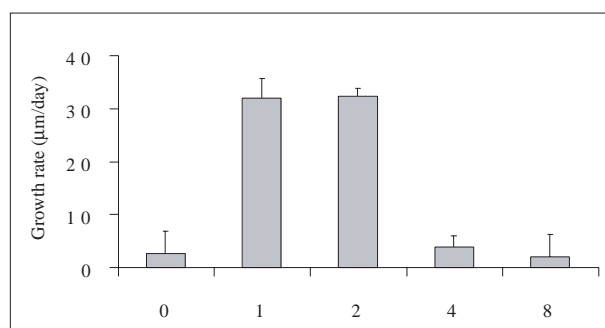
Growth of larvae at 1 and  $2 \times 10^4$  cells/ml differed from 0, 4 and  $8 \times 10^4$  cells/ml ( $p < 0.005$ ; Table 2). Growth of starved larvae and growth of larvae between 4 and  $8 \times 10^4$  cells/ml did not differ significantly, as was the case between 1 and  $2 \times 10^4$  cells/ml ( $p > 0.005$ ). Larvae at 1 and  $2 \times 10^4$  cells/ml grew an average of  $32 \pm 7.3$  and  $32 \pm 3.1$   $\mu\text{m}/\text{day}$ , respectively (Fig. 3). Larvae at these two concentrations reached a total length of 899 and 866  $\mu\text{m}$  respectively on day 10 (Table 3).

**Table 2.** Analysis of variance (ANOVA) results for growth rate of *Holothuria scabra* larvae over 10 days and p-values for Least squares means at 0, 1, 2, 4, and  $8 \times 10^4$  cells/ml of the algae *Isochrysis galbana* ( $p < 0.005$ ).

Source	DF	SS	MS	F value	Pr>F
Model	4	12387	3097	22.99	0.0001
Error	10	1347	135		
Corrected total	14	13734			
LS Means	1	2	4	8	
0	0.0001	0.0001	0.8151	0.887	
1		0.96	0.0001	0.0001	
2			0.0001	0.0001	
4				0.7077	
8					

**Table 3.** Summary of measurements of growth and survival of *Holothuria scabra* larvae at different concentrations of the algae *Isochrysis galbana*.

Conc. $\times 10^4$ cell/ml	Length $\mu\text{m}/\text{day}$ Day 10	Growth rate $\mu\text{m}/\text{day}$	Initial no. per litre	Final no. per litre	% Survival
0	654	2.64	478	263	55
1	899	32	462	373	81
2	866	32	437	467	107
4	642	3.78	468	294	63
8	568	1.95	433	293	68



**Figure 3.** Mean growth rate ( $n = 3$ ) of *Holothuria scabra* larvae measured over eight days at concentrations of 0, 1, 2, 4, and  $8 \times 10^4$  cells/ml of the algae *Isochrysis galbana* (mean  $\pm$ S.E.).



## Development

Highest proportions of late auricularia larvae were observed at 1 and  $2 \times 10^4$  cells/ml on day ten while little or no development to late auricularia occurred in other algal concentrations (Fig. 4). On day eight there was a slight increase in the numbers of early auricularia larvae recorded at 4 and  $8 \times 10^4$  cells/ml due to inaccuracies in distinguishing between early and abnormally developing larvae.

After two days, only early auricularia were present in all concentrations. Mid-auricularia were seen at varying levels in all concentrations from days four to ten, and late auricularia was observed only at days eight and ten (Fig. 4). From four to ten days the proportion of mid-auricularia larvae did not change markedly. However, early auricularia were observed less frequently at 1 and  $2 \times 10^4$  cells/ml.

## Larval quality

The physical appearance of larvae differed between algal concentrations. The appearance of larvae was assessed to provide an indicator of the effects of the culture environment on larval quality (Fig. 5). Early auricularia appeared uncompromised during the first few days in each algal concentration.

However, by the time larvae reached mid-auricularia there were differences in larval form (Fig. 5; a1, b1, and c1). Two features were obvious in distinguishing the quality of larvae; the appearance of the dermis of larvae and the curvature and degree of folding in the lateral arms. A minimal amount of folding and a pointed apex, together with an anterior-posterior contraction of the body length indicated an extreme of larval abnormality (Fig. 5; c, c1 and c2). This occurred predominantly at  $8 \times 10^4$

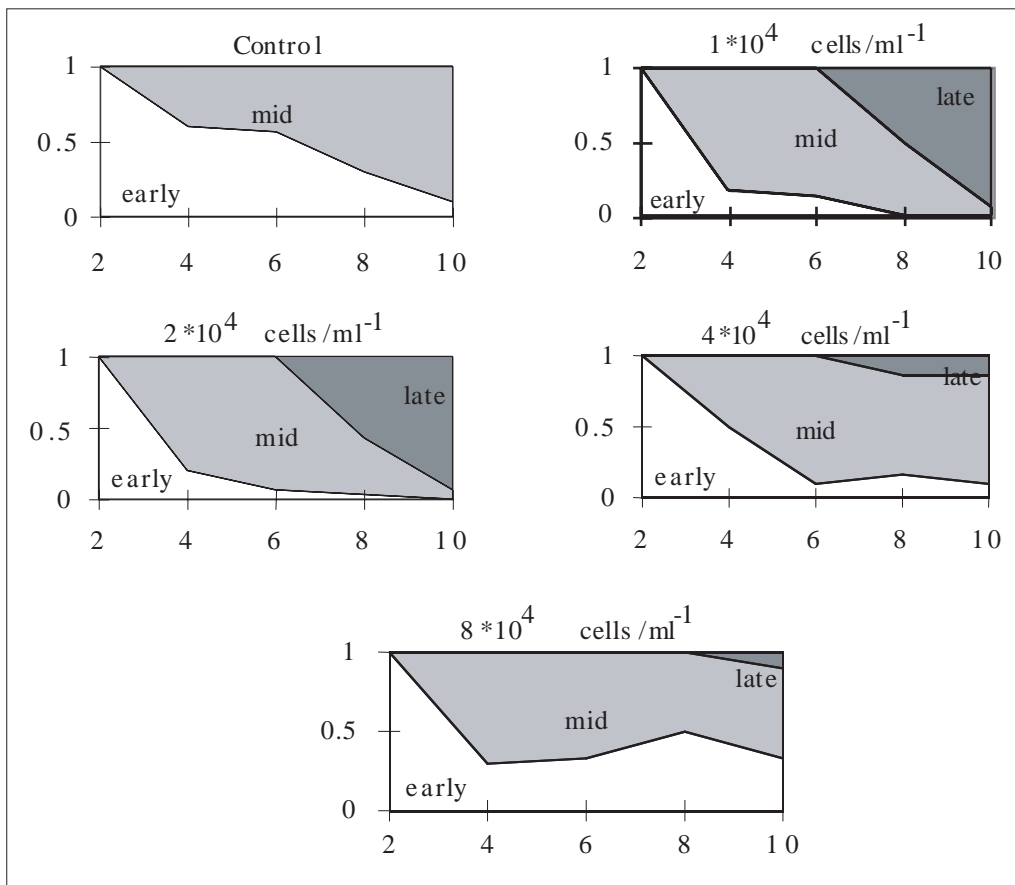


Figure 4.

Development of *Holothuria scabra* larvae observed over ten days at concentrations of 0, 1, 2, 4, and  $8 \times 10^4$  cells/ml of the algae *Isochrysis galbana*, (three replicates pooled for each concentration; mean  $\pm$ S.E.; n = 30 to 50 larvae per day/bowl).

cells/ml but was less frequent at other concentrations. Discolouration of the dermis could be seen (Fig. 5; b1 and b2) but did not always occur with other abnormalities described above. Some variation in pH occurred at 4 and 8 x 10<sup>4</sup> cells/ml (7.5 to 8.5), which may have reflected variation in the level of un-ionised ammonia, affecting the quality of larvae.

Early stages of *H. scabra* larvae were affected by the placement of the aeration pipette as they were not strong enough to swim out of eddies created at the base of the bowl. The pipette was situated about 2 cm off the bottom of each bowl and larvae were placed in bowls at 60 to 72 hour's post-fertilisation as they were better able to maintain their position in the water column.

## Discussion

In the present study optimal levels of algae for maintaining the quality of larvae in culture were 1 and 2 x 10<sup>4</sup> cells/ml or 2 to 4 x 10<sup>4</sup> cells/larva/day. Increasing the algal concentration to 4 and 8 x 10<sup>4</sup> cells/ml did not result in increased growth and development but compromised the quality of larvae and the culture environment.

It was likely that the maintenance of excess concentrations of algae disrupted the process of filtration, ingestion and digestion in *H. scabra* larvae. When observing the state of the gut of *H. scabra* larvae, James et al. (1994) considered a concentration of 2 to 3 x 10<sup>4</sup> cells/ml to be optimal for growth and development. Ito (1995) states that in the early devel-

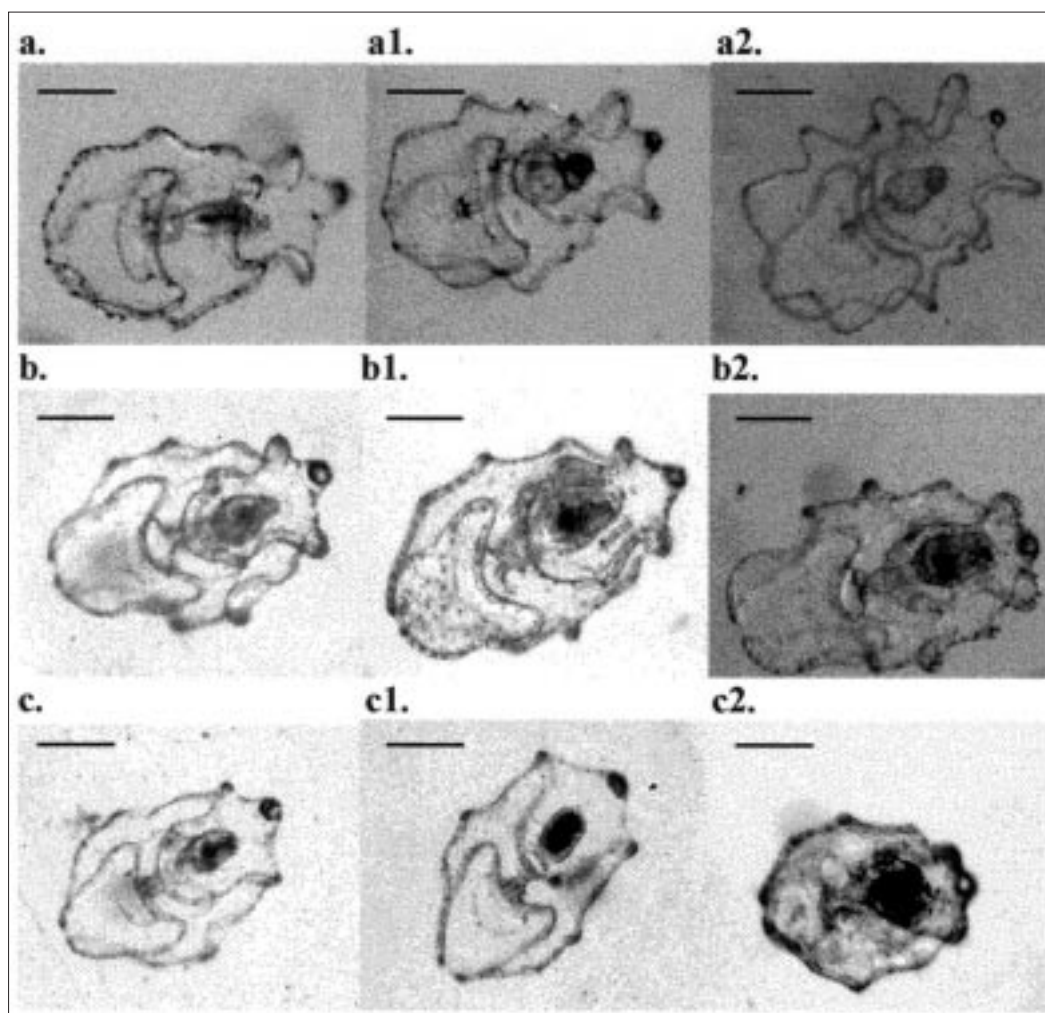


Figure 5.

