

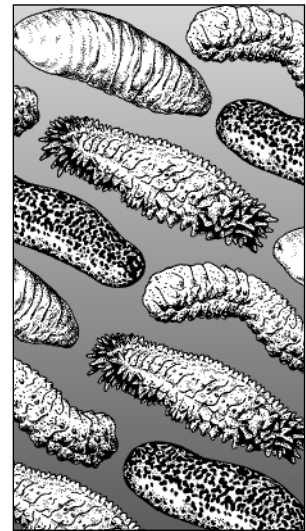


Secretariat of the Pacific Community

# BECHE-DE-MER

Number 24 — July 2006

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 has been less than six months since the last issue of this bulletin was published, and, as you will discover in these pages, we have nevertheless received enough new information to publish a very rich issue.

We begin with an article by Hearn who presents some baseline information on the warty sea cucumber *Stichopus horrens* from the Galápagos (p. 3). Following the collapse of the *Isostichopus fuscus* fishery in the Galápagos Marine Reserve, fishers are now focusing on *Stichopus horrens*, which has been illegally fished since 2004. It is hoped that if a legal fishery for this species is opened, the baseline information collected will be used to manage it sustainably.

We continue with two articles from the Middle East. Dar and Ahmad write about the feeding selectivity and ecological role, as sediment "reworkers", of three shallow water holothurians, *Holothuria atra*, *H. hawaiiensis* and *Bohadschia vitiensis*, in the Red Sea (p. 11). Tehranifard et al. examine the reproductive cycle of *Stichopus hermanni* from Kish Island, Iran, and share their results with us (p. 22).

As demonstrated in several past issues of this bulletin, sea cucumber aquaculture is well developed in some parts of the world such as China, but is still in its infancy in the Pacific Islands region. Ivy and Giraspy work in eastern Australia at the only commercial sea cucumber farm operating in the tropical Pacific region. They evaluate the potential of *Holothuria scabra versicolor* (golden sandfish) for mass culture in a hatchery (p. 28). Conscious that the development of sea cucumber farming will likely lead to the introduction of new diseases, Yasoda et al. discuss the possibility of using probiotics as an "eco-friendly" biological method against pathogens (p. 45).

Lavitra et al. assess the abundance of echinoderm larvae, including sea cucumber larvae, in the lagoon of Toliara Great Reef in south-west Madagascar (p. 35). The authors collected more than 9000 echinoderm larvae, and their sampling clearly shows seasonal variability among the different larval forms. Sea cucumber larvae are poorly studied, and this article comes as a useful addition to the scientific literature.

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Minimum size for live sea cucumbers is often used as a management tool, but measuring live sea cucumbers can be a real challenge. Laboy-Nieves and Conde devised a new approach: submerging specimens in cold (~ 8° C) seawater to obtain maximum contraction in less than 10 seconds (p. 39).

Following the publication of the SPC *Pacific Island sea cucumber and beche-de-mer identification cards* — which focused on 22 commercially important species in the Western Pacific — a need was identified for a guide that would include major commercially valuable species from other parts of the world. Toral, from the Charles Darwin Foundation (Galápagos), has initiated a project to develop such a guide (p. 49) with the collaboration and support of FAO, which will publish it. The project presents the perfect venue for gathering available information on sea cucumber biology, ecology, processing and markets, including photos and descriptions of commercial species at different levels of processing.

We continue to publish observations about natural sea cucumber spawnings with descriptions in this issue from the Mediterranean Sea (p. 52) and from La Reunion in the Indian Ocean (p. 53). And as usual, we also include a section on Abstracts and Publications (p. 55).

I remind you that this and all previous issues of the bulletin are available in pdf format on SPC's website at: <http://www.spc.int/coastfish>. The 600+ article and abstract titles that have been published so far in the Bulletin can also be searched by title, author name(s), scientific name, region or country in the database put together by SPC's Fisheries Information Section, and available on SPC's website at: [http://www.spc.int/coastfish/news/search\\_bdm.asp](http://www.spc.int/coastfish/news/search_bdm.asp).

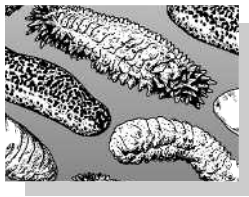
Finally, I draw contributors' attention to the new "Instructions to authors" that have been issued by SPC's Fisheries Information Section (<http://www.spc.int/coastfish/news/SIG-instructions.pdf>). This bulletin now receives many more submissions than can be fitted in a 50–60 page issue. This "success" may be partly attributed to two factors: 1) sea cucumber fisheries have recently expanded (and often collapsed) in many parts of the world, making them a hot topic of fisheries management, and to our knowledge, the BDM bulletin is the sole existing publication fully dedicated to the subject; and 2) the bulletin's longevity and high quality attracts spontaneous submissions from authors.

But, this relative success has a downside. The human and financial resources required to produce two lengthy issues each year (and BDM is only one of seven information bulletins published by SPC's Information Section) are significant. Consequently, we must look for ways to reduce the associated workload. Therefore, we will now only be accepting articles that require minimal English language revision and editing, and which adhere to SPC's Instructions to authors. If you plan to submit an article to this bulletin, and your native language is not English, we strongly recommend that you hire an English language editor to review your work before submitting it. If this is not possible or practical, then we suggest you ask an English-speaking colleague to check your manuscript prior to submission. This will help to ensure that your paper is not rejected for publication.

The *Beche-de-Mer Information Bulletin* has now reached its 17<sup>th</sup> year of existence and we must make sure it safely rides towards and beyond its 20<sup>th</sup> anniversary. I trust that you will help us achieve this goal.

