

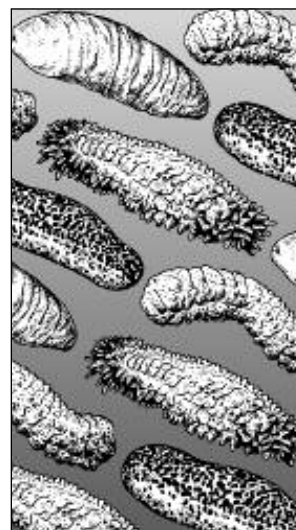


Secretariat of the Pacific Community

# BECHE-DE-MER

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



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## Editorial

It is a great pleasure to present the 22<sup>nd</sup> issue of the *SPC Beche-de-mer Information Bulletin*, which is particularly rich and diverse.

I draw your attention to the new database of all articles and abstracts published in the Bulletin to date. This was recently put together by SPC's Fisheries Information Section, and is available on SPC's website at: [http://www.spc.int/coastfish/news/search\\_bdm.asp](http://www.spc.int/coastfish/news/search_bdm.asp). The database includes close to 560 article and abstract titles that can be searched by title, author's name(s), scientific name, region or country. Each search result is presented with a hyperlink that allows downloading in pdf format. Your comments and suggestions on how to improve this useful tool are most welcome.

This issue of the Bulletin includes articles focusing on many different areas; in some cases, this is the first time research from these locales has appeared in the Bulletin (e.g. the Comoros, featured in the article by Samyn et al., p. 14; Mauritius, addressed by Laxminarayana, p. 48; and Sri Lanka, addressed by Terney, p. 24). These articles highlight the current interest in sea cucumbers in the western Indian Ocean. Contributions about Madagascar by Rasolofonirina and Jangoux (p. 6) and Mayotte by Conand et al. (p. 19) also serve to illustrate the progress in sea cucumber research in this region.

Identification of sea cucumber species is difficult. With CITES' conservation concerns, the issue of identifying processed specimens has become critical. One identification method involves special preparation of the body wall to examine the calcareous spicules. This method has been used in the Galapagos for *Isostichopus fuscus* (see article by Toral on p. 3).

Purcell presents advances from the important WorldFish Center, which is developing technologies for sandfish restocking (see article on p. 30).

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We continue to publish articles on asexual reproduction by fissiparity. Purwati presents her recent findings with *Holothuria atra* and *H. leucospilota* (p. 11) and Kohtsuka et al. write about their observation of a *Stichopus horrens* specimen undergoing fission (p. 23).

For this issue, we have not received answers to the different questionnaires presented in previous issues of the Bulletin, but we hope to receive your contributions for the next issue on the following important fishery-related subjects: overexploitation, sexual reproduction, recruitment and juvenile observations in the wild.

Proceedings of the 11<sup>th</sup> International Echinoderm Conference are now published as: Proceedings of the 11<sup>th</sup> International Echinoderm Conference, Munich, Germany, October 6–10, 2003: Heinzeller, T. and Nebelsick, J.H. (eds), Taylor and Francis Group, London, 633 p. You can order this publication through Taylor and Francis website (<http://www.tandf.co.uk/books/>, click on “Catalogue” in the menu on the left side and enter Echinoderms Munchen in the search box). Several abstracts of published papers are included in the Abstracts and New Publications section on p. 55 of this Bulletin.

The location of the 2006 International Echinoderm Conference has not yet been decided. We hope to be able to give this information in the next issue of the Bulletin.

All previous issues of the Bulletin are available from SPC’s website at: <http://www.spc.int/coastfish/News/BDM/bdm.htm> (English and French versions).

The latest issue of the “Echinoderm Newsletter” is available at: <http://www.nmnh.si.edu/iz/echinoderm>. And, as usual, you can access the Echinoderm Portal from: <http://www.nrm.se/ev/echinoderms/echinportal.html.en>

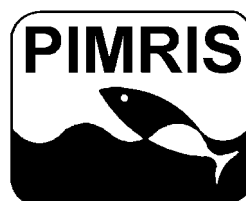
**Chantal Conand**

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*The views expressed in this Bulletin are those of the authors and are not necessarily shared by the Secretariat of the Pacific Community or the European Union.*



PIMRIS is a joint project of five 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 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.



# new info

beche-de-mer

## The use of calcareous spicules for the identification of the Galápagos sea cucumber *Isostichopus fuscus* on the international market

M. Verónica Toral-Granda<sup>1</sup>

### Introduction

About 40 species of commercial sea cucumbers are harvested throughout the world's oceans (Conand 2004). Once on the international market, the dried (processed) form of some species can be almost impossible to distinguish from others (Conand 2004). Sea cucumber taxonomy is based on anatomical features, such as the shapes and combination of microscopic spicules that are the skeletal components in the body wall of all sea cucumbers. These spicules can take widely different shapes, such as rosettes, C-shaped rods, buttons, and plates (Fig. 1) (Hickman 1998).

The sea cucumber *Isostichopus fuscus* (Ludwig, 1875) has been harvested commercially in the Galápagos Islands since 1991, where it has become the most important fishery resource (Murillo et al. 2004); it is commercially depleted in mainland Ecuador (Camhi 1995). Illegal fishing activities have been continuous in the Galápagos (Piu 1998, 2000; Martínez 1999; Sant 2004), fuelled mainly by international demand for this species and the economic needs of the local fishing sector. Although it is not among the most valuable sea cucumber species (Conand 2004), the Galápagos sea cucumber has been recog-

nized as an important food item in the Chinese market (J. Chen, Yellow Sea Fisheries Research Institute, China, pers. comm).

In the field, this species can easily be recognized by its thick, firm brownish body wall covered with blunt yellow papillae (Fig. 2) (Hickman 1998). However, once processed, it looks similar to some other species of the family, including *S. horrens*, which is harvested illegally together with *I. fuscus* (Arellano 2004). The spicules in the body wall of *I. fuscus* take the form of tables and C-shaped rods while the tube feet produce spicules shaped like end-

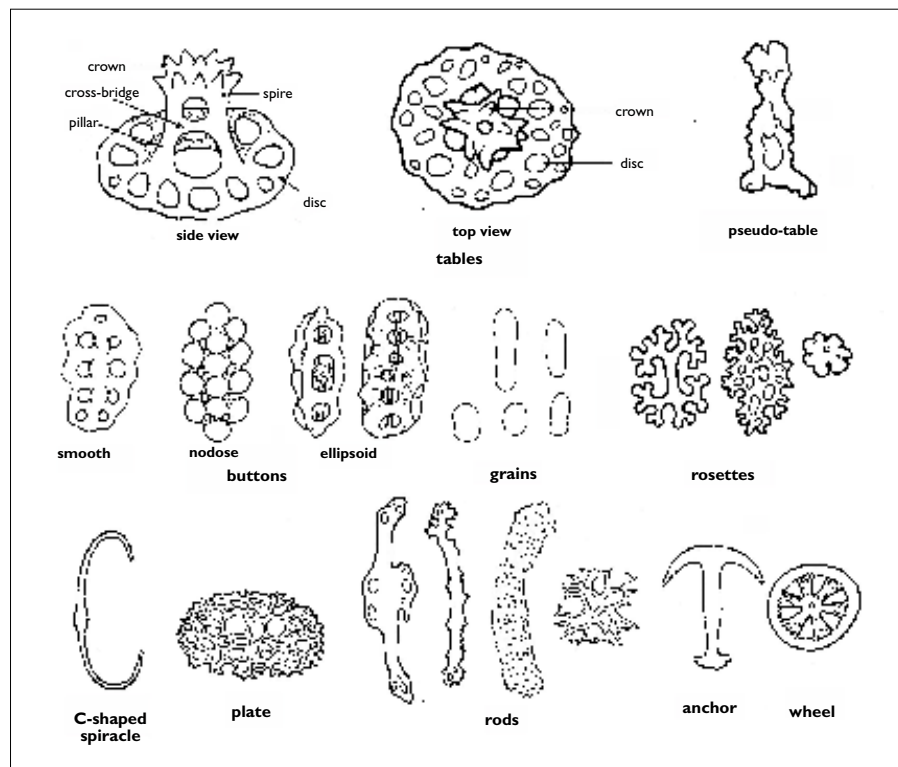


Figure 1. Different types of calcareous ossicles present in holothurians.

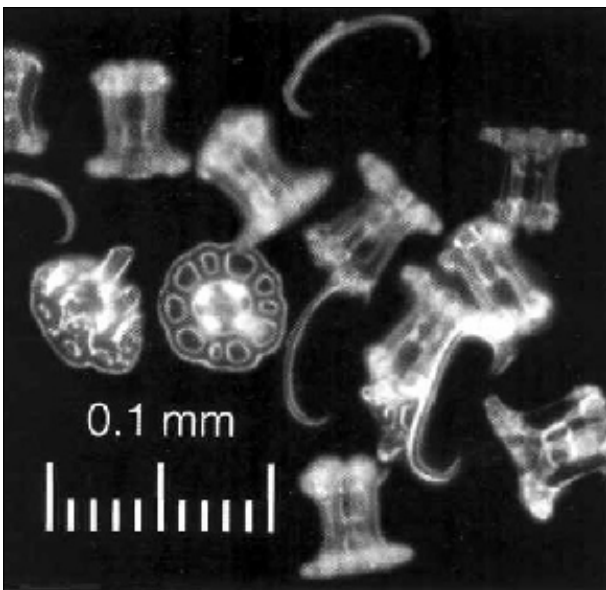
Source: Conand 1998

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**Figure 2.** The Galápagos sea cucumber *Isostichopus fuscus*. Image: Cleveland P. Hickman



**Figure 3.** Tables and C-shaped rods characteristic of the body wall of *Isostichopus fuscus*. Image: Cleveland P. Hickman

plates and perforated supporting tables (Fig. 3) (Hickman 1998).

In October 2003, *I. fuscus* was included in CITES Appendix III, as a result of a valuable initiative by the Ecuadorian government to diminish the illegal trade in this species. This listing gave rise to the need to develop identification procedures that would enable positive identification of this species by officials at major trading ports and in different countries, thus deterring the illegal trade in *I. fuscus*, and supporting conservation efforts.

This paper aims to illustrate the technique used to extract the spicules as a means to aid the beche-de-mer scientific community, custom officers and the international market. It also hopes to show the advantages of this methodology for the detection of illegal shipments, hence benefiting conservation efforts of this taxon.

## Methodology

Thirty *I. fuscus* specimens (10 fresh, 10 in brine and 10 dried) were obtained from the Galápagos National Park Service; all had been impounded by the Park Service. A slice 1 cm<sup>2</sup> x 1 mm thickness was dissected from the dorsal epidermis of each individual. Following the methodology described by Hickman (1998), each sample was placed in a small test tube with 3 ml of commercial bleach (NaOCl). Samples were left for approximately 30 min or until the body wall had dissolved and the ossicles settled to the bottom, resembling fine white sediment. By means of a pipette, the precipitated spicules were transferred to a microscope slide, covered with a cover slip and examined with a microscope at 100 x magnification (Hickman 1998).

## Results and discussion

In all samples, the calcareous spicules remained intact and fully distinguishable, regardless of whether the animal was fresh, salted or dried. No changes in the proportion, size or shape of the different spicules were observed between the different processing stages.

Commonly used taxonomic characters include the shape and composition of the spicules embedded in the body wall, as well as other morphological characters such as the presence or absence of Cuvierian tubules and the shape of the calcareous ring (Uthicke et al. 2004). In the case of *I. fuscus* in the Galapagos, only the spicules were used, proving their usefulness to identify this species under different processing methods. The spicules remained intact, with little fractioning (especially of the plates), and the proportion of different types of spicules did not differ between the processing stages.

Although their harvest is illegal, large quantities of *Stichopus horrens* are caught in the Galapagos Marine Reserve (Arellano 2004); these sea cucumbers are then traded at much reduced prices, or on the black market. Using the proposed body wall methodology, we observed the existence of rosettes and large tack-like ossicles on *S. horrens* body walls that are non-existent in *I. fuscus* (Fig. 3) (Hickman 1998); this will facilitate identification of which species has been caught, which is useful both for management and enforcement.

Several methods have been used to try to identify different species of sea cucumber while live or fresh (i.e. molecular phylogeny) (Uthicke and Benzie 2003, Uthicke et al. 2004), morphology (Cherbonnier 1980), visual (Conand 1990), and skeletal features (Cherbonnier 1980, Conand 1990, Uthicke et al. 2004); these have been useful in most

cases. The methodology presented here targets dried specimens that are already on the international market, the identification of which should be done in a precise and timely manner by custom officers. In the case of *I. fuscus*, this procedure enables quick and easy identification, is cost-effective and can be performed by biologically untrained personnel. Further studies should be done to compare the spicules of *I. fuscus* among populations in different countries, so as to fully establish this method's usefulness.

Examination of calcareous spicules in other commercial sea cucumber species can benefit conservation efforts for these species worldwide. Care must be exercised when relying exclusively on this procedure to identify a species, however, as the composition of the body wall spicules has not yielded positive results in all species analysed (Uthicke et al. 2004, Uthicke et al. in press). The procedure is cost-effective and yields rapid results, and hence the feasibility of performing such examinations in time constrained situations, and by untrained personnel. A catalogue identifying the key external characteristics as well as the spicules present in each of the commercially important species could be of great help to aid conservation efforts and encourage legal trade in these species.

### Acknowledgements

Dr Cleveland Hickman Jr. kindly granted permission to reprint the photographs presented in this document. Dr Steve Purcell greatly improved the document and Anita Sancho and Tom Poulson provided useful comments and input.

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# Appearance and development of skeletal structures in *Holothuria scabra* larvae and epibiont juveniles

Richard Rasolofonirina<sup>1,2</sup> and Michel Jangoux<sup>2,3</sup>

## Abstract

Various types of calcareous structures have been observed in *Holothuria scabra* sea cucumber larvae and epibiont juveniles at various ages and stages of development. An unpaired calcareous body can be observed as early as the dipleurula stage (less than two days after fertilisation); it is located in the posterior part of young larvae (36 h), grows, changes appearance (from a star, it becomes a half-sphere) and can still be found in epibiont juveniles. The madreporite appears in older auricularia (10 days) in the form of a curved stalk located at the base of the water ring canal; it develops to form a screened sphere in pentactulae and juveniles. Tables are recognisable in doliolaria larvae (15 days) and the initial formation of the peripharyngeal calcareous ring can be observed in pentactulae (17 days). The first podia rods and perforated disks appear in 20-day-old juveniles and anal plates from day 22 onwards, but button spicule only develop at the end of the epibiont life stage (45 days).

## Introduction

The calcareous parts of a sea cucumber's tegument, oral tentacles and podia are the most important taxonomic features. However, these parts change during growth and can be very different in juveniles and adults of the same species (Féral 1980; Cutress 1996; Massin et al. 2000). In particular, these differences involve size or architecture of the structures (e.g. bigger or smaller, more complex or simpler), but they can also be linked to the early disappearance or late appearance of some structures. Massin et al. (2000) described in detail the wide range of spicules found in *Holothuria scabra* juveniles. However, apart from what can be found in Mortesen's general and fairly dated works (1921, 1937, 1938), there is almost no information available on calcareous structures in sea cucumber larvae (see Hamel et al. 2002; Sewell and McEuen 2002). This paper looks into such structures in the pre- and immediately post-metamorphic phase of *H. scabra*. Its purpose is to identify the exact time they appear, describe them and follow the growth of young larvae to the epibiont juvenile stage.

## Materials and methods

The larvae and juveniles analysed came from fertilisation and breeding efforts carried out at the "Aqua-lab" in Toliara (southwest coast of Madagascar) (Jangoux et al. 2001; Rasolofonirina 2004). The formation and development of calcareous structures were monitored on a daily basis, from the gastrula stage through to the end of the juveniles' epibiont stage (i.e. about 50 days after fertilisation, when the juveniles began burrowing [en-

dobionts]). To do this, batches of several dozen larvae were set in 70% ethanol, placed in water glasses and soaked for 10 minutes in a 10% bleach solution. The digesta were then rinsed six times in distilled water, dried, coated with gold and examined with a scanning electron microscope (JEOL JSM-6100). The same procedure was used for juveniles except that they were left longer in the bleach solution, and were monitored under a microscope (followed by digestion of the fleshy tissues). Calcareous structures in the larvae were located using a photon microscope with a polarizing filter. Locating them in whole juveniles was facilitated by soaking the juveniles in distilled water. The specimens then became turgid and the calcareous structures could be easily seen using a binocular or photon microscope.

## Results

Various calcareous structures observed in larvae and epibiont juveniles and the exact stage of development they appeared are shown in Table 1.

### Calcareous structures in larvae

#### Calcareous body

The calcareous body is the first structural element to differentiate (Fig. 1A). It appears just 36 hours after fertilisation, in the posterior part of the larva blastocoel. It takes the form of a five-arm star about 13 µm in diameter (Fig. 2A). The star grows by multiplying and branching off its arms (Fig. 2B) and takes the form, in the late auricularia stage (10 days), of a half-sphere about 50 µm in di-

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**Table 1.** *Holothuria scabra*: type and order of appearance of calcareous structures in larvae and epibiont juveniles. (B = button; BT = tentacle rods; BP = podia rods; CC = Calcareous body; CCP = peripharyngeal calcareous ring; DTP = podia terminal disk; M = madreporite; PPA = perforated anal plate; PPP = perforated podia plate; T = table, + = this structure exists).

Age (days)	Stage of development	Type of calcareous structure									
		CC	M	CCP	Spicules						
					T	BT	BP	DTP	PPA	PPP	B
1.5	Dipleurula	+									
3	Auricularia	+									
10	Auricularia	+	+								
15	Doliolaria	+	+	+	+						
17	Pentactula	+	+	+	+						
20	Juvenile	+	+	+	+	+	+	+	+		
22	Juvenile	+	+	+	+	+	+	+	+	+	
45	Juvenile	+	+	+	+	+	+	+	+	+	+

ameter whose flat side is facing forward. The calcareous body in auricularia larvae takes the form of relatively smooth but spiny protuberances that gradually appear on its rounded side (posterior side) (Fig. 2C), while the flat side maintains a fairly even aspect (Fig. 2D). Throughout development, the calcareous body stays in the same place (i.e. in the anterior lobe of larvae and the posterior part of the bodies of juveniles) (Fig. 1A and 1C).

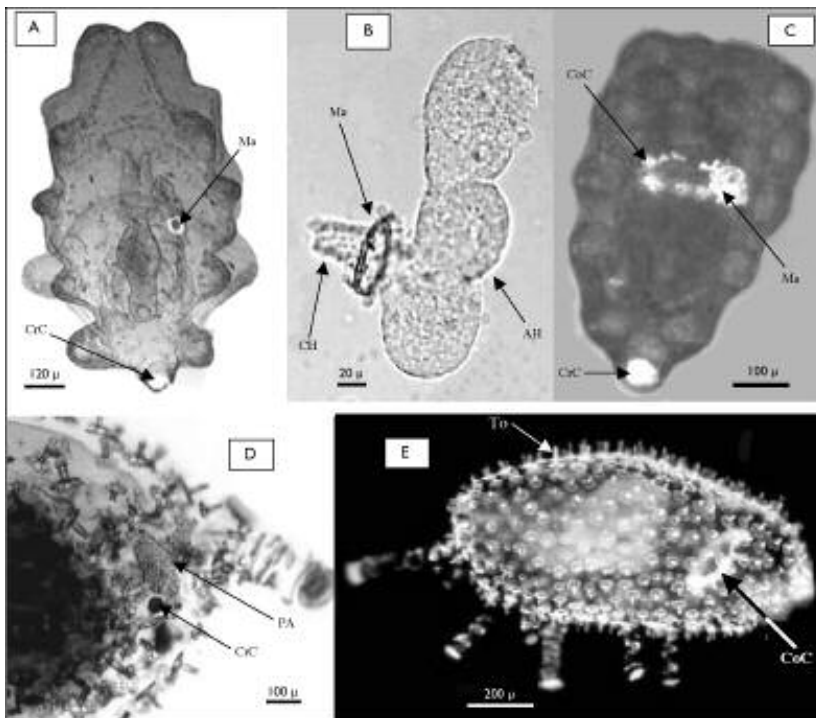
#### Madreporite

The madreporite is the second calcareous formation to differentiate in larvae. It appears in the late auricularia stage (10 days), when it can be observed in the left dorsal-lateral part of the larval body, in the form of a curved stalk about 40  $\mu\text{m}$  long. This stalk is located at the base of the water ring canal (Fig. 2E). It lengthens during development to form a ring surrounding that canal (Fig. 1B). The ring then branches off to form a sort of hollow sphere with screened walls measuring from 45  $\mu\text{m}$  (doliolaria stage) to 50  $\mu\text{m}$  (25-day-old juveniles) in diameter (Fig. 2F).

#### Peripharyngeal calcareous ring

The peripharyngeal calcareous ring appears beginning with the doliolaria stage (Fig. 1C). It surrounds the larva's oesophagus and is made up of 10 side-by-side pieces — five radial and five interradial.

Each part forms from a main rod that stretches out and branches off at both ends. In pentactulae the interradial pieces are not compact but rather formed from an unpaired median stem, from the ends of which joined stems grow (Fig. 3A). In juveniles, the stems become more nu-



**Figure 1.** *Holothuria scabra*.

Position of calcareous structures in auricularia (A,B) and doliolaria (C) larvae and in epibiont juveniles (D,E); (AH = Axohydrocoel, CH = water-ring canal, CoC = Calcareous ring, CrC = Calcareous body, Ma = madreporite, PA = anal plate, To = table).

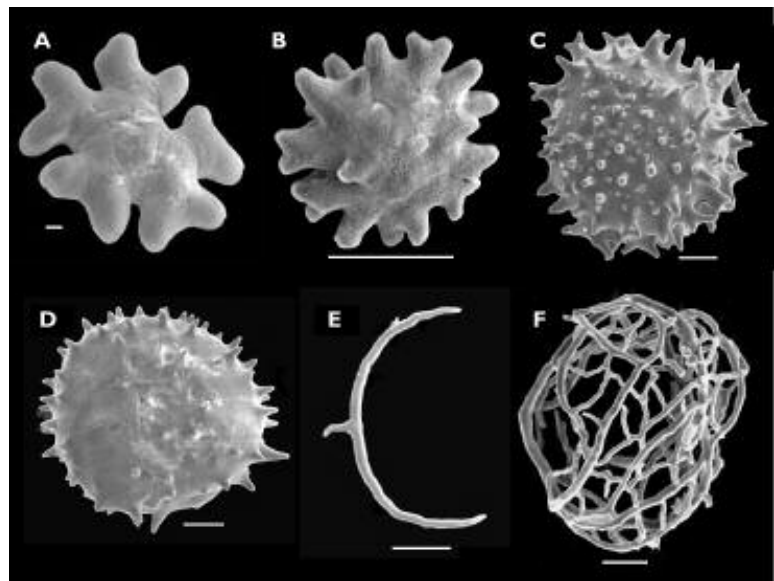
merous and massive. In older juveniles, these stem fuse and the ends of each piece become compact. The calcareous ring increases in size as specimens grow.

### Tables

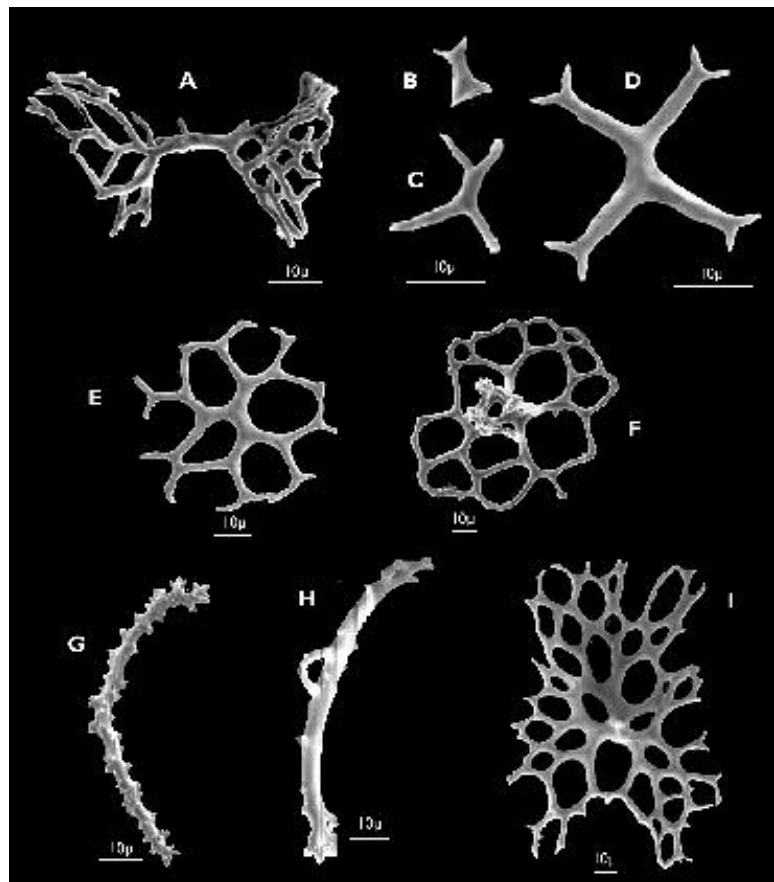
Tables are made up of a base plate and spire. These are the first type of intra-tegument spicules to form in *H. scabra*. They appear in the doliolaria stage in the form of small bobbin-like structures 7  $\mu\text{m}$  long (Fig. 3B). These bobbins grow longer and their free ends branch out to form a cross with four arms (primary arms) (Fig. 3C). The ends of the primary arms then also branch out to make secondary (Fig. 3D), and then tertiary arms. The latter get closer to each other as they grow and end up touching and fusing into pairs to form closed rings and a perforated disk (Fig. 3E). Four protuberances then grow out in the middle of each of the primary arms. They develop the same number of stalks that lengthen vertically to then touch laterally with short traversal junctions laid out like the rungs of a ladder, and the overall structure forms the spicule's spire (Fig 3F). Complete formation of this type of spicule takes about four days; it then reaches a height of about 70  $\mu\text{m}$ . The tables of doliolaria and pentactulae always appear in groups, with the first complete tables found only in juvenile specimens. In these juvenile specimens, they can be seen along the entire length of the tegument and in the walls of the podia, papillae and the oral tentacles (Fig. 1E). The table's spires are always facing outside, forming a row of small knobs on the tegument.