Quality of *Holothuria scabra* larvae reared in different concentrations of the alga *Isochrysis galbana*. a: normally developing larvae at early, mid-and late auricularia in moderate amounts of algae (1 and 2 x 10<sup>4</sup> cells/ml). b: abnormal development of larvae at increased algal concentrations (4 x 10<sup>4</sup> cells/ml). c: abnormal development of larvae in excessive amounts of algae (4 x 10<sup>4</sup> cells/ml; scale bar approximately 200 µm).

opment of larvae of the sea cucumber *Stichopus japonicus*, a concentration of  $0.5 \times 10^4$  cells/ml was adequate but that this was increased to around  $3 \times 10^4$  cells/ml late in the larval cycle. It was likely that the survival of *H. scabra* larvae in the absence of algae resulted from the use of stored nutrients. Archer (1996) found that larvae of the sea cucumber *S. mollis* eventually stopped feeding when concentrations of algae continuously exceeded  $0.6 \times 10^4$  cell/ml as ingestion rates peaked at 18.2 cells/min at this level.

Ito (1995) indicated that the growth in length and width of the stomach may be an important indicator of larval quality especially late in development. In the present study the shape of the gut of most larvae in moderate amounts of algae was spherical, but in increased amounts of algae this was more variable and the gut was often contracted along the lateral axis (Fig. 5 c1 and c2).

Some asymmetry in development of the lateral folds of larvae was evident in excess amounts of algae (Fig. 5 b and c). If larvae were not developing normally, there was a greater chance of observer error in recording developmental stages as early, mid- or late auricularia. The lack of a skeletal structure in holothurian larvae made it difficult to distinguish transition to subsequent larval stages. In the present study large numbers of abnormally developing larvae stayed in the water column for the duration of the experiment and in some instances exhibited normal developmental features but had contracted lateral folds. In cultures containing excess food the quality of the lateral folds, symmetry of shape, growth in total length and the shape of the gut best indicated the response of larvae to the culture environment.

The observation and recording of development of the left and right somatocoel was difficult in cultures containing excess food. In the early auricularia larva the hydrocoel is connected to the left somatocoel, which breaks from the hydrocoel during larval development and divides into the left and right somatocoel (Balsler et al. 1993). The timing of breakage of the left somatocoel from the hydrocoel and the right somatocoel from the left somatocoel may indicate the transition of larvae to subsequent stages of auricularia development.

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## Sea cucumber retail market in Singapore

Chantal Conand<sup>1</sup>

The retail market for sea cucumbers is active in Singapore and many shops are selling diverse dried sea products (Fig. 1). The price for sandfish *Holothuria scabra* was around SGD 180 per kg in October 2000 [Note from editor 1SGD ≈ 0.58 USD].

In the main town market, there are many sellers of sea cucumbers already soaked. It is difficult to determine the species in that case. They sell for between SGD 14 and 18 per kg (Fig. 2) and an assortment of 6-7 pieces at SGD 2 (Fig. 3).



Figure 1.

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Figure 2.



Figure 3.



# Review of sandfish breeding and rearing methods

Rayner Pitt<sup>1</sup>

## Summary

*Sandfish (Holothuria scabra) are economically important warm-water sea cucumbers. Generally, a few collected animals are found to be ripe at most times, with one or two spawning peaks in the year. Spawning is most reliably stimulated by temperature changes, but at best only about one-third of large, freshly-collected animals can be induced. Larval rearing to settlement has repeatedly been achieved in earlier work using cultured phytoplankton, Chaetoceros spp, Skeletonema spp and Isochrysis galbena. More recently a schedule, feeding Chaetoceros muelleri, Chaetoceros calcitrans and Rhodomonas salina, at a rate increasing from 20,000 to 40,000 cells/ml 'muelleri-equivalent', has been used. Settlement is promoted by the use of conditioned plates and early nursery carried out using these plates in conditioned outdoor tanks, sometimes under partial shade. After reaching about 20 mm (1g) juveniles can be transferred onto fine sand for further nursery. Growth of juveniles (and adults) appears to depend largely on photosynthetic production of much of their food, even when prepared feeds are added, and drops at stocking levels above about 225 g/m<sup>2</sup>. This may present a bottleneck in broodstock management and in large-scale nursery production for farming or restocking. The available data suggests that adult animals can grow at about 2 g/day.*

## Introduction

Among the warm-water sea cucumbers, sandfish (*Holothuria scabra*) has attracted interest as a candidate species for breeding and farming, or as an overexploited species that could benefit from stock enhancement programs (Conand 1998a, 1998b). Sandfish are often found in lagoons or somewhat estuarine areas near mangroves or on seagrass beds, suggesting that they may tolerate a wider range of conditions (of salinity, temperature and eutrophication) than deep-water species. Their diet is believed to consist largely of benthic algae and bacteria associated with organic detritus, which they extract by ingesting and excreting large quantities of substrate. This leads to hopes that low-cost feeding regimes can be developed. Their liking for substrates of sand or sandy silt/mud means that they may also fit into pond or pen culture systems as benthos processors in polyculture. Because they are slow-moving animals living in shallow inshore waters, sandfish are easily overfished.

The dried product (beche-de-mer) produced from sandfish is of high-value, and often constitutes one of the most important exported species from fisheries in small island developing states. However the weight loss during processing of beche-de-mer is about 95% (Shelley 1985; Conand 1989, 1990; Preston 1990). Little is known about the environmental tolerances of sandfish (for growth rather than just survival) or their compatibility with other cultured species, and there is surprisingly little published data on growth rates and productivity.

Several groups have claimed success in sandfish breeding. These include the Central Marine

Fisheries Research Institute, Cochin, India (James et al 1994; James 1996); Mariculture Development Centre, Lampung, Sumatra, Indonesia; Research Station for Coastal Fisheries, Gondol, Bali, Indonesia (Dr Ketut Sugama, pers. comm.); and ICLARM Coastal Aquaculture Centre (CAC), Guadalcanal, Solomon Islands (Battaglione and Seymour 1998; Battaglione et al 1999). Numerous others have looked at aspects of the ecology, behavior, gonadal maturity, processing and pond or pen culture of the species. The aim of this short review is to present an outline of these findings in a compact form which will be useful for those considering carrying out culture work on this species. A comprehensive review on all aspects of the biology of *Holothuria scabra* has been prepared by Hamel et al (in preparation, 2000).

## Ripeness and fecundity

The sexes cannot be distinguished externally until the start of spawning. Gonosomatic Index (GSI) and spawning studies usually show that some animals are ripe at most times in the year, with one or two seasonal peaks usually reported.

Ong Che and Gomez (1985) in Batangas, Philippines, found spawning peaks in mid-year (June–July) with high sea temperatures, and at the end of the year (either December–January or October–November) with cooler water, and rapid ripening after spawning. Conand (1989, 1993) identified in New Caledonia, from morphological and gonad-index variations, a first well-marked peak from December to February, followed by a smaller more variable one between August and October. Tuwo (1999) in southwest Sulawesi, Indonesia

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found mature gonads throughout much of the year but in increasing numbers from June to October and from February to April. Krishnaswami and Krishnan (1966) report on two breeding periods in the Krusadi Islands, Gulf of Myanmar, India in July and October.

Morgan (1999) looked at a sandfish population in a subtropical area at latitude 27° South (Stradbroke Island, Queensland, Australia) and found a GSI peak in November. While gonads and oocytes were developing from June to December the bodywall lost weight. He was able to induce spawning in all animals, suggesting a more marked seasonal pattern in this region. However (Morgan 2000) broodstock held indoors generally lost condition and many died.

Battaglione et al (in review, 2000) working with batches of, on average 28 large animals (usually of at least 500 g each) freshly collected from the Vona-vona lagoon, Western Province, Solomon Islands found peak egg production in September when 35% of the animals spawned. Males spawned in every month of the year except February. Females failed in May and December. An average of 1.9 million eggs were produced per spawning female. Collection of fertilized eggs was possible on 46% of attempts. Spawning was somewhat easier during the last quarter lunar phase, and most frequent during the afternoon, evening or night before midnight (though this may have been due to the transport schedule). On average 1.9 females and 3.6 males spawned from a particular batch.

In practical terms, this means that batches of about 30 large animals freshly collected from the wild were needed to have an even chance of getting a few million eggs, but the odds were improved by working at or shortly before the month of peak ripeness.

### Broodstock collection and spawning stimulation

James (1996) collected broodstock from commercial landings, selecting large healthy individuals that had not eviscerated. He then held them in broodstock tanks, where natural spawning sometimes occurred during the months of peak ripeness, though it is not clear whether this was generally shortly after collection or after some months of culture. He mentions the possibility of obtaining fertilized eggs by sacrificing animals and dissecting out ripe translucent ovaries, drying them in the shade 'for some time', puncturing the ovary in a dish of seawater and introducing sections of ripe testis. He also describes a technique for obtaining eggs from live broodstock in which the water was drained

from the broodstock tank, animals were dried in the shade for about half an hour, then subjected to a powerful jet of water for a few minutes and then put back into the tank with water, which led to spawning within 2–3 hours. However, he recommends thermal stimulation, whereby animals are subjected to a 3–5 degree water temperature rise, as the best method.

In the western Solomon Islands, animals were collected by snorkeling or SCUBA in the morning and taken in an insulated container by boat to the field station (Nusa Tupe, near Gizo, Western Province) with occasional water changes but no aeration. Collection and transport times were 3–5 hours. At the field station, depending on flight availability, they were stored for a few hours in a flowing seawater tank and then packed individually in plastic bags with 500–1000 ml seawater. Between 15 and 25 bags were sent in an insulated box for the approximately two-hour flight and subsequent one-hour road journey, generally arriving at the hatchery in the evening, some 8–10 hours after collection.