**Chantal Conand**

PS: Please note that, following its usual three-year rhythm, the next Echinoderm Conference will be held in August 2006 on the campus of the University of New Hampshire, Durham, NH (USA) (more information at: <http://www.iec2006.unh.edu>).



## new info

beche-de-mer

### Baseline information on the warty sea cucumber *Stichopus horrens* in Santa Cruz, Galápagos, prior to the commencement of an illegal fishery

Alex Hearn<sup>1</sup> and Fernando Pinillos

#### Abstract

With the depletion and collapse of the fishery for the sea cucumber *Isostichopus fuscus* in the Galápagos Marine Reserve, attention is now being focused on the warty sea cucumber *Stichopus horrens*, which has been illegally fished since 2004. This study presents the results of surveys carried out at a site in the south of Santa Cruz Island before illegal harvesting began, and constitutes the only baseline information available to date on this species. *S. horrens* displays more diurnal variation than *I. fuscus*, and may be present in densities greater than 100 individuals per 100 m<sup>2</sup> at night. Total length of individuals ranged from 9–31 cm, with a mean size of 20 cm.  $L_{\infty}$  was estimated at 37.7 cm and Z/K was equal to 4.95. No recruitment was observed. The length–weight relationship was linear but displayed considerable variation, suggesting that a minimum landing size may not be appropriate for this species. Before such a fishery is opened, lessons need to be learned from the failure to sustainably manage the *I. fuscus* fishery, and a management plan, including marketing aspects, should be developed.

#### Introduction

The Galápagos Marine Reserve, created in 1998, straddles the equator at 600 nautical miles off the coast of Ecuador (Fig. 1). Its location, in the path of the warm Panama Bight from the north, the cool Humboldt Current from the south, and the nutri-

ent-rich, cold upwelling Cromwell Current from the west make it unique in the variety of species and communities found over a relatively small area (Edgar et al. 2004).

As a multi-use marine reserve, fishing is permitted, although this is restricted to artisanal fishing carried out by registered local fishers. In the early 1990s, as a response to the collapse of the fishery for the sea cucumber *Isostichopus fuscus* on the coast of continental Ecuador, there was an influx of fishers to Galápagos, where this species was not traditionally exploited. In 1994 an experimental fishery was opened with a catch quota of 500,000 individuals over a period of two months. However, the season was closed only six weeks later and the final catch was estimated somewhere between 8 and 12 million individuals (DeMiras et al. 1996). After four more years of illegal fishing and political pressure, the sea cucumber fishery for *Isostichopus fuscus* was finally opened on a seasonal basis with a series of regulations in 1999. After only seven years of legal fishing, despite regulations (Toral

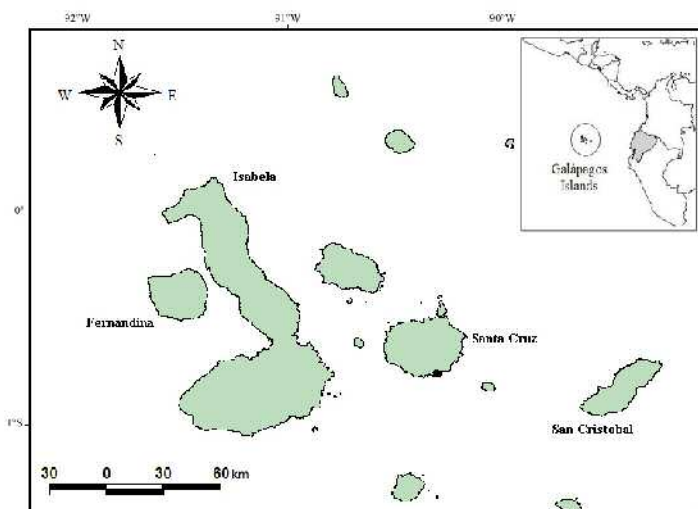


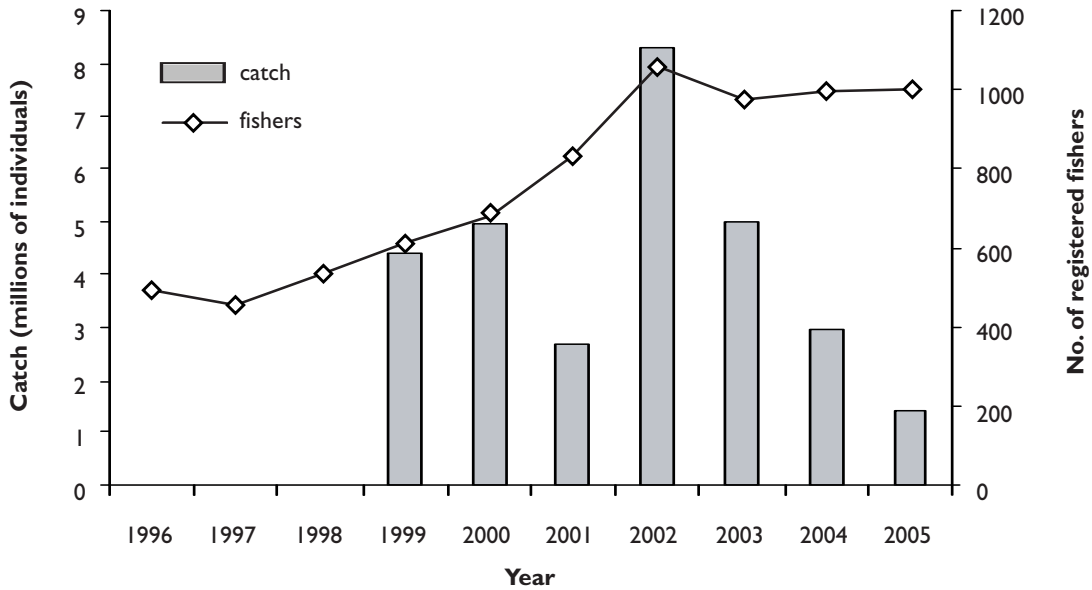
Figure 1. Galápagos Islands.