### Calcareous structures in juveniles

All the calcified structures that appear during the larval stage continue to exist in epibiont juveniles. Still others begin to differentiate at the very outset of the juvenile stage.



**Figure 2.** *Holothuria scabra*: Calcareous body (2A–D) and madreporite (2E–F) in dipleurula (2A; age 36 h) and auricularia (2B; age 45 h et 2E; age 11 days) larvae and epibiont juveniles (2C,D,F; age 30 days). Scale: A (Bar = 1  $\mu\text{m}$ ); B–F (Bar = 10  $\mu\text{m}$ ).



**Figure 3.** *Holothuria scabra*: Interradial piece of the peripharyngeal calcareous ring of a 17-day-old pentactula larva (3A); table development stages (3B–F), tentacle rod spicule (3G) and podia rod spicule (3H); perforated anal plate (3I). Scale bar = 10  $\mu\text{m}$  (A–I).



### *Podia and tentacle rods, podia terminal disks and anal plates*

All these structures appear at the same time (i.e. on day 20 after fertilisation). The podia and oral tentacles rods are curved. They are slightly (podia) to highly (tentacles) spiny, but podia rods are shorter (about 75 µm as compared with 95 µm for the tentacle rods) (Fig. 3G,H).

The podia terminal disks are pierced circular spicules made up of a web of joined trabeculae. They form at the apex of each podium and reach some 110 µm in diameter in 27-day-old juveniles.

The anal plates are formed from two large perforated spicules found in the posterior part of juveniles near the anus (Fig. 1D). They appear in 22-day-old juveniles. They do not have the same size or the same shape: on day 27, the right anal plate is about 220 µm long and 160 µm wide, as compared with 170 µm and 115 µm for the left plate (Fig. 1D,3I). The anal plates develop in the same way as the table base plates, i.e. from a main structure that branches out, forms primary and then tertiary arms that join and fuse to form closed rings (Fig. 3I).

### *Button-type spicules*

The buttons appear in juveniles at the end of the epibiont stage (40 to 50-day-old juveniles that measure 10–15 mm in length). Appearance of these buttons seems to depend more on the specimen's size than its age; they are observed in higher densities in the largest specimens.

## Discussion

In *Holothuria scabra*, most intra-tegument spicules differentiate after metamorphosis as tables, and are the only type of spicule observed in an initial form at the end of the larval stage (doliolaria larvae). In contrast, everything suggests that the unpaired calcareous body, which appears very early (36-hour-old larvae), is a truly larval formation. It does, in fact, disappear in juveniles some time after metamorphosis (Mortensen 1937, Massin et al. 2000). Other calcified structures, that are not spicules, develop early on (madreporite in the auricularia; the calcareous ring in doliolaria) and continue to develop and exist in post-metamorphic specimens.

The existence of a calcareous structure in the posterior part of larvae, known as the calcareous body, in many different Holothuriidae species, including *Holothuria scabra*, was mentioned by Mortensen (1921, 1937, 1938; also see Ramofafia et al. 2003).

Depending on the species and stage of development, the calcareous body takes on a variety of shapes (e.g. rounded, flat, star-shaped or with several lobes) (Mortensen 1937, 1938). In *H. scabra*, the shape differentiates at the very outset of the larval stage and appears as a star-shaped structure that gradually becomes a half-sphere. The calcareous body can be found throughout the larval stage and can still be seen in epibiont juveniles (also see Massin et al. 2000). Although this calcareous body does exist in the larvae of many species, its role remains a mystery. (It may help to stabilise the larvae during movement.) Auricularia, doliolaria and pentactula larvae do, in fact, swim by rotating around their anterior-posterior axis (vertical axis), which goes through the calcareous body. Moving larvae always keep their posterior part (where the calcareous body is located) down when they are moving vertically up or down, as if they were carrying ballast (Rasolofonirina 2004).

The development of spicules in *H. scabra* juveniles was studied by Massin et al. (2000). The authors reported that in juveniles measuring 0.9 mm in length, the peripharyngeal calcareous ring is made up of 10 unfused pieces (5 big pieces that are presumed to be radial and 5 smaller, so-called interradial pieces). They also mentioned the existence of large perforated plates in juveniles measuring less than 6 mm in length but they did not indicate their position or numbers; these were probably anal plates. According to them, these plates disappear in specimens measuring more than 6 mm in length, whereas we observed them in 45-day-old juveniles (about 18 mm in length). The existence of such anal plates in juvenile of the species *Holothuria floridana* was reported by Edwards (1909). Edwards did not talk about what happens to them but theorized that these plates were the remnants of the anal teeth that can be observed in *Actinopyga* species.

## Acknowledgements

Work was carried out through the financial support of the *Coopération Universitaire au Développement* (CUD, Belgium) as part of the project *Echinoculture tropicale à Madagascar* (Tropical echinoderm farming in Madagascar). We warmly acknowledge the technical assistance of Mr J.M. Ouin and Mr. Pol Postiau. Contribution of the *Centre Interuniversitaire de Biologie Marine* (CIBIM).

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## Fission inducement in Indonesian holothurians

P. Purwati<sup>1</sup> and S.A.P. Dwiono

### Introduction

Fissiparous holothurians are capable of reproducing asexually through binary fission. Inducing fission in order to double individual numbers would be a promising alternative for holothurian re-population, as suggested by Lokani et al. (1996). Fission is described as asexual reproduction in which an adult splits into two individuals, A anterior and P posterior. Induced fission is only successful if both A and P survive and grow into intact individuals.

Only 10 species of holothurians are known to be fissiparous. Among those, four are common in tropical Indonesian waters: *Holothuria atra*, *H. leucospilota*, *H. edulis* and *Stichopus chloronotus*.

Research on fission of sea cucumbers in their natural habitat has indicated that the fission plane is specific for each species. The body of *H. atra* splits at 44% (Chao et al. 1993) and *H. leucospilota* at 20–33% of its body length when measured from the anterior end (Townsend and Townsend 1973; Conand et al. 1997; Purwati 2004).

Fission has been observed in natural stocks but has rarely been observed under specific conditions (i.e. laboratory). Uthicke (2001) published his observations when he brought a fissioning *S. chloronotus* individual to the laboratory. Reichenbach and Holloway (1995) induced fission in eight holothurians species. Their results showed a higher survival rate when the rubber bands were placed on the animal's natural fission plane. Inspired by this information, we conducted induced fission experiments that we present here.

### Methods

The target species *H. atra* and *H. leucospilota* were collected from the village Teluk Kombal, Mataram, in west Lombok. Prior to fission inducement, 20 individuals of *H. atra* and 20 individuals of *H. leucospilota* were kept overnight in a tank with aerated and filtered fresh seawater in order for them to empty their gut contents.

The experiment took place from June–August 2004, at the Marine Bioindustry Technical Implementation Unit Mataram, RC Oceanography, LIPI, in Indonesia.

Initially, four different items — nylon string, cable tie, rubber band and rubber bicycle inner tube — were used to tighten up the animal's body. Results showed that the bicycle inner tube was the most suitable, as it was flexible and harmless to the animal's skin. Nylon string and cable ties created open wounds on the skin, triggering evisceration. Rubber bands were difficult to put on, and scratched and injured the skin when placed too tightly.

In the following experiment, we used rubber from bicycle inner tubes. The rubber was placed tightly around each animal, and animals were then placed in a small basket with a small amount of seawater to relax. Each band was then tightened around the sea cucumber, at 40–50% posteriorly for *H. atra* and 25–30% anteriorly for *H. leucospilota*.

Constricted individuals were reared in small buckets filled with fresh, filtered seawater and slowly aerated. Ten individuals of each species were reared separately, while the rest were kept together. When fission occurred, sand-filtered seawater was flowed over the animals (temperature and salinity similar to natural seawater). No food was added. This treatment continued until morphological recovery was completed. When a new anal aperture or mouth started to appear, the animals were moved outdoors and reared in concrete tanks with 10 cm of sand substrate and water flowing for four to six hours a day. Water was fully changed each week.

### Results and discussion

Figure 1 shows the inducing techniques used in this experiment. When inducement injured the animals, evisceration was the usual response. Even so, fission could still occur and commonly resulted in an open wound on the fission plane. When the inducement position was 25–30% posteriorly on *H. atra* specimens, A and P specimens

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with open wounds were common. Inducement at 40–50% posteriorly produced at least 90% survival of A and P specimens.

Induced individuals did not show twisting as normally occurs in specimens undergoing natural fission. Occasionally, viscera appeared on the fission plane of either A or P after splitting (Fig. 2). But the wound healed not long afterwards. The fission process took several hours to three days, and signs of regeneration appeared two to three weeks after fission. Figures 3 and 4 show obvious signs of re-growth.

These preliminary results support previous studies that indicate fission can be induced. Even though fission only allows doubling of a sea cucumber stock, its advantages are: inducement can be done on any number of individuals; survival rates can be very high, as the produced individuals are already adapted to the habitat and begin growing from a relatively large size; threats (predation, etc.), which normally occur during the larval and juvenile stages, are reduced; and costs and technology are low.

Lokani et al. (1996) mentioned that fission can be induced in non-fissiparous *H. scabra*. During our experiment, we induced *Bohadschia marmorata* to divide using the same technique; nearly all induced individuals survived and recovered. For future work, we will focus on the regenerating efforts of fission products.



**Figure 1.** Induced fission of *Holothuria atra*.

## Acknowledgements

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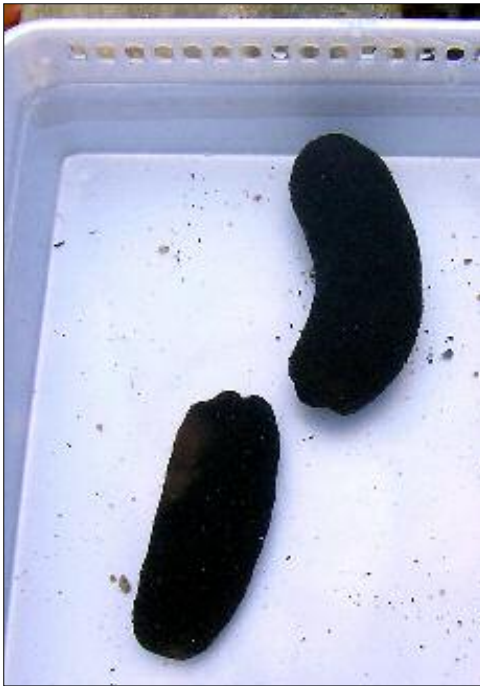
**Figure 2.** Result of inducement (a), and P individuals (b) of *Holothuria atra*.



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**Figure 3.** Regenerating individuals of *Holothuria atra*: 4 weeks after fission (left) and 9 weeks after fission (right).



**Figure 4.** Regenerating individuals of *H. leucospilota*. New mouth part (left) and anal aperture (right), 9 weeks after fission.



## Sea cucumbers of the Comoros Archipelago

Yves Samyn<sup>1</sup>, Didier VandenSpiegel<sup>2</sup> and Claude Massin<sup>3</sup>

### Abstract

Sea cucumbers have been harvested for centuries in the Far East. Overexploitation, coupled with increasing demand has led to local depletion of certain standing stocks. *De novo* investigation at Grande Comore (one of the four main islands of the Comoros Archipelago) allows re-appraisal of local holothuroid biodiversity. Comparison with neighbouring areas allows extrapolation of holothuroid species richness to the rest of the archipelago. The current exploitation of holothuroids has been documented and there are definite signs of overexploitation. Conservation measures are urgently needed if exploitation of sea cucumbers in this area is to become sustainable in the near future.

### Introduction

Holothuroids have been harvested for human consumption for centuries (Conand and Byrne 1993; Conand 2004). While harvesting was formerly concentrated in the Far East, market demand has shifted the exploitation grounds towards the western Indian Ocean during the last two decades (Marshall et al. 2001). Current exploitation has expanded to the point that sea cucumber populations in many parts of the Indo-Pacific are declining rapidly, with local extinctions being recorded (Samyn 2003; Thandar and Samyn 2004).

Very few scientific data are available concerning holothuroid biodiversity and the exploitation thereof for the four islands that constitute the Comoros Archipelago (including Grande Comore, Anjouan and Moheli, which constitute the Federal Islamic Republic of the Comoros, and Mayotte, which is an overseas "Communauté Territoriale" of France). Cherbonnier (1988) has documented some isolated findings from Mayotte and Grande Comore; VandenSpiegel and Samyn (internal report) made a preliminary inventory of the sea cucumbers of Grande Comore; and Pouget (2003, 2004, 2005) did the same for Mayotte, although focussing more on commercial species. No information is currently available for Anjouan and Moheli.

The aim of the present paper is threefold: 1) recapitulate existing information regarding Comorian holothuroid biological diversity; 2) predict total Comorian holothuroid biodiversity by comparing the richness of the neighbouring areas (North Mozambique and the northwest of Madagascar) to that of the Comoros; and 3) document the current unbridled exploitation of these animals in the Comoros.

The present paper should aid local authorities as well as the general public in understanding the need to protect and sustainably manage this ecologically and economically important, but fragile, natural resource.

### Inventory of holothuroid biodiversity in the Comoros Archipelago

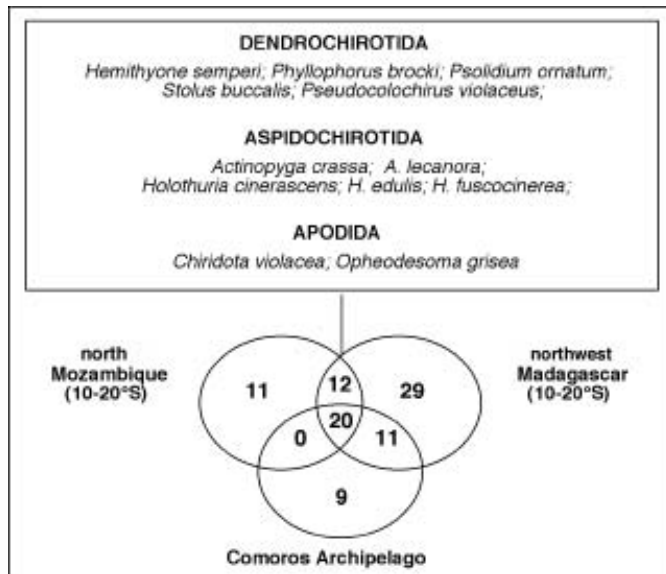
Our current knowledge of sea cucumber biodiversity of the Comoros Archipelago is poor, as few studies have been devoted to these islands. Table 1 documents the 40 species currently known from the Comoros. Our *de novo* investigations have contributed significantly to this total, adding 19 new records. It is striking that the majority of the species (77.5%, or 31 out of 40) belong to the order Aspidochirotida, while the other two orders, Apodida and Dendrochirotida, are represented by 10% (4 out of 40) and 12.5% (5 out of 40 species), respectively.

In order to get a more complete sense of Comorian holothuroid species richness, we analysed any existing species that are common to both the east (northwest Madagascar) and west (north Mozambique) of the Comoros, but which have not yet been reported from the Comoros. This gap analysis revealed that 12 additional species are potentially present in the Comoros (Fig. 1). With these potential species included, the systematic composition is adjusted to: Aspidochirotida,  $\pm 69.25\%$  (36 out of 52 species), Apodida,  $\pm 11.50\%$  (6 out of 52 species) and Dendrochirotida,  $\pm 19.25\%$  (10 out of 52 species). These proportions correspond relatively well to what has been reported for other tropical areas in the Indo-Pacific (Levin 1999; Massin 1999; Samyn 2003; Thandar and Samyn 2004), although apodids appear underestimated.

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**Table 1.** Shallow water holothuroids documented from the Comoros Archipelago. Pers. observ. means species was collected by our team in 2003 or 2004.

Taxon	Reference	Taxon	Reference
<b>APODIDA Brandt, 1835</b>		<i>H. (Metriatyla) scabra</i> Jaeger, 1833	Pouget 2003, 2004, 2005
<b>Chiridotidae Oestergren, 1898</b>		<i>H. (Microthele) fuscogilva</i> Cherbonnier, 1980	pers. observ.
<i>Polycheira fusca</i> (Quoy & Gaimard, 1833)	Cherbonnier, 1988	<i>H. (M.) fuscopunctata</i> Jaeger, 1833	Pouget 2004; pers. observ.
<b>Synaptidae Oestergren, 1898</b>		<i>H. (M.) nobilis</i> (Selenka, 1867)	Pouget 2003, 2004, 2005; pers. observ.
<i>Euapta godeffroyi</i> (Semper, 1868)	pers. observ.	<i>H. (Platyperona) difficilis</i> Semper, 1868	pers. observ.
<i>Synapta maculata</i> (Chamisso & Eysenhardt, 1821)	pers. observ.	<i>H. (Selenothuria) parva</i> Krauss in Lampert, 1885	Cherbonnier 1988
<i>Synaptula recta</i> (Semper, 1868)	pers. observ.	<i>H. (Stauropora) pervicax</i> Selenka, 1867	Cherbonnier 1988
<b>ASPIDOCHIROTIDA Grube, 1840</b>		<i>H. (Theelothuria) maculosa</i> Pearson, 1913	pers. observ.
<b>Holothuriidae Ludwig, 1894</b>		<i>H. (Thymiosycia) impatiens</i> (Forskål, 1775)	Cherbonnier 1988
<i>Actinopyga</i> sp.	pers. observ.	<i>Pearsonothuria graeffei</i> (Semper, 1868)	Cherbonnier, 1988; pers. observ.
<i>A. echinites</i> (Jaeger, 1833)	Pouget 2004	<b>Stichopodidae Haeckel, 1896</b>	
<i>A. mauritiana</i> (Quoy & Gaimard, 1833)	Cherbonnier 1988; Pouget 2003, 2005; pers. observ.	<i>Stichopus chloronotus</i> Brandt, 1835	Cherbonnier 1988; Pouget 2003, 2004, 2005; pers. obs.
<i>A. miliaris</i> (Quoy & Gaimard, 1833)	pers. observ.	<i>S. hermanni</i> Semper, 1868	pers. observ.
<i>A. obesa</i> (Selenka, 1867)	Cherbonnier 1988; pers. observ.	<i>S. horrens</i> Selenka, 1867	pers. observ.
<i>Bohadschia atra</i> Massin et al., 1999	Cherbonnier 1988; Pouget 2005; pers. observ.	<i>Thelenota ananas</i> (Jaeger, 1833)	Pouget 2003, 2004, 2005; pers. observ.
<i>B. cousteaui</i> Cherbonnier, 1954	pers. observ.	<i>T. anax</i> H.L. Clark, 1921	pers. observ.
<i>B. marmorata</i> Jaeger, 1833	pers. observ.	<b>DENDROCHIROTIDA</b>	
<i>B. subrubra</i> (Quoy & Gaimard, 1833)	Pouget 2005; pers. observ.	<b>Cucumariidae Ludwig, 1894</b>	
<i>B. vitiensis</i> (Semper, 1868)	Pouget 2003, 2004, 2005	<i>Pentacta tesselara</i> Cherbonnier, 1970	pers. observ.
<i>Holothuria (Halodeima) atra</i> Jaeger, 1833	Pouget 2003, 2005; pers. observ.	<i>Havelockia turrispinea</i> Cherbonnier, 1988	Cherbonnier 1988
<i>H. (Lessonothuria) hawaiiensis</i> Fisher, 1907	pers. observ.	<b>Phyllophoridae Oestergren, 1907</b>	
<i>H. (L.) pardalis</i> Selenka, 1867	Cherbonnier, 1988; pers. observ.	<i>Thyone comata</i> Cherbonnier, 1988	Cherbonnier 1988
<i>H. (L.) verrucosa</i> Selenka, 1867	pers. observ.	<b>Sclerodactylidae Panning, 1949</b>	
<i>H. (Mertensiothuria) hilla</i> Lesson, 1830	pers. observ.	<i>Afroccumis africana</i> (Semper, 1868)	pers. observ.
<i>H. (M.) leucospilota</i> (Brandt, 1835)	Cherbonnier 1988; pers. observ.	<i>Ohshimella ehrenbergi</i> (Selenka, 1867)	pers. observ.



**Figure 1.** Gap analysis indicating potential Comoros Archipelago holothuriid species. Such gap analysis is appropriate given the fact that the northwest Madagascar Current connects the west coast of Madagascar with mainland Africa (Mozambique); the Comoros are situated between the latter areas, and the archipelago's fauna should resemble that of the areas to the west and east (see Thandar and Samyn 2004 for the oceanography of the region).

## Exploitation of sea cucumbers in the Comoros Archipelago

### 1. Harvesting from the wild

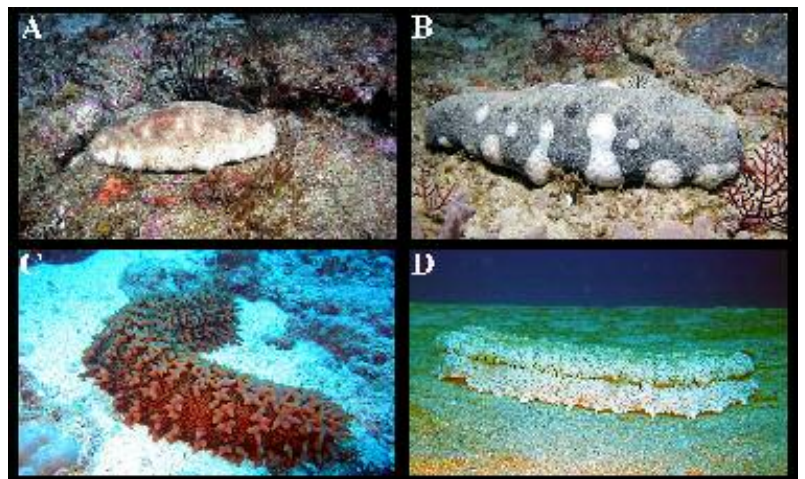
The first national strategy and action plan for the conservation of biological diversity of the Federal Islamic Republic of the Comoros (2000, see <http://www.biodiv.org/world/map.asp>) contained an interesting account of the country's sea cucumber resources. This legal document concisely mentions the dangers associated with unbridled exploitation of sea cucumber stocks. More recently, VandenSpiegel and Samyn (internal report) expressed their concern that excessive exploitation of this fragile resource would inevitably lead to depletion. A recent survey by the latter authors, aided by one of us (CM) as well as a local NGO (AIDE, Association pour l'Intervention et le Développement de l'Environnement) and the responsible governmental ministry (DGE, Département Général de l'Environnement), came to the same conclusion.

As in the whole of the Indo-Pacific, the most sought-after species belong to Aspidochirotida. Target species include Holothuriidae [several *Actinopyga* spp., *Holothuria (Microthele) fuscogilva* Cherbonnier, 1980, *H. (M.) nobilis* (Selenka, 1867)] and Stichopodidae [*Stichopus chloronotus* Brandt, 1835, *Thelenota ananas* (Jaeger, 1833) and *T. anax* H.L. Clark, 1921] (Fig. 2).

We note that abundance of several of the above mentioned species appears to have decreased from November 2003 to October 2004, although at present this observation cannot be supported with hard data.

### 2. Processing into beche-de-mer

The process used to prepare species for the beche-de-mer trade is a simplified version of the process used throughout the Indo-Pacific (Conand 1990). Freshly collected specimens are gutted with a longitudinal incision, boiled for one hour, and dried in the sun. Contrary to other regions, the body wall of the specimens is not scraped to remove excessive ossicles. Burrowing of treated specimens in order to speed the process is also not carried out. Figures 4 and 5 illustrate the processing technique for some of the harvested species.



**Figure 2.** Commercial species belonging to the order Aspidochirotida. At Grande Comore, the main harvested species are: A) *Holothuria (Microthele) fuscogilva* Cherbonnier, 1980; B) *Holothuria (Microthele) nobilis* (Selenka, 1867); C) *Thelenota ananas* (Jaeger, 1833); and, D) *Thelenota anax* H.L. Clark, 1921.