Frequently, the stress of collection and transport were sufficient to induce spawning to start the same evening or night. Otherwise, further stimulation was tried either that night or on the following days, with animals generally held in a low 2000 l tank of static aerated seawater. The use of sun-warmed water to raise the temperature by up to 5 degrees was the most effective. Water changes, water jetting, short-term drying and refilling with cooled seawater and addition to the tank of 0.1 g/l of a commercially available powdered *Schizochytrium* feed (Algamac-2000 Bio-Marine, Hawthorne, California, USA) for one hour were all sometimes used.

### Broodstock maintenance

James et al (1994) speaks of holding 20–30 animals on six inches of sand in a one-tonne tank with daily water changes and fortnightly sand changes, and feeding them once a week with a little freshly ground algal paste. In 1996, he recommended stocking 15–20 animals on 100 mm of mud in a one tonne tank containing 800 l of water, which is completely changed daily, and giving daily feedings of 50 g of a preparation of prawn head waste, soya bean powder and rice bran (6.5% protein). Apparently, animals held under these conditions could be used for spawning over several months and often spawned when the water was changed.

Battaglione (pers. comm.) says that animals held at low density on sand in tanks with continuously flowing seawater, fed powdered dried algal preparations and prawn pellets, would sometimes ripen and could be spawned more than once.

Morgan (1999) found weight losses of about 20% per month for unfed animals on sand in a tank (no stocking density data), reduced to about half by feeding prawn and lucerne pellets. Holding animals for up to five weeks did not appear to affect fecundity but reduced egg hatch rate.

## Spawning and incubation

Side to side rolling movements, and lifting and swaying of the front end of the body, are signs that spawning may be beginning. Males usually spawn first, releasing a stream of sperm for many minutes or even hours. Females often show a bulging of the front of the body where the gonopore emerges; egg release is less continuous but may also wax and wane over an hour or more. In order to prevent excessive sperm damaging the eggs, animals of either or both sexes were often removed from the spawning stimulation tank and put in smaller containers of 10–60 litres once spawning started. (The risk that they may not start to spawn again after this transfer is greater with females.) Both eggs and sperm appeared to remain separately viable for an hour or more.

Both James (1996) and Battaglione et al. (in review, 2000) advise washing the eggs (which sink) to remove excess sperm. An alternative is to fertilize the eggs using a sperm suspension of known strength (counted on a haemocytometer cell). A final concentration of about 20,000 sperm per millilitre of water containing eggs appears to be low enough to avoid the need for washing. On at least one occasion, eggs that had been fertilized in this way and then left aerated overnight in a 60 l container were in better condition the next day than those that had been carefully washed on a sieve (50 or 80 microns) and stocked in rearing tanks.

Stocking levels of about 0.5–1 eggs/ml are indicated by James (1996), in tanks of about 800 l with light to medium aeration. Perhaps higher densities would be possible with well-handled, clean batches of eggs in good water; Battaglione (pers. comm.) suggests up to 2.5 eggs/ml. In Solomon Islands, conical-bottomed 200 l or flat-bottomed 600 l cylindrical fibreglass tanks were usually used for hatching and larval rearing.



*H. scabra*

James (1996) suggests about 24 hours are required for hatching (to the motile gastrula stage) and 48 hours until the appearance of early auricularia, the first feeding stage. Ramofafia et al (2000, in review) on the other hand, say that at 25–27 °C hatching to the swimming gastrula occurs after 12 hours and the transition to auricularia after 30 hours.

## Larval rearing

At CAC opaque 200 l or translucent 600 l tanks with central drains and internal standpipes were used indoors, either with translucent lids or uncovered, and with bright 12-hour artificial illumination. This consisted of one or two 20 W fluorescent tubes per tank at a height of about a metre from the water surface. Water was pumped ashore from about 12 m depth, passed through pool and then cartridge filters down to (nominal) one micron. Sometimes UV treatment was also used. Temperatures were typically 27–29 °C, salinities 32–37 ppt. Aeration, from a single central airstone, was generally light in the early stages.

Water in the hatching tanks was usually unchanged for the whole of day 1, although conspicuous pink or yellow bacterial patches on the tank floor were removed by siphoning. Tanks were drained completely on day 2 (counting the day on which fertilisation occurred, usually in the evening or before midnight, as day zero) and the early auricularia larvae were collected on 80 micron sieves immersed in bowls. Care was taken that the flow rates were kept low (not more than about 10 l/min. on a cylindrical 300 mm diameter sieve) and that the larvae remained in water at all times. They were transferred by beaker periodically into aerated buckets (briefly stirred for counting aliquots) before stocking into clean rearing tanks of the same type. Suitable larval stocking density is given by James (1996) as 0.3–0.7/ml, by Battaglione and Bell (1999) as 1/ml.

Feeding has to begin on day 2. James (1996) mentions mixed diatom culture of *Chaetoceros* spp and *Skeletonema* spp plus *Isochrysis galbana* or the latter alone, maintaining a level of 20,000–30,000 cells/ml in the rearing tank. At CAC, various larval rearing trials were carried out and the feeding schedule was still under investigation at the end of 1999, but a synthesis of the experiences to date is shown in Table 1, adapted from Battaglione (pers. comm.). In the latest batches there were some indications that *C. calcitrans* could have been omitted and one tank which received only the mixed dry diets Algamac and Livic (Riken Vitamin Co. Tokyo, Japan) yielded a few pentacularia.

Larval tanks were usually completely drained down (as described above) every second day. Counts were made and larvae were then re-stocked

in clean tanks and fed a mixture of three algal species. The feeding rate was gradually increased as larvae developed. There were no counts made of residual food and no additional feeding on the alternate days. The three species, *Chaetoceros muelleri*, *Chaetoceros calcitrans* and *Rhodomonas salina* were fed on an 'equal biomass' basis. As the cells of the three species differ in size the number of cells of *C. calcitrans* fed had to be increased (divide by 0.75) and of *R. salina* reduced (divide by 3). The feeding rate given is the 'muelleri equivalent' of the total mixture. (Thus if on a particular day a feeding rate of 30,000 cells/ml was required it would consist of about 10,000 cells/ml of *C. muelleri*, 13,000 cells/ml of *C. calcitrans* and 3,300 cells/ml of *R. salina*). At CAC algae were generally produced indoors using artificial light and air-conditioning, in autoclaved flasks or carboys.

On about day 7 diatom plate conditioning was started. Stacks of plates similar to those used in abalone culture were put outdoors under partial shade (50–75%), in shallow tanks supplied with continuously-flowing seawater filtered to one micron to prevent the development of large numbers of copepods. (At CAC, plates were of corrugated fibreglass roofing material cut into rectangles of about 300 mm x 400 mm and assembled into stacks of four with 30 mm spaces. There was a surface area per stack of about 1m<sup>2</sup>.) About 0.25–0.5 m<sup>2</sup> of plate surface was used per 100 l of larval rearing tank, and 4–7 days allowed for a biofilm to develop on the plates. James (1996) used extracts of sargassum to induce development of biofilm. There are also more managed techniques of benthic diatom culture on plates; these have not been tried.

Under good conditions, late auricularia with pronounced oil globules could be seen from day 8, some non-feeding doliolaria from day 10, and the first pentacularia from about day 12. At this stage conditioned plate stacks were put into the tanks, after rinsing in filtered seawater to remove any copepods. Batch changes were usually stopped, and continuous or semi-continuous water flow started, at a rate of 1–3 tank volumes per day. Outlet screens were of 80 or 120 microns. Microalgae continued to be fed daily, mainly for the

less developed larvae. A daily supplement of a dry algal food blended in water; Algamac, *Tetraselmis* sp. (Cell Systems, Cambridge, UK), Livic or a mixture (as available) was added at a rate of about 0.05 g per 100 l of tank water.

Plates can remain in the larval rearing tanks as long as some competent auricularia are present and food availability does not limit the growth of the pentacularia and juveniles (which appear from about day 20). Eventually, the relatively small surface area and low light levels mean that the juveniles are likely to outgrow the supply of suitable food, assumed to be at this stage largely benthic or attached diatoms, or other settled algal cells.

If accurate counts and weight measurements are needed, juveniles can be detached by the use of 0.5–1% potassium chloride solution in seawater. This technique was quantified by Battaglione and Seymour (1998) who found that a 10 minute bath in 1% KCl caused less than 2% mortality in juveniles of 2–20 mm (although as many as 17% of the larger sizes eviscerated), and rapid reattachment occurred after they were put back in normal seawater. Alternatively, the plates can be taken outdoors without detaching the juveniles and counts made if needed by inspecting the individual (separated) plates. When indoor rearing tanks are drained, the use of a 1% KCl spray helps detach animals from the tank walls and floor.

## Nursery

In a long series of experiments, Battaglione et al (1999) looked in detail at the conditions for rearing hatchery-bred juveniles of different sizes. Their main findings were:

1. One-month juveniles were nursed in bare conditioned fibreglass tanks (plus many plates) at a density of about 400/m<sup>2</sup> of tank floor and wall area and fed Algamac at 1g per m<sup>3</sup>. They grew from mean length 1.8 mm to mean length 13 mm in four weeks, with an average survival of 34%.
2. Juveniles were transferred into small, non-conditioned indoor aquaria with or without sand at different ages (from one to two months) and sizes (3–10 mm). They were fed Algamac at

10% of initial biomass. Survival increased markedly with size at transfer, from 52% at 3 mm to 87% for larger juveniles. Survival did not vary significantly with the substrate but growth was better on sand.

**Table 1.** Feeding rates for larvae of *Holothuria scabra*

Days (after spawning)	Feeding rate (cells/ml)	Treatment	Stage	Other feeds
2	20,000	batch water change	early auricularia	
4	20,000	"	auricularia	
6	25,000	"	auricularia	
8	30,000	"	late auricularia	
10	35,000	"	first doliolaria	
12 onwards	40,000 once daily	12 hours flow	first pentacularia	plates + dry algae



3. Small juveniles (1.5 mm) were stocked in bare concrete tanks (with a few plates) at two densities (167 and 558/m<sup>2</sup> of tank including walls) and fed either Algamac or Livic at 10% of initial biomass. Survival differences (15.7% at low, 5.9% at high densities) were apparently not significant. Juveniles fed Livic initially grew faster than those fed Algamac but, by the end of the two-month experiment, length (av. 19.5 mm), weight (av. 1.1 g) and survival were not significantly different between the two diets.
4. Four-month juveniles (av. 1.6 g) were stocked in concrete tanks at a density of 5/m<sup>2</sup> of floor plus walls (or about 10/m<sup>2</sup> of floor), with or without sand. They were fed Algamac at 1% or 10% of (adjusted) bodyweight. After two months, animals under the best treatment (sand and high food) all survived and averaged over 60 mm and 23 g. Sand was significantly better than no sand for growth (and perhaps for survival) but effects of feeding rate were not significant.
5. One-month 1 g juveniles were reared in outdoor fibreglass tanks at stocking densities of 7.5/m<sup>2</sup> of floor plus walls (25/m<sup>2</sup> of floor), with or without sand, and with or without 70% shade cover. They were fed Algamac at 0.2 g/day. After two months, weights were significantly different: the shaded juveniles averaged 7.3 g with sand and 11.5 g without, while unshaded juveniles averaged 12.5 g without sand and 14.1 g with sand.
6. The experiment described above was repeated a year later with 0.9 g animals at 1.8 times higher stocking levels, with double shade covers or without shade. Both shaded treatments grew very slowly (1.4 g with sand and 2 g without), while unshaded juveniles survived (95%) and grew better (10.8 g without sand and 12.1 g with sand).
7. Five-month 1 g juveniles were stocked at three densities (7.5, 15 and 30/m<sup>2</sup> of floor plus walls or 25, 50 and 100/m<sup>2</sup> of floor) in outdoor fibreglass tanks with sand. They were fed Algamac at two levels, 1% and 10% bodyweight per day (adjusted fortnightly) for eight weeks. Final weights were inversely related to density (low 17.8 g, medium 11.8 g, high 7 g) but feeding level had no significant effect.
8. Juveniles of 0.8 g were stocked at 15/m<sup>2</sup> of floor plus walls (50/m<sup>2</sup> of floor) in outdoor tanks that either had or had not been conditioned for two weeks prior to stocking, with either beach sand or coral sand substrate. They

were either fed Algamac at 10% bodyweight daily (adjusted after four weeks) or unfed. After eight weeks the fed, conditioned, coral sand treatment juveniles were the largest but all groups' averages were within the size range 6.6–9.9 g and none of the differences were considered significant.