The study site (●) is at the south end of Santa Cruz Island.

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and Martinez 2004; Altamirano et al. 2004; Shepherd et al. 2004; Hearn et al. 2005a; Toral et al. 2005), the *I. fuscus* resource has been overexploited to the point of commercial extinction (Fig. 2), and the number of registered fishers has increased to around 1000 (Fig. 2).

As a result of the decline and collapse of the *I. fuscus* fishery, there has been increasing pressure to allow extraction of other species of sea cucumber. One alternative, which was identified by the fishing sector in 2004, is the warty sea cucumber, *Stichopus horrens* Selenka, 1867 (Fig. 3).



**Figure 2.** Official annual catch of sea cucumber *Isostichopus fuscus* since the fishery was opened in 1999, and number of registered fishers in the Galápagos Marine Reserve (source: Galápagos National Park Service and Charles Darwin Foundation archives).



**Figure 3.** The warty sea cucumber *Stichopus horrens* (photo: Alex Hearn)

*Stichopus horrens* is found in the Pacific Ocean, from Malaysia to the Society Islands, French Polynesia, and from southern Japan and Hawaii to New Caledonia (Massin et al. 2002), as well as in the Galápagos Archipelago, where it is found on rocky substrates from 5–20 metres depth (Hickman 1998). It is cryptic and lethargic by day, when it finds shelter in cracks, caves and crevasses in the rocky substrate. At night, it emerges to feed and may be found in large aggregations (Hearn et al. unpubl. data).

This species is subject to commercial exploitation in other parts of the world, such as Madagascar (Rasolofonirina et al. 2004) and Malaysia (Baine and Choo 1999) where it is exploited for the medicinal properties of its coelomic fluid, known as “gamat”. In Galápagos, illegal shipments of *S. horrens* were detected during the *I. fuscus* fishing season in 2004. Since then, a widespread illegal fishery has developed (Hearn et al. 2004; Toral et al. 2005) and the Galápagos National Park Service has seized a number of shipments of *S. horrens* (Table 1). At the same time, the local fishing sector has applied pressure through legal channels and the local Participatory Management Board has called for a baseline study to be carried out in order to assess whether this species can sustain a fishery. However, after two years of illegal fishing, it is unlikely that any study carried out in the short-term future on size structure and abundance will truly reflect the unexploited population.

**Table 1.** Illegal *Stichopus horrens* shipments and camps raided by the Galápagos National Park Service (source: Galápagos National Park Service archives).

Date	Site	Island	Number of <i>S. horrens</i>
07 Oct. 04	Puerto Ayora	Santa Cruz	20448
03 Jun. 05	illegal campsite	Santa Cruz	5934
27 Jul. 05	BP Calipso	At sea	1355
14 Oct. 05	illegal campsite	Santiago	1752
19 Oct. 05	illegal campsite	Isabela	7141
20 Oct. 05	illegal campsite	Isabela	3986
09 Jan. 06	illegal campsite	Santa Cruz	13000
23 Feb. 06	Bellavista	Santa Cruz	13343
<b>Total</b>			<b>66959</b>

The aim of this document is to report the findings of a pilot study carried out in 2003 in the south of Santa Cruz Island as the only existing information on this species in Galápagos prior to the commencement of illegal extraction.

## Materials and methods

Surveys were carried out at a depth of 6 metres at Punta Estrada in Academy Bay, Santa Cruz Island

(S 00.7622; W 090.3019), central Galápagos Archipelago (Fig. 1). The seafloor in this area is characterised by a sloping bed of lava rocks, below a coastline of cliffs.

In order to study the diurnal variability in abundance of *S. horrens* in comparison with that of the commercial species, *I. fuscus*, two permanent transects of 100 m<sup>2</sup> were established in Academy Bay. Abundance of both *S. horrens* and *I. fuscus* was determined at the following times: 00:00, 06:00, 08:00, 12:00, 18:00 and 20:00. Each transect was carried out twice for each time regime. Both datasets were normalised to show the comparative changes throughout the day for each species.

Nocturnal circular surveys covering 100 m<sup>2</sup> were carried out in September, October and December 2003 and January 2004, following the methodology of Richmond and Martinez (1993). One circular survey was carried out per month in a fixed 100-m<sup>2</sup> area, in which all individuals of *S. horrens* were collected by a diver and measured underwater in order to reduce handling, as this species is known to eviscerate immediately when gripped by hand (Kohtsuka et al. 2005). Total length (dorsal distance from the mouth to the anus) was recorded for each individual.

Approximately 25 individuals from outside the transects were collected each month and taken to the laboratory, where the following measurements were taken for morphometric analyses: total length ( $\pm 1$  mm), maximum circumference ( $\pm 1$  mm), total wet weight ( $\pm 1$  g), volume ( $\pm 1$  mL) and gutted wet weight (the total weight once the gonads, viscera and coelomic fluids have been removed,  $\pm 1$  g) (see Conand 1990).