Images: A by B. Van Bogaert; B by T. Schilis; and C and D by D. VandenSpiegel



### 3. Farming as a sustainable approach

Chinese immigrants control the harvesting and processing of sea cucumbers in the Comoros. They have the expertise to process sea cucumbers into an exportable commercial product, and are aware of the significant retail value. Interviews with fishermen, Comorian environmental authorities and policy makers, in addition to personal observations, however, indicate that sea cucumbers are currently exploited in a detrimental manner. Various observations support this conclusion: 1) some high value commercial species such as *Holothuria scabra* were not found, even though some ideal habitats were encountered during our

surveys; 2) seagrass beds at Grande Comore are nearly devoid of sea cucumbers; 3) densities of species appear to have dropped since our first survey in November 2003; 4) fishing is done at ever increasing depths (scuba divers now readily descend to 70 m or more); 5) some processed specimens are so small that they will fail to satisfy the retail market in the Far East; and 6) high as well as low value species are processed.

Fortunately, there is some positive news. Chinese fishermen, fully aware of their destructive fishing activities, have recently begun farming some of the high value species. We hope they will be successful in their initiative.



**Figure 3.** Freshly gutted specimens of A) *Holothuria (Microthele) fuscogilva* Cherbonnier, 1980; B) *Thelenota anax* Clark, 1921; and C) an as yet undescribed color morph of *Holothuria (Microthele) nobilis* (Selenka, 1867). All images by D. VandenSpiegel



**Figure 4.** After gutting, specimens are boiled for one hour (A) and dried in the sun (B, C). Image A & C by D. VandenSpiegel, B by A. Soifa



**Figure 5.** Achieving durability in sea cucumber exploitation through farming initiatives. A) Tank for rearing sea cucumbers; B) *Holothuria (Microthele) nobilis* (Selenka, 1867); and C) *Thelenota ananas* (Jaeger, 1833). All images by D. VandenSpiegel

## Towards sustainable exploitation of Comoran sea cucumbers

In early 2003, the Government of the Federal Islamic Republic of the Comoros sought the assistance of Belgian specialists to train local scientists in sea cucumber identification and fishery management. Such education was indeed urgently needed, as exploitation by (predominantly) Chinese fishermen was pushing local stocks to extinction. Financially supported by the *Commission de l'Océan Indien* (COI) and the Belgian Development Cooperation (DGDC), we have responded by documenting the sea cucumber biodiversity of the Comoros through *de novo* sampling as well as by reviewing already existing museum collections. In addition, with the further support of the Belgian Development Cooperation, we trained two local scientists in the taxonomy and monitoring of sea cucumbers, equipped a laboratory with basic infrastructure and appropriate literature, and established a reference collection. These actions have allowed the establishment of a "Point focal holothurians" that is housed at the NGO AIDE in Moroni (Comoros).

## Conclusion

The holothuroid fauna of the shallow Comoran waters was poorly known prior to our studies, which have expanded the number of known species of sea cucumbers from 22 to 40. Furthermore, gap analysis allowed the prediction of 12 additional species. Total shallow water holothuroid species richness thus seems to average around 50 species. Until all four islands of the Comoros have been adequately sampled, however, this figure should be viewed as an underestimate.

We take this opportunity to express our deepest concern in regard to the present, rather blind, over-exploitation of sea cucumbers in the Comoros.

## Acknowledgements

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## Sea cucumber inventory in Mayotte, southwest Indian Ocean

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### Introduction

Recently, the number of echinoderm inventories in the southwest Indian Ocean has increased, and such inventories usually include a section on sea cucumbers. We should mention, for example, those carried out in Kenya and in Tanzania by Samyn (2003), Rodrigues by Rowe and Richmond (2004) and Reunion Island by Conand and Mangion (2002). A major inventory of sea cucumber species in Madagascar was published by Cherbonnier (1988), which included references to specimens in Mayotte, thus providing a rich working base. In addition, a new species was described in Madagascar (Massin et al. 1999).

Sea cucumber fisheries are growing worldwide, leading to overexploitation in most tropical Indo-Pacific countries (Conand 1999, 2004a). For that reason, FAO recently held a symposium on exploitation, management and aquaculture (Lovatelli et al. 2004) and CITES has also focused attention on conservation of these species at a number of its meetings (2003 Conference of the Parties in Chile, 2004; Meeting in Malaysia, 2004; Animals Committee Meeting in Bangkok [see [www.cites.org](http://www.cites.org) and Conand 2004b]).

Mayotte is about 8 million years old and is the oldest of the four main island islands that make up the Comoros Archipelago (Grande Comore, Mohéli, Anjouan and Mayotte). With a surface area of 374 km<sup>2</sup> and a 185-km-long coastline, Mayotte has two main islands (Petite Terre and Grande Terre) and 30 smaller islands spread across a lagoon with an area of more than 1000 km<sup>2</sup>. Mayotte's EEZ covers 73,600 km<sup>2</sup> and includes the Banc de la Zélée (Zelee Bank). Natural and human pressure on marine resources has been growing steadily in Mayotte, including coral bleaching, which destroyed 90% of the corals in 1998. Since then, efforts by the local government to sustainably manage reef resources have led to the creation of the Mayotte Lagoon Management Plan (PGLM) (CAREX et al. 2002), to a project to make six noteworthy marine areas into nature reserves, and to preliminary work on identifying "natural marine areas of interest for their ecology, fauna and floral" (ZNIEFF), in line

with the overall methodology used in France (Guillaume 2000). It was against this background that work to compile existing data was carried out in 2004. Designed as a pilot project, this study focused on several taxonomic groups, including echinoderms; the goal was to test operational protocols, with the real field inventory to be conducted later by specialists.

An initial article by Pouget (2004) directly addressed sea cucumbers of commercial interest in Mayotte (a topic that had never before been studied), and presented the characteristics of this fishery. Pouget (2005) then presented information on the distribution of the major species and displayed study sites on a map.

This paper discusses a new inventory conducted with a rapid method using different types of data.

### Method

This approach combines those used:

- in French Overseas Departments with "ZNI-EFF-Mer" typologies (Guillaume 2000),
- to define noteworthy habitats and marine areas described in detail in Andrefouët's works (2002),
- as part of the Mayotte Lagoon Management Plan (CAREX et al. 2002), and
- in the *Atlas préliminaire des espaces naturels* (Exploratory Atlas of Natural Spaces) (Valentin and Vanssay 2004).

Echinoderms are one of the key groups used to characterize the habitats of Mayotte's marine flora and fauna.

The methodological approach consisted of compiling a list of existing works (scientific publications and reports) that mention sea cucumbers. In addition to the above-mentioned publications, there were a number of reports (ARVAM et al. 1997; ARVAM 1997; CAREX 2001; Thomassin 1997). During the period 1994 to 2005, 153 days of fieldwork were carried out by ARVAM (and its partners), during which a large number of underwater photos were taken; these were later linked to the "BDMay" information base in Excel format. Given

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the methodology's obvious limits from the point of view of systematic identification of species, we created the following validation scale:

- 1: species mentioned in a publication by a well-known taxonomist,
- 2: species mentioned in report by a non-specialist author, and of a species that has been verified or identified (by photo) by a specialist,
- 3: species mentioned in a report, but deemed doubtful by a specialist; also includes those that must be later identified using an actual specimen,
- 4: species mentioned in a report but which probably does not exist in Mayotte, so not included here.

The BDMay database will include three major types of information for each sea cucumber observation (and for the other taxonomic groups as well):

- systematic data (family, genus, species, common name)
- spatial data (geomorphology of the seafloor, habitats, GPS point, depth)
- bibliographic references (reports, publications).

## Results

The pilot sea cucumber database is shown in Table 1; the purpose of this work was not to con-

**Table 1.** BDMay sea cucumber database.

Validity*	Family	Genus	Species	Author, year	Pictures	Market value**
1	Cucumariidae	<i>Havelockia</i>	<i>turrispinea</i>	Cherbonnier, 1988	-	-
1	Cucumariidae	<i>Thyone</i>	<i>comata</i>	Cherbonnier, 1988	-	-
1	Holothuriidae	<i>Actinopyga</i>	<i>echinites</i>	(Jaeger, 1833)	x	2
1	Holothuriidae	<i>Actinopyga</i>	<i>mauritiana</i>	(Quoy & Gaimard, 1833)	x	2
1	Holothuriidae	<i>Actinopyga</i>	<i>miliaris</i>	(Quoy & Gaimard, 1833)	-	2
1	Holothuriidae	<i>Actinopyga</i>	<i>obesa</i>	(Selenka, 1867)	-	3
2	Holothuriidae	<i>Bohadschia</i>	<i>atra</i>	Massin et al., 1999	x	3
2	Holothuriidae	<i>Bohadschia</i>	<i>marmorata</i>	(Jaeger, 1833)	-	3
1	Holothuriidae	<i>Bohadschia</i>	<i>similis</i>	(Jaeger, 1833)	-	3
1	Holothuriidae	<i>Bohadschia</i>	<i>subrubra</i>	(Quoy & Gaymard, 1833)	x	3
1	Holothuriidae	<i>Bohadschia</i>	<i>vitiensis</i>	(Jaeger, 1833)	x	3
1	Holothuriidae	<i>Holothuria</i>	<i>atra</i>	(Jaeger, 1833)	x	3
2	Holothuriidae	<i>Holothuria</i>	<i>fuscogilva</i>	(Cherbonnier, 1980)	x	1
1	Holothuriidae	<i>Holothuria</i>	<i>fuscopunctata</i>	(Jaeger, 1833)	x	3
2	Holothuriidae	<i>Holothuria</i>	<i>nobilis</i>	(Selenka, 1867)	x	1
1	Holothuriidae	<i>Holothuria</i>	<i>pardalis</i>	(Selenka, 1867)	-	-
1	Holothuriidae	<i>Holothuria</i>	<i>pervicax</i>	(Selenka, 1867)	-	-
1	Holothuriidae	<i>Holothuria</i>	<i>impatiens</i>	(Forsk., 1775)	-	-
1	Holothuriidae	<i>Holothuria</i>	<i>scabra</i>	(Jaeger, 1833)	-	1
1	Holothuriidae	<i>Holothuria</i>	<i>scabra versicolor</i>	(Conand, 1986)	x	1
1	Holothuriidae	<i>Pearsonothuria</i>	<i>graeffeii</i>	(Semper, 1868)	x	3
1	Stichopodidae	<i>Stichopus</i>	<i>chloronotus</i>	(Brandt, 1835)	x	2
1	Stichopodidae	<i>Stichopus</i>	<i>hermani</i>	(Semper, 1868)	x	2
1	Stichopodidae	<i>Thelenota</i>	<i>ananas</i>	(Jaeger, 1833)	x	1
2	Stichopodidae	<i>Thelenota</i>	<i>anax</i>	(Clark, 1921)	x	2
1	Synaptidae	<i>Euapta</i>	<i>godeffroyi</i>	(Semper, 1868)	-	-
1	Synaptidae	<i>Synapta</i>	<i>maculata</i>	(Chamisso & Eysenhardt, 1821)	-	-

\* Validity: 1 to 3 (see text)

\*\* Market value: 1: high value, 2: medium and 3: low, according to Conand (1999)

duct true taxonomic assessments. Of the 27 species listed, 22 had Level 1 validity, 2 species still need to be confirmed, and 3 species were added to the database through the use of photos.

The photos of a few common large-sized species are shown in Figure 1 (A to F).

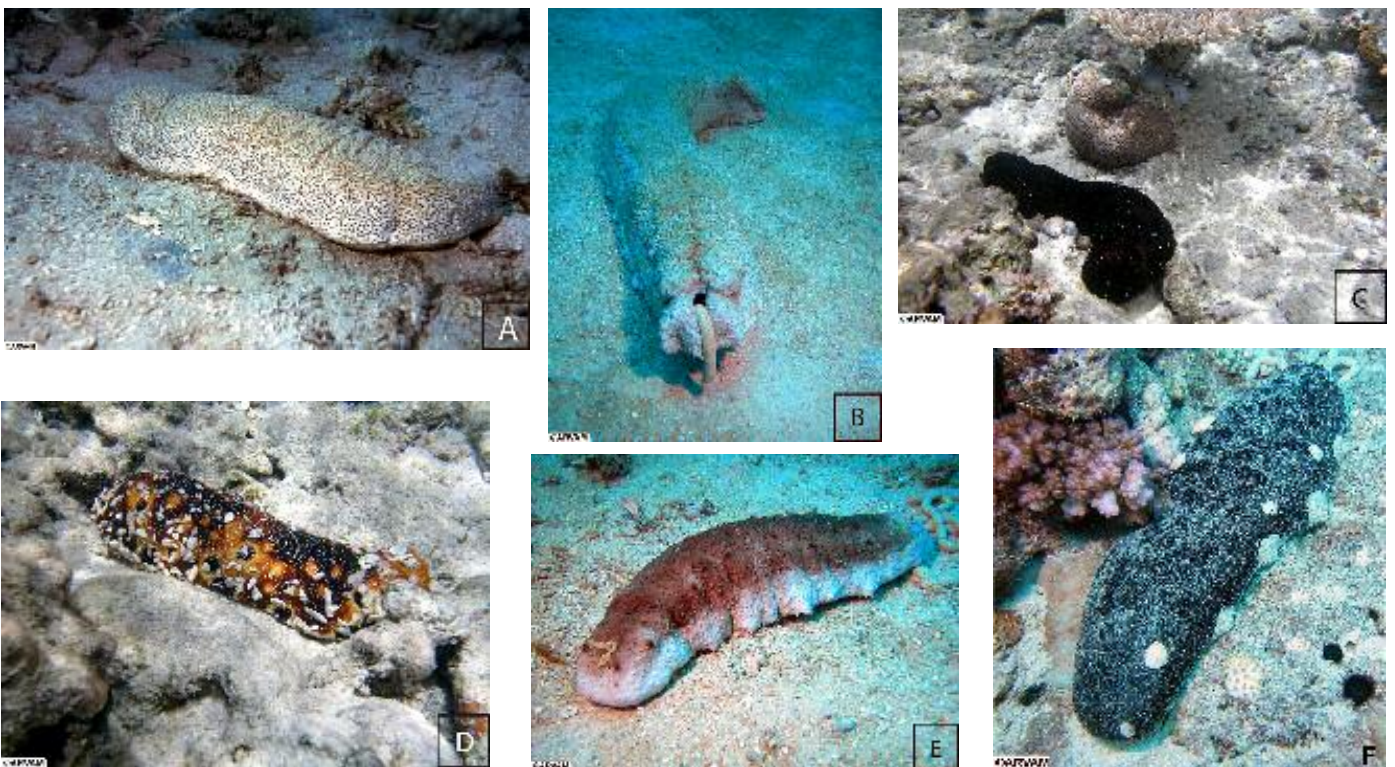
### Discussion and conclusions

Before the ZNIEFF inventories for Mayotte were carried out, methodological work was undertaken so as to optimise these efforts. This work was based on a review of existing information (species and sites). During this work, we realised that several prerequisites had not yet been fulfilled, such as creating a single recognised list of flora and fauna and creating a list of Mayotte's marine habitats to supplement the one that exists for the French Overseas Departments. Based on the work done on echinoderms, it was shown that consolidating available data would make it possible to begin the actual inventory under the best possible conditions. Such data provide the bases of an information system on the species' spatial distribution, which may make it possible to determine more precisely those species that should be considered decisive in terms of the ZNIEFF, ap-

proach, and determine those that need specific management/conservation measures (protected habitats, regulations, etc.). The list of sea cucumber species given here is still provisional. Small-size or cryptic species are not listed and require additional collection work. Using photos to identify species made it possible to illustrate 25 species with almost absolute certainty. This work allowed us to add three species that had not been reported previously. Other photo collections exist and should be analysed.

Information validation is an important issue for future studies. While using photographic data can provide a rapid assessment method (as long as a referencing procedure is adopted that integrates baseline information on substrata and locations), it cannot replace actual taxonomic work, for which precise specimens must be collected, set, identified, and archived.

The use of *in situ* photos distributed to a large range of agencies and governments may make it possible to better define the habitats of the various species. Field prospecting should also be carried out, focusing on those reef flats that are relatively easy to access but which have not yet been studied in detail.



**Figure 1.** Some large holothurians species common in Mayotte: A) *Holothuria fuscopunctata*; B) *Thelenota anax*; C) *Bohadschia atra*; D) *Bohadschia subrubra*; E) *Holothuria nobilis*. All images: © ARVAM

Gaps do exist in this work: we should point out that there are other publications and reports on Mayotte (mentioned by Thomassin 2004) that were not available for this phase of the study. The various activities planned during implementation and scientific control of ZNIEFF-Mer in Mayotte will be continued; this is an original and timely approach, and should be effective, as is sought by the local government. It should make it possible to supplement and correct the inventory in an iterative and constructive manner, and allow comparison, in a relevant way, of Mayotte's marine biodiversity with that of the other Comoros islands, the Mozambique Canal and the Indian Ocean. This is a vital step towards gaining a better understanding of the issues involved in marine and coastal resource conservation and the operational measures that need to be taken.

### Acknowledgements

This prospecting work was part of the assignment the Direction de l'Agriculture et de la Forêt (DAF) of Mayotte gave to the Mascarin National Botanical Conservatory (CBNM)–ARVAM group for implementation of the scientific management of the ZNIEFF in Mayotte. We would particularly like to thank V. Boulet, Director of the CBNM.

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## Observation of asexual reproduction by natural fission of *Stichopus horrens* Selenka in Okinawa Island, Japan

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Asexual reproduction, by fission, of *Stichopus horrens* Selenka in its natural habitat was observed in Okinawa, Japan during December 2004. Some of the tropical sea cucumber species that reproduce by fission are also known in Japan (Emson and Wilkie 1980; Smiley et al. 1991; Conand et al. 2002). However, detailed studies have not been made. Though this *S. horrens* is known to proliferate by asexual reproduction, the photograph below is the first photograph of fission of this species in Japan. Therefore, it is valuable and important for reproductive studies of sea cucumbers in Japan.

**Species:** *Stichopus horrens* Selenka, 1867

**Location:** Nakagusuku Bay, Awase of Okinawa Island (26°17'13.7"N and 127°52'42.4"E), Pacific Ocean

**Date and time:** 14 December 2004, 10:30 am.

**Depth:** 19 m

**Bottom:** Soft mud with fine sand and silty sediment

**Notes:** This species is known to immediately autotomize when gripped by hand. It can be seen on the photographs that the mouth of the anterior individual is large, and the mouth of the posterior one is small (Fig. 1). The mouth of the individual issued from the posterior part will be newly formed (Fig. 2).



**Figure 1.** *Stichopus horrens* in the process of fissioning into two individuals (dorsal view).

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**Figure 2.** Close up of the same individual's ventral side, showing the small newly formed mouth on the posterior part.

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## Present status of the sea cucumber fishery in southern Sri Lanka: A resource depleted industry

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### Introduction

Sri Lanka is a small tropical island situated between 5°55' and 9°55'N and 72°42' and 81°52'E, south of the Indian subcontinent. The country has a total land area of 65,000 km<sup>2</sup>, including inland water bodies and 62,705 km<sup>2</sup> excluding inland water bodies. Although the country's coastline is about 1739.3 km long, the continental shelf area is 30,000 km<sup>2</sup>, which is relatively narrow and small in area when compared with other island nations. The island's coastline contains highly productive ecosystems such as mangroves, coral reefs, sea-grass beds and marshy lands. These coastal ecosystems are a valuable resource for the people of the country, particularly for coastal communities. Coastal ecosystems have been utilized as a food source and income-generator for centuries and the rate of exploitation is increasing at an alarming rate. Collection and export of coastal ecosystem-associated organisms have contributed substan-

tially to the foreign exchange earnings of the country. The collection of sea cucumbers for export is one such industry.

A sea cucumber fishery has existed in the northern parts of the island for many years, but in the south, along the coast from Negombo to Dondra (Fig. 1) the fishery began only about 10 years ago. Regrettably, overexploitation with no effective management measures has led to its complete collapse.

### History of the sea cucumber fishery in southern Sri Lanka

Sea cucumbers are locally known as *muhudu kekiri* or *atta* but are not used locally as a food item or for any other purpose. As with most sea cucumber producing countries, production is not meant for local consumption but rather for export to Asian countries (Conand 1990). There are nearly 200

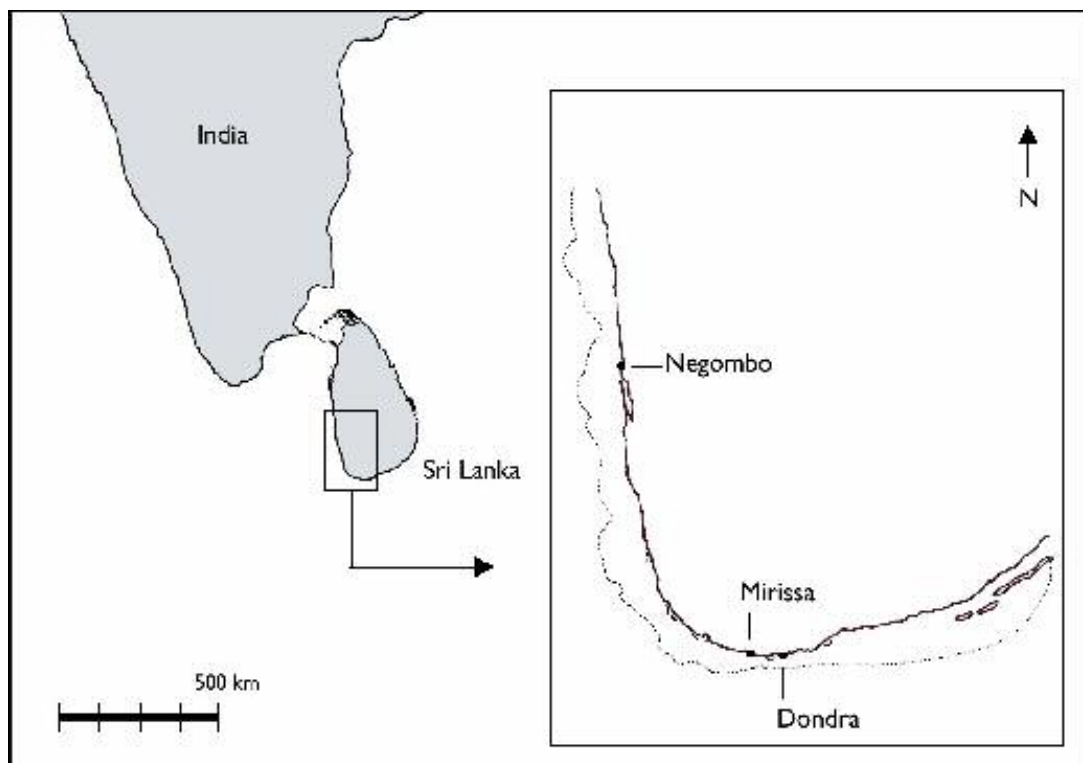


Figure 1. Major sea cucumber landing sites along the southern coast of Sri Lanka.

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**Figure 2.** *Holothuria edulis*.  
Image: Dr Malik Fernando



**Figure 3.** *Holothuria atra*.  
Image: Dr Malik Fernando

known species found in the waters around Sri Lanka. About 75 species have been shown to be present in shallow waters while nearly 50 species can be collected from the inter-tidal areas (Clark and Rowe 1971). Although sea cucumbers were abundant along the shallow coastal waters they were not harvested in the south until buyers from Singapore created a demand. The buyers primarily purchased two species, *Holothuria edulis* (Fig. 2) and *H. atra* (Fig. 3), for very low prices. Although buyers paid only one rupee per animal, fishermen earned a considerable amount of money because of the organism's abundance in shallow coastal waters. The price increased up to five rupees per animal as supplies dwindled. Harvesting at first was done by hand while wading or using snorkel gear in shallow water. As the shallow areas were fished out, scuba gear was used to exploit increasingly deeper sea cucumber beds. Over the past three to four years, the sea cucumber fishery in the shallow areas off southern Sri Lanka declined rapidly and finally collapsed. The fishermen and divers of these areas then turned to distant sea cucumber beds.