9. Juveniles of 7.5 mm, (0.5 g) were stocked in larger concrete tanks (conditioned for two weeks) with or without sand, at 27.6/m<sup>2</sup> of floor plus walls (40/m<sup>2</sup> of floor) and fed Algamac at 3% of initial biomass daily. After three months, those on sand were significantly larger than those without sand (27.2 g compared to 7.2 g), but survival on sand was significantly poorer (12.4% compared with 67.2%). A few of the largest surviving juveniles of about 40 g each were stocked on fresh sand at 5/m<sup>2</sup> of floor and reared unfed for another year. Of these, 92% survived but they did not grow.

Conclusions and recommendations based on this work were as follows. Diatoms and attached algae are an important source of food for juveniles up to at least 50 mm in length. They therefore need to be grown in outdoor tanks with plenty of light. Growth slows when densities exceed about 200–225 g/m<sup>2</sup> and this is only slightly alleviated by supplementary dry algal feeding; however individuals stunted by high density later appeared to grow normally when given better conditions. Transfer of juveniles onto a sand substrate should be delayed until they reach about 20 mm length or 1 g live weight, but thereafter growth and survival are better on sand than in bare tanks. Growth of larger juveniles was approximately linear and averaged 0.5 mm or 0.2 g/day.

Therefore two nursery phases in outdoor tanks are needed; the first up to about 1 g size in bare tanks, the second on sand. For both stages there is currently no effective prepared diet; they appear to depend largely on food produced by photosynthesis and are density-limited.

For early nursery at CAC fibreglass tanks of 2.2 m diameter and 70 cm water depth were supplied with a continuous flow of about 6 l/min., ie 2–3 changes per 24 hours, filtered to 1 micron. A single shade cover was often used, and additional covers could be put on if filamentous algae threatened to dominate. Juveniles were usually fed a daily suspension of dried algae (at around 1 g/m<sup>3</sup>) and sometimes cultured phytoplankton if available (at up to 40,000 cells/ml) but, in the absence of an effective diet, stocking levels had to be kept low, or tanks periodically thinned, for growth to be maintained.

## Grow-out

There are accounts from Indonesia and India about farmers practicing culture in ponds or pens based on collected small sandfish. However, there is a dearth of real data concerning the growth and survival of larger animals in tanks, ponds or pens. An experiment in India in which juveniles of 67 g stocked in a concrete well ring (top closed with a net, bottom embedded in the mud of a prawn pond) reached 284 g in six months is reported by Battaglione (1999) who apparently also saw commercial pond culture in south Sulawesi, Indonesia. He obtained good growth of juveniles in a prawn nursery tank containing sand and mud; 0.7 g/day at low and 0.3 g/day at high density with 93% survival over 20 weeks. However, larger animals stocked in a prawn growout pond disappeared without trace.

A group from the Research Institute for Marine Fisheries, Jakarta (RIMF 2000; Basuki pers. comm.) stocked about 1000 collected sandfish (av. 46 g) in a 10 x 20 m shallow water net pen on a sandy seagrass area off Kongsu Island, Pulau Seribu archipelago, Jakarta Bay, Indonesia. Subsequent monthly samplings gave average weights of 101, 113 and approximately 150 g, but until a complete harvest is carried out it is possible that part of this apparent growth could be due to selective escape or mortality of smaller individuals, or to the greater ease of finding big ones when sampling. Another pen culture trial is believed to have been carried out in Sabah, East Malaysia by an Indonesian MSc student working with Ko-Nelayan (Sabah Fishermen and Fisheries Development Corporation); however at the time of visiting (May 2000) no data were available.

In Nha Trang, Vietnam (Nguyen Chinh 1995; N.T. Xuan Thu pers. comm.) 100 collected sandfish of 55–160 g (av. 68 g) were reared in a 50 m<sup>2</sup> concrete tank on sand and fed shrimp pellets 2–3 times per week. After three months 85 animals of 140–440 g (av. 350 g) were harvested. This appears to be a rare instance of good growth at high density. In nearby Cam Ranh 48 animals of 40–220 g (av. 90 g) were stocked with (fed) shrimp postlarvae in a 200 m<sup>2</sup> nursery pond on a mud and sand substrate. After three months 34 were harvested at 160–645 g (av. 353 g).

More recently in the same area, several hundred sandfish (most in the range 50–500 g) were bought from fishermen and stocked in ponds and pens. Preliminary results (R. Pitt, 2000, in preparation) indicate apparent growth rates in ponds of 2.2–3.2 g/day (varying inversely with stocking density in the range 106–170 g/m<sup>2</sup>). Survival was excellent in

ponds (88–97%) until the start of the wet season, which was rapidly followed by massive mortalities. In a pen stocked at about 500 g/m<sup>2</sup> there was 98% survival but virtually no growth, while growth and survival were both fair (1.7 g/day and 90%) in a pen holding about 390 g/m<sup>2</sup> of sandfish.

Growth and survival data of wild or released cultured animals in the natural environment is also scarce. Shelley (1985) sampled sandfish in Bootless Bay near Port Moresby, Papua New Guinea for over a year. From size frequency measurements he estimated growth rate as 14 g/month and reef production of sandfish as nearly 500 kg/year. Battaglione released large adults collected elsewhere in Ndoma Bay near CAC where none had been found previously; most could be found many months later in the same vicinity. Hamel et al (2000, in preparation) released hatchery-reared juveniles on different substrates in areas of NW Guadalcanal, Solomon Islands where no sandfish had previously been found and were apparently able to observe very high growth rates of 10–15 cm/month on subsequent visits.

On the other hand, Dance (pers. comm.) had difficulty finding hatchery-bred animals (mainly in the 25–35 mm) soon after release. From 15% to 95% were missing after only 24 hours on two different seagrass release sites near Gizo, Solomon Islands. In further trials, Dance et al (2000 in review) released batches of hatchery-bred and nursed sandfish juveniles of 20–74 mm (av. 36 mm) on fine sand, or silt and fine sand areas in coral reef-flat or mangrove-seagrass sites. They then observed survival and fish behaviour at the sites. On the coral reef-flat sites mean survival was as low as 37.5% one hour after release and total mortality occurred at two of three sites within 48 hours. At mangrove-seagrass sites, only 0–5% were injured or eaten within an hour, and 70% were found alive after three days. Predation, by triggerfish, emperors and breams (Balistidae, Lethrinidae and Nemipteridae) could be prevented by the use of a small enclosure, but growth and longer-term survival were not measured.

## Prospects and problems

### *Broodstock management*

In principle, eggs can be obtained either from wild or captive broodstock. However where populations are already depleted accessible wild stocks of large animals may be hard to find. Methods for maintaining adequate captive stocks which can be reliably spawned have not yet been demonstrated. In the absence of effective prepared diets, quite large areas of tanks or pens are likely to be needed. If ex-

isting ponds (for example shrimp ponds) prove suitable for broodstock maintenance, and in particular if sandfish are found to be compatible with locally farmed species (both biologically and in terms of management) this may provide an economical solution.

### Larval rearing

The use of larger tanks could have many benefits. They require relatively less labour. Physically and biologically they are more stable so a low-cost building or even just a roof should suffice. It may be possible to replace complete drain-down water changes by partial batch changes or continuous-flow, further reducing labour costs. Daylight can probably replace high levels of artificial lighting.

### Phytoplankton

It should be possible to produce some or all of the algae in outdoor cultures. This would save a great deal of the capital and running costs involved in autoclaving large volumes of water, lighting the cultures artificially and removing the heat produced by the lights. Cultures have to be kept sufficiently free of contamination to survive for long enough to be useful, and also to prevent them becoming sources of unwanted organisms which can infest larval rearing tanks. Since water for algae culture is to be fertilized and then stored it probably needs more careful treatment than that for larval rearing, for example additional filtration, UV treatment, chlorination-dechlorination or heating to 60–80°C.

### Nursery

Large outdoor tank areas are currently needed because there is as yet no effective prepared diet. For early nursery about 3–5 m<sup>2</sup> (of bare floors plus walls) are required per thousand 20 mm (~1 g) juveniles although some space can probably be saved by using additional conditioned plates and by frequently grading out the larger animals. For later nursery, 30–50 m<sup>2</sup> of tanks with fine sand would be required per thousand 50 mm (~10 g) juveniles, which might be a suitable size for on-growing in ponds. Correspondingly more tank space will be needed if bigger animals are required for pen culture or release. This is likely to be prohibitively expensive unless ponds, pens or seabed cages can replace tanks at later stages in the nursery process.

### Farming

Big areas of ponds have been constructed for shrimp farming, in Asia and elsewhere. Many shrimp farms now struggle with serious disease

problems. Other species and different systems of culture are of interest if they can reduce these problems and provide alternative crops. Sandfish ingest large amounts of substrate and may improve water conditions by removing organic detritus. Even if only a small fraction of existing shrimp ponds can be maintained at salinities suitable for sandfish, and if systems can be developed to farm them either alone or in polyculture with fish, crustaceans or molluscs, the production potential will be considerable.

### Restocking

Apart from the limited experiments described by Dance et al. (2000) and Hamel et al. (2000), there is still very little information on the survival of released animals. ICLARM has recently received funds from the Australian Centre for International Agricultural Research (ACIAR) to develop optimal release strategies for cultured sandfish in restocking programs. Arrangements are being made to do this work in New Caledonia in collaboration with the Provincial Governments, IFREMER, the Australian Institute of Marine Science and Melanesian fishing communities. The project will focus on identifying the optimum size-at-release, stocking density, release habitat and times for release. The possibility of using commercial shrimp farming ponds to mass-produce juvenile sandfish will be investigated.