Assuming that the population is in equilibrium, and that the size distribution is not affected by exploitation, the Powell-Wetherall method can be used to estimate  $L_\infty$  and  $Z/K$ , where  $Z$  is the total mortality rate (Powell 1979; Wetherall et al. 1987; Sparre and Venema 1992). This method is based on Beverton and Holt's (1956) equation, which estimates  $Z$  in a steady state population:

$$Z = K [(L_\infty - L)/(L - L')] \quad (1)$$

Where  $L$  is the mean length of individuals of size  $L'$  and greater, and  $L'$  is a length for which all individuals of that length and longer are fully exploited. There is a linear relationship between a series of arbitrary cut-off lengths  $L'$  and corresponding mean lengths  $L$  of fully exploited size classes, so that:

$$L - L' = a + (b \times L') \quad (2)$$

Where  $Z/K = -(1 + b)/b$  and  $L_\infty = -a/b$

## Results

### Length–weight relationship

*Stichopus horrens* displays a high degree of plasticity and is able to rapidly shrink or elongate. Daytime specimens (not included in this analysis) were usually found in crevices or under stones and were highly contracted. The length–weight relationship for individuals in a relaxed state displays a loose positive correlation, with considerable variation around mean (Fig. 4).

### Diurnal patterns of abundance

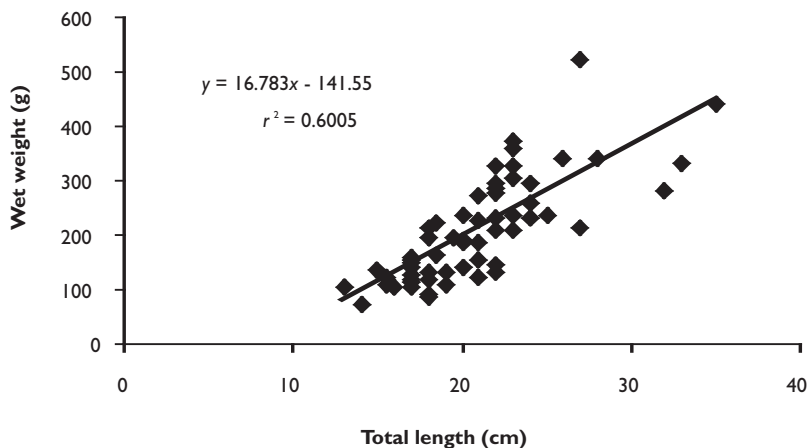
During the daytime, the abundances of both *S. horrens* and *I. fuscus* were similar and low (around 7 individuals per 100 m<sup>2</sup>). For both species, abundance increased at night, although for *I. fuscus* the increase was small, whereas for *S. horrens* the increase was on an order of magnitude (Fig. 5).

### Size structure and density

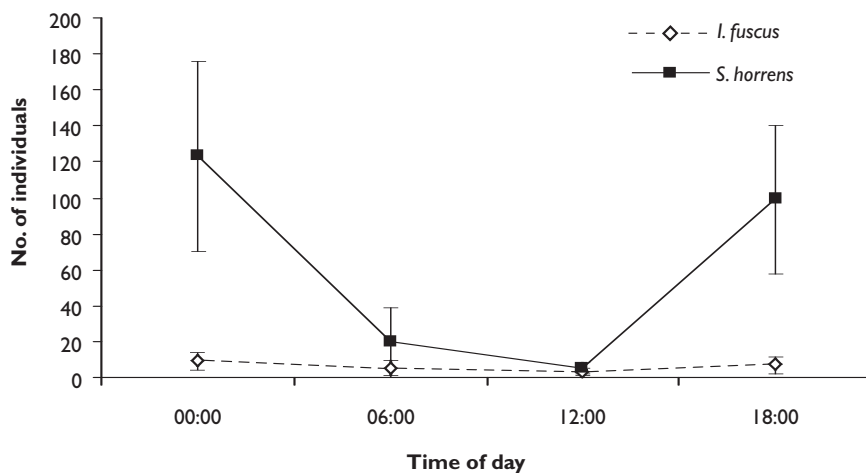
The size frequency distribution displayed by *S. horrens* each month from September 2003 to January 2004 (N = approximately 100 individuals each month) was normal and stable. The smallest individual measured 9 cm, the largest 31 cm. Mean length overall was 19.6 cm. No modal progression or recruitment events were observed (Fig. 6).

According to the size frequency distribution, the first fully recruited length group was 20–21.9 cm. Powell-Wetherall analysis of the fully recruited size groups provided estimates of  $L_{\infty}$  equal to 37.7 cm and  $Z/K$  equal to 4.95, with an  $r^2$  value of 0.967 (Fig. 7).

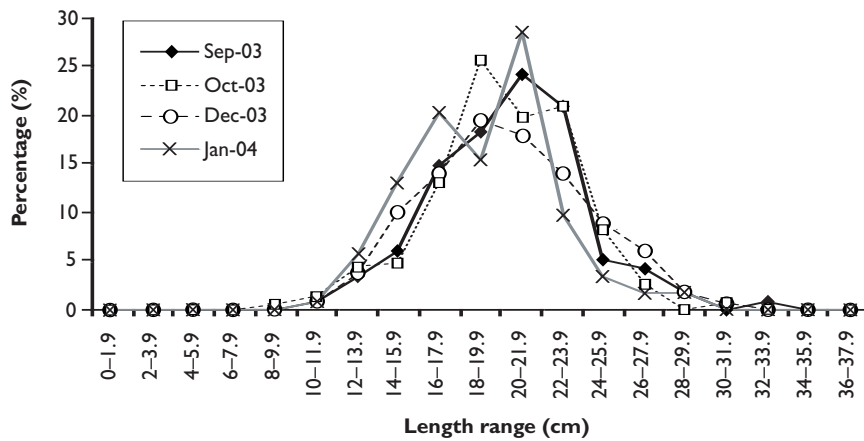
In all four surveys, the density of *S. horrens* exceeded 100 individuals per 100 m<sup>2</sup> (Fig. 8). Individuals were generally found fully extended, on rock surfaces, and appeared to be highly mobile in comparison with the more sedentary individuals of *I. fuscus*.