**Table 1.** Holothurians present in Sri Lankan waters. Source: Summarized from Clark and Rowe (1971).

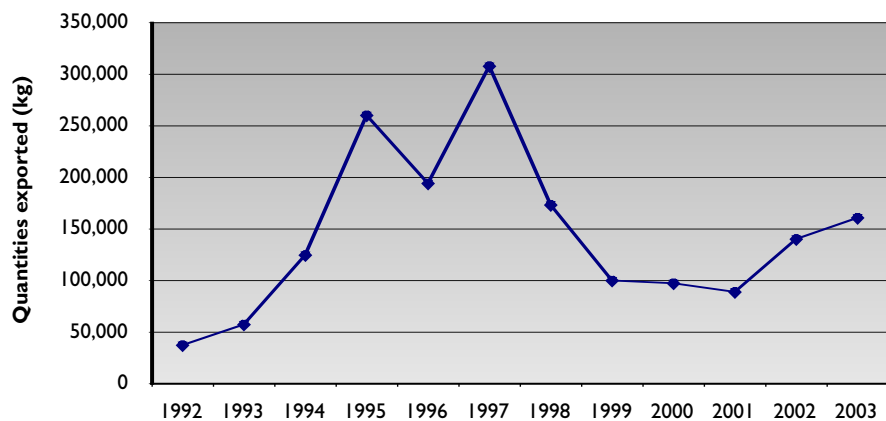
CLASS HOLOTHUROIDEA		
<p><b>Family Holothuriidae</b></p> <p><i>Actinopyga echinites</i>  <i>Actinopyga lecanora</i>  <i>Actinopyga mauritiana</i>  <i>Actinopyga miliaris</i>  <i>Actinopyga serratidens</i>  <i>Bohadschia argus</i>  <i>Bohadschia marmorata</i>  <i>Bohadschia tenuissima</i>  <i>Bohadschia vitiensis</i>  <i>Holothuria (Halodeima) atra</i>  <i>Holothuria (Halodeima) edulis</i>  <i>Holothuria (Lessonothuria) glandifera</i>  <i>Holothuria (Mertensiothuria) fuscocinerea</i>  <i>Holothuria (Mertensiothuria) leucospilota</i>  <i>Holothuria (Mertensiothuna) pervicax</i>  <i>Holothuria (Mertensiothuria) scabra</i>  <i>Holothuria (Microthele) nobilis</i>  <i>Holothuria (Platyperona) difficilis</i>  <i>Holothuria (Selenkothuria) erinaceus</i>  <i>Holothuria (Selenkothuria) moebii</i>  <i>Holothuria (Semperothuria) cinerascens</i>  <i>Holothuria (Semperothuria) imitans</i>  <i>Holothuna (Theelothuria) kurti</i>  <i>Holothuria (Theelothuria) spinifera</i>  <i>Holothuria (Thymiosyca) hilla</i>  <i>Holothuria (Thymiosyca) impatiens</i></p>	<p><b>Family Stichopodidae</b></p> <p><i>Stichopus chloronotus</i>  <i>Stichopus naso</i>  <i>Stichopus variegatus</i></p> <p><b>Family Psolidae</b></p> <p><i>Psolus complanatus</i></p> <p><b>Family Cucumariidae</b></p> <p><i>Havelockia herdmani</i>  <i>Hemithyone semperi</i>  <i>Pentacta armatus</i>  <i>Pentacta quadrangularis</i>  <i>Pseudocolochirus tricolor</i>  <i>Staurothyone rosacea</i>  <i>Stolus buccalis</i>  <i>Stolus conjugens</i>  <i>Thyone papuensis</i>  <i>Trachythyone imbricata</i>  <i>Trachythyone typica</i></p> <p><b>Family Phyllophoridae</b></p> <p><i>Actinocucumis typicus</i>  <i>Ohshimella ehrenbergi</i>  <i>Phyllophorus (Phyllophorella) parvipedes</i>  <i>Phyllophorus (Phyllothuria) cebuensis</i>  <i>Phyllophorus (Urodemella) brocki</i></p>	<p><b>Family Caudinidae</b></p> <p><i>Acaudina molpadioides</i></p> <p><b>Family Synaptidae</b></p> <p><i>Opheodesoma grisea</i>  <i>Synapta maculata</i>  <i>Synaptula recta</i>  <i>Synaptula striata</i></p> <p><b>Family Chirotidae</b></p> <p><i>Polycheira rufescens</i></p>

## Import and export trade

Sea cucumber collection in Sri Lanka “is rooted in antiquity, when Arab and Chinese merchants employed local inhabitants of western, northern and eastern maritime regions, to gather and cure the (animal) for them” (Adithiya 1969). The sea cucumber fishery in these western, northern and eastern waters flourished in later years to service increased demands from East Asian countries such as Singapore, Taiwan and Hong Kong. As shallow areas were fished out, the ready availability of scuba gear in the 1990s enabled exploitation of deeper habitats. Published Sri Lanka Customs Department statistics for the whole island show steep rises and declines in quantities exported (Fig. 4).

These fluctuations appear to correspond with the discovery of new sea cucumber beds and their subsequent depletion, as a result of unrestricted and intensive collection (Rajasuriya 1999).

Suitable shallow habitats for sea cucumbers are restricted to the southern parts of the island. Consequently, the collapse of the fishery in the south has led fishermen and divers from these areas to seek other, more distant areas where they can harvest sea cucumbers; fishing in these areas (which include the Chagos Archipelago, the Laccadive Islands and the Andaman Islands) is usually done illegally. This practice has also been reported in other parts of the island. Customs statistics for the whole country suggest that importation of sea cucumbers commenced in 1996, with small quantities from the Maldives (collected by Sri Lankans) for processing and re-export. Starting with 3 kg in 1996 the imported quantity gradually increased to 23,609 kg in 2000. The source of imports has not been established, but probably reflects clandestine operations. It should be noted that all available trade values are in dry weights after processing. Hence, the actual wet weight of the catch is at least 10 times as high as the dry weight, because 10 kg of fresh sea cucumbers yield less than 1 kg of dry product after processing (Conand 1990).



**Figure 4.** Beche-de-mer exports from Sri Lanka.

(Source: Sri Lanka customs external trade statistics and Anonymous 1999, 2000)

**Table 2.** Sea cucumber exports (kg) from Sri Lanka, 1996–2003.

	1996	1997	1998	1999	2000	2001	2002	2003
Singapore	73,266		88,959	30,905	16,479	19,739	25,519	47,223
Taiwan	27,457		68,330	45,112	39,626	48,649	50,593	44,866
Hong Kong	69,803		46,424	22,001	29,530	14,205	40,057	40,746
<b>Total</b>	<b>170,526</b>	<b>307,578</b>	<b>203,713</b>	<b>98,018</b>	<b>85,635</b>	<b>82,593</b>	<b>116,169</b>	<b>132,835</b>

Source: Sri Lanka customs external trade statistics and Anonymous 1999, 2000

## The main sea cucumber markets

Sea cucumbers are a delicacy in the Far East; the Chinese consume them in processed form while the Japanese and Koreans eat them fresh (James 2001). They are also used in the production of oils, lotions, cosmetics and tablets (Baine and Sze 1999). Exports from Sri Lanka are usually in the processed form: the dried product is called beche-de-mer or trepang (Conand 1998).

The major export destinations for sea cucumbers are Singapore, Taiwan and Hong Kong. Singapore has been the dominant buyer from Sri Lanka since 1999. Taiwan and Hong Kong are the second and third largest markets, respectively (Table 2). The mean weight of annual exports to these three countries since 1997 is 49,633 kg ( $\pm 76,498$  SD). The actual wet weight for the mean amount exported is approximately 4,966,330 kg.

## Present status of the fishery

At present, Sri Lankan fishermen from the south are exploiting sea cucumber beds in distant parts of the Indian Ocean. Although fishermen from Negombo on the west coast started the fishery in the southern part of the island, fishermen and divers from the southern fishing towns of Mirissa

and Dondra now dominate (Fig.1). Around 35 boats are engaged in the sea cucumber fishery along the southern coast. Fishermen use multi-day operating craft (MDOC) and global positioning systems (GPS) to navigate far from shore (Fig. 5). Boats range from 10.7–15.2 m (35–50 ft), but all use 45 hp inboard engines. These boats are usually four to six year old and modified to accommodate 10 to 12 crew members. The crew consists of a skipper, divers, a cook, a compressor operator, and an electricity generator operator. At times these boats operate in groups of three or four; by doing so they are able to maximise their profits by carrying fewer support personnel (such as cooks and compressor operators) and a larger number of divers.



**Figure 5.** Multi-day operating crafts used in the sea cucumber fishery. Some of these boats operate in distant areas such as the coastal waters of the Chagos Archipelago, Laccadive and Andaman islands.  
Image: Terney Pradeep Kumara

Fishing for sea cucumbers in the south is mostly done by opportunistic tuna fishermen. Whenever exploitable sea cucumber beds are located, the fishermen switch to that fishery; and when the beds are fished out, they revert to tuna fishing. The duration of each activity depends on the sea cucumber population and the number of boats and divers participating in the fishery.

The harvesting method depends on the depth of the fishing ground. Snorkelling gear is used in shallow water, and the animals are collected by hand and placed into a net bag. Sometimes, divers use small hand nets to collect sea cucumbers from gullies and crevices on rocky bottoms. In deeper water, scuba gear is used. The filled net bags are sent to the surface using lifting bags filled with air that are retrieved by the boat's crew. The catch is washed and stored on ice in the hold. Scuba divers complain that they now need to dive to increasing depths to

find holothurians, leading to diving-related accidents. Divers also complain that they are constantly forced to look for new fishing grounds, as described by Conand et al. (1997) for Madagascar.

Examination of the fresh sea cucumbers (using photo guides as a reference) as well as the cured product, indicates that there are three principal species collected by Sri Lankan divers from the south. These sea cucumbers have been given local names based on their appearance. *Holothuria fuscogilva* (white teatfish) is called *prima* locally. It occurs in deeper waters, around 60–90 feet deep, and is common on coral slabs near reef passages or at the foot of reef slopes (James 2001). This is a high value species, and good quality animals command a price of around Sri Lankan rupees (LKR) 1200 each<sup>1</sup>. *Holothuria nobilis* (black teatfish) is known locally as *polanga*. This species is found in shallow reef beds and on turtle grass beds to a depth of 15 feet (James 2001). The market value is LKR 400 each. *Holothuria spinifera* (brown sandfish) has the local name *gal atta* or *weli atta*. This species has a comparatively low market value ranging from LKR 40 to 50.

The majority of the catch goes through collectors or middlemen for processing, which is mainly a cottage industry involving families of collectors and middlemen. During processing, sea cucumbers are gutted, cleaned, cooked in boiling water for half an hour and dried (Figs. 6, 7 and 8). Processed products are then stored in plastic boxes and channelled to the export market.

### Future development of the industry

The sea cucumber fishery in the south is not expected to grow much beyond the current level; it is instead likely to decrease in the near future due to the scarcity of the resource. Demand will continue to rise, however. As a result, it is expected that new species will be introduced to the market. The depletion of wild sea cucumber stocks may have the effect of increasing the value of those that remain, so that low value species become medium value, and medium value species become high value. Although the principal markets for beche-de-mer are East Asian countries, there is a possibility that the market will expand towards Europe, America and Canada, where many East Asians live. Improved processing and cooking methods, and increased awareness of the antiviral, antitumoral, anticancerous, and pro-fertility properties of this product could also increase demand (James 2001).

1. LKR 100 ≈ USD 9.8 (July 2005)





**Figure 6.**  
Straining fresh sea cucumbers.  
Image: Dr Malik Fernando

**Figure 7.**  
Laying out *Holothuria scabra*  
in the sun to dry.



**Figure 8.**  
*Holothuria scabra* left to sun dry.  
Image: Dr Malik Fernando

### Management measures

There are no effective management measures in place to ensure sustainability of the sea cucumber fishery in Sri Lanka. Although most sea cucumber fishermen are aware of the negative impacts of the fishery and the rate of resource depletion, the high revenue that this fishery brings, the low amount of fishing effort required, and the scarcity of alternate sources of income of the same magnitude, drive

them towards harvesting all available sea cucumbers in the shortest possible time.

There is a lack of coordination at different levels of government, particularly at the provincial level where authorities do not have the required knowledge or understanding to manage the environment (Rajasuriya et al. 1995). Monitoring stocks and trade in sea cucumbers has been severely hampered in the past due to lack of expertise to identify

both live animals and the cured products at the point of export. This matter has been addressed, however, by the National Aquatic Resources Agency, which now has an ongoing programme to identify harvested species. The Fisheries Ministry has also set up a working party to investigate all aspects of sea cucumber extraction, with the intention of ensuring sustainability of the fishery. Considerable work needs to be done as almost nothing is documented about the diversity and distribution of sea cucumbers in Sri Lanka at the present time.

Sustainable use can be achieved through management of the resource. Replenishment of stocks by artificial culture and re-introduction is an option to consider. Financial constraints are the major obstacle to the implementation of an effective management system. Primary management costs are likely to be those associated with enforcement, provision of technical assistance, training, monitoring, and evaluation. These problems can be resolved with better understanding and dialogue between scientists and politicians.

### Conclusion

The sea cucumber fishery in southern Sri Lanka has collapsed due to overexploitation. As a result, Sri Lankan fishermen and divers exploit sea cucumber beds well away from Sri Lankan waters as an illegal fishery because of the high demand and the high income provided by the industry. Factors that limit the sustainability of the industry in Sri Lanka are inadequate information about current stocks, exploitation rates, fishing grounds, and absence of resource management regulations and awareness programmes. Research into improved processing techniques and possible culturing techniques are advisable.

### Acknowledgements

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## Developing technologies for restocking sandfish: Update on the WorldFish–SPC project in New Caledonia

Steve Purcell<sup>1</sup>

The WorldFish Center project to develop strategies for restocking sea cucumbers is now in its fourth year. Originally planned for Solomon Islands, the project was relocated to New Caledonia and has been hosted and partnered by the Secretariat of the Pacific Community (SPC). Its main purpose is to develop optimal release strategies for the restocking of sandfish (*Holothuria scabra*) (see Purcell 2004). Multi-lateral funding for the project has come from grants by the Australian Centre for International Agricultural Research (ACIAR), the three provinces of New Caledonia, the Government of France and the ATSE Crawford Fund. Following an initial year of project rebuilding and hatchery construction, the team produced sandfish in three successive years in sufficient numbers for the experiments. In 2003 and 2004, we completed most of the field and hatchery experiments on sandfish, on topics including:

- methods for broodstock maturation and spawning
- methods for rearing juveniles
- growth of sandfish in earthen (shrimp) ponds
- optimal methods for transporting juveniles for restocking
- optimal habitats for release
- best times of the day for release
- co-culture of juvenile sandfish with juvenile shrimp.

Experiments in 2004–2005 at the hatchery north of Noumea have refined new methods, which the WorldFish Center pioneered (by Rayner Pitt) in Vietnam, to grow sandfish juveniles in net enclosures within earthen shrimp ponds up to larger sizes for release. Two types of net enclosures are used: “hapas”, of 670 mm mesh, and “bag nets”, of 1 mm mesh. The experiments determined the best size at which to transfer the newly settled juveniles from the hatchery to hapas, how to increase their growth rate, what food they should be given in bag nets (Fig. 1), and in which situations to feed. The growth of juveniles within bag nets in ponds was faster than in those within bag nets in the sea. The new methods furnish a more cost-effective mode to scale up the production of juveniles and provide solutions to the hatchery-space constraints when mass-producing juveniles. Hatchery production of sandfish was kept to an experimental scale, this

year totalling 20,000 juveniles, allowing resources to be spread to the field research.



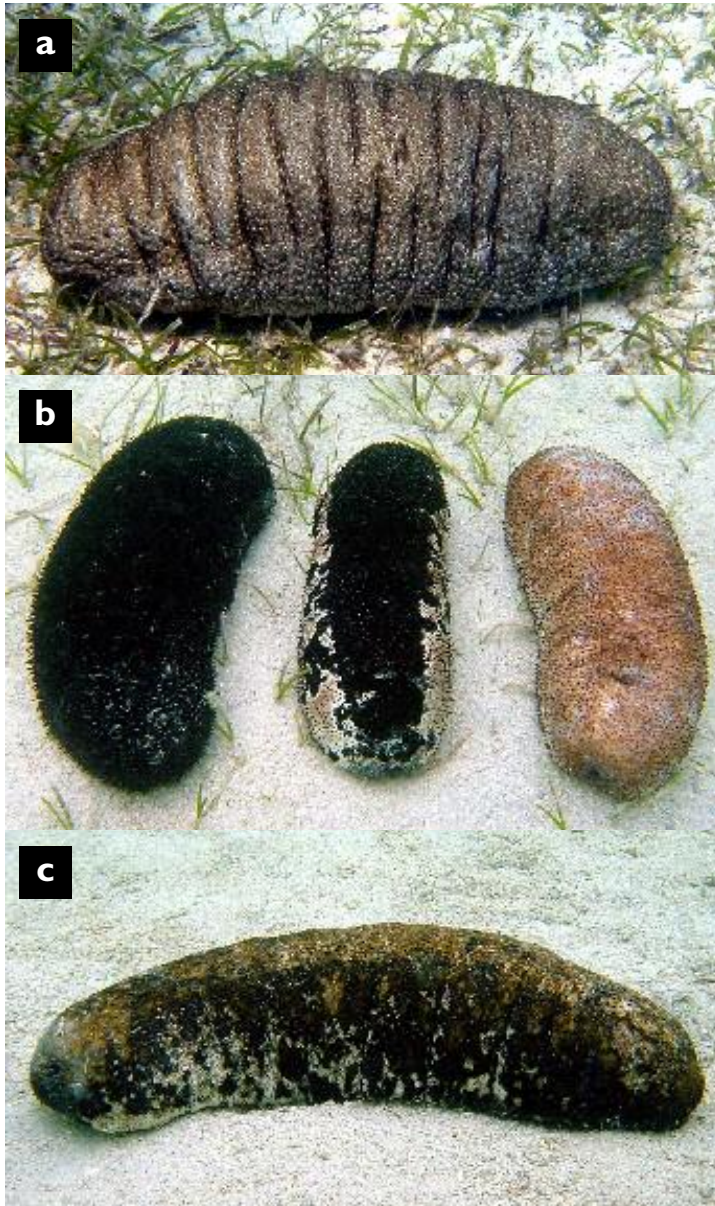
**Figure 1.** Bag net enclosures (1 mm mesh), 2 m x 2 m x 1 m high, set up in an earthen pond and used to grow sandfish juveniles from around 0.5 g to larger sizes (1–20 g) for release into the wild.

Following a collaborative study on the population genetics of sandfish along the main island of New Caledonia, La Grande Terre (Uthicke and Purcell 2004), we conducted further sampling and genetic analyses. These showed that the putative subspecies *Holothuria scabra* var. *versicolor* (golden sandfish) is actually a separate species, but can naturally hybridise with *Holothuria scabra* (sandfish) (Uthicke et al. in press). Figure 2 illustrates the two species and one hybrid morph.

As part of a commitment to the Provincial Government, we conducted broad-scale surveys of sea cucumbers using the “manta tow” technique in all three provinces in New Caledonia (Fig. 3). The Loyalty Islands Province had generally good stocks of reefal species, particularly black teatfish, which were in densities up to 244 adults ha<sup>-1</sup>. Surveys for sandfish along La Grande Terre showed that some sites had healthy populations (up to 1016 adults ha<sup>-1</sup>) while populations at other sites were depleted, apparently from a history of heavy fishing pressure. It is encouraging that the Northern Province is now developing management regulations to limit the harvest and trade of sea cucumbers.

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**Figure 2.**

Underwater photographs of:  
 a) *Holothuria scabra*,  
 b) black, blotchy and beige colour morphs of a new species previously called *H. scabra* var. *versicolor*, and  
 c) a blotchy hybrid of the two putative phenotypes, distinguished by colour pattern and length of papillae and identified by DNA sequencing.

**Figure 3.**  
 A diver conducts a transect survey (2 m x 200 m) using the manta tow method. A boat tows the manta board, with attached data sheet, at ~2 km h<sup>-1</sup>. GPS is used to control distances, and transect width is reduced to 1 m for surveys at shallow or turbid sites.



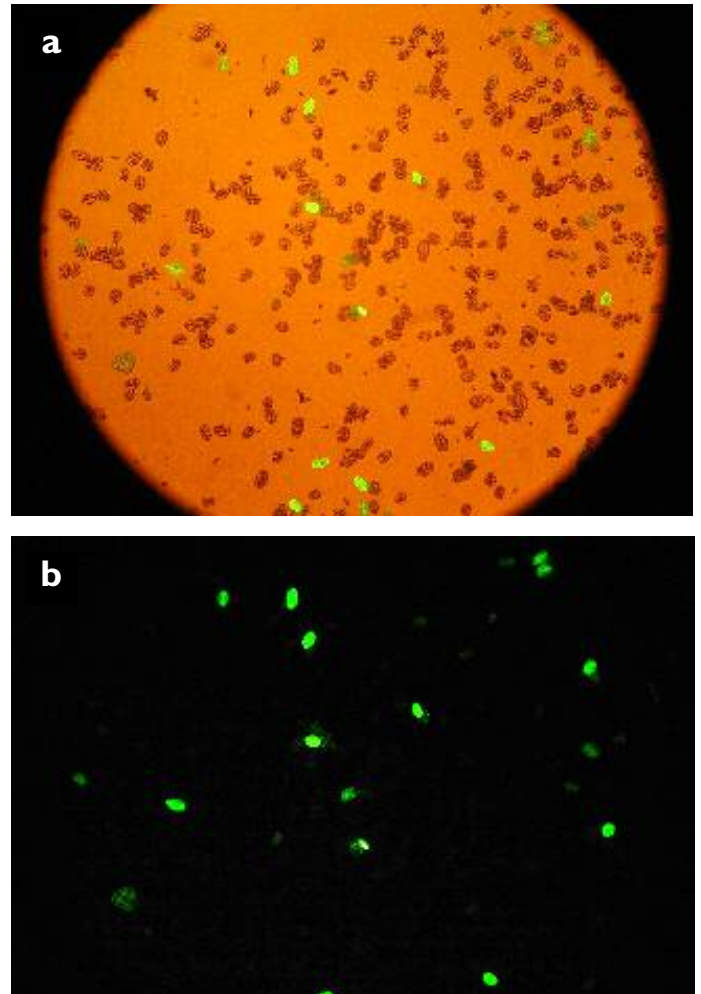


As an adjunct to the stock assessment surveys, we measured the *in situ* movement and burrowing behaviour of wild sandfish adults and released juveniles. These data, along with estimated growth rates, furnished an individual-based model of the dispersal of sandfish from release sites, which was then used to predict the requisite size of no-take zones for restocking (Purcell and Kirby in review).

Several complementary studies were completed to develop cheap, simple, methods for marking juvenile sea cucumbers so they can be distinguished from wild animals following their release. After short trials comparing the viability of five methods, we tested two fluorochromic markers and injected wire tags into sandfish juveniles held in enclosures in an earthen pond. Breaking new ground, the study showed that tetracycline and calcein permanently stained the spicules of sandfish and fluorescent spicules can be detected from skin samples after at least one year (Fig. 4a and b). Further experiments established the best protocols for tagging the juveniles and these studies are now in preparation for publication. The two markers stain the spicules different colours; this allows up to three groups to be tagged (with each marker separately and with both combined). The tagging methods will be invaluable for demonstrating the success of restocking in “mixed-stock” scenarios (i.e. when both released and wild animals could be present).

As a means of gauging the viability of restocking using the release methods developed in 2003 and 2004, we planned a final, “large-scale release experiment”, employing the release of juvenile sandfish into large sea pens. To develop the methodology for that experiment, we started a pilot study in July 2004 by installing a large sea pen (500 m<sup>2</sup>) at each of two sites, and releasing 2000 juvenile sandfish into each pen. The sea pens are simply a short plastic mesh fence, without cover, to limit juvenile emigration from the natural habitat. Although mortality was high during the first 1–2 months after release, the rate of mortality thereafter became much lower, when the juveniles reached ~50–100g (Fig. 5). Following the trends in Figure 5, sandfish survival is estimated at 5–9% after 18 months from release, when they would be expected to reach maturity.

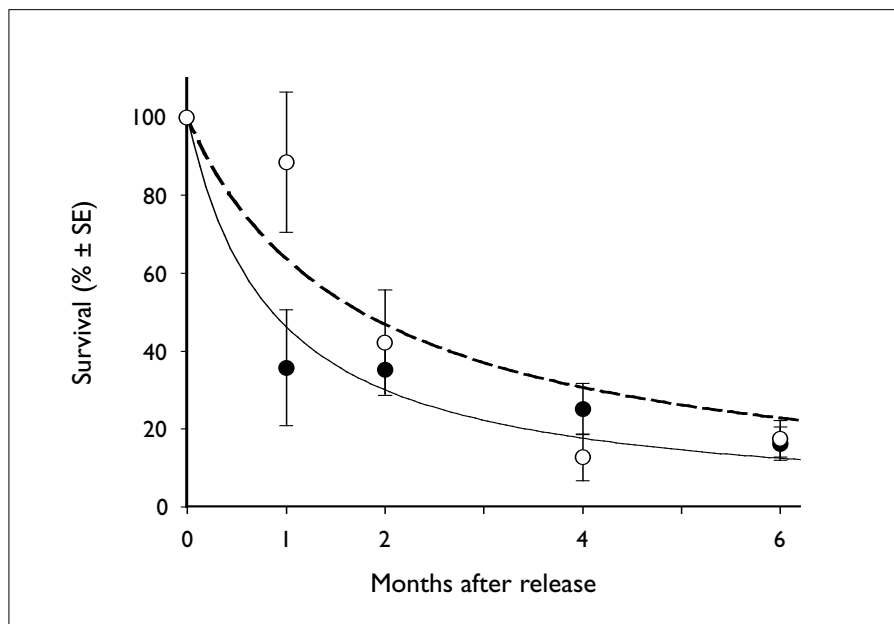
Based on the success of that pilot study, the large-scale release experiment was launched in April 2005. It involves 12 sea pens (each 500 m<sup>2</sup> in area) and an initial 9000 hatchery-produced sandfish juveniles, batch tagged in three size classes. At each of the four sites along La Grande Terre, sandfish juveniles were released into three sea pens, each with a different release density (0.5, 1, and 3 juveniles m<sup>-2</sup>). Quadrat



**Figure 4.** Photos under epifluorescence microscopy of spicules from a juvenile sandfish, tagged with calcein 12 months prior: a) stained and unstained spicules under normal and fluorescent light; b) the same field of view under fluorescent light only, showing stained spicules only.

sampling will be used to monitor juvenile survival and growth every two months for one year. The experiment will allow us to compare survival between size classes and among the three release densities, to see if survival in the long term is higher at lower densities than used in the pilot study. Because the experiment involves four sites (roughly 50–100 km apart), the results will give a range in survival and time frame for juveniles to reach maturity and commence spawning; the ecological goal of restocking. A cost-benefit analysis will be made, and only then can the utility of restocking with hatchery-produced juveniles be weighed against other forms of management (Bell and Nash 2004).