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# The Galapagos sea cucumber fishery: a risk or an opportunity for conservation?

Priscilla C. Martinez<sup>1</sup>

The increasing demand for sea cucumbers from Asian markets and their over-exploitation in the Western Pacific has caused this fishery to move into the eastern Pacific as far as the Galapagos in recent years. The Galapagos Islands are known worldwide for their unique fauna and flora. About 97% of the terrestrial area of the Galapagos was declared a National Park in 1959, and the marine and coastal environments were declared a Marine Reserve in 1986. Since the establishment of the Galapagos National Park, the Government of Ecuador has made efforts to maintain the integrity of the islands and to protect their unique environments. This contributed to the declaration of the Galapagos Islands as a World Heritage Site by UNESCO in 1978, and later in 1985 its recognition as a Biosphere Reserve. However, new pressures from the mainland threaten the well-being of the Galapagos, including the sea cucumber fishery.

The exploitation of sea cucumbers started in the Galapagos in the early 1990s and concentrated on one species, *Stichopus fuscus*. This new fishery was introduced by Asian entrepreneurs that settled in mainland Ecuador around 1989. After depleting *Stichopus fuscus* populations along the coast of Ecuador, they moved their operations to the Galapagos, carrying with them a flux of people looking for new opportunities. From the beginning, this fishery developed without baseline biological data, and without a monitoring plan. It has had an enormous socioeconomic impact, resulting in increased human migration to the Galapagos, illegal fishing, and violations of the regulations of the National Park. For all these reasons the Government of Ecuador in 1992 prohibited fishing for sea cucumbers in the Galapagos by a presidential decree.

Nevertheless, an illegal fishery continued. In 1994, an experimental two month artisanal fishery was allowed. This harvest was closed one month after it started, due to a lack of commitment by the fishers to the rules imposed by the National Park and Fisheries authorities.

Sea cucumber fishing was then banned in the Galapagos. But the clandestine fishery continued, due to poor enforcement. Meanwhile, population studies of the sea cucumber *Stichopus fuscus*, by researchers at the Charles Darwin Research Station,

revealed a continuous decline in the number of sea cucumbers. The scientific and conservation community urged the Government of Ecuador to dedicate more effort to the conservation of the Galapagos Marine Environment.

As a response, in 1996, a consensus-based, participatory management process, with representatives of various stakeholders was established. The stakeholders include the fishing, tourism, science and education sectors, and National Park authorities. This group has maintained regular meetings to review and develop sustainable management and conservation policies. Their work resulted in the approval of a special law for the Management of the Galapagos Islands, signed by the president of Ecuador in 1998, and the second Marine Management Plan of the Galapagos, which was legally approved by the executive in 1999. With these important achievements, the Galapagos now have a legal framework for conservation and resource management in the Marine Reserve.

In the meantime, the artisanal fishing sector continued to petition the Government for the reopening of the sea cucumber fishery. Their petition was accepted, and a two month sea cucumber fishery was opened during April and May 1999. The Galapagos National Park Service, the Charles Darwin Foundation, the National Fisheries Institute, and the Ecuadorian Navy carried out the control and management of this fishery with the collaborative support of the Artisanal Fisheries sector. This arrangement made it possible to organise an inter-institutional working team to plan, coordinate, control and follow up the whole fishing activity and the commercialisation of the product.

Approximately 795 fishermen and 222 artisanal vessels participated in this fishery. A total of 4,401,657 sea cucumbers were exported from the Galapagos during this two month harvest season, representing a total of over 122 metric tonnes of dry product, worth USD 3.4 million. There were many lessons, both positive and negative, learned during this first fully organized and monitored sea cucumber fishery, and a very important point was that a new fisheries management and control system was established and respected by most fishermen and local community. This was a big step forward.

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This year, a new sea cucumber season was included in the fishing calendar, from May to July. On the basis of past experience, many efforts have been made to conduct this fishery with a better management and control system. A fixed quota and a zoning plan with “no take” areas have been established. However, just a few days before the sea cucumber season started, a group of fishermen revolted in protest against the rules established by the authorities. They took by force the National Park and Charles Darwin Research Station offices on Isabela Island, and removed tortoises from the rearing center as ransom in an attempt to get the rights to fish more sea cucumbers. Fortunately, the government kept to the rules established by the management authorities and the threat was unsuccessful. The tortoises were recuperated and actions have been taken against those responsible for this criminal act.

Although, this type of event caused instability and raised questions about the management process, not all the fishermen support this kind of deed. The leader of the fisheries cooperative of Santa Cruz Island declared his rejection to this action and expressed his total support to the participatory management process. There is a long way to go in this slow process but a basis for conservation has been established in the Galapagos.

It is hoped that this new strategy of conservation based on participatory management will not be another human experiment with nature, but the beginning of a solid basis for protection of the Galapagos as a whole.

## The process of asexual reproduction by transverse fission in *Stichopus chloronotus* (greenfish)

Sven Uthicke<sup>1</sup>

### Introduction

*Stichopus chloronotus* (greenfish) is generally considered a low value beche-de-mer species. However, due to overfishing of high commercial species worldwide (Conand and Jacquemet 2000) it is likely to become more important in tropical fisheries. This species is one of eight aspidochirotide species known to have asexual reproduction by transverse fission in addition to sexual reproduction by broadcast spawning (Harriott 1980; Conand et al. 1998; Uthicke 1997; Uthicke et al. 1999). Asexual reproduction is a seasonal event mainly occurring in winter and is an important means of population size maintenance in this species (Uthicke 1997; Uthicke et al. 1998). Therefore, information on this reproductive mode is important for the sustainable management of the fishery for this species.

Most holothurian species with asexual reproduction follow the “twisting-and-stretching” mode (Emson and Wilkie 1980): the anterior and posterior sections slowly rotate in opposite directions, resulting in a constriction in the holothurian. In a second step, the two halves slowly move in opposite directions, until the bodywall tears at the constriction and the two halves become completely separated.

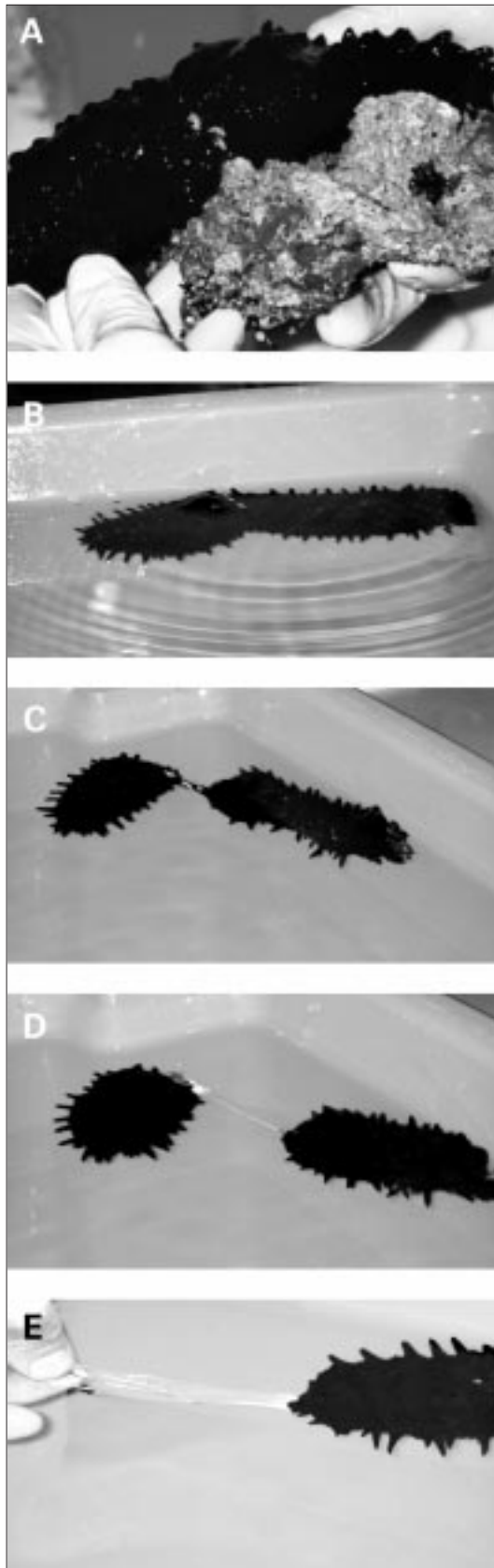
This process had not been previously observed in *S. chloronotus*. Observations on this process are reported here.

### Observations

During routine surveys of holothurian populations on Lizard Island (Great Barrier Reef) on 8 June 2000 at 14h00, I observed on the shallow reef flat a specimen of *S. chloronotus* that appeared constricted slightly anterior to the middle and showed some white tissue at the constriction (Fig. 1A). I carefully collected this individual and transported it to a nearby aquarium with running seawater. After some initial activity, this specimen remained nearly stationary on the wall of the aquarium for about four hours. At 19h30, the constriction became slightly more distinct, and the animal started to move (Fig. 1B). Shortly after, the posterior half of the individual remained stationary, while the anterior end continued to move forward. This resulted in a more distinct constriction (Fig. 1C). At this point, the bodywall at the fission site was nearly liquid, and the two body parts separated apparently without effort, remaining connected only with a string of mucus for about 30 seconds (Fig. 1D). The entire process of fission lasted only for about five minutes. The bodywall at the fission

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site remained a liquid or mucus like consistency (Fig. 1E) for at least two more hours. The following morning, the bodywall had its normal consistency and the wounds at both ends were nearly entirely closed.



This process took place during a period of intense asexual reproduction in the field. In a nearby population of *S. chloronotus* I observed 15.5% of all specimens being products of asexual reproduction just three days before the animal described here was collected (Table 1). Only four days later, this percentage had increased to 23.2 (Table 1). Most of these individuals appeared recently devided, and only one animal was in the process of regenerating (one anterior section with regenerating posterior end; sensu Conand et al. 1998).

### Discussion

To my knowledge, this is the first description of the process of asexual reproduction in *S. chloronotus*. However, the observations are based on only one individual and were done in an aquarium, and should thus not be over interpreted.

Asexual reproduction in the individual *S. chloronotus* did not follow the twisting-and-stretching mode, as described for species of the genus *Holothuria* (Emson and Wilkie 1980), which may take up to several hours (personal observations). Instead, the mechanical properties of the body wall allow this organ to become semi-fluid, and the sections may separate rapidly, apparently with minor effort simply by forward movement of the anterior section.

The mechanical properties of the bodywall of many holothurians have fascinated physiologists for a long time, and are well described for *S. chloronotus* (Motokawa 1982, 1984). Connective tissue in holothurians (and other echinoderms) is named “catch-connective-tissue” (Motokawa 1984) or

**Table 1:** Observations on the frequency of fission products (“post-fission”) in a population of *Stichopus chloronotus* on Lizard Island, Great Barrier Reef.