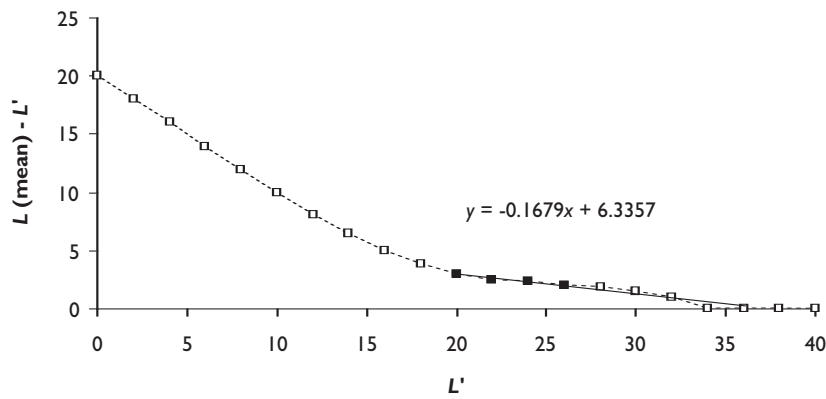
**Figure 4.** Length-weight relationship for *Stichopus horrens* in relaxed state (n = 59).



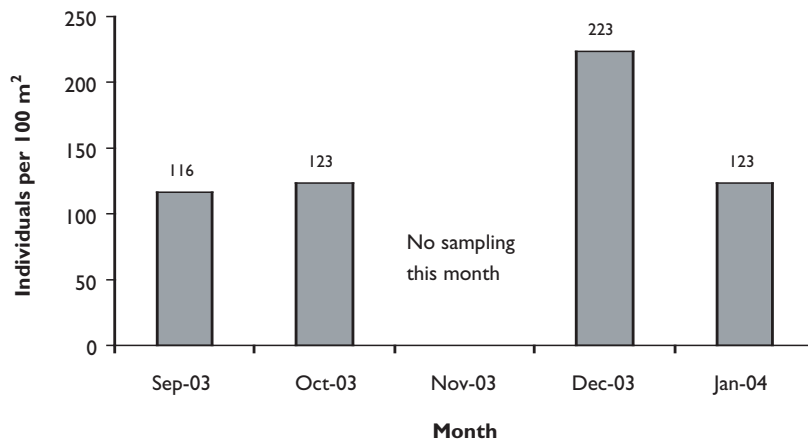
**Figure 5.** Mean (with 95% confidence intervals) abundance of *Stichopus horrens* and *Isostichopus fuscus* over a 24-hour period in Academy Bay, Santa Cruz Island, using visual diver census.



**Figure 6.** Monthly size frequency distribution for *S. horrens* at Academy Bay, Santa Cruz Island.



**Figure 7.** Powell-Wetherall plot for *S. horrens*, linear regression based on filled points for determination of  $L_{\infty}$  and  $Z/K$ .



**Figure 8.** Population density of *S. horrens* in number of individuals per 100 m<sup>2</sup> area in Academy Bay, Santa Cruz Island.

## Discussion

Sea cucumber fisheries generally follow the pattern of rapid expansion followed by collapse. Included in this pattern is the serial depletion of high value to low value species (Conand 1990, 2004, in press; Uthicke and Benzie 2000). Galápagos does not appear to be an exception to this (Shepherd et al. 2004; Hearn et al. 2004, 2005a; Toral et al. 2005). The reasons behind these patterns are both biological and economical. Biologically, sea cucumbers are slow moving, easy to catch, slow growing and with poorly understood recruitment dynamics. The fishery for *I. fuscus* in continental Ecuador, which collapsed in the early 1990s, has still not recovered (Altamirano et al 2004; Toral-Granda and Martinez 2004, Toral et al. 2005), and holothurian fisheries worldwide are generally poorly managed and in decline (Lovatelli et al. 2004; Bruckner in press).

The economic activity of sea cucumber extraction in Ecuador has been promoted by investors from the Far East, who pay low prices initially for an abundant resource and often subsidise fishers' operations. As the resource declines, they are prepared to pay higher prices, and once an area is exhausted, the merchants move to a new area. This results in social and economic problems in communities that have grown to depend on an unrealistically high income for a short period of time (Christy 1995; Carranza Barona and Andrade Echeverría 1996). In Galápagos, the search for alternative employment or resources for sea cucumber fishers has been marked by unrealistic expectations, illegal activities (such as shark finning) and frequent outbreaks of civil unrest (Hearn et al. 2004; Shepherd et al. 2004).

The eventual opening of a legal fishery for *S. horrens* must be analysed against the background of the inherent governance capacity of the Galápagos Marine Reserve (GMR) and the failure to manage *I. fuscus* in a sustainable fashion. In this sense, the lack of response to the Participatory Management Board's call for the fishing sector to elaborate and present a feasibility study regarding exploitation of *S. horrens* is most likely a result of the ease with which the illegal fishery can be carried out, thus eliminating the sense of urgency for legalization. One fisherman explained to the authors that he could collect around 40 crates of *S. horrens* (equivalent to some 4000 individuals) in one night, for which he received USD 2000.

Currently, illegal *S. horrens* are processed in the same way as *I. fuscus* — boiled, salted and dried. However, in certain parts of Malaysia, *S. horrens* is used for its coelomic fluid (termed gamat water),

which has medicinal uses (Baine and Choo 1999). As part of a feasibility study for Galápagos, market options should be explored, not only to determine the highest value product from the species, but also to avoid the economic dependence of local fishers on the merchants and middlemen.