If the large-scale release experiment indicates that restocking sandfish through the release of hatchery-produced juveniles is economically viable, this



**Figure 5.** Plots of the estimated survival ( $\pm$  SE) of juveniles in open sea pens at two sites in New Caledonia from random quadrat surveys. Fitted lines are non-linear regressions of survival v time for the two sites (Ouano, dotted line; Népoui, solid line).

strategy will provide a way forward for restoring breeding populations that were overfished to levels too low to allow natural recovery. WorldFish hopes to embark on the Phase 3 project of the programme in July 2006, which will seek to carry out broad-scale restocking of sandfish in several Pacific Island countries. The purpose of such restocking would be to rebuild a breeding population to generate larval supply to depleted fishing grounds rather than a “put-and-take” fishery. Clearly, better management of sea cucumbers is needed as a precursor to restocking. Encouragingly, Pacific Island nations (e.g. Papua New Guinea, Solomon Islands, Fiji) are making strong moves to implement such management. In places where management can safeguard the means of restocking, this research will be a basis by which livelihoods and household income of coastal fishers can be restored.

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## An external check for disease and health of hatchery-produced sea cucumbers

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### Abstract

Sea cucumber diseases that arise in the hatchery can cripple production and undermine restocking programmes. A rapid protocol for the external examination of juvenile sea cucumbers was developed in order to screen for disease and poor health. After selecting a random sample of juveniles from the entire group, each individual is examined for one minute under low-power microscopy. Six external criteria are checked, including abnormalities of the mouth, anus, papillae, body colour, and signs of excess mucus, unhealed lesions, parasites and macroassociates. If more than 5% of screened animals were “unhealthy” or more than 2% were “diseased”, then the whole group from which the sample was derived should be considered unfit for grow-out or restocking. In such cases, the handling and environmental conditions in the hatchery should be improved and the entire group quarantined and treated. The protocol sets a standardised procedure for checking large numbers of juveniles for many infectious diseases, and is a starting point for further development of standardised protocols.

### Introduction

Production of hatchery-reared sea cucumbers has gained global interest, and is underway in Australia, China, Ecuador, Kiribati, Madagascar, Malaysia, New Caledonia and Vietnam. Regardless of whether juveniles are to be used for restocking, stock enhancement or land-based grow-out, diseases can arise in the hatchery and cripple production. Identifying diseases in the hatchery is a precondition for their treatment and a prerequisite for releasing juveniles into the wild. Until recently, however, little was known about sea cucumber diseases.

Diseases can be biomolecular (e.g. hereditary), induced by pathogens, or arise from abiotic factors (Kinne 1980). The United Nations-FAO funded workshop in China (October 2003), “Advances in Sea Cucumber Aquaculture and Management”, improved the collective knowledge of biotic diseases and parasites affecting sea cucumbers. Wang et al. (2004) reported that various pathogens, including bacteria, fungi and other parasites, could affect *Apostichopus japonicus* larvae and juveniles in culture conditions. Clinical signs of infections include lesions starting around the mouth or anus, whitish ulcerations on the skin or papillae, excessive mucus on the body, skin discolouration, and changes in behaviour and appearance (e.g. the infected animals can become thin, weak and sluggish). Eeckhaut et al. (2004) provided a list of bacteria, protozoans and metazoans (e.g. flatworms, gastropods and crustacean parasites) that can potentially cause disease in sea cucumbers. They also

reported a contagious bacterial disease of *Holothuria scabra* that begins as a white lesion close to the anus, followed by a lesion that progresses quickly over the body. Microscopy and biomolecular techniques have proved instrumental in identifying the bacterial species associated with that disease, called “skin ulceration disease” (Becker et al. 2004). Symptoms of many of the diseases reported by Wang et al. (2004) and Eeckhaut et al. (2004) can be seen externally by low-power microscopy so it should be possible to screen juveniles for visible symptoms of diseases prior to transfer to grow-out ponds or into the wild.

Aside from true biotic diseases, some sea cucumber illnesses can be viral, chemical or from poor culture environment. For example, excess aeration or inappropriate temperatures cause illness and death in sea cucumber larvae (Hamel et al. 2001; Wang et al. 2004). In New Caledonia, copper wire placed in tanks was sufficient to kill juveniles within days (S. Purcell, unpubl. data). Illnesses may be subtle with no lesion or malformation. Instead, the animals become unhealthy and the symptoms could include sluggishness or change in body colour. Illness from chemical contamination could reduce feeding in juveniles and make them more prone to predation when they are released into the wild.

The impacts of releasing diseased or unhealthy juveniles into the wild can be indirect or direct. First, contagious diseases from hatchery-reared sea cucumbers can spread to native stocks, competitors or predators. For example, some bacterial strains can be infectious to other invertebrates

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and to fish (see Becker et al. 2004). Second, the reduced fitness of released animals can cause unexplained mortality, if they die soon after release, or can make them more prone to predation if their natural mechanisms to avoid predation (e.g. chemical deterrents, burrowing behaviour or camouflage) are diminished.

A screening procedure is needed to reduce the risk of releasing diseased animals into the wild. Here we present a protocol for the rapid checking of sea cucumber juveniles under a dissecting microscope, guidelines for determining whether juveniles are fit for release, and some specific criteria for disease screening of *Holothuria scabra*.

## Disease and health check

### Pre-screening preparations

In order for disease screening to reflect the status of juveniles in the group, random sampling of juveniles from the entire pool of juveniles at the hatchery is essential. As a guide, the number of juveniles in the sample should be no less than the square root of the total number of juveniles in the batch.

1. Randomly sample a group from the batch of cultured juveniles.
2. Place each live juvenile in a Petri dish with seawater and examine under a dissecting microscope (Fig. 1a).
3. Following the procedures below, and verifying each point in the proceeding checklist for each animal, score any abnormalities on a proforma in two categories: “unhealthy” or “diseased”. Unhealthy juveniles are those with symptoms

of stress or mishandling, whereas “diseased” juveniles show symptoms of infection by pathogens.

### Screening protocol

The following points relate to various indicators of health and disease. Checking each point individually, the complete screening of all points should take around 1 minute per juvenile.

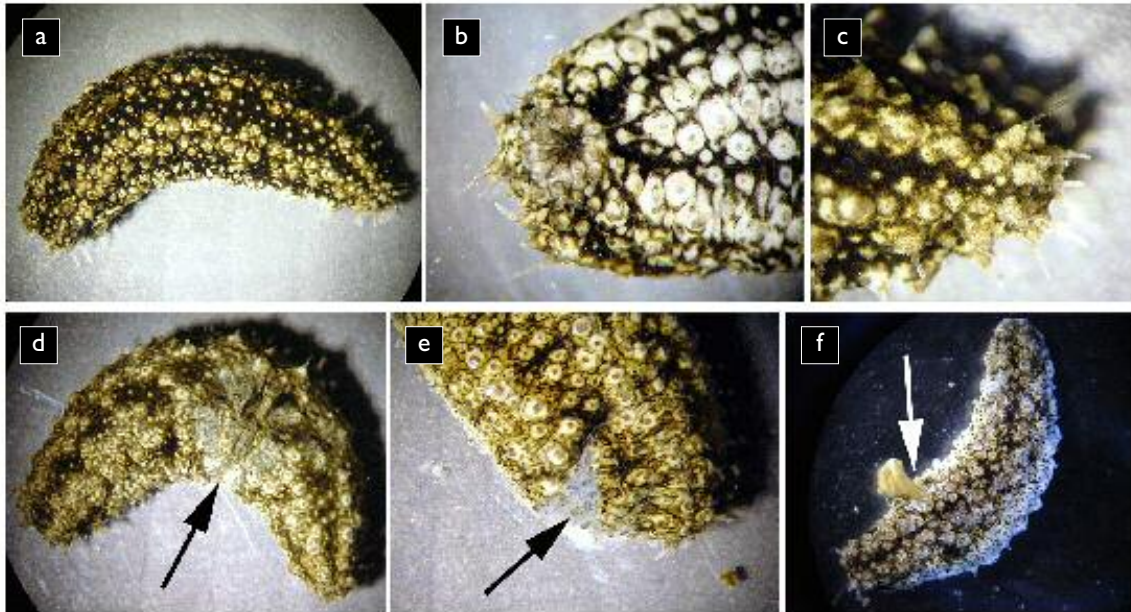
- I. Confirm that the skin and papillae colouration is “normal” and healthy. *H. scabra* juveniles from the Pacific can be black or display mottled colouration of greenish beige, brown and grey, with black speckling (Fig. 1b). The papillae can appear wart-like, which is normal (Figs. 2a, b, c). There should be some thinly scattered podia (tube feet) on the dorsal surface, and many more ventrally. The podia will appear as a dark spot when retracted. Tube feet can be coloured dark yellow, black or grey-brown, and are arranged irregularly. The ventral surface should be generally more whitish or cream coloured (Fig. 2b). Juvenile sea cucumbers that are handled and then placed back into seawater may bloat with water. This is a normal sign of mild stress but the bloating should subside within one hour. Juveniles with body colour that is unusually pale should be scored as “diseased” for precautionary reasons.
- II. Check any malformation, or abnormal retraction, of the mouth or the area around the anus. Refer to taxonomic literature on the normal arrangement of papillae (e.g. Conand 1998). The mouth and oral tentacles, if visible, should be normal in colour; in *H. scabra*, these should be



**Figure 1.**

- (a) Checking juveniles for signs of disease using a low-power (dissecting) microscope.  
 (b) Varied colours and colour patterns of healthy juvenile sandfish *Holothuria scabra* from the WorldFish hatchery in New Caledonia (approx. 2–20 g).





**Figure 2.**

- (a) A healthy juvenile sandfish (approx. 1 g or 20 mm) under low-power microscope.  
 (b) Ventral surface and mouth of healthy juvenile sandfish.  
 (c) Dorsal surface and anus of healthy juvenile sandfish.  
 (d) Juvenile sandfish with healed lesion on the middle of the dorsal surface, indicated by arrow.  
 (e) Open lesion, exposing white muscle tissue (indicated by arrow), near anus of juvenile sandfish.  
 (f) Extreme injury in juvenile sandfish with part of intestine protruding out of open lesion on dorsal surface, indicated by arrow.

yellowish-grey (Fig. 2b). Juvenile sandfish should not have any white spots near the anus (Fig. 2c). Score as “diseased” if abnormal.

- III. Juveniles sometimes feel slightly slimy. However, juveniles with excessive mucus on the skin should be scored as “unhealthy”.
- IV. Check the entire body (including buccal region and anus) for any fungal or bacterial infections. Juveniles with white spots, whitish fluffy patches or cysts on the body should be scored as “diseased”.
- V. It is not unusual for juveniles in the hatchery to have some scars from handling in the transfer among tanks or outdoor enclosures, so juveniles with healed scars (e.g. Fig. 2d) can be considered healthy. Check the entire body for open lesions (e.g. Fig. 2e and 2f). Score as “unhealthy” if the skin is damaged and not closed.
- VI. Confirm that juveniles are not infested by macroassociates such as polychaetes, marine nematodes (roundworms), crabs, eulimid gastropods or copepods. A few copepods can be present on healthy individuals but a density of more than 5 copepods or nematodes on a small juvenile (1–5 cm long) is abnormal. Score as “unhealthy” if infested.

### **Checklist for each individual**

A table including cells for each of the points in the *Screening Protocol* should be used to record the date of screening, number of juveniles and scores for each point. Score each point for each juvenile (e.g. with a tick for okay and a cross for “unhealthy” or “diseased”) as a record that each point was examined. Individuals with one “unhealthy” or “diseased” score in the following checklist have failed the screening.

#### *Disease*

- Skin and papillae colouration is “normal” and healthy.
- No white spots, malformation or abnormal retraction of the mouth or anus.
- Oral tentacles, if visible, are yellowish-grey.
- Ventral surface should be generally more whitish or cream-coloured.
- No fungal or bacterial infections, involving white spots or fluffy patches on the body.

#### *Health*

- No prominent layer of mucus on the skin.
- Body with no open lesions or scars with un-

- sealed epidermis.
- No infestation of macroassociates.

### Post screening

Once each juvenile in the sample has been screened and points scored, the final step is to assess the “fitness” of the complete group at the hatchery, based on the screened sample of juveniles, and take actions if the screening indicates unacceptable proportions of unhealthy or diseased juveniles.

1. Determine proportional score of the group. Pass the total group as “fit” if no more than 5% of screened animals were scored as “unhealthy” and if no more than 2% of screened animals were scored as “diseased”. These guidelines permit some occurrence of stressed juveniles, as this may be unavoidable during handling at the hatchery, but places stricter control on potential risks of diseases.
2. If possible, take photos via the microscope of any “unhealthy” or “diseased” juveniles. Preserve any “diseased” juveniles in 100% analytical-grade alcohol and label accordingly. These preserved specimens can be analysed later.
3. If the screening suggests that the group is unhealthy, improve the handling and environmental conditions in the hatchery and allow sufficient time for recovery. If the screening shows an unacceptable proportion of “diseased” juveniles, the entire group should be quarantined and a disease treatment applied. Treatment of disease should start with improving water quality and stocking conditions (e.g. see Battaglione et al. 1999), then consider an application of chemicals, such as a low-dosage of formalin-based products, or antibiotics, such as oxytetracycline at 1–5 ppm. Do not use copper-based disease remedies. Re-screen a further random sample of juveniles no earlier than two weeks after treatment.
4. Do not attempt to release juveniles from groups that have had recent unexplained mortality. The deaths may be due to disease and surviving animals that appear to be healthy could be carriers of disease.

### Discussion

This protocol will not provide a definitive or comprehensive certification that sea cucumbers are disease-free because some protozoans and metazoan parasites occur in the digestive tract and coelom and do not manifest clear external symptoms (Eeckhaut et al. 2004). However, the protocol is useful for rapidly checking a large number of juveniles for many infectious diseases, and is a

starting point for further development of standardised protocols.

Hatcheries should not rely on the use of these protocols alone to avoid the transfer of diseased or unhealthy sea cucumbers. Precautions should be taken throughout the culture cycle to reduce the risk of diseases. These precautions include:

- 1) keeping the number and biomass of animals within reasonable limits (Eeckhaut et al. 2004);
- 2) maintaining good water quality and limiting temperature extremes to minimise stress on juveniles;
- 3) handling and transferring juveniles gently to minimise abrasions that break the external mucous layer and epidermis, exposing tissue to infection.

Also, some infections arise when bacteria are at unacceptably high levels and when the sea cucumbers are stressed or wounded. For example, lesions and death of juvenile and adult *H. scabra* occurred in earthen ponds in New Caledonia where dissolved oxygen and temperature could not be controlled.

If disease is found, the infected animals should be quarantined and treated or destroyed. One overarching comment raised by pathologists that we consulted was the need to establish a database of sea cucumber pathogens. In this regard, a study of diseases, and their prevalence, in natural populations of sea cucumbers would be invaluable. An additional need is for a repository of reference material detailing normal histology of healthy sea cucumbers. Accordingly, Pierre Becker (Université de Mons-Hainaut, Belgium) has requested samples of infected individuals so that the infectious agent can be identified by microscopic and/or biomolecular methods. A database of pathogens and resulting diseases will be a valuable tool for progressing a better understanding of diseases of cultured sea cucumbers.

### Acknowledgements

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## An incidence of parasitic infestation in *Holothuria atra* Jaeger

M.K. Ram Mohan<sup>1</sup> and D.B. James<sup>2</sup>

The reproductive cycle of the sea cucumber *Holothuria atra* was studied at Tuticorin, on the southeast coast of India (8°45'N, 78°12'E). Specimens were collected from Tuticorin Bay from November 1997 to April 1999. Collected specimens were cut open by making an incision through the anal portion to open up the coelom, exposing the viscera (including gonads) for the purpose of studying the gonadal reproductive stage. During the course of study, two parasitic gastropods were found in the cloacal chamber of one specimen, which was at an indeterminate stage of sexual maturity.

This gastropod, according to Waren (1983), belongs to the genus *Megadenus* sp. The classification is as follows: Class: Gastropoda; Subclass: Prosobranchia; Order: Caenogastropoda; Superfamily: Eulimoidea; Family: Eulimidae; Genus: *Megadenus*.

The parasites, which were noticed during September 1998, were embedded in the wall tissue of the cloacal chamber. The shell breadths of the gastropods were 2.8 mm and 2.2 mm, the smaller one being the male. The sea cucumber weighed 160 g. During the study, a total of 994 specimens were examined, and infestation of parasites in the cloacal chamber was found in only one animal. Hence, it could be assumed that about 0.1% of the population was infested by the above-mentioned parasite.

Jones and James (1970) reported a gastropod parasite, *Stilifer* sp., from the cloacal chamber of *H. atra*. They recovered 13 gastropods from 8 infested specimens, after examining 1359 specimens. The infestation rate was 0.58%. Further studies on the physiological implications of parasitic infestations are required, as effects such as castration could affect and influence the parameters of the sea cucumber population in a particular locality.

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## The recent status of sea cucumber fisheries in the continental United States of America

Andrew W. Bruckner<sup>1</sup>

### Introduction

Sea cucumber fisheries occur in waters of the United States off the Pacific coast states of California, Oregon, Washington and Alaska, and the Atlantic coast state of Maine. In the Pacific, commercial harvest focuses on two species in the family Stichopodidae, *Parastichopus californicus* (giant red sea cucumber) and *P. parvimensis* (warty sea cucumber). In the Atlantic, commercial fisheries target a single species in the family Cucumariidae, *Cucumaria frondosa*. Commercial fisheries in the US were first established in Washington state (1971), followed by California (1978), Alaska (1981), Maine (1988), and Oregon (1993). Sea cucumbers are selectively collected by hand using scuba or hookah, and non-selectively harvested with bottom trawls, scallop chain sweeps and urchin drag gear. Sea cucumber fish-

eries are managed by each state, using a variety of management measures such as permitting and licensing requirements, spatial and temporal closures, harvest quotas, seasonal fishing periods, and gear restrictions (Table 1).

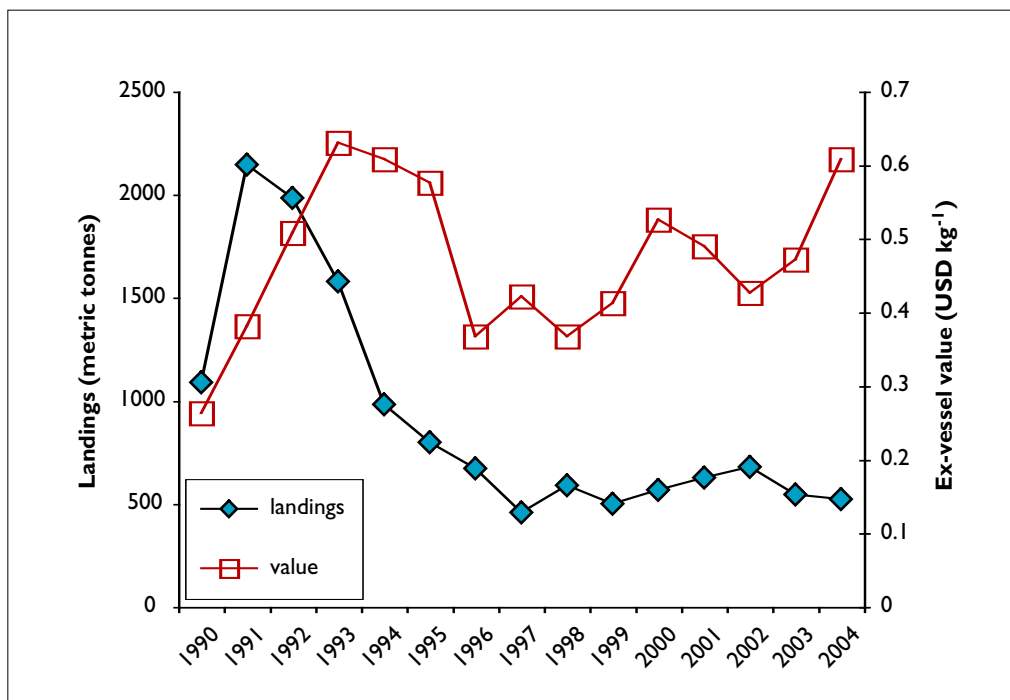
Maine currently has the largest sea cucumber fishery (based on wet weight), followed by Alaska, Washington and California, respectively; minimal harvest occurs in Oregon. Total harvests for California and Washington peaked in 1991 (2144 metric tonnes [t]) and have subsequently declined to 500–600 t per year (Fig. 1). According to FAO statistics, the total fisheries production for US temperate sea cucumber fisheries between 1992 and 2001 was 18,127 t, with a maximum harvest of 4583 t in 2000 (Table 2). Most sea cucumbers are exported to Hong Kong, Chinese Taipei, mainland China and Korea, with limited consumption in

**Table 1.** Controls and enforcement measures for sea cucumber fisheries in US temperate waters.

Location	Licensing	Reporting	Validation
Alaska, USA	Divers registered and permitted.	Dive/harvest logbook with date, location (GPS), depth, bottom time, quantity.	Divers can only obtain permits for urchins or sea cucumbers but not both.
Washington, USA	Limited entry; 190 divers in 2000.	Logbooks with daily reporting of catch to avoid exceeding quota.	Must submit logbooks every month with data on date, depth location and amount (number and weight) collected.
Oregon, USA	Licenses issued up to 2003, with only two divers requesting a license.	Fish receiving tickets (dock ticket) required from sea cucumber dealers with fisherman's name, location, date and amount.	Cucumbers are listed under developmental fisheries species list category B. As of 2004 the fishery no longer requires a permit.
California, USA	Separate annual permits for each gear type: In 2004 95 dive permits and 24 sea cucumber trawl permits were issued.	Dive and trawl fisheries target different species and areas; Trawl fishery near port of Los Angeles; dive fishery near Santa Barbara.	Limit permits by requiring a minimum landing of 50 lbs during the previous year. Permits can be transferred if the permit holder held a valid sea cucumber permit for any four permit years and landed at least 100 pounds of sea cucumbers in each of those permit years.
Maine, USA	In 2004, 10 endorsements.	Harvester Logbooks.	Limit licenses to fishermen that landed >250,000 lbs in a previous year. No incidental take allowed, only take through targeted, licensed fishery.

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**Figure 1.** Sea cucumber landings (t) and ex-vessel value (USD kg<sup>-1</sup>) between 1990 and 2004 for California and Washington.

**Table 2.** Sea cucumber production and export. Production is in metric tonnes (t) (FAO FishStat Plus v. 2.3; Hong Kong SAR import statistics).

	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001
U.S. production	<0.5	481	472	2141	729	1779	-	2406	3732	4583	1804
Hong Kong imports										181.57	89.74

**Table 3.** Recent landings (t) and value (USD ,000) of sea cucumbers in California.  
\* In 2001 and 2002 species of *Parastichopus* were not separated.  
Source: California Department of Fish and Game (CDFG) 2005.

	2001	2002	2003
Red sea cucumbers (t)	*	*	132.6
Warty sea cucumbers (t)	*	*	209.2
Sea cucumbers total value (USD ,000)	325.5	429.5	344.1

**Table 4.** Sea cucumbers landings (kg) and number of permits issued in Oregon, 1995–2003. As of 2004, permits are no longer required.

	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Landings (kg)	0	0	3295	0	3.28	132	15.9	0	312	120.6
Harvesters			5		2	1	2		2	
Permits issued	15	3	7	9	2	7	8	4	2	0

Chinese markets in the US; a small portion of the processed product is also used locally for nutritional supplements and arthritis treatments.

## US sea cucumber fisheries

### California

A commercial fishery for *P. californicus* and *P. parvimensis* was established in 1978 using scuba diving and trawls. Most trawl effort is concentrated in southern California, and collection is by hand using scuba in northern California. Until 1997 about 75% of the annual catch was from the southern California trawl fishery. Declines in harvest from southern California have occurred in recent years, partially due to prosecution of illegal trawl fishers, which reduced the total number of trawl fishermen. Beginning in 1997, divers who held sea urchin and abalone permits shifted their efforts to sea cucumbers. The dive fishery has increased substantially, and now accounts for 80% of the total harvest (Rogers-Bennett and Ono 2001).