	05 June 2000		09 June 2000	
	N	%	N	%
Total observed	110		112	
Intact individuals	93	84.5%	86	76.8%
Post-Fission	17	15.5%	26	23.2%

**Figure 1:** Process of asexual reproduction in *S. chloronotus*. An individual shows a slight constraint in the body wall and some tissue damage (A). Once the animal becomes active (B), the anterior section moves away from the posterior section (B), until they are only connected by a thin string of mucus (C, D) and finally separate after about five minutes. The bodywall at the fresh “wounds” remains liquid for several hours (E).



“mutable collagenous tissue” (Wilkie 1984). These tissues may contract or expand nearly instantaneously without the action of muscles, probably under control of the nervous system (Wilkie 1984). *S. chloronotus* is a primary example for these properties. When rubbing animals of this species, the whole bodywall may disintegrate within minutes, and handling therefore often poses a problem for beche-de-mer fishermen.

The function of the catch connective tissue may mainly be associated with locomotion. *S. chloronotus* was shown to escape through shedding of a fraction of the bodywall when attacked by gastropods (Kropp 1982), which may also be facilitated by the connective tissue. It appears that, at least in *S. chloronotus*, another important function of the catch connective tissue is to aide in asexual reproduction by transverse fission and to warrant rapid wound healing.

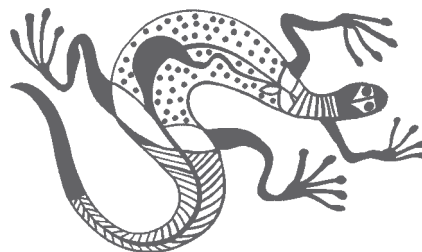
The main fission period for *S. chloronotus* on the Great Barrier Reef (Uthicke 1997) and on La Réunion Island (Conand et al 1998) is in winter. The findings presented here confirm that fission activity in June is very high. In fact, the very small number of individuals in the process of regeneration suggest that fission may have commenced just prior to the beginning of the observations. I previously inferred that asexual reproduction in *S. chloronotus* occurs mainly at night (Uthicke 1997). Although I observed the first indications in the early afternoon, the observations presented here seem to corroborate this. However, due to the rapid speed of the process, and the fact that I only observed one individual, it cannot be excluded that some fission during daytime may have been overlooked.

## Acknowledgements

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## Spawning observations

communicated by Sumaitt Putchakarn<sup>1</sup>

During my last field trip to Koh Samet (“Koh” means island), eastern coast of Thailand on 22–24 September 2000, I observed holothurians spawning. The species was *Holothuria (Thymioscycia) impatiens*. I found both females and males. I think this species might couple one by one because I did not see any holothurians in a 2–3 meter area (if I am right). The distance between males and females was about 80 centimeters and the size of the holothurians was about 15 centimeters in length.

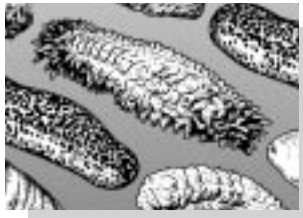
I found this appearance on the afternoon of 23 September, which is the late rainy season in Thailand. There was heavy rain in the morning but the sea was calm with no current. It was my first time to observe holothurians spawning.

Three pictures of sperm released by these holothurians are on the webpage:

<http://www.nrm.se/ev/dok/thaiechinod.html.en>.

This web page is maintained by Dr. Sabine. Unfortunately, I could not take any photos of females.

I would appreciate it if anybody can give me information on the coupling of holothurians in their natural habitat and discuss holothurian spawning.



## correspondence beche-de-mer

### 1- From Jerry Comans

(Project Officer, Hervey Bay Dugong And Seagrass Monitoring Programme, 22 Byron St, Scarness, Hervey Bay, Australia 4655)

I am Project Officer for a seagrass watch program in Hervey Bay, Queensland, Australia. Can you advise if you have any information on the relationship of beche-de-mer to seagrass meadows? In our area we

know that when seagrass dies out, the beche-de-mer disappear. Conversely would the depletion of stocks have an impact on seagrass meadows?

### 2- From Norman Reichenbach

(nreichen@liberty.edu)

Hope you are well. I am working on amphibians these days. You may already be using or know about elastomer tagging methods. We are using them on amphibians for permanent marks and they are working well.

It seems to me these should be tried on sea cucumbers. I think it might be a way to permanently mark them. The company that markets the elastomer has

a web site: [www.nmt-inc.com](http://www.nmt-inc.com). You can contact Mary Woodgate who works for the company.

You should be able to inject it directly into the muscle mass near the surface where it would be visible. The company sends out a free test kit to try it out. Unless there is a good way to permanently mark the sea cucumbers, I think it's worth a try. They test on different organisms to see if their product works.

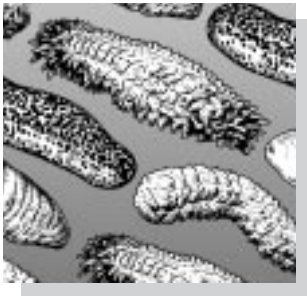
<sup>1</sup> Institute of Marine Science, Burapha University, Bangsaen, Chonburi 20131. Thailand. Tel.: +66 38 391671-3; Fax : +66 38 391674; E-mail: [sumaitt@bucc4.buu.ac.th](mailto:sumaitt@bucc4.buu.ac.th) or [sumaitt@dolphin.BIMS.buu.ac.th](mailto:sumaitt@dolphin.BIMS.buu.ac.th)

They also have some other marking methods that involve a tiny wire placed in the organism that can be detected with a scanner which energizes the

wire to send a signal to the scanner with the number encoded in the wire. I have not used the latter technique.

### 3- From Peter Howard (phishypete@yahoo.com)

Please email any information on the commercial viability of the cucumbers found in the waters off the Virgin Islands.



abstracts, publications,  
workshops & meetings  
beche-de-mer

## 1. Abstracts

### Induction of spawning in the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea)

Andrew Morgan  
(a.morgan@auckland.ac.nz)

(Source: *Journal of the World Aquaculture Society*. 31(2):186-194. (2000))

With the advent of sea cucumber aquaculture in the South Pacific region a reliable method is needed to induce large numbers of animals to spawn in captivity. Broodstock of the sea cucumber *Holothuria scabra*, collected from Stradbroke Island, Moreton Bay (27°30'N, 153°24'E) Australia, during the reproductive season from October to January, and used in spawning trials. During the 1997/98 summer between one to five weeks of captivity, 100% of animals were induced to spawn in four trials at dusk on or close to a new or full moon, using 9 males and 9 females contained in a Reln tank and 30 cm of filtered sea water, using a 3 to 5°C temperature shock. *H. scabra* was induced to spawn in small numbers during the 1996/97 summer despite a marked degree of weight loss and all animals spawned during the 1997/98 with minimal loss of weight. The difference in the number of spawned eggs between animals of similar size and mean numbers of spawned eggs in consecutive trials decreased the longer animals were held in captivity before spawning. The hatch rate of eggs was reduced significantly for broodstock held for more than one month. Hatch rate and numbers of spawned eggs are important indicators of egg viability of broodstock maintained in captivity for an extended period.

### The auricularia-to-doliolaria transformation in two aspidochirote holothurians, *Holothuria mexicana* and *Stichopus californicus*

Thurston C. Lacalli and Jenifer E. West

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(Source: Communicated by Communicated by Vicki Pearse Editor, *Invertebrate Biology*)

The fragmentation and rearrangement of the ciliary bands that occurs during the auricularia-to-doliolaria transformation is described for the non-feeding auricularia larva of *Holothuria mexicana* and the more typical planktotrophic auricularia of *Stichopus californicus*. The ciliary band of the auricularia larva runs along a series of ridges that project from the sides of the body. Fragmentation results from a loss of ciliary band cells from the zones between the ridges. The remaining fragments then reorient, elongate, and fuse to form the 5 circumferential bands of the doliolaria. The fate of the band cells lost during this process could not be determined with certainty, but they disappear after being sequestered beneath the epithelium for a time, probably through histolysis. Cell counts indicate that significant numbers of cells are also lost from the ridges. Normal swimming ceases just before transformation begins, probably because the nerve supply to all or parts of the band is disrupted, and this may play a role in initiating morphogenesis.

### Ossicle change in *Holothuria scabra* with a discussion of ossicle evolution within the Holothuriidae (Echinodermata)

Claude Massin<sup>1</sup>, Annie Mercier<sup>2,3</sup> and Jean-François Hamel<sup>3</sup>

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(Source: *Acta Zoologica (Stockholm)* 81: 77–91 (January 2000))

Ossicles of *Holothuria (Metriatyla) scabra* Jaeger, 1833 vary mainly in early juveniles between 0.9 and 15 mm long. While ossicles were not observed in auricularia and doliolaria larvae, which instead possessed elastic balls, ossicles were present in late pentactulae. Specimens 0.9–1.5 mm long have tables with tall spire (4–5 cross beams), no buttons, and large irregular perforated plates. Specimens 5–6 mm long have tables with moderate spire (2–4 cross beams) and a few smooth buttons. Specimens 9–16 mm long have tables with low spire (1–2 cross beams) and knobbed buttons. From 30 mm, ossicles are similar to those of adults, with more buttons and fewer tables. Several features of the ossicles of early juveniles, including their size, shape and prevalence, are unique to the species. Comparison with holothurian juveniles of other species indicates that presence of tables with tall spire and absence of buttons are plesiomorph characters in the evolution of the Holothuriidae.

### *Cucumaria flamma*, a new species of sea cucumber from the central eastern Pacific (Echinodermata: Holothuroidea)

Francisco A. Solís-Marín and Alfredo Laguarda-Figueroa

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(Source: *Proceedings of the Biological Society of Washington* 112(4):778–786. 1999.)

*Cucumaria flamma*, n. sp. from the central eastern Pacific is of medium size with mouth and anus terminal, directed upward; a conspicuous deep antero-posterior groove in the dorsal skin extending from near base of tentacles to anus. Ventral skin ossicles are knobbed buttons and smooth perforated plates, dorsal skin ossicles are smooth perforated plates and smooth buttons. Distributed from Mazatlán, Sinaloa, México to Isla de la Plata, Ecuador from 4 to 12 m on rocky substrata.



## Mitotic activity of cells during muscle regeneration in a holothurian *Eupentacta fraudatrix*

I.Yu. Dolmatov and T.T. Ginanova

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(Source: *Russian Journal of Development Biology*, Vol. 29, No. 6, 1998, pp. 282-285. Translated from *Ontogenez*, Vol. 29, No. 6, 1998, pp. 459-462. Original Russian Text © 1998 by Dolmatov, Ginanova.)

The mitotic activity of cells during regeneration of the longitudinal muscle bands in a holothurian *Eupentacta fraudatrix* was studied by blocking cell division by colchicine. The regenerating tissues contained mitotically dividing cells. The highest mitotic index was noted in the coelomic epithelium covering the muscle. There, the mitotic index gradually increased in the course of regeneration (from  $0.45 \pm 0.23\%$  on the tenth day to  $1.18 \pm 0.45\%$  on the 17th day) and then fell. The mitotic index in the muscle was low during the entire period of observation: no more than  $0.19 \pm 0.07\%$ . Thus, the mitotic activity in the regenerating tissues is low and does not correspond to a high level of thymidine incorporation in the cells. The results obtained suggest that, despite the presence of mitoses, the contribution of cell proliferation to restoration of the longitudinal muscle bands in *Eupentacta fraudatrix* is low.