The results of these preliminary surveys show a size structure characterised by an absence of juveniles, with a modal size of around 20 cm, and potentially high densities, which may compare with estimated virgin densities of *I. fuscus* (Shepherd et al. 2004). However, a management plan for the extraction of this species should consider the relative enforceability of the regulations that were applied to *I. fuscus*. Management measures such as a minimum landing size (MLS) may not be appropriate for a species that displays such high levels of plasticity, and for which a size at maturity is unknown. The provisional coastal zoning scheme (Danulat and Edgar 2002) is aimed at creating space for different stakeholders (fishers, tourism and science) and is not appropriate for the management of benthic resources. Clear rules on how to establish catch quotas from survey results should be drafted. Despite the problems associated with individual transferable quotas (ITQs) for *I. fuscus* in 2001 (Murillo et al. 2003), some form of private rights should be explored, in order to avoid the race to fish, which characterised the collapse of *I. fuscus*.

The Galápagos Marine Reserve Management Plan is based on the Precautionary Principle, and aimed at allowing sustainable use of its resources. To date, its most important fisheries are severely depleted, and little has been done to manage them sustainably (Toral et al. 2005; Hearn et al. 2005b). Unless there is a shift in awareness of the users themselves, legalising a fishery for *S. horrens* under the current management regime is likely to result in another link in the chain of successive collapses in the Galápagos Marine Reserve.

## Acknowledgements

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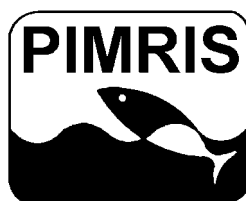


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# The feeding selectivity and ecological role of shallow water holothurians in the Red Sea

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## Abstract

This study examined the feeding selectivity and ecological role of the most dominant holothurian species — *Holothuria atra*, *Holothuria hawaiiensis* and *Bohadschia vitiensis* — on the tidal flats and in the shallow waters in the Red Sea at Hurghada, Egypt. The three species exhibited a wide range of variations in their gut sediment contents, depending on their total weight. Medium-weight individuals, which require considerable food for growth and gonad development, were found to consume more gravel than light- and heavy-weight individuals. The selective behaviour of the holothurians illustrates that gravel and coarse sands are the main sediment components in the animals' guts during the different seasons. During the spawning period (early summer to autumn), the animals tended to assimilate fine sediments more than coarse sediments, perhaps due to their increasing need for organic matter.

Sediment reworking processes by the different species varied during the year and depended on individual numbers, food availability, individual sizes and local conditions. *H. atra* showed the highest sediment reworking in July (summer) and November (autumn), *H. hawaiiensis* in autumn/winter (September and December, and *B. vitiensis* mainly from summer (May to August). It was observed that the reworking lasted from late spring to the end of autumn. This period covers the two essential phases of holothurian reproduction: maturation and spawning.

## Introduction

The deposit feeding holothurians are the dominant megafauna, in terms of both number and biomass, in many littoral ecosystems (Coulon and Jangoux 1993) and on sheltered marine shallow water substrates (Conde et al. 1991).

Variability in food supply is a major controlling factor in the population dynamics of benthic animals, in particular holothurians. Deposit feeders are among the most important consumers of detritus on the ocean floor, playing an important role in the removal, recycling and repackaging of nutrients, especially organic matter (Jumars and Self 1986). The way in which the various species feed on the top layer of sediments is highly variable, depending on their tentacles and gut morphology (Roberts et al. 2001). Holothurians feed by either ingesting material on the surface of the substrate or by swallowing nutrient-laden sediments. The sediments ingested by deposit feeding holothurians comprise mainly inorganic compounds (coral debris, shell remains, coralline algae, foraminiferal tests, inorganic benthos remains, and silicates), organic detritus (seagrass, algae, dead and decaying animals), microorganisms (bacteria, diatoms, protozoan and cyanophytes), or the faecal pellets of other animals or their own faecal pellets (Massin 1982; Moriarity 1982).

The quantity and quality of organic matter varies from year to year depending on numerous factors, including underlying sediments, and possibly pollution levels (Dar 2004). The three holothurian species examined in this study — *Holothuria atra*, *Holothuria hawaiiensis* and *Bohadschia vitiensis* — are known to feed more rapidly during the day than at night and continuous feeding may be necessary to keep sediments moving through the gut (Hammond 1982).

The objectives of this study were to investigate:

- 1) holothurian feeding behaviour throughout the year,
- 2) feeding selectivity habits of holothurians,
- 3) the most effective periods of sediment reworking by holothurians, and
- 4) the relationship between reproductive seasons and the type of sediments consumed.

## Materials and methods

### Site morphology and field investigations

Three different sites on the shallow tidal flat at Hurghada, Egypt were chosen for sampling (Fig. 1). Each one had an abundance of three holothurians: *Holothuria atra*, *H. hawaiiensis* and *Bohadschia vitiensis*.

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**Site I:** 4 km north of the National Institute of Oceanography and Fisheries (NIOF). This site has a relatively heterogeneous thin sand bed mainly from the biogenic origin of coral debris and shell remains. This site shows healthy and widespread coral reef patches and seagrass beds with water depth varying between 0.5 m at low tide and 1.50 m at high tide.

**Site II:** an area sheltered from intense wave action and in deeper water than Site I. The area surrounding this site is considered a natural sedimentation basin due to its morphology. This site has a thin, loose sediment layer partially covered with seagrass and macroalgae.