Annual landings remained under 40,000 kg until 1982, when the principal trawl areas shifted from Los Angeles area ports to the Santa Barbara Channel. Currently, most fishing is in the Los Angeles and Santa Barbara areas, with limited harvest off San Diego and Bodega Bay. Between 1983 and 1990 annual landings fluctuated between about 20,000 and 60,000 kg. In 1991, over 261,871 kg were harvested. Combined trawl and dive harvest peaked in 1996 at 380,703 kg with an ex-vessel value of USD 582,370 (Rogers-Bennett and Ono 2001). Although there are fewer dive and trawl permits in recent years, the sea cucumber harvest has remained at levels close to that reported in 1996 (Table 3).

Since 1992–1993, a special permit has been required for harvesting sea cucumber, at a cost of USD 250 per year. Permit recipients must have landed a minimum of 20 kg during the previous four-year period. In 1997, separate permits were issued for each gear type, with a limit on the total number of permits issued. A maximum of 111 dive permits and 36 trawl permits were issued in 1997, and this declined to 95 dive permits and 24 trawl permits in 2004. There are no restrictions on catch, but trawling is prohibited in the Trawl Rockfish Conservation Areas (water from 30 fathoms to 150–250 fathoms (depending on latitude) along the mainland coast, shoreline to 200 fathoms around most islands, except the Farallon Islands, where the fishery is closed from the shoreline to 10 fathoms) (CDF&G, 2005). Other trawl fisheries have a total trip limit of 136 kg of bycatch, which includes sea cucumbers.

### Oregon

Oregon's sea cucumber fishery began in 1993. The primary target is *P. californicus*, with most collection done by hand using dive gear. Harvest by trawl was also allowed, but required an experimental gear permit (McCrae 1994). Oregon Department of Fish and Wildlife placed sea cucumbers within the Developmental Fisheries Program, which was developed in 1993 to allow for the controlled development of new commercial fisheries. Under the first year of the program 44 permits were issued, but only 9 divers were active, landing 2335 kg of sea cucumbers. In 1994, 22 divers landed 4777 kg of sea cucumber. Between 1995 and 2004, there was little or no harvest in Oregon waters, with exception of 1997 (Table 4).

Permits were issued to sea cucumber fishermen until 2003, when sea cucumbers were moved to category B of the Developmental Fisheries Program species list, which includes those species with less potential for a viable fishery. For 2004 and beyond, sea cucumber harvest no longer requires a developmental fishery permit (McCrae pers. comm.).

### Washington

The Washington State sea cucumber fishery is based on one managed species, *P. californicus*, with commercial and tribal fisheries concentrated around the San Juan Islands and in Port Angeles. Commercial harvest primarily involves hand collection using scuba or hookah, with lower levels of harvest by an experimental trawl fishery. Sea cucumbers may also be collected while diving for personal use, with a daily limit of 10 animals. An additional 13 non-classified sea cucumber species are also collected at low levels in Washington State waters for research and public aquaria under Washington Department of Fish and Wildlife (WDFW) Scientific Collecting permits. The average statewide CPUE has increased since 1996 and has reached a historic high, possibly as a result of a smaller, more efficient fleet. There are currently 46 licensed commercial divers, with a license reduction program initiated in 2002, with the goal of reducing the total number of licenses to 25.

The sea cucumber fishery was established in 1971 and occurred without restrictions until 1987. In the early and mid-1980s commercial harvest was relatively low (125–181 t year<sup>-1</sup>) and the value was low (USD 0.06–0.13 kg<sup>-1</sup>). The total harvest began to increase in 1988 (952 t) and peaked in 1992 (1880 t). This increase was associated with an increase in the value of sea cucumber (from USD 0.21 kg<sup>-1</sup> in 1988 to USD 0.87 kg<sup>-1</sup> in 1993). However, the dramatic increase in harvest increased concerns of the po-

tential for overexploitation and a seasonal harvest and specific harvest districts were subsequently adopted, along with the adoption of a total harvest quota in 1994.

Current management measures for the Washington State sea cucumber fishery include closed seasons, spatial closures, licensing of collectors, and an annual quota. Seven area closures for the dive fishery have been established in the current management plan, including two that are closed for human health reasons and a prohibition on trawling in shrimp areas. Other regulations for the trawl fishery include 1) a ban on trawling in waters less than about 20 m deep; 2) temporal closures during soft-shell Dungeness crab (reproductive) periods; 3) specific fishing locations; and 4) restrictions on gear type and size, including maximum beam width for beam trawl gear and minimum mesh size for otter trawl gear. The commercial dive fishery is open year round.

Harvesting in Washington State occurs under a cooperative management agreement with treaty tribes. There are five management regions in Puget Sound, with about 50% of the total quota allocated for state harvest and 50% tribal harvest. The annual statewide harvest guideline (1997–2002) was 427,690 kg, with a total estimated available commercial biomass of 5.58 million kg. As a precautionary approach, the 2003–2004 quota has been reduced by 15% of that calculated for 1997 until more recent biomass estimates are completed (Table 5). The quota has been determined using the Schaefer (1954) surplus production model based on estimates of biomass from catch-effort data, video surveys, and dive surveys (Bradbury 1994).

Fish receiving tickets are submitted to Washington State Department of Fish and Wildlife after each fishing trip. These data are used to determine when the annual tribal and state commercial harvest quota is reached. Fishermen also submit monthly harvest logs that include the date, vessel name or

boat registration number, location fished, pounds landed, average depth of harvest, number of divers, and total diver hours spent fishing. Based on submitted logbooks, annual landings of cucumbers currently have an ex-vessel value of about USD 1 million.

## Alaska

Sea cucumbers were used as a traditional food source and for subsistence along the northwest coast, including south Alaska since at least 1804. Harvest occurred primarily in the spring, summer and fall during minus tides, either from shore or boat in shallow waters, using a pole (called a *yein* or *yaanu* stick, which is about 2.5 m in length with a cross stick attached at one end; Lawrence 1977) to lift sea cucumbers from behind rocks or from eelgrass beds. Total subsistence harvest during the 1980s varied between communities with a maximum annual harvest of 4386 kg harvested by one community, with up to 51% of the households in an individual community involved in the fishery (Mathews et al. 1990).

The commercial sea cucumber fishery for *P. californicus* began in 1981 in Southeast Alaska as an experimental fishery, and in 1987 around Kodiak Island. One or two permits were issued per year between 1981 and 1986, with only one vessel reporting landings. Sea cucumbers are collected by hand using scuba or hookah, typically at depths of 3–20 m, with no restrictions on the use of mixed gases or saturation diving. Fishermen historically used small skiffs and operated as a day fishery. There has also been a recent increase in the use of larger vessels with two divers and a crewman and living quarters, which have extended the range and duration of fishing trips.

The dive fishery in both locations was initially based only on a permit system, but was closed in May 1990, to allow resource management agencies sufficient time to evaluate the status of sea cucumber re-

sources and fishing pressure, and develop a management plan. This plan relies on a quota developed based on historic production, fisheries performance, and biannual survey data of population biomass with a total harvest rate of 6%. The harvest rate is estimated to be 50% of maximum sustainable yield (MSY), calculated using a surplus production model that incorporates 1) an estimate of virgin population size; 2) a reduction of the quota to 50% of the harvest rate derived from the model; and 3) with

**Table 5.** Harvest guidelines for sea cucumbers in Washington (Anonymous 2003).

Management Region	2003–2004 harvestable surplus (kg)
San Juan	295,372
Strait of Juan de Fuca	70,755
Central Puget Sound (26C)	17,280
Central Puget Sound (remaining areas)	906
Hood Canal	3,084
South Puget Sound	30,840
TOTAL	372,055

**Table 6a.** Total landings of sea cucumbers (t), fishing effort and landed value for Kodiak Island in Alaska, 1993–2001. Source: Alaska Department of Fish and Game (ADF&G) Preliminary Alaska Commercial Shellfish Catches and Ex-vessel Values

	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Landings (t)	256.1	187.6	65.8	73.7	60	64.6	52.7	52.7	69.2	77.1	63.5	58.9
No landings	487	269	60	93	65	55	36	56	73	*	*	*
Permits issued	50	86	21	31	26	16	19	20	18	*	*	*
Value (USD kg <sup>-1</sup> )	0.42	0.54	0.57	0.57	0.53	0.54	0.54	0.68	0.57	0.57	0.73	0.86

\* data unavailable

another 30% reduction to account for field sampling variability. In addition, there is no allowable harvest in areas with biomass estimates below a threshold of 1 kg m<sup>-1</sup> of shoreline (Woodby et al. 1993).

The Southeast Alaska Sea Cucumber Commercial Fisheries Management plan was completed in October 1990. The management plan established 18 areas closed to fishing and annual guideline harvest levels of 6.4% of the total sea cucumber biomass taken on a 3 year rotational basis. There is also a seasonal closure (April–September), and a limit of 3 fishing days per week, and trip limits for each vessel. Fishing effort increased from 1990 to a maximum of 424 divers in 1995–1996 season. Beginning in 1996, a moratorium on the dive fishery was imposed, limiting the number of divers able to participate in the fishery to 472. To be eligible for a permit, individuals must have commercially harvested sea cucumbers at least once between 1992 and 1996. There were 235 permits issued for the 2001–2002 season (Hebert and Pritchett 2002). The quota for 2001–2002 was 646,466 kg; the actual harvest was 652,477 kg, with an estimated ex-vessel value of USD 2,517,289 (Table 6b). For the 2002–2003 season, an additional 58,000 kg were added to the quota to accommodate for collection within three newly identified collection areas (ADF&G 2005).

The commercial fishery was reopened in Kodiak in 1991 under a new management plan. The plan establishes 1) a closed season from May through September to protect spawning aggregations, and 2) fifteen large harvest refugia within the managed area. Since 1995, fishing periods have also been reduced to three days per week to allow analysis of fishing performance and monitor progress towards the established harvest guidelines (Ruccio and Jackson 2002). Starting in the 2002–2003 season, four additional areas in Kodiak district and three other areas in the Aleutian Islands were open to ex-

**Table 6b.** Total landings of sea cucumbers (t), number of divers and ex-vessel value in millions of USD from 1986–2001 in Southeast Alaska. Source: Alaska Department of Fish and Game (ADF&G) Preliminary Alaska Commercial Shellfish Catches and Ex-vessel Values.

Year	Landings (t)	No divers	Ex-vessel value (USD million)
1986	15.44	7	0.007
1987	29.51	11	0.014
1988	363.51	57	0.169
1989	1051.58	205	0.969
1990	364.78	143	0.472
1991	394.62	187	0.697
1992	566.82	240	0.988
1993	437.42	320	0.995
1994	599.75	261	2.361
1995	604.23	424	1.846
1996	411.32	294	1.169
1997	405.85	226	1.458
1998	478.80	219	1.636
1999	711.98	200	3.06
2000	525.44	220	2.583
2001	652.48	235	2.517
2002	743.90	*	2.870
2003	743.89	*	2.670
2004	771.11	*	2.500

\*unknown

perimental fisheries, with a guideline harvest level of 2268 kg for each area (Ruccio and Jackson 2002).

Sea cucumber divers fishing in waters surrounding Kodiak are licensed and must obtain a Commercial Fisheries Entry Commission interim use permit. Fish tickets are required from fishermen, operators of commercial fishing vessels, processors and buyers; each fishing vessel with a fish ticket must provide logbooks that include coordinates of fishing areas (Ruccio and Jackson 2002). Annual quotas have been established for each area as guideline levels of harvest, currently amounting to 113,759 kg divided among Kodiak (90,719 kg) and Chignick



(23,040 kg). The number of permits reached a maximum in 1986 (86 permits), with 18 permits issued in 2001. Harvest in the 2000–2001 season amounted to 69,216 kg (Ruccio and Jackson 2002). Prices have fluctuated between USD 0.42 kg<sup>-1</sup> and 0.68 kg<sup>-1</sup> with a total landed value in 2001 of about USD 190,000.

### Maine

The Maine sea cucumber fishery is a low value/high volume fishery that targets *Cucumaria frondosa*. The fishery began in 1988 with one operator, and expanded in 1994 when Asian markets for this species emerged. Fishermen use boats ranging from 12–30 m in length equipped with either scallop chain sweeps or light urchin drag gear. The gear is limited to 167 cm width and 7 m length and has a head bail constructed of less than 3.8 cm round steel stock. On a typical day, each boat harvests 70–200 t of sea cucumbers. Catch per boat per day was about 7212 kg, with an average of 16 (±5) tows per day. There are currently 16 endorsements, although only three are active (Feindel 2002).

In the mid-1990s, the industry employed 75–100 individuals who processed sea cucumbers, and 15–20 fishermen; annual harvest was 453.542–1360.512 t. Landings had increased to over 3600 t in 1999 and over 4080 t in 2000. In 2001, landings decreased to 1140 t (Table 7). The decline was associated with the closure of two of the three processing plants (Feindel 2002). The level of harvest nearly doubled in 2002 (2850 t) and again in 2003 (4470 t) with a smaller decrease in 2004 (4650 t). The total value of the fishery was USD 0.56 million in 2003 and USD 0.51 million in 2004.

Regulations were implemented in March 2000 under the 1999 Sustainable Development of Emerging Fisheries Act. The Act included restrictions that limit the fishing season (closures between 1 July and 31 September), define gear size, and establish a maximum number of endorsements, with licenses given only to individuals that had sold 100,000 kg during the previous year. Licensed fishermen are required to submit log-

books that include information on catch, time at sea, area fished, and catch value (Feindel 2002). The number of sea cucumber endorsements have ranged from 10–13 per year between 2001–2004. These new regulations were intended to address concerns of possible depletion of the resource as interest in the fishery peaked, and to address gear conflict issues between sea cucumber and lobster fisheries.

### Domestic consumption and international trade

Traditional methods of preserving of sea cucumbers include drying, smoking, canning or freezing. Sea cucumbers are prepared for consumption by being gutted and then boiled or roasted. Sea cucumber processing plants are located in Maine, Massachusetts and Washington. The primary commercial products derived from sea cucumbers include the internal muscle bands and the dried body. Processing of sea cucumbers involves removing the end with the tentacles, slitting the body lengthwise to remove the viscera, and scraping the muscles off the body wall. The body and muscles are typically boiled, dried and salted before export, while lesser quantities are marketed as a frozen, pickled or live product. The byproduct of processing from *C. frondosa* is marketed in the United States as a nutritional supplement providing chondroitin (*NutriSea*), is also sold as a treatment for arthritis for people (*ArthriSea* and *SeaCuMAX*) and pets (*Sea Jerky*) (Coastside Bio Resources, Stonington, Maine), and is marketed as compost in Maine (Feindel 2002).

Most of the sea cucumbers harvested in the US Pacific are exported to Hong Kong, Chinese Taipei, mainland China and Korea. Exports of *Parastichopus* spp. were worth USD 0.07 kg<sup>-1</sup> in the early 1980s, which had increased to USD 0.82 kg<sup>-1</sup> in 2005; processed sea cucumbers can sell for up to USD 9 kg<sup>-1</sup> (wholesale). Total ex-vessel revenue for Washington and California fisheries has varied from USD 1,000,073 in 1999 to a maximum of USD 4,848,999 in 1993, with most revenue associated with the Washington State fishery in the early

**Table 7.** Summary of available information on commercial landings of sea cucumbers in the United States in metric tonnes (t), live weight. Data for Alaska, California and Washington are from Pacific Fisheries Information Network (2005).

	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Alaska	693.5	786.7	670.0	485	465.8	543.4	764.6	578.0	721.7	821.0	807.4	830.01
Oregon	2.3	4.8	0.0	0.0	2.9	0.0	0.003	0.1	0.01	0	0.31	0.12
California	265.8	293.0	267.6	381.0	193.0	341.0	272.0	291.0	325.5	429.5	344.1	260.0
Washington	1281.3	684.4	529.1	290.0	248.2	239.3	228.0	275.0	300.1	249.1	199.0	262.2
Maine		1451.0	1950.0	1270.0	453.0	2359.0	3630.0	4080.0	1141.0	2845.0	4467.0	4650.0

1990s; the ex-vessel value fluctuates substantially due to annual variations in total harvest and high variability in the price received for each landed kg (Fig 1). In Maine, fishermen are paid USD 0.05–0.06 per unprocessed *C. frondosa*. Internal muscle bands and the dried body wall are the primary export products, and are currently worth about USD 1.59 kg<sup>-1</sup>. After harvest the animals are either loaded onto refrigerated trucks at the dock and shipped to Seattle for processing, or they are processed in Maine and shipped directly to mainland China, Hong Kong, South Korea, Singapore, Chinese Taipei and Japan. Chinese markets in San Francisco, New York and other cities within the United States also purchase a portion of the sea cucumber catch.

The US west coast also serves as a transshipment point for sea cucumbers originating in Latin America en route to Asia. Los Angeles, California is the primary port of entry for the aquarium trade, with most sea cucumbers originating in Southeast Asia. The genus *Pseudocolochirus* is the dominant import, although several smaller species such as *Pentacta anceps* and *Colochirus robustus* are also available. One Caribbean species, *I. badionatus*, is commonly imported and also collected in US waters.

### Conservation concerns

There have been some problems associated with sea cucumber fisheries, including the potential for overexploitation, habitat damage, bycatch, illegal fishing activities, and conflicts with other resource uses. In many locations sea cucumber landings increased rapidly following exploratory phases, but some fisheries have also experienced decreases in the number of sea cucumbers landed per dive, and overexploitation has been reported from some areas. Fishery sustainability is a major concern for US sea cucumber populations, particularly for stocks that lack stock assessments. Several states are implementing sea cucumber monitoring programs, and there has also been an increase in research efforts to better understand sea cucumber reproduction, growth, recruitment, distribution and abundance (Shroeter et al. 2001; Cameron and Frankboner 1989). For example, fishery-independent monitoring conducted in the Channel Islands and Santa Barbara Islands off California has revealed a decline in sea cucumber populations within fished areas since 1990, with populations that are 50–80% smaller than that observed in sites that were fished (Rogers-Bennet and Ono 2001).

Illegal fishing has been reported from California. This was primarily associated with 16 trawl fishermen that fraudulently obtained sea cucumber permits, and has been subsequently addressed.

During the development of the sea cucumber fishery in Southeast Alaska, concerns existed about potential conflicts between subsistence and commercial harvesters. These conflicts have been largely addressed through the implementation of a fishery management plan, which established conservative harvest rates in specific areas that for subsistence preference be closed to commercial harvest. Other long-term management options that have been proposed include identifying subsistence areas that should be closed to commercial harvesting (Mathews et al. 1990).

The primary concern associated with the Maine sea cucumber fishery is that most of the fishing effort is concentrated in a few sites in three locations in eastern Maine, and there are anecdotal reports that some sites have been fished out. Substantial amounts of bycatch are associated with the fishery in muddy and gravel environments, while bycatch is low in rocky areas where the species forms dense aggregations (Feindel 2002).

Impacts associated with the use of non-selective gear, such as trawls, include potential habitat damage and bycatch. These impacts are being minimized through 1) implementation of area closures for trawling in sensitive habitats (Washington, California and Alaska); 2) seasonal or spatial closure to protect other species (e.g. shrimp) during key life history stages such as reproductive periods (Washington and Alaska); and 3) restrictions on the size and type of bottom tending gear that can be used in the fishery (Maine).

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## Management of Queensland sea cucumber stocks by rotational zoning

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Globally, concerns have been raised about the unsustainable rates of harvest of tropical sea cucumber stocks. The sedentary nature of these creatures and the comparatively clear shallow waters they inhabit makes them susceptible to over exploitation.

Government authorities in Queensland (Australia) have expressed concern that localized depletion of sea cucumber stocks may be occurring on the Great Barrier Reef (GBR). The sea cucumber fishing industry does not support these concerns; however, to allay fears that localized depletion is occurring, industry members have decided to implement the Rotational Zoning System (RZS), which is a new approach to sea cucumber fisheries management.

Prior to the introduction of the RZS on 1 July 2004, sea cucumber stocks in Queensland were managed by a mixed-species total allowable catch (TAC) of 380 tonnes (t), with around 5 vessels operating. Given the size of the GBR this figure is considered very conservative; the Torres Strait fishery (an area only around 20% the size of the GBR) has a recommended TAC in excess of 420 t.

The RZS allows for all coral reef areas open to harvesting to be divided into 156 zones. The size of the zones varies from approximately 100 square nautical miles (nm<sup>2</sup>) to 200+ nm<sup>2</sup> (1 nm<sup>2</sup> = 3.43 km<sup>2</sup>). A maximum of 15 d fishing zone<sup>2</sup> y<sup>-1</sup> is allowed, and only 52 zones are open each year. This allows limited harvesting of a zone once every three years. Zones are allocated according to an operator's share of the TAC; i.e., someone with 10% of the TAC will receive 10% of the zones open to fishing in that year.

By allocating operators their own zones each year, each location will be fished a maximum of once every three years. Parts of some zones may not be targeted even when they are open to fishing, as the design of the RZS makes it extremely difficult to fish an entire 100+ nm<sup>2</sup> zone in 15 days.

Size limits for all sea cucumber species were increased so that no harvesting occurs until the animals are well past sexual maturity, thus giving them time to spawn at least once prior to harvesting.

Some species have been allocated their own TAC. For other species, catch trigger points have been set. Once a trigger point is reached, the sea cucumber industry must carry out a stock assessment of that particular species to determine an adequate TAC. We have recently completed our first stock assessment for an individual species.

Rotational Zoning has come at a cost to the sea cucumber industry. Given all the restraints of the system, catches are expected to be down from previous years. However, because Australia is the only developed country with tropical sea cucumber stocks, we see a bright future for this fishery as developing nations continue to overfish their sea cucumber resources.

The Rotational Zoning System is an initiative of the Queensland Sea Cucumber Industry Association and has the full support of the Queensland Fisheries Service, Great Barrier Reef Marine Park Authority and Commonwealth Department of Environment and Heritage.

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## Induced spawning and larval rearing of the sea cucumbers, *Bohadschia marmorata* and *Holothuria atra* in Mauritius

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### Abstract

Two species of sea cucumbers, *Bohadschia marmorata* and *Holothuria atra* have been induced to spawn by thermal stimulation. The larval stages of these sea cucumbers were reared on diet of unicellular algae, artificial feed and seaweed paste. The pentactula stage was reached in 20 days. Survival rates obtained up to the pentactula stage were 12.5% and 6.4% for *B. marmorata* and *H. atra*, respectively.

### Introduction

In Mauritius, sea cucumbers are regularly collected by fishers, mainly for domestic consumption. The loss of critical stocks of sea cucumber is likely to have a significant impact on the ecosystem condition and the adjacent marine environment, as a whole. Therefore, there is an urgent need for developing a technology for seed production and culture of sea cucumbers in Mauritius. Although about 1400 species have been identified worldwide, there is little information about holothurian species in Mauritius. Conand (1998) mentioned 11 edible species in the southwestern Indian Ocean region, including Mauritius. Luchman et al. (2001) carried out a study to assess the distribution and abundance of the holothurians in the lagoon at Preneuse (west coast) and in Baie du Cap (south coast) in Mauritius. The dominant species of sea cucumbers found in Mauritian waters are the chalkfish, *Bohadschia marmorata* and the lollifish, *Holothuria atra*. Among these two species, *B. marmorata* has a better commercial value and grows to a larger size (2.0 kg) than *H. atra* (1.5 kg).

Hence, the present work was undertaken in order to develop appropriate technologies for the breeding, seed production and culture of the two species of sea cucumbers, *B. marmorata* and *H. atra*. The results obtained are presented and discussed in this paper.

### Materials and methods

The work was carried out at the Albion Fisheries Research Centre, Mauritius. Sea cucumber broodstock were collected from the wild at low tide, and stocked in 1-tonne tanks filled with a sandy substratum of six inches thickness to enable the sea cucumbers to bury in the sand. The water in the tanks

was changed everyday and sand was changed every fortnight. Fresh seaweed was ground into a paste in a mixer and put in water at least twice a week. The sea cucumbers thrived on the organic matter present in the mud. The algal paste settled to the bottom and was consumed by the sea cucumbers along with the mud. Fifteen to twenty adults were kept in each tank. The methods used for inducing spawning in these two species of sea cucumbers are described below.

i) *Thermal stimulation*: The temperature of the seawater was reduced by 3–5°C by the addition of ice, and the sea cucumbers were then introduced into this tank. After 5 minutes, the sea cucumbers were introduced into another tank filled with filtered seawater at normal temperature (3–5°C higher than the first tank temperature). A rise of 3 to 5°C was enough to induce spawning. The males spawned first, which induced the females to release their eggs.

ii) *Stimulation through drying and powerful jet of seawater*: This method was used with breeders that were conditioned for more than one week in the hatchery. The sea cucumbers were dried in the shade for 30 minutes. Then, the specimens were subjected to a powerful jet of seawater for 30 minutes. After 1–1.5 hours, the specimens began to move up the tank wall and began to show swaying movements. The males released the sperms first and then, 30 minutes later, the females started reacting. The anterior region of the female became bulged due to the inside pressure. The eggs were released in powerful jets intermittently.

The success achieved for *B. marmorata* and *H. atra* in inducing spawning and larval rearing are described separately.