## Regeneration of longitudinal muscle bands in juvenile holothurians

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Institute of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences, ul. Pal'chevskogo 17, Vladivostok, 690041 Russia

(Source: *Russian Journal of Development Biology*, Vol. 29, No. 5, 1998, pp. 188–194. Translated from *Ontogenez*, Vol. 29, No. 5, 1998, pp. 354–361. Original Russian Text © 1998 by Dolmatov, Eliseikina.)

Regeneration of longitudinal muscle bands after their transaction in one month old holothurians *Eupentacta fraudatrix* was studied using light and electron microscopy. At this age, the muscles are not yet terminally differentiated and are at the third stage of their development. Myoepithelial bundles are formed in the area of lesion at the expense of the coelomic epithelium cells located nearby, while the myocytes per se do not take part in this process. Regeneration proceeds without mitotic cell division. On the whole, the mechanism of muscle restoration in one month old holothurians is similar to the initial stage of definitive myogenesis in this species and the muscle formed is structurally similar to that at the third stage of normal development. The conclusion was drawn that during regeneration of an organ which has not yet completed its development, the stage is restored at which this organ was at the moment of lesion.

## Sea cucumbers in demand

(Source: *Seafood New Zealand* 69 (July 2000))

A bizarre species and the last thing you would think had any commercial value, but products from the sea cucumber *Stichopus mollis*, along with a number of other temperate and tropical sea cucumbers are considered a delicacy in Japan and China and fetch high prices. Non-sustainable harvest levels in the Asia Pacific have seen the exploitation of species in temperate fisheries become a viable industry.

## Reproduction in the apodid sea cucumber *Patinapta ooplax*: semilunar spawning cycle and sex change

Tomoyuki Kubota

Department of Biology, Faculty of Science, Kagoshima University, Kagoshima 890, Japan

Source: *Zoological Science* 17: 75–81 (2000)

Many fertile individuals of the apodid holothurian *Patinapta ooplax*, living in the intertidal area of the Ichiki Fishing Port in southern Kyushu, Japan, spawned during the two days after every full and new moon, probably the first and second days, in the period from the middle of July to the end of August during 1990,

1992 and 1993. Matured individuals were divided into three sexual types: males, hermaphroditic males with an early stage of oocytes, and females, using a dissecting microscope. The distribution frequency and gonadal histology of these sexual types indicate that some individuals changed from male to female or in the reverse direction at two-week intervals between spawnings, and suggest that some change first from male to female, and then back to male during the main breeding season. In addition, it was found that during the main breeding season, synchronous gametogenesis occurred in association with the sex changes, and that the period from the initiation of spermatogonia proliferation to sperm release during the same season was two weeks, and the period from the initiation of oocyte growth to egg shedding was probably slightly longer than two weeks.

### Cuvieran tubules in tropical holothurians: usefulness and efficiency as a defence mechanism

Jean-François Hamel <sup>a</sup> and Annie Merciera <sup>b</sup>

<sup>a</sup>Society for the Exploration and Valuing of the Environment (SEVE), 655 rue de la Rivière, Katevale (Québec), Canada J0B 1W0;

<sup>b</sup>International Center for Living Aquatic Resources Management (ICLARM), Coastal Aquaculture Centre, P.O. Box 438, Honiara, Solomon Islands

(Source: *Mar. Fresh. Behav. Physiol.*, Vol. 33, pp. 115–139)

The tropical holothurians, *Holothuria leucospilota*, *Bohadschia argus* and *B. marmorata* responded to tactile stimulation by expelling Cuvieran tubules in proportion to the intensity of the stimulation. They were able to target the stimulated area with variable success depending on the location of the stimulus. Field surveys showed that 2.3–6.1% of *H. leucospilota* presented signs of having recently used their Cuvieran tubules and laboratory experiments revealed that they released tubules in response to several natural predators. The tubules did not adhere nor cause any distress to fish, but proved effective in discouraging attacks. Crabs, molluscs and echinoderms were entangled and also efficiently repelled. *H. leucospilota* without tubules were wounded and even killed by predators that were usually discouraged by tubule discharge. Conversely, after having induced the release of tubules once, 96% of the predators placed in the presence of *H. leucospilota* three days later remained at a distance. Released tubules that did not adhere to any surface were quickly retracted, while regeneration of a complete set of tubules took 15–18 days. The release of Cuvieran tubules by tropical holothurians therefore appears to be a sensitive defence mechanism. Data on *H. leucospilota* further suggest that they are readily used against predators in the field.

### Stress and deviant reproduction in echinoderms

John M. Lawrence and Joan Herrera

(Source: *Zoological studies* 39(3):151–171 (2000))

Normal reproductive characteristics in echinoderms include non-maternal nutrition with a planktonic feeding development stage, sexual reproduction with gonochorism, seasonal reproduction, and no protection of young by secondary metabolites. Deviant reproductive characteristics include maternal nutrition of the development stage that may be planktonic, demersal, or brooded; hermaphroditic sexual reproduction; intermittent or continuous low-level reproduction; protection of young by secondary metabolites; and asexual reproduction. We tested the hypothesis that stress, which causes a decrease in capacity for production, was a factor responsible for deviant reproductive characteristics by comparing taxa from the subphylum to species levels. In the examples used, deviant reproduction occurred in the taxa for which stress could be predicted. Although other factors undoubtedly affect reproductive characteristics, the analysis indicates that stress must be considered an important one.

## Settlement preferences and early migration of the tropical sea cucumber *Holothuria scabra*

Annie Mercier <sup>a,b,c</sup>, Stephen C. Battaglene <sup>b</sup>, Jean-François Hamel <sup>a</sup>

<sup>a</sup> Society for the Exploration and Valuing of the Environment (SEVE), 655 rue de la Rivière, Katevale, Québec, Canada J0B 1W0

<sup>b</sup> International Center for Living Aquatic Resources Management (ICLARM), Coastal Aquaculture Centre, P.O. Box 438, Honiara, Solomon Islands,

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(Source: *Journal of Experimental Marine Biology and Ecology* 249:89–110 (2000))

Settlement and post-settlement processes of the sea cucumber *Holothuria scabra* Jaeger were studied in the laboratory. Independent and paired choice experiments revealed that several substrates could induce metamorphosis into pentactulae, but that specific substrates favoured settlement. Leaves of seagrass *Thalassia hemprichii*, with or without their natural bio-film, yielded the highest settlement rates (4.8–10.5%). *T. hemprichii* was preferred as a settlement substrate over sand, crushed coral, several other plant species and artificial seagrass leaves with or without a bio-film. Only settlement on the seagrass, *Enhalus acoroides*, was similar to that recorded for *T. hemprichii*. In the absence of a substrate, the larvae delayed settlement for nearly 96 h and survival was less than 0.5%. Sand and crushed coral, either alone or together, induced settlement from < 1.5% of the available larvae. The pentactulae found on sand, coral and in bare containers were 10–35% smaller than those on *T. hemprichii* leaves. Soluble extracts from *T. hemprichii* and *E. acoroides* successfully induced metamorphosis and settlement on clean plastic surfaces. Newly settled juveniles remained on the seagrass leaves for 4–5 weeks before migrating to sand at around 6 mm in length. Prior to this, the juveniles spent 4–5 days moving on and off the leaves. Once on the sand, the juveniles became deposit-feeders, but did not show the typical burrowing behaviour of older specimens until they reached around 11 mm in length. The larvae of *H. scabra* appear to actively select seagrass leaves, possibly through chemical detection. We hypothesise that larvae settling on seagrass have an increased chance of growth and survival because they are provided with a suitable substrate on which to grow, and a bridge to sand substrates as they become deposit-feeders.

## Induction of spawning in the sea cucumber *Holothuria scabra* (Echinodermata: Holothuroidea)

Andrew David Morgan

School of Marine Science, University of Queensland, Australia

(Source: *Journal of the World Aquaculture Society*, Vol. 31, No. 2. (June 2000))

With the advent of sea cucumber aquaculture in the South Pacific region, a reliable method is needed to induce large numbers of animals to spawn in captivity. Broodstock of the sea cucumber *Holothuria scabra*, collected from Stradbroke Island, Moreton Bay (27°30'N, 153°24'E) Australia, during the reproductive season from October to January, were used in spawning trials. During the 1997–1998 summer between one to five weeks of captivity, 100% of animals were induced to spawn in four trials at dusk on or close to a new or full moon, using nine males and nine females contained in a Reln tank and 30 cm of filtered sea water, using a 3–5°C temperature shock. *H. scabra* was induced to spawn in small numbers during the 1996–1997 summer despite a marked degree of weight loss, and all animals spawned during 1997–1998 with minimal loss of weight. The difference in the number of spawned eggs between animals of similar size and mean numbers of spawned eggs in consecutive trials decreased the longer animals were held in captivity before spawning. The hatch rate of eggs was reduced significantly for broodstock held for more than one month. Hatch rate and numbers of spawned eggs are important indicators of egg viability of broodstock maintained in captivity for an extended period.



## Consumption and assimilation of salmon net pen fouling debris by the Red Sea cucumber *Parastichopus californicus*: implications for polyculture

Molly O. Ahlgren

Science Division, Sheldon Jackson College, Sitka, Alaska 99835 USA

(Source: *Journal of the World Aquaculture Society*, Vol. 29, No. 2. (June 1998))

Fouling debris composed of fish feces, excess fish food, algae, and other particulate organic matter can create environmental problems for aquaculture facilities that rear fish in ocean net pens. Accumulations of organic debris can clog the nets and restrict water circulation which in turn can stress fish. Experiments in which red sea cucumbers *Parastichopus californicus* were allowed to feed inside floating net pens at a salmon rearing facility in Southeast Alaska showed that sea cucumbers consumed fouling debris and cleared a significant amount of surface area on the nets ( $P < 0.0001$ ). Sea cucumbers assimilated amino acids and other organic matter from fouling debris two or three times more efficiently than from their natural sediment diet. Muscle development of sea cucumbers consuming fouling debris inside the net pens was also significantly greater than that of sea cucumbers feeding in their natural environment ( $P < 0.0003$ ). This work suggests that polyculture operations in which commercially important detritivores, like the red sea cucumber, are grown in net pens along with salmon could possibly convert the net from self-fouling to self-cleaning and could turn fouling debris into a marketable product (sea cucumber biomass).