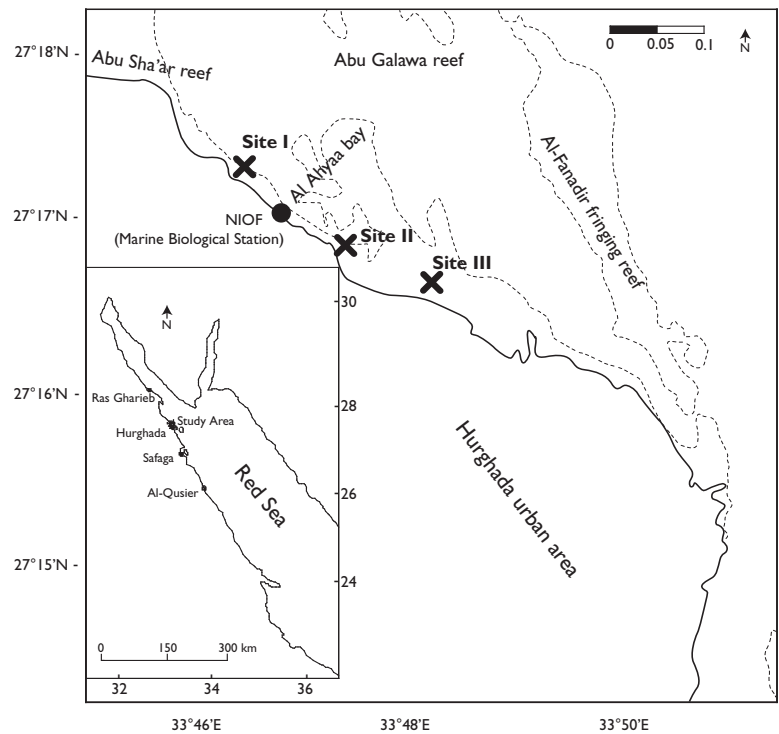
**Site III:** 4 km to the south of NIOF. This site has a homogeneous thick sediment layer composed of a mixture of biogenic and terrestrial sands. This site has high turbidity, especially on windy days, and very few coral patches as compared with Sites I and II.

Between April 2003 and March 2004, at least 10 individuals of each holothurian species were collected monthly and randomly using quadrates (10 m x 10 m) from the selected sites. Also, five sediment samples were collected from each site to compare sediment types between the sites and the gut contents of the sea cucumbers at those sites.

### Analyses

The collected individuals were weighed in order to estimate total weight under natural conditions. The gut contents of each individual were dried and weighed. Sieving and grain size analysis were applied to the gut contents to evaluate the different fractions of gut contents using a one- $\phi$  interval sieve set according to Folk (1974). Benthic sediment samples were dried and sieved in order to study the degree of divergence or coincidence between them and the gut contents of the animals. Seven fractions were obtained: gravel ( $\phi_{-1}$ ), very coarse sand ( $\phi_0$ ), coarse sand ( $\phi_1$ ), medium sand ( $\phi_2$ ), fine sand ( $\phi_3$ ), very fine sand ( $\phi_4$ ) and mud ( $\phi_5$ ). Each fraction was weighed and expressed as a percentage of total weight.

The sediments and gut contents were grouped into three categories: coarse sediments ( $\phi_{-1} + \phi_0 + \phi_1$ ),



**Figure 1.** The study area and the three selected sites.

medium sediments ( $\phi_2 + \phi_3$ ), and fine sediments ( $\phi_4 + \phi_5$ ). This grouping is more efficient for determining sediment variations in the different sites and animal guts, and consequently, the grouping gives an indication of the actual behaviour of the animal feeding in these sites.

The total organic matter (TOM) content in the gut contents and sediments were determined as the ignition weight loss at 550°C (Yingst 1976; Brenner and Binford 1988), and expressed as (mg g<sup>-1</sup>).

### Results and discussion

The sediments at the investigated sites comprise biogenic gravel, sand and mud. At site I, the average percentage of gravel was 23.83%, sand 75.34%, and mud 0.82%. At site II, the average percentage of gravel was 18.53%, sand 80.05%, and mud 1.44%. And at site III, gravel averaged 23.03%, sand 76.12%, and mud 0.85%. The TOM content of sediments averaged 47 mg g<sup>-1</sup> at site I, 41.6 mg g<sup>-1</sup> at site II, and 45 mg g<sup>-1</sup> at site III. At site I, an average of 55.17% of all sediment belonged to the coarse sediment group, 43.22% to the medium sediment group, and 1.77% to the fine sediment group. The averages for the different sediment groups at site II was 45.93% coarse, 46.80% medium and 7.29% fine;

and at site III it was 71.37% coarse, 24.21% medium and 4.42% fine (Table 1).

### *Holothuria atra* and *H. hawaiiensis*

The body weights of *H. atra* individuals varied between 50 g and 590 g. The highest body weight was

recorded in April at 590 g, while the lowest weight (50 g) was recorded in February. July showed the highest average weight of gut contents (58.26 g) accompanied with the highest average percentage of gut contents (33.72%) in relation to total weight, while the lowest average (13.28 g) was recorded in June with the lowest gut content percentage (2.10%) (Table 2).

**Table 1.** Sediment fraction and group percentages and the total organic matter (TOM) contents of the sea floor sediments at the study sites.