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## ***Bohadschia marmorata***

### **Induced spawning**

This species was induced to spawn by both of the methods described above, although successful egg fertilization was only obtained with the thermal stimulation method. The water temperature was reduced to 25°C and after 5 minutes the sea cucumbers were transferred to a tank where the seawater was at 30°C. The males released their sperm after 10 minutes, which induced the females to spawn after one hour.

Fertilization took place in the water. After the eggs and sperm were released, the breeders were removed from the tank. The eggs were washed several times in order to remove excess sperm. Two of the females released 624,000 eggs. The eggs are spherical, white, and visible to the naked eye. After fertilization, the first polar body appeared within 20–25 minutes. The first cleavage took place after 20 minutes. Early gastrula was formed after 45 minutes. In 4 hours, the blastula — which is oval and motile — was fully formed. After 50 hours, early auricularia larvae were formed. The total number of early auricularia larvae obtained was 450,000. The hatching rate was 72.1%.

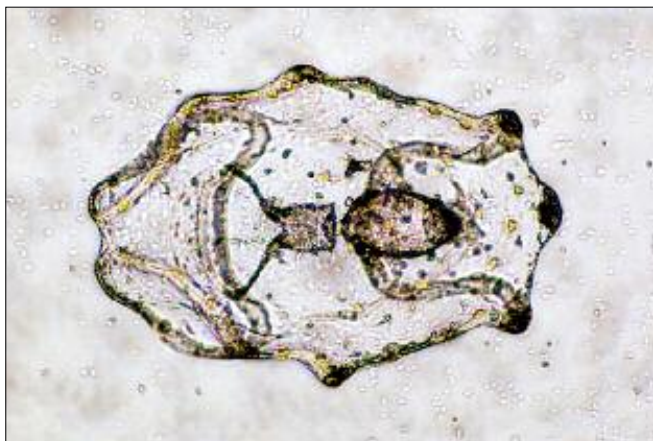
### **Larval rearing**

The early auricularia larvae (Fig. 1) were stocked in 1-t FRP tanks, filled with 750 litres (L) of filtered sea water. Stocking density was 200 larvae L<sup>-1</sup>. Larvae were fed with unicellular algae, and the algal concentration in the rearing tanks was maintained at 20,000–25,000 ml<sup>-1</sup>. The quantity of phytoplankton was increased or decreased depending on the quantity of food in the stomach of the larvae. This was checked every-day before feeding.

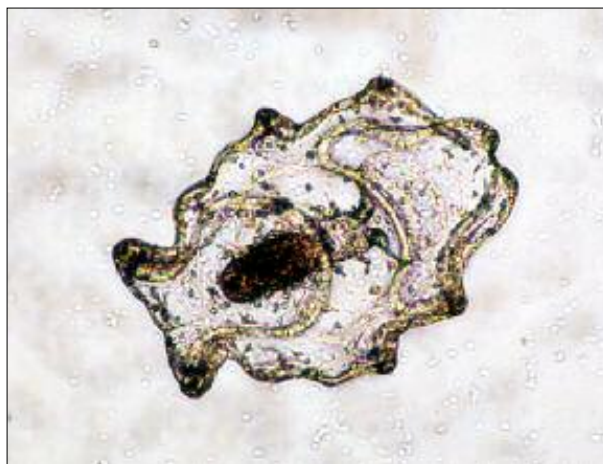
Water was changed every day by keeping a sieve inside the tank with a mesh size of 80 µm. Sediments at the bottom of the tank were siphoned out completely every three or four days.

Seawater in the larval rearing tanks ranged from 27–30°C, pH ranged from 8.1–8.3, and salinity between 34 and 36 ppt. Continuous aeration was provided.

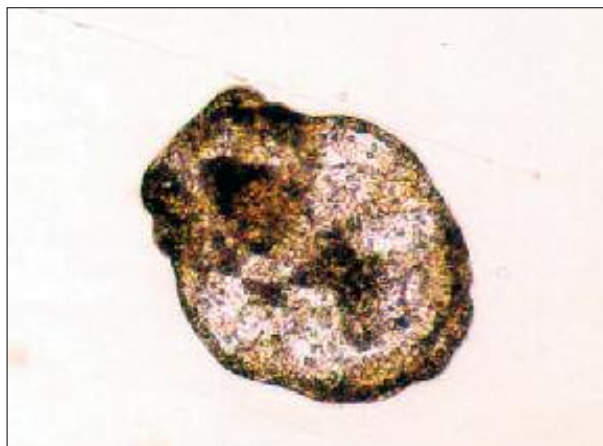
Seven to eight days after stocking, late auricularia (Fig. 2) were formed. The auricularia is slipper-shaped, transparent and pelagic. It has a preoral loop anteriorly, and an anal loop posteriorly. The



**Figure 1.** Early *B. marmorata* auricularia larva.



**Figure 2.** Late *B. marmorata* auricularia larva.



**Figure 3.** Late *B. marmorata* doliolaria larva.

digestive tract consists of a mouth, an elongated pharynx and a sacciform stomach. The early auricularia measures on average 480 µm. The late auricularia measures on average 420 µm. On the day 12, the auricularia larvae metamorphosed to doliolaria larvae (Fig. 3).

The doliolaria is barrel-shaped, with five bands around the body. It measures on average 370 µm. This stage is short, lasting three to four days after which the larvae transformed into a creeping stage known as pentactula.

The pentactula is tubular with five tentacles at the anterior end and a singular tube foot at the posterior end. The pentactula measures on average 560 µm and feeds actively on benthic algae and other detritus matter.

When the larvae reached the doliolaria stage, "settling plates" were put in the tank. The settling plates consisted of polythene sheets that were kept in advance in a tank filled with seawater. Seaweed extract was added, and this eventually stuck to the polythene sheets. Every day, seawater was changed and fresh seaweed extract was added. After a week, the polythene sheets were covered with a fine coat of algal extract and this served as a good settling base for the larvae.

### **Holothuria atra**

Methods for broodstock collection, maintenance, spawning induction and larval rearing of *Holothuria atra* are similar to those used for *B. marmorata*.

### **Spawning induction**

*H. atra* was induced to spawn by thermal stimulation and also by drying and using a powerful jet of seawater. Fertilized eggs were obtained by using the thermal stimulation method.

Bbreeders were first introduced into the seawater with a temperature of 27°C. After 10 minutes, the sea cucumbers were introduced into a tank containing seawater with a water temperature of 30°C. Within 5 minutes, the male released sperm which induced one of the females to release eggs after 30 minutes. This female spawned 800,000 eggs, which hatched into 750,000 early auricularia after 48 hours. The hatching rate was 93.8%.

### **Larval rearing**

The early auricularia larvae were stocked in 1-t FRP tanks at the stocking density of 330 L<sup>-1</sup>. The procedures used for larval rearing were similar to those followed for *B. marmorata*.

Seawater in the larval rearing tanks ranged between 26 and 28°C, and had a pH of 8.2–8.4, and a salinity of 34–35 ppt.

After 10 days of stocking, the late auricularia larvae were formed. At this stage, the shape is similar to *B. marmorata*'s. The early auricularia measured an average of 440 µm. The late auricularia measured an average of 404 µm. On the day 15 day, the auricularia larvae metamorphosed into doliolaria larvae.

The barrel-shaped doliolaria larvae measured on average 360 µm. This stage was short and lasted 4–5 days. Larvae then transformed into pentactula, a tubular shape with five tentacles at the anterior end and a tubefoot at the posterior end. The pentactula measured on average 550 µm and fed on benthic algae and other detritus.

As in the case of *B. marmorata*, "settling plates" were put in the tank. The details of induced spawning and larval rearing are summarized in Table 1.

The survival rate of larvae up to the pentactula stage was 13.2% for *B. marmorata* and 6.4% for *H. atra*.

### **Discussion**

Successful spawning and larval rearing has been achieved only in some species of sea cucumbers. *Apostichopus japonicus* juveniles were produced in Japan more than 60 years ago (Inaba 1933) and were successfully reared (Imai et al. 1950). Subsequently, Shuxu and Gengeheo (1981) and Li (1983) reported on the breeding and culture of this species in China. James et al. (1988) produced sandfish, *Holothuria scabra* juveniles. Chen and Chan

**Table 1.** Details of spawning events and larval rearing of *B. marmorata* and *H. atra*.

Species	No. of broodstock used	Date of spawning	No. of eggs	No. of early auricularia larvae	No. of pentactula larvae
<i>Bohadschia marmorata</i>	8	04-02-05	624,000	450,000	59,400
<i>Holothuria atra</i>	12	26-02-05	800,000	750,000	48,000

(1990) reported on the larval development of *Actinopyga echinites* and James et al. (1993) on the spawning of *A. mauritiana*. Hamel et al. (1993) studied the gametogenesis and spawning of the sea cucumber *Psolus fabricii*. Artificial induction of oocyte maturation and development in *H. leucospilota* and *H. paradis* was reported by Mayurama (1980). Ramofafia et al. (1995) achieved the spawning and early larval rearing of *H. atra*. Breeding of *H. scabra* was reported by Pitt and Duy (2004) and Morgan (2000). Breeding of *Isostichopus fuscus* in Ecuador was reported by Mercier et al. (2004). Hamel and Mercier (2004) investigated the role of perivisceral coelomic fluid (PCF) in the spawning induction of holothurians. Hamel and Mercier (1996) induced gamete release in *Cucumaria fondosa* by manipulating temperature and light. The use of a powerful jet of water on drying sea cucumbers also triggered spawning in *H. scabra* (James 1994) and *Parastichopus japonicus* (Liu et al. 2004; Wang and Yuan 2004) and also in the present study on *B. marmorata* and *H. atra*. In the present study, fertilized eggs of both species were only obtained when the thermal stimulation method was used. Battaglione et al. (2002) were able to trigger spawning in 10% of mature *H. fucogilva* females by adding a solution of dried algae, *Schizochrytrium* sp. (Algamac). As there has so far been no published work on the induction of spawning and larval rearing of the sea cucumber *B. marmorata*, this is the first report on induced spawning and larval rearing of this species. *B. marmorata* larvae were reared on a diet of unicellular algae, *Chaetoceros calcitrans*. After settling, the late larval stages were fed on the algal extract present on the "settler plates" and in the artificial feed. The species of algae that have been fed to the larvae of sea cucumbers *A. echinites*, *P. californicus* and *S. californicus* include: *Isochrysis galbana*, *Phaeodactylum tricornutum*, *Dunaliella salina*, *D. tertiolecta* and *Pavlova lutheri*. Battaglione et al. (1999) fed *H. scabra* larvae with the microalgae, *C. mulleri*, *C. calcitrans*, *P. salina*, and *Tetraselmis chuii*. James (2004) fed *H. scabra* larvae with cultures of *I. galbana* and mixed cultures of *Chaetoceros* sp. Morgan (2001) observed that at a concentration of 1 and  $2 \times 10^4$  cells ml<sup>-1</sup> of the alga *I. galbana*, growth and development of larvae increased substantially. At higher algal concentrations, larval survival was less and growth and development were inhibited. Ramofafia et al. (1995) fed *H. atra* larvae with *Tetraselmis* sp. and artificial feed, and reared the larvae to the doliolaria stage. In the present study, pure culture of unicellular algae, algal extract and artificial feeds were used. The concentration of unicellular algae was maintained at 20,000 to 25,000 cells ml<sup>-1</sup> in the rearing tanks.

Future experiments on larval rearing of *B. marmorata* and *H. atra* are planned using a variety of microal-

gae such as *I. galbana*, *C. calcitrans* and other species of microalgae to obtain better survival rates.

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## Correspondence beche-de-mer

### Correspondence from M. Verónica Toral-Granda (25 June 2005)

**Subject:** Collaboration needed to produce an identification guide for sea cucumbers

Dear colleagues,

During previous meetings that covered commercial sea cucumbers, one of the major concerns raised was how to identify the various species once they have been processed (i.e. in brine, sun dried, smoked, etc.). This issue has been identified as a major handicap for implementing better conservation policies. In addition, custom agents (in various importing countries) who deal with processed products find it almost impossible to detect illegal cargo. Illegal shipments continue to constitute a major conservation problem for certain countries and have detrimental effects on sea cucumber populations.

Much talk has been given to identifying a procedure that will allow quick and reliable identification of processed beche-de-mer products. Based on my experience with the conservation and management of *Isostichopus fuscus* in Ecuador, and the fact that this is the only species of sea cucumber that has been listed in CITES Appendix III, I looked into the use of calcareous ossicles as a helpful tool to identify this species in the international market. In the case of *I. fuscus*, the ossicles remain intact despite the type of processing, hence they are a very useful tool for identification. I undertook similar work on *Stichopus horrens* (illegally caught and traded in Ecuador) and came up with the same results. The procedure for obtaining the ossicles was simple: i) a 1–2-cm slice, 1 mm in thickness, was collected from the dorsal area; ii) each sample was placed in a small test tube with 3 ml of commercial bleach (NaOCl) and properly labelled for further identification; iii) samples were left for approximately half an hour, or until the body wall had dissolved and the ossicles had settled onto the bottom as white fine sediment; iv) the white fine sediment was transferred to a microscope slide by a pipette, and covered with a cover slip and examined with a microscope at 100 x magnification. Such work could be undertaken in a few hours, yielding the results needed for an identification guide.

An identification guide, which would also be of benefit for conservation efforts, should include photos of: i) live animals; (ii) processed animals (in different forms); and iii) calcareous ossicles. Such a guide could be produced for all commercially important species.

Some of you attended the FAO-ASCAM workshop (Dalian, China in October 2003) and the CITES workshop (Kuala Lumpur, Malaysia, March 2004); some of you are also regular contributors to SPC's *Beche-de-mer Information Bulletin*, thus your feedback is important. Also, any suggestions you may have would be greatly appreciated.

In this first step, I would like to ask all of you who are interested in collaborating on this initiative to reply to me, so that I can determine the level of support to be expected, and define the next steps. I have contacted FAO regarding funding possibilities (for compiling the information, and publishing the guide as an FAO Fisheries Technical Paper in joint collaboration with the Charles Darwin Foundation).

Many thanks in advance for your help and support with this initiative. Please forward this message to whomever you think could assist with this project. I look forward to hearing from you.

Best regards,

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**Correspondence from M.F. Nievales\* (2 February 2005)**

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I'm from the Philippines doing research on *Holothuria scabra* and I would like to share a surprising observation of the early sexual maturity of one specimen of this species.

The pictures below show a hatchery-raised individual about seven months old. I have been keeping it as a "pet" in a plastic tank in our lab. Figure 1 shows this individual a week before it eviscerated. Figure 2 shows the same individual on the day it eviscerated (five days after parts of its body wall collapsed and I had to fix it in formalin). Figure 3 shows the eviscerated organs and the tubules of the testis indicated by arrows. These tubules bear mature swimming spermatozoa when examined under the microscope. The last measurement taken of the individual some 18 days prior to evisceration showed that it weighed 36 g and had a length of 8.7 cm. Had it not eviscerated, I would have thought it was a juvenile or subadult. The reproductive biology of *Holothuria scabra* in western Visayas, central Philippines — where the parents of this individual were collected for induced spawning trials — had been investigated two decades prior. It was then estimated that the size at first sexual maturity should be about 19 cm — and a drained weight of 130 g. So, I was really surprised that this cultured individual could already be mature at around 9 cm. It should be noted, however, that for the northwestern Philippine population — which we have used in the hatchery to do spawning trials and juvenile grow-out during the last three years or so — we did use, and successfully induced spawning with, broodstock that were under 100 g.



**Figure 1.** Seven-month-old *Holothuria scabra*.



**Figure 2.** Same individual (indicated by arrow) after evisceration.



**Figure 3.** Eviscerated organs (arrows indicate testis tubules).



## Abstracts & publications

beche-de-mer

### Reproductive aspects, larval and juvenile rearing of *Holothuria (Theelothuria) spinifera* Theel

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**Source:** Summary of PhD thesis

As in many Indo-Pacific countries, sea cucumbers form an important part of a multispecies fishery in India. Beche-de-mer exported from India during the period 1992–2000, varied from 10.5 to 140 tonnes (t) and ranked first among the dried marine products. The beche-de-mer industry mainly depends on two species of *Holothuria*: *H. scabra* and *H. spinifera*. Beche-de-mer exports have declined from 70 t in 1996–1997 to 3.8 t in 2001. Due to overexploitation, the Ministry of Environment, Government of India, has banned both the fishing and export of sea cucumbers since June 2001.

Considering the role of *H. spinifera* in the commercial fishery, and the fact that it has been indiscriminately exploited, knowledge on reproductive aspects and seed production through a hatchery system is essential for enriching the natural population of this species. Therefore, a study of *H. spinifera* was undertaken and the salient results are summarized below.

Of the 294 mature *H. spinifera* studied, 52.1% were females and 59.3% were males; the sex ratio was 1:1. The occurrence of 90% mature animals during September–October and 100% in April indicated the biannual reproductive activity. The mean gonadal index of males and females was 1.4 and 2.0, respectively. The peak gonadal index during September–November and the minor peak in February–March indicated biannual gametogenic activity. Gonochoric gonad consisted of 17–490 tubules of varying lengths from 9–51 mm, with uniform development. The tubule length in mals (33–51 mm) was significantly ( $t = 2.537$   $p < 0.05$ ) greater than that of females (24–47 mm). The white colour of the tubules in the indeterminate and spent stages changed to creamy white in mature males and dark yellow in mature females. The tubule diameter of 1–1.2 mm in the females was significantly higher ( $t = 3.921$ ;  $p < 0.01$ ) than that of the males. The mean oocyte diameter was 148  $\mu\text{m}$  and the number of oocyte per tubule varied from 2233 and 29,667 with a mean value of 7938. The number of oocytes per tubule was significantly related to the tubule length ( $r = 0.2903$ ;  $p < 0.01$ ) and also to the oocyte diameter ( $r = 0.923$ ;  $p < 0.01$ ). The absolute fecundity ranged from 36.1 to 5195  $\times 10^3$ , with a mean of 1739  $\times 10^3$ . Out of this, the actual reproductive output is 1660  $\times 10^3$ , which is equivalent to 5.4 g ovary weight. The mean fecundity index was 1770. The relative fecundity to eviscerated weight was 10.8  $\times 10^3$  and that of gonad weight was 307  $\times 10^3$ .

The mean value of oxygen consumption in *H. spinifera* was 0.012 ml  $\text{g}^{-1}$  (dry wt)  $\text{h}^{-1}$  and the ammonia excretion was 12.45  $\mu\text{g g}^{-1}$  (dry wt)  $\text{h}^{-1}$ . The rate of oxygen consumption and ammonia excretion did not vary during the maturity stages but varied with length and dry weight of the animals. The mean values of protein, carbohydrate and lipid content in the body wall of *H. spinifera* were 17.8, 2.8 and 1.3%, respectively. The highest concentration of these organic constituents was during the maturity stages, and their decline during the post-spawning periods indicated their role as the source of energy during gametogenesis.

In *H. spinifera*, spawning was induced by powdered feed. The larval cycle lasted for about 10–15 days, with a pelagic larval stage: auricularia (809  $\mu\text{m}$ ) up to 10 days; non-feeding floating doliolaria (468  $\mu\text{m}$ ) on 10–12<sup>th</sup> day; and the settling pentactula (330  $\mu\text{m}$ ) on 13–15<sup>th</sup> day. On day 20, 200 juveniles attained 1 mm, and on the day 80 day, 55 juveniles attained 30 mm for the first time in the hatchery. The larval growth rate was 49–58  $\mu\text{m day}^{-1}$ . Algamac, at a concentration of 0.5g 500  $\text{L}^{-1}$ , was observed to be the best inducing agent for larval settlement. A sharp increase in the growth rate of juveniles (1.5 mm  $\text{day}^{-1}$ ) was observed when they were fed with *Spirulina*. A survival rate of 73.3%, 16  $\mu\text{m day}^{-1}$  growth of stomach and 80% of late auricularia development with normal symmetrical shape suggested that  $2 \times 10^4$  cells  $\text{ml}^{-1}$  was the optimum algal



feed concentration for larvae. Among the single algal diet, a high larval growth rate of  $59 \mu\text{m day}^{-1}$  was observed in the larvae that were fed with *Chaetoceros calcitrans*, whereas greater survival (68%) occurred in the larvae that were fed with *Isochrysis galbana*. Among the combination of algal feed, *I. galbana* and *C. calcitrans* yielded better growth rates ( $43 \mu\text{m day}^{-1}$ ) and survival rates (73%). Highest survival rates (90.8% and 72.3%), growth rates ( $62.6 \mu\text{m day}^{-1}$  and  $33.6 \mu\text{m day}^{-1}$ ) and development of doliolaria (100%) and late auricularia (100%) indicated that suitable rearing conditions for *H. spinifera* larvae consisted of water temperatures of 28–32°C, salinity 35 ppt and pH 7.8. The maximum growth rate for length ( $0.52 \text{ mm day}^{-1}$ ) and weight ( $0.08 \text{ g day}^{-1}$ ) with *Spirulina*, as supplemented in the feed, indicated that 4% *Spirulina* could be supplemented as an additional protein source along with fine sand and *Sargassum* powder. Gonochoric gonad, biannual reproductive cycle, high fecundity and amenable for production of juveniles through hatchery techniques, show the suitability of *H. spinifera* as a candidate species for farming.



*Holothuria spinifera*

## Abstracts from presentations made at the 11<sup>th</sup> International Echinoderm Conference, Munich, Germany, 6–10 October 2003

### Population biology of shallow water holothuroids and ophiuroids from Raine Island and Moulter Cay, Northern Great Barrier Reef

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Echinoderms are a conspicuous component of the invertebrate fauna of Raine Island and Moulter Cay, Northern Great Barrier Reef (GBR). Reefs around these islands support high densities of the commercially important holothuroid, black teatfish *Holothuria whitmaei*. The densities of *H. whitmaei* recorded were on the higher end of those measured for the Northern GBR, supporting previous findings that no-take (green) zones are effective in protecting stocks of commercial holothuroids. The seagrass habitat at Raine Island had the highest densities recorded for *H. whitmaei* and the specimens from this habitat were smaller than those in the lagoon. It is suggested that the seagrass habitat may be an important settlement and nursery area for these holothuroids. A survey of ophiuroids in the rubble zone on the Raine Island reef flat revealed an assemblage of at least 8 species. Ophiuroids were more abundant under the largest boulders. *Ophiocoma scolopendrina* and *O. dentata* were the most common ophiuroids with *O. scolopendrina* being more abundant near shore and *O. dentata* being more abundant further from shore. The lack of co-occurrence of these ophiuroids under the same boulder might suggest the presence of antagonistic interactions, but a coefficient of interspecific association indicated a random distribution for these species.

### Diversity of echinoderms at Raine Island, Great Barrier Reef

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Echinoderms are a conspicuous and diverse component of the invertebrate fauna of the waters around Raine Island, in the Far Northern Great Barrier Reef (GBR). Detailed surveys of the shallow waters around the island revealed the presence of 111 echinoderm species: 11 asteroids, 24 crinoids, 8 echinoids, 27 holothuroids and 41 ophiuroids. In general, the echinoderm fauna of Raine Island is typical of the outer Great Barrier Reef, although aspects of the fauna differed from other areas of the Far Northern GBR. Several species were recorded for the region for the first time. These included an asteroid of the genus *Ophidiaster* that appears most similar to *O. perrieri*, a species known only from the western Indian Ocean, and *Microcyphus maculatus*, an echinoid previously only known in Australia from Heron Island in the far south of the GBR. Several potentially new species were also found. These included two *Ophiocoma* species, a new fissiparous *Ophiacantha*, an *Ophiarachnella* species and an unusual colour morph of the crinoid *Comanthus*

*parvicirrus*. A surprising observation was the absence of the brittle star *Ophiarachnella gorgonia* and crinoids in the genus *Himerometra*. These taxa are common elsewhere in the region. The abundance of the commercial sea cucumber *Holothuria nobilis*, a species on the decline elsewhere due to over-fishing, attests to the conservation value of Raine Island Marine Park.

### **Estimation of the evolution of the Cuvierian tubules, defense organs in the family Holothuriidae, by the character mapping method and by ultrastructural analyses**

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2. University of California, Santa Barbara, USA

Cuvierian tubules are specialized defense organs occurring exclusively in some holothuroid species from the family Holothuriidae. Within the family, these organs differ greatly in terms of their morphology and their mode of functioning. The goal of this work was to determine the evolutionary path of Cuvierian tubules by the character mapping method and by ultrastructural analyses. A fragment of the mitochondrial genome corresponding to two genes was first sequenced for 20 species of Holothuriidae (3 *Actinopyga*, 3 *Bohadschia*, 12 *Holothuria*, 1 *Labidodemas semperianum* and 1 *Pearsonothuria graeffei*) and the relationships between these species were estimated from the molecular data obtained. The methods used to reconstruct those relationships were the neighbour joining, the maximum parsimony and the maximum likelihood. The consensus phylogenetic tree indicates that: (1) the genus *Actinopyga* is monophyletic and was the first to diverge from the rest of the family, (2) the second diverging group was a clade comprising the 3 *Bohadschia*, *P. graeffei* and 4 *Holothuria*, (3) within this clade the genus *Bohadschia* is monophyletic, (4) the remaining clade comprises the other species of *Holothuria* and *L. semperianum*, (5) the genus *Holothuria* is paraphyletic. The analysis of the different characteristics of Cuvierian tubules from the viewpoint of this phylogenetic tree strongly suggests that the common ancestor of the Holothuriidae had Cuvierian tubules and that those tubules were ramified, non-adhesive, non-expellable and non-stretchable; that those tubules have evolved to give the non-ramified, adhesive, expellable and stretchable tubules; and that the loss of Cuvierian tubules has occurred several times independently during evolution.