## Aspects of the reproductive cycle of the sea cucumber *Holothuria scabra* (Echinodermata: holothuroidea)

Andrew David Morgan

(Source: *Bulletin of Marine Science*. 66(1): 47–57. (2000))

The economically valuable sea cucumber, *Holothuria scabra* (sandfish) is distributed throughout the Indo-Pacific in subtidal estuarine habitats and exploited heavily for markets in the Asia-Pacific region. With interest growing in the aquaculture of this animal, it is essential to understand the mechanisms regulating the reproductive cycle and refine husbandry practices. From July 1996 to December 1997 the reproductive cycle of male and female *H. scabra* was followed for a local population at Stradbroke Island, Moreton Bay (27°30'N, 153°24'E) Australia. The gutted weight gonad index peaked in November of both years while no obvious progression of oocyte size was noticed. The gametogenic cycle coincided with a period of body wall weight loss from mid-winter to mid-summer. During gametogenesis there was a continuous presence of mature oocytes that were either reabsorbed or spawned during or prior to the vitellogenic period from September to November. It was likely that stored nutrients in the body wall were used for gametogenesis during the latter part of winter and that in response to its environment, oocyte production was regulated by phagolytic activity. If at any point in the gametogenic period, naturally occurring oocyte vitellogenesis in collected animals could be bolstered in some individuals and the onset of maturation (gametogenesis) triggered in others then individual *H. scabra* could be induced to spawn out of season.

## The sea cucumber fishery of Saipan, Northern Mariana Islands

Trianni, M.

Commonwealth of the Northern Mariana Islands Division of Fish and Wildlife. Email mtsdfw@itecnmi.com

(Source: 9th International Coral Reef Symposium, Bali 10/00, Abstracts. p. 209)

A sea cucumber fishery targeting the surf redfish, *Actinopyga mauritiana* and the blackteatfish, *Holothuria nobilis*, occurred in the island of Saipan in the Commonwealth of the Northern Mariana Islands (CNMI) during 1996 and 1997. A pre-harvest stock assessment was not conducted and the fishery was managed based on catch-effort statistics. The fishery was temporarily halted in early 1997 due to declining CPUE. A subsequent analysis of catch-effort statistics was conducted using three depletion models; the Leslie, DeLury, and an unbiased likelihood estimator derived from the Leslie, termed the Akamine model. These models indicated that the remaining population numbers in the fishery management units were consider-

ably harvested, with 78% to 90% of the initial population sizes taken. The fishery was subsequently shut down and a post-harvest survey conducted by the CNMI Division of Fish and Wildlife supported the depletion model analysis results. Results from the depletion models varied, with the Leslie and DeLury failing to produce valid results for all management units. The Akamine model was preferred for any future depletion estimation analyses, although a pre-harvest stock assessment along with the collection of harvest statistics was concluded essential for coherent management.

### **Overfishing of holothurians on the GBR: the effects on population structure of *Holothuria nobilis*, and likely sources of new recruitment**

Sven Uthicke\* and John A.H. Benzie

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(Source: 9th International Coral Reef Symposium, Bali 10/00, Abstracts. p. 209)

Decreasing catch rates for *H. nobilis* on the GBR prompted management agencies to close the fishery on this species in October 1999. We surveyed densities and size structures of *H. nobilis* at 20 reefs and investigated the generic connectivity between reefs using allozyme and mitochondrial DNA (mtDNA) analyses. Densities of *H. nobilis* on four reefs protected from fishing were about 4 times higher compared to 16 reefs open to fishing and the average weight of the animals was significantly smaller on fished reefs. The study of 7 polymorphic enzymes revealed no significant population differentiation between reefs separated by distances up to 1300 km. This may indicate a high potential for larval dispersal and that larvae from populations or regions which were not fished could re-colonise overfished reefs. However, conclusions based on the allozyme data may be flawed because the allozymes may not be in evolutionary equilibrium due to the relatively young age of the reef. Preliminary studies using higher resolution genetic markers (mtDNA sequences) have indicated that population differentiation may exist even on small geographic scales. Therefore, a recommendation on the scale on which the beche-de-mer fishery on the GBR should be managed can only be made after final analyses of the mitochondrial markers.

### **Asexual reproduction in holothurian (holothuroidea): a comparison between Pacific (GBR, Australia) and Indian Ocean (La Réunion) populations of *Stichopus chloronotus*. (poster)**

Conand C.\* & Uthicke S.

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(Source: 9th International Coral Reef Symposium, Bali 10/00, Abstracts. p. 300)

*Stichopus chloronotus* is a widespread holothurian species, in the Indo-Pacific which often occurs in high population densities. Its asexual reproduction by fission was monitored in several populations from the Great Barrier Reef (GBR, Australia, Pacific Ocean) (Uthicke, 1997) and from La Réunion fringing reefs (France, Indian Ocean) (Conand et al., 1998). The results obtained at these locations are compared, to investigate similarities in the fission pattern between the two geographic regions. Fission rates showed distinct seasonality, with maxima occurring during the cold season (May to July), both in La Réunion and the GBR. The processes of external and internal regenerations have been described in view to understand the resumption of the nutrition and the sexual reproduction after fission. Annual fission rates and population densities are positively correlated in all populations studied. In contrast, annual fission rates and modal sizes in the populations are negatively correlated. The consequences of fission in terms of density and size of the individuals are discussed. Several abiotic factors, which may differ between species, have been hypothesized to (part of the word missing)ger fission in holothurians. For *S. chloronotus* we suggest that food availability and population densities may be involved in the regulation of asexual reproduction. However, the comparison of more stations remains necessary to substantiate this hypothesis.

### Reproduction and growth of *Isostichopus fuscus* (Echinodermata: Holothuroidea) in the southern Gulf of California, México

M.D. Herrero-Pérezrul, H. Reyes Bonilla, F. Garcia-Dominguez and C.E. Cintra-Buenrostro

(Source: *Marine Biology* 1999:521–532)

The reproduction and growth of *Isostichopus fuscus* (Ludwig, 1875) at Isla Espiritu Santo, Baja California Sur, México from July 1992 to September 1993 and from August 1996 to July 1997 were analyzed. The reproductive cycle was monitored by using histological analysis. Five gonadal stages were described according to cell types present in the gonad: undetermined, gametogenesis, maturity, spawning and post-spawning. This holothurian reproduces annually during summer (July to September), when sea surface temperature reaches 27°C. The gonad index is related to the gonadal stages. The first sexual maturity for *I. fuscus* was calculated at 367 g (4 to 5 years old) according to the von Bertalanffy equation, and nearly all the specimens at Isla Espiritu Santo were 5 to 7 years old (including some individuals over 35 years old). Data of the length-weight relationship showed that *I. fuscus* grows allometrically at the studied site ( $b = 1.83$ ). The mortality rate was relatively high ( $M = 0.51$ ), indicating that in a typical cohort, 40% of the recruits does not survive the first year, and less than 20% reaches maturity.

### Population density, distribution and consumption rates of three corallivores at Cabo Pulmo Reef, Gulf of California, Mexico

Héctor Reyes-Bonilla & Luis Eduardo Calderon-Aguilera

(Source: *Marine Ecology*, 20(3-4): 347-357 (1999))

The influence of Corallivores on coral community structure of eastern Pacific reefs has been considered less important than that of abiotic oceanographic factors. The data that support this assumption, however, are only available for Central American reefs. To assess the role of predation on hermatypic corals in a different regional reef environment, the abundance, spatial distribution and consumption rate of three corallivores: the echinoid *Eucidaris thouarsii* (Valenciennes), the asteroid *Acanthaster planci* (Linnaeus) and the teleostean fish *Arothron meleagris* (Bloch & Schneider), were estimated at Cabo Pulmo reef, Gulf of California, Mexico (23°25'N, 109°25'W). Statistically, the abundances of the species did not change in any sections of the reef (mean values: *E. thouarsii*, 0.17 indiv/m<sup>2</sup>; *A. planci*, 1.9 indiv/m<sup>2</sup>; *A. meleagris*, 39 indiv/ha). The average daily individual consumption rates of coral were calculated at 1.83 g CaCO<sub>3</sub>/m<sup>2</sup> for *E. thouarsii*, 118.4 cm<sup>2</sup> for *A. planci*, and 16.38 g CaCO<sub>3</sub>/m<sup>2</sup> for *A. meleagris*, and were lower than those reported for Central American reefs. Considering the mean estimated carbonate production (7.9 kg CaCO<sub>3</sub>/m<sup>2</sup>/a), corallivores eliminate less than 4% of the coral standing stock of Cabo Pulmo reef. The low corallivore population density and consumption rates, together with high local coral cover, indicate that corallivores are not key factors determining scleractinian abundance in this marginal reef.

### Hatchery and Culture Technology for the sea cucumber, *Holothuria scabra* Jaeger, in India

D.B. James

(Source: *Naga, the ICLARM Quarterly* (Vol. 22, No. 4) (October-December 1999))

The seed of the sea cucumber *Holothuria scabra* Jaeger is being produced at the Central Marine Fisheries Research Institute in India. This article describes the techniques being used in the production of the seed and the experiments being carried out for the rearing of juveniles. Trials to grow juveniles in hatcheries on prawn farms have shown spectacular results that are both cost efficient and environmentally friendly.



## Cultural of tropical sea cucumbers for stock restoration and enhancement

S.C. Battaglione

(Source: *Naga, the ICLARM Quarterly* (Vol. 22, No. 4) (October-December 1999))

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 relative 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 ( $\pm 0.03$  s.e.) and range from 0.2 to 0.8 mm/day, 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.

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### Sea cucumber farming in New Zealand

Andrew Morgan

(Source: *Austasia Aquaculture* – 54 (August/September 2000))

### A glimpse into some sea cucumbers in Panay, Philippines

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(Source: *SEAFDEC Asian Aquaculture* Vol. XXII No. 3 (May-June 2000))

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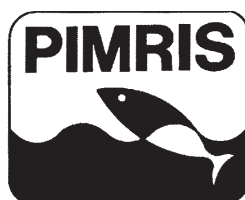
## 2. MEETINGS

**1- The 9th International Congress on reproduction and development (ICIRD)** will be held at Grahamstown (Rhodes University, South Africa) from 15 to 20 July 2001. For more information, please visit the following web site: [www.rhodes.ac.za/conferences/icird2001/](http://www.rhodes.ac.za/conferences/icird2001/)

**2- The 6th European Conference on Echinoderms** will be held from 3 to 7 September 2001 in Banyuls France. For more information, please visit the following web site:

[http://www.obs-banyuls.fr/web/departs/feral/biolpop/6thECE/6th%20ECE\\_accueil.htm](http://www.obs-banyuls.fr/web/departs/feral/biolpop/6thECE/6th%20ECE_accueil.htm)

PIMRIS is a joint project of 5 international organisations concerned with fisheries and marine resource development in the Pacific Islands region. The project is executed by the Secretariat of the Pacific Community (SPC), the South Pacific Forum Fisheries Agency (FFA), the University of the South Pacific (USP), the South Pacific Applied Geoscience Commission (SOPAC), and the South Pacific Regional Environment Programme (SPREP). This bulletin is produced by SPC as part of its commitment to PIMRIS. The aim of PIMRIS is to improve



Pacific Islands Marine Resources  
Information System

the availability of information on marine resources to users in the region, so as to support their rational development and management. PIMRIS activities include: the active collection, cataloguing and archiving of technical documents, especially ephemera ('grey literature'); evaluation, repackaging and dissemination of information; provision of literature searches, question-and-answer services and bibliographic support; and assistance with the development of in-country reference collections and databases on marine resources.