### **Functional morphology of the tentacles in the apodid holothuroid *Synapta maculata***

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*Synapta maculata* is a long snake-like holothuroid inhabiting seagrass beds of the Indo-West Pacific. It has 15–16 pinnate tentacles, each comprising a central stem bearing 30–40 pairs of pinnules, which are located in a plane tangential to the mouth. The tentacle's inner tissues are organized in such a way that the stem and each pinnule can move independently from the others. This makes the tentacles exceedingly prehensile, being able to wrap around seagrass leaves or press against a flat surface. The internal surface of the tentacles (i.e., facing the mouth) is smooth while the external surface is covered with bulges. It is this surface that is applied on the substratum during feeding. The external epidermis is mainly made up of a typical echinoderm duo-gland adhesive system involved in the catching of food particles. An additional cell type may also be observed in the tentacular epidermis: the vesicular cells. These cells, packed with large vesicles, each containing one spherule, are conspicuous in the tentacle epidermis, being present on both the inner and outer surfaces, but preferentially located on the tentacle margin. It is proposed that vesicular cells could be defensive cells containing a toxic material. The tentacles of *S. maculata* appear therefore to be well adapted for efficient deposit feeding: their large adhesive outer surface and great mobility allow a maximal capture of food particles while the presence in their epidermis of defensive cells would deter tentacle predators and thus allow the animal to optimize its feeding time.

### **Sea cucumber diversity and resources in Brunei, Borneo Island**

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A wide diversity of holothurians occurs on the coastal reefs of Brunei, with 14 beche-de-mer species being recorded in significant numbers and densities. Asexual fission apparently contributes to the high population densities of *Holothuria atra* and *Holothuria edulis*. In the genus *Bohadschia*, four species are recognized

and two of them are considered to be new to science. There is some evidence of illegal harvesting but surveys indicate that sea cucumber resources on Brunei reefs are, unlike those on most tropical Indo-Pacific reefs, relatively non-depleted. Given the limited domain of reef sites in Brunei territorial waters, stocks of commercial species are probably not sufficient to support a viable national fishery but the resource of high-value commercial species is of importance, not only in terms of biodiversity, but as a viable natural breeding population and a source of broodstock for mariculture research.

### A review of the genus *Synallactes* (Echinodermata: Holothuroidea: Synallactidae)

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The family Synallactidae Ludwig, 1894 holds mostly deep-sea forms and it is the least-studied large taxon among the deep-sea cucumbers. The synallactids are one of the most characteristic animals of the deep ocean. The Synallactidae, as presently recognized, comprises approximately 131 species currently named. The genus *Synallactes* Ludwig, 1894, embraces approximately 22 species. Five of these species occur in the Atlantic Ocean, the rest inhabit the Pacific (10 species), Indian Ocean (5 species) and Antarctic Ocean (2 species). A checklist of *Synallactes* species and an original and current name combinations list are presented.

### Other abstracts

#### Rapid growth and bioturbation activity of the sea cucumber *Holothuria scabra* in earthen ponds

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**Source:** Proceedings of Aquaculture Australasia 2004, Sydney, Australia, September 2004

Sea cucumbers are valuable export products. Technology on their culture is expanding rapidly to help meet market demand in China, and to help relieve exploited fisheries. The sandfish, *Holothuria scabra*, is the most valued of tropical species. It is the focus of a restocking research project in New Caledonia, funded by ACIAR, provincial governments and the government of France. Sandfish are deposit-feeding detritivores of shallow muddy/sandy habitats, thus earthen ponds provide suitable conditions. Pond grow-out could reduce the costs involved in producing juveniles for restocking and provide a valuable and novel crop.

Hatchery-produced juveniles and wild-caught broodstock sandfish grew rapidly in earthen ponds in New Caledonia (Table 1). No additional feed was given, so animals had only the existing organic matter in the sediments, averaging 4.0 to 6.2 % by wt., and autochthonous production. Densities were 78–128 g m<sup>-2</sup>, under the reported growth-limiting threshold of 225 g m<sup>-2</sup>.

One kg is an appropriate size for processing for exportation. Therefore, the culture cycle in ponds could be 1.5 to 2 years.

In-situ measurements showed that the bioturbation of pond sediments, particularly by burrowing, is substantial. Sandfish averaging 919 g ( $n = 4$ ) displace 1087 ( $\pm 296$ ) cm<sup>3</sup> of sediment by burrowing, which is a natural diurnal behaviour. Burrows are shallow in ponds due to hard underlying sediments. Feeding was periodic and variable but, as a guide, animals averaging 1.0 kg excreted 1.6 g ( $\pm 1.3$  g) sediment h<sup>-1</sup>.

Pond grow-out of sandfish appears technically feasible. Sandfish could act as bio-remediators for pond aquaculture by eating unused organic deposits, but conversion to soluble nitrogenous excreta should be considered. Bioturbation by sandfish could benefit shrimp culture. An experiment rearing juvenile sand-

**Table 1.** Growth of sandfish broodstock and hatchery-produced juveniles (asterisks) in ponds.

Av. start wt. (g)	<i>n</i>	Duration (months)	Growth rate (g ind <sup>-1</sup> month <sup>-1</sup> )
1375	43	12	8.2
702	132	5	69.8
378	37	7.5	71.6
262	31	11.5	46.2
22 *	55	1	35.0
26 *	62	1	30.1

fish with shrimp showed promise for successful co-culture. However, co-culture is not a likely panacea for reduced pond management, since ponds with sandfish can also become eutrophic with anoxic sediments. Future research should examine inter-specific interactions, verify bio-remediation effects, and determine optimal management of co-culture systems.

### **Long-term changes in reproductive patterns of the holothurian *Oneirophanta mutabilis* from the Porcupine Abyssal Plain**

*E. Ramirez-Llodra, W.D.K. Reid and D.S.M. Billett*

**Source:** Marine Biology (2005) 146:683–693

Time-series studies (1989–2002) in the NE Atlantic have shown large-scale changes in the composition and structure of the benthic community on the Porcupine Abyssal Plain. Radical changes in the abundance of some species in 1996 led to a significant shift in the way in which organic matter was reprocessed at the seabed. This article examines the reproductive processes of the holothurian *Oneirophanta mutabilis* collected during the time series. The reproductive biology of *O. mutabilis* is reviewed. No males were evident in any of the samples. The sex ratio (females: “no-gender”) was biased significantly towards no-gender individuals. The maximum egg size was 650 µm and there was no evidence of synchrony in reproduction. Significant changes in the oocyte-size distribution and the fecundity of *O. mutabilis* were noted through time, coinciding with the time of greatest faunal change in the benthic community. There was an increase in the proportion of previtellogenic oocytes and a decrease in the proportion of mature vitellogenic oocytes in 1997 and 1998, in parallel with a significant decrease in fecundity of the post-1996 samples. Samples from 2002 showed a reversal in the reproductive trends, with an increase in the proportion of mature vitellogenic oocytes and fecundity. The results are discussed in relation to large changes in abundance of the epibenthos on the Porcupine Abyssal Plain. It is suggested that the superabundance of certain megafaunal species on the abyssal seafloor affected the availability of trophic resources for *O. mutabilis*, leading to the changes in its reproductive effort.

### **Regenerating holothurian tissues as a source of cells for long-term cell cultures**

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**Source:** Marine Biology (2005) 146:5

Cell cultures of marine invertebrates can provide simple and adequate model systems for studying different aspects of complex biological processes, with the additional advantage of allowing study under controlled experimental conditions. In the present study an attempt was made to establish long-term cell cultures from a regenerating intestine of the sea cucumber *Apostichopus japonicus* and showed that cells obtained at different stages of gut regeneration display different behaviors in culture. Cultured holothurian cells were viable for a long period of time, and displayed a high level of activity after 1 month in vitro. However, only cells of primary intestinal cultures, obtained 14–16 days after evisceration, were involved in active proliferation. Furthermore, the cells undergo mitosis, and a very intensive growth period was observed during the first week of culture. Some cells (conceivably, enterocytes) were able to grow in suspension only, whereas others (presumably, coelomic epithelial cells, which form the outer layer of the gut in intact animals) grew as a multilayered mass on the polylysine surface. In contrast, cultures of intestinal cells from both intact and regenerating animals at other stages of regeneration displayed no proliferative activity. This seems to be due to specific events that take place in the regenerating gut tissues 14–16 days after evisceration. In addition, under TEM, we found that an active morphogenetic reorganization of both the gut coelomic and digestive epithelia occurred on the 14<sup>th</sup> day of regeneration. This was accompanied by cell dedifferentiation, migration and proliferation. Maximum numbers of both dedifferentiating and redifferentiating cells were also registered on the 14<sup>th</sup>–16<sup>th</sup> days of regeneration.

### **Resurrection of *Bohadschia bivittata* from *B. marmorata* (Holothuroidea: Holothuriidae) based on behavioral, morphological, and mitochondrial DNA evidence**

*Ron Clouse, Daniel Janies and Alexander M. Kerr*

**Source:** Zoology (2004) 28

Behavior, color, body size, spicules, and mitochondrial DNA were examined in two morphs from the *Bohadschia marmorata* (De Holothuriis. Dissertatio Inauguralis [1833]) species complex in Micronesia to test



whether they are conspecific. This complex consists of eight morphs that have been described as separate species and combined in various ways for over a century. We examined the classic *B. marmorata* type and the type originally described as the species *B. bivittata* (Mitsukuri 1912); *B. bivittata* was combined with *B. marmorata* by Panning (Mitteilungsanmeldung Hamburgischen Zoologischen Museum und Institut, Hamburg 49 [1944] 1). Several observations and phylogenetic analysis led us to conclude that *B. marmorata* and *B. bivittata* should return to their status as separate species. First, *B. marmorata* lives in shallow areas with strong currents, and *B. bivittata* lives on open sand between corals in deeper water. Second, the coloration of *B. bivittata* is distinct from *B. marmorata*, and although specimens collected on Yap Island differed from Mitsukuri's original description of *B. bivittata*, no specimens were collected with coloration intermediate between *B. bivittata* and *B. marmorata*. Third, spicules are more highly branched, perforated, and spiked in *B. bivittata* than in *B. marmorata* (and, in our study, spicule complexity did not correlate with body size). Finally, our phylogenetic analysis, based on partial nucleotide sequences of 16 s, 12 s, and COI mitochondrial genes, resulted in a tree — (*Pearsonothuria graeffei* (*Bohadschia marmorata*), (*B. argus* (*B. bivittata*))) — which shows that *B. marmorata* and *B. bivittata* are not even sister species, with *B. bivittata* more closely related to *B. argus*. Support for the clades for each *Bohadschia* species was strong but the clade containing *B. argus* and *B. bivittata* had weaker support. Color and spicule examinations made of preserved *B. marmorata*-complex specimens from the Indo-Pacific as well as behavioral observations in the field also support the resurrection of *B. bivittata*.

### **Reproductive biology of *Holothuria leucospilota* in the Cook Islands and the implications of traditional fishing of gonads on the population.**

D.J. Drumm and N.R. Lonergan

**Source:** New Zealand Journal of Marine and Freshwater Research 39(1):141–156

This study describes the reproductive biology of *Holothuria leucospilota* in Rarotonga, Cook Islands and assesses the effect of traditional fishing on survival and reproduction. Monthly collections of *H. leucospilota* from January 1998 until March 1999 revealed that gametogenesis and spawning were synchronous between the sexes and spawning occurred annually during the summer months. Spawning during the first year had already started at the beginning of the study in January and lasted until April, whereas in the second year, spawning started in November and finished in February, possibly because of higher water temperatures in this year. Our data indicate that the incision of the body wall and gonad removal had no impact on the survival of *H. leucospilota* in cages. However, their body weight, general sheltering and feeding behaviours were affected by gonad removal. Although the body wall of fished animals healed within 7–14 days, the gonads had just started to regenerate after 41 days, suggesting that the spawning of fished individuals would have been greatly delayed, possibly until the following year.

### **Natural hybridization does not dissolve species boundaries in commercially important sea cucumbers**

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**Source:** The Linnean Society of London, Biological Journal of the Linnean Society, 2005, 85

The study of species boundaries in areas of sympatry provides important insight into speciation processes. We investigated whether (i) two sympatric holothurians, *Holothuria scabra* and *H. s. var. versicolor* constituted species, and (ii) specimens of intermediate phenotype hybrids. Results from allozyme and 16S mtDNA sequence analyses indicated these two sea cucumbers to be distinct but young biological and phylogenetic species. Several private allozyme alleles existed and a Bayesian analysis grouped varieties into separate clusters. MtDNA sequences hardly varied within each taxon, and nine single bp changes were diagnostic between these two taxa. Allozyme allele frequencies in individuals of intermediate phenotype were intermediate to those of *H. scabra* and *H. s. var. versicolor*, most private alleles were present and heterozygote frequencies were higher than in either species. Ancestry coefficients models for these individuals were close to 0.5, indicating that the two taxa contributed equally to their genome. MtDNA sequences were identical to those of either species. We conclude that individuals of intermediate phenotype represent F1 hybrids. The presence of hybrids demonstrates that the opportunity for introgression exists, but is not realized, as backcrossing and introgression were not supported by the data. Thus, the genetic integrity of either holothurian species remains intact through an unknown postzygotic mechanism, possibly hybrid sterility.

## Hatchery of sea cucumber *Holothuria scabra*

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The sea cucumber (*Holothuria scabra*), widely known as Teripang Pasir in Indonesia, has become one of the country's export commodities. High marketing demand and relatively high prices have encouraged people to exploit this sea cucumber intensively. Currently, the main source of sea cucumber is natural exploitation. Although sea cucumber culture activities have begun in some places, they cannot develop quickly because seed is unavailable in sufficient quantities.

Availability of quality seed production in sufficient quantity is the starting point of successful sea cucumber culture activities. Generally, sea cucumber hatchery activities include broodstock management, larva rearing to juvenile stage, and live food culture. Sea cucumber broodstock consists of individuals of 400 g or more, which are reared in coastal ponds that are not influenced by fresh water. In coastal ponds, the gonad maturity process can occur year round, so spawning induction can be done at any time.

Larval rearing is done in fiberglass or concrete tanks of 1.0–4.0 m<sup>3</sup>; nursery tanks are 0.5–1.0 m<sup>3</sup>. For daily feed, larvae are given *Chaetoceros* sp., *Isochrysis* sp., *Phaeodactylum* sp. and *Nannochloropsis* sp. After 60 days, larvae become juveniles and are transferred to nursery tanks. They are then fed with benthic algae, dominated by diatoms. Harvesting occurs when the sea cucumber reaches 7 cm, which corresponds to a rearing period of around 3 months.

**Table 1.** Stages of larval development

Stage	Age (day)	Size (µm)	
		Length	Width
Early auricularia	1	400–500	300–350
Late auricularia	10–13	800–1100	600–800
Doliolaria	13–30	500–650	300–430
Pentactula	16–40	600–5000	250–1000

## The opportunity of sea cucumber *Holothuria* sp. culture in Indonesia

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Sea cucumbers have high economic value and represent significant potential for cultivation. Recent scientific research in China shows that sea cucumbers and their processed products can cure certain forms of cancer, such as lymphoma and breast cancer.

In other countries, sea cucumbers have been overfished and their natural populations have sharply depleted. In order to increase their production, the promotion of their cultivation has become an important part of mariculture activities. The main market for sea cucumbers are China and Japan. While there is a potential market for sea cucumbers, there has been relatively little effort in Canada to develop the culture technology to rear them in a controlled manner. A few trials were conducted for sea cucumber culture, but no significant development to pilot scale and commercial projects has yet taken place. Considering the potential for sea cucumber culture in Indonesia, effective and efficient culture technology applications are needed.

Of the many methods tried for inducing sea cucumber breeding, only some have proved successful, while the rest had only limited success. One method combines desiccation and flowing water.

This method is commonly used to produce sea cucumber larvae on a commercial scale. The operation starts at about 17.00 h, when the water in the temporary stocking tank is drained away and the spawners exposed to air for 30–60 minutes. After this, they are jetted with water for about 5–10 minutes. After about 1.5–2.0 h, the spawners move upwards, become restless and toss their head from side to side frequently. The males begin to spawn first, followed about half an hour later by females. Fertilization rates as high as 95% can be obtained with this method.

To manage natural sea cucumber resources, enhancement is needed, particularly aspects: (1) protection of broodstock and their larvae; (2) release of larvae and transplantation of broodstock, and (3) improvement of ambient environment. These measures have proved successful in the enhancement of sea cucumber productivity.

## Étude de la variabilité génétique des holothuries en Tunisie par analyse allozymique

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**Source:** Abstract from Master Thesis

Specimens of holothurians were collected along the Tunisian littoral. They were first identified using morphological, anatomical and microscopically criteria. Samples were then investigated by allozyme electrophoresis of five polymorphic loci. Cluster statistical analysis and parameters describing genetic variability, confirm morphological analysis, detect an important genetic variation, and indicate a high gene flow between *Holothuria polii* populations.

## Evolutionary diversification of holothuriid and synaptine holothuroids onto coral reefs

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An organism's potential to adapt to a novel environment depends in part upon its history of evolving features needed in previous environs. Aspidochirote and apodan holothuroids are diverse and ubiquitous groups of echinoderms inhabiting a derived habitat, scleractinian coral reefs. These two groups provide replicate radiations into an identical ecosystem, allowing one to test the question: How has the evolutionary history of organisms prior to their expansion in the tropics constrained or facilitated their diversification onto coral reefs? Ancestral character reconstructions using ecological data and a new phylogeny based on morphology and several molecular markers suggest that aspidochirote coral reef sea cucumbers evolved from deepwater epibenthic ancestors, while reef apodans arose from littoral infaunal forms. Much ecological novelty evolved *in situ* as species expanded into the numerous new microhabitats afforded by coral reefs. Associations between habitat choice and diel patterns of activity have evolved multiple times. Finally, despite high local species diversity in the tropics, expansion onto coral reefs does not appear to have been an adaptive radiation facilitated by the evolution of a "key innovation," as diversification was not accompanied by an increase in net speciation rates.

## Phylogeny of Holothuroidea based on 18S and histone 3 DNA sequences

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We present a phylogeny of 30 species of Holothuroidea based on PCR-amplified complete 18S-ribosomal and partial histone-3 gene sequences. Estimated maximum parsimony and maximum likelihood topologies using POY direct optimisation strategies are largely congruent with a previous phylogeny based on morphological characters. For example, as first hypothesised by Carl Semper in 1868, Apodida (as currently defined) is sister to all other holothuroids. Molpadida is sister to Dendrochirotida. The new estimates also differed in several important ways. Synallactidae, a morphologically diverse group, appears to be paraphyletic. Also, surprisingly, the deepwater order Elaspodida appears polyphyletic with the deimatids grouping within synallactids at the base of the aspidochirote clade. Points of contention are defined by short internal branches co-eval with a rapid radiation of holothuroids and other marine organisms in the early Mesozoic.

### **Behavioral-ecological, morphological and mtDNA evidence to resurrect *Bohadschia bivittata* (Mitsukuri) from the *B. marmorata* (Jaeger) species complex**

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Behavior, habitat selection, body size, color, spicule complexity and mtDNA were used to examine taxonomic relationships among specimens from the *Bohadschia marmorata* species complex. Spicules were more highly branched, perforated, and spiked in *bivittata* specimens than those of the classic *marmorata* type. Moreover, spicule complexity did not correlate with body size. Phylogenetic analyses of partial nucleotide sequences of 16S and 12S ribosomal genes were done with the hypothesis that cladistic groupings would reflect morphological similarity instead of geographic proximity. This hypothesis was supported by the clustering of *bivittata*-looking specimens despite being from different Micronesian islands. The resulting tree, (*Pearsonothuria graeffei*, (*B. marmorata*, (*B. argus*, *B. bivittata*))), indicated that *marmorata* and *bivittata* are not even sister species, with *bivittata* more closely related to *argus*. These results were corroborated by color and spicule examinations made of specimens from this complex in the National Museum of Natural History (Washington DC USA) collection and by behavioral observations made in the field.

### **Sea cucumber fisheries: Assessing resource status for better management in the tropical Pacific**

K. Friedman, T. Skewes and M. Kronen

**Source:** Paper presented at the International Coral Reefs Symposium, Okinawa, 2004

Despite being active for over two centuries, sea cucumber fisheries in the tropical Pacific are not well documented or understood. Heavy fishing pressure has depleted stocks of valuable species and places a greater need for management based on reliable stock estimates. However, visual surveys of sea cucumbers generate estimates of stock size and structure with varied effectiveness. The precision of assessments can be improved markedly when the multispecies nature of stocks, their low density, spatial aggregation and habitat specificity is accounted for in survey design. In addition, socio-economic assessments of these fisheries can provide a complimentary understanding to the instantaneous evaluation of stock size supplied by in-water surveys. Socio-economic assessments of the actual level of resource exploitation take account of variations in the commercial structure of these fisheries, and the presence, absence and/or fluctuations in regulations, marketing, and prices. Research agencies in Australia and the Pacific Islands have developed various approaches to assess the status of sea cucumber fisheries through resource and socio-economic assessment on a number of spatial scales, using tested and novel survey techniques. These approaches are compared and contrasted within the current understanding of sea cucumber stocks, to identify practical and reliable strategies for assessing these fisheries, and to build a framework for integrating current knowledge into advanced assessment techniques.

### **The past and present of the coral reef fishing economy in Madagascar: Implications for self-determination in resource use**

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**Source:** Nobuhiro Kishigami and James Savelle (eds.) 2005. Indigenous Use and Management of Maritime Resources (Senri Ethnological Studies 67), Osaka: National Museum of Ethnology, 237–258.

In coral reef areas of southwestern Madagascar, sea cucumbers have been a major source of cash income for Vezo fishermen throughout the 1990s and 2000s, when the national economy was opened to global markets. At the research site in Morombe region, however, the resource had already been depleted around 1980, when cash and commodity economies rapidly penetrated rural life. This is why Morombe fishermen began, around 1992, seasonal fishing with base camps 100–250 km away from the village. From Ampasilava village, for example, 55.6 % of adult males went to remote fishing camps during 1995–96, for 86.15 days in average, in order to dive for sea cucumbers and to net sharks.

This article describes seasonal camp fishing in remote areas, as well as regular fishing near the village, and reveals the importance of the former in the fishermen's household economy. In the latter part, it describes



the development of Ampasilava fishing life since the 1970s, calling attention to the fishermen's mobility and migration as ways of solving their economic predicament. For effective management of fishery resources in general, it proposes, fishermen should share information of national and global socioeconomic change through local public meetings, rather than demarcate fishing grounds to limit entry, which narrows the fishermen's determination.

### **Transportation methods for restocking of juvenile sea cucumber, *Holothuria scabra***

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**Source:** Aquaculture (in press)

The sandfish, *Holothuria scabra*, is a heavily exploited sea cucumber species. Minimising stress in the transportation of hatchery-produced sandfish juveniles to release sites is critical for successful restocking. Replicate groups ( $n = 4$ ) of 20 hatchery-produced juveniles (1–5 g) were held in plastic bags with oxygen under 6 transport durations (0, 2, 4, 8, 12 and 24 h), two media (water or saturated sponge), and two temperature regimes (ambient and cool). Subsequent deaths and sand-burrowing of the groups in release chambers were monitored for 5 days. Juveniles eviscerated or died only in treatments at ambient temperature on sponge for 24 h. Oxygen consumption in bags was reduced at cool temperature. On the first day after release, the normal sand-burrowing was suppressed in juveniles held for 12 and 24 h, suggesting that pre-release acclimation for one day at field sites would benefit restocking. After the initial "shock" of transport, handling stress appeared to increase burrowing behaviour for several days. Hatchery-produced sandfish also proved hardy for transport in high densities (100 and 200 juveniles per bag) and transportation stress will be minimised in seawater held at cool, constant temperature.

### **Taxonomy of the monotypic genus *Koehleria* Cherbonnier, 1988 (Echinodermata: Holothuroidea: Cucumariidae)**

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**Source:** Zootaxa 652:1–11 (2004)

Voucher material recently collected from the 2-Mile Reef at Sodwana Bay (Republic of South Africa), allows the evaluation of the monotypic genus *Koehleria* Cherbonnier, 1988 and its species *K. unica* Cherbonnier, 1988 collected from Tuléar (Madagascar). Cherbonnier's (1988) conclusion, that *Koehleria* is most related to the also monotypic genus *Pseudocolochirus* Pearson, 1910 is correct, but the differences between *Koehleria* and *Pseudocolochirus* are too small to justify the retention of *Koehleria*. *Koehleria* is relegated herein to the synonymy of *Pseudocolochirus*. Such a step decreases the velocity with which monotypic genera are currently described within the Dendrochirotida.

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