Sample	Gravel (%)	Sand (%)	Mud (%)	( $\emptyset_{-1} + \emptyset_0 + \emptyset_1$ ) (%)	( $\emptyset_2 + \emptyset_3$ ) (%)	( $\emptyset_4 + \emptyset_5$ ) (%)	TOM (mg g <sup>-1</sup> )
<b>Site I</b>	1	24.22	73.26	2.52	48.93	45.64	55.00
	2	24.49	74.65	0.85	59.5	39.03	51.00
	3	26.93	72.81	0.26	74.55	25.90	43.00
	4	26.08	73.60	0.32	48.74	49.98	45.00
	5	17.45	82.40	0.15	44.11	55.53	41.00
	Average	23.83	75.34	0.82	55.17	43.22	47.00
	SD	3.74	4.00	0.99	12.22	11.41	5.83
<b>Site II</b>	1	15.45	83.92	0.72	47.60	44.65	55.00
	2	17.38	80.47	2.15	39.73	53.33	51.00
	3	11.19	87.98	0.83	28.46	61.29	34.00
	4	24.56	72.30	3.10	60.91	33.40	33.00
	5	24.07	75.56	0.38	52.97	41.32	35.00
	Average	18.53	80.05	1.44	45.93	46.80	41.60
	SD	5.74	6.29	1.15	12.45	10.80	10.53
<b>Site III</b>	1	27.39	71.81	0.80	75.91	21.63	64.00
	2	26.07	73.84	0.09	77.7	21.08	41.00
	3	22.85	76.15	1.00	66.93	28.95	32.00
	4	14.29	84.05	1.66	54.9	32.17	35.00
	5	24.55	74.76	0.69	81.43	17.24	53.00
	Average	23.03	76.12	0.85	71.374	24.214	45.00
	SD	5.17	4.70	0.57	10.64	6.14	13.32

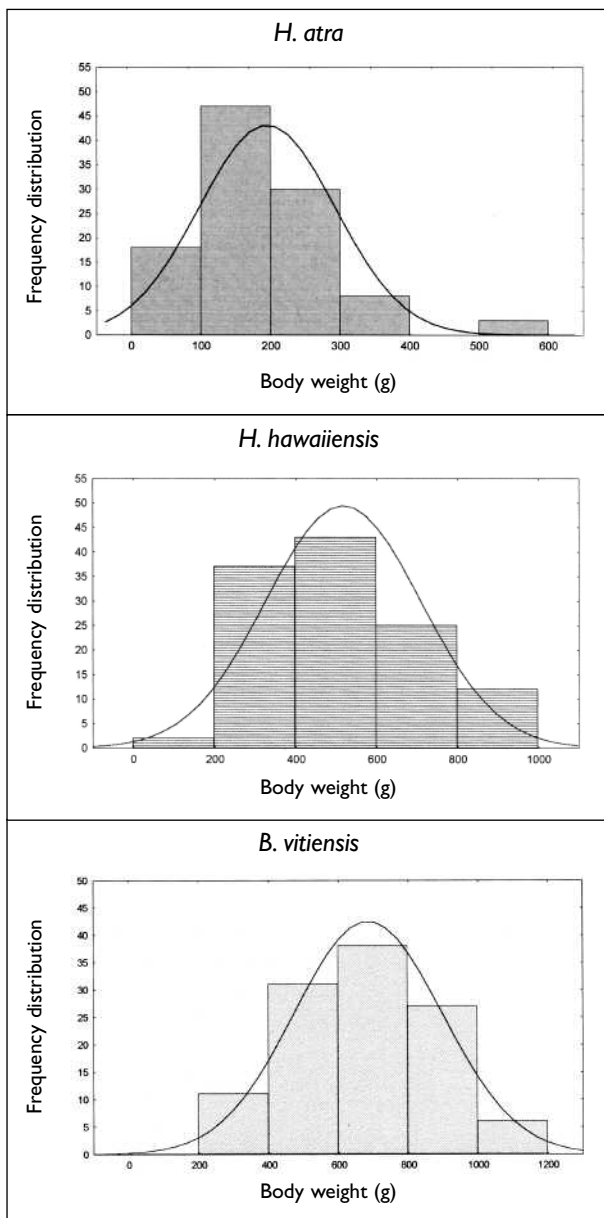
**Table 2.** Body weight, gut sediment percentages, reworking sediment load, and TOM content (mg g<sup>-1</sup>) for *Holothuria atra*.

		Body weight (g)	Sed. weight (g)	Sed. (%)	Re. load (kg y <sup>-1</sup> )	Gravel (%)	Sand (%)	Mud (%)	TOM (mg g <sup>-1</sup> )
<b>Spring</b>	Apr-03	315.88	22.98	7.99	18.28	19.89	78.86	1.25	65.00
	May-03	369.17	29.20	8.43	23.23	17.54	81.35	1.12	67.80
	Jun-03	209.00	13.28	6.32	10.56	27.21	70.34	2.45	63.00
	Average	298.01	21.82	7.58	17.36	21.55	76.85	1.61	65.27
	SD	66.60	6.55	0.91	5.21	4.12	4.71	0.60	1.97
<b>Summer</b>	Jul-03	178.00	58.26	33.72	46.36	3.15	95.18	1.67	70.30
	Aug-03	225.00	22.92	10.24	18.24	31.56	66.30	2.08	58.40
	Sep-03	277.50	17.52	6.75	13.94	16.13	80.73	3.15	51.50
	Average	226.83	32.90	16.91	26.18	16.95	80.74	2.30	60.07
	SD	40.64	18.07	11.97	14.38	11.61	11.79	0.62	7.77
<b>Autumn</b>	Oct-03	206.00	30.15	14.56	23.99	2.97	90.97	6.06	64.20
	Nov-03	121.00	19.16	16.15	15.25	31.75	65.69	2.56	51.40
	Dec-03	133.50	18.89	14.94	15.03	28.13	69.46	2.41	63.80
	Average	153.50	22.73	15.22	18.09	20.95	75.37	3.68	59.80
	SD	37.47	5.24	0.68	4.17	12.80	11.14	1.69	5.94
<b>Winter</b>	Jan-04	135.00	21.02	16.55	16.73	37.05	61.57	1.38	65.10
	Feb-04	115.50	18.15	18.72	14.44	18.58	76.91	4.51	60.60
	Mar-04	141.25	18.07	12.98	14.38	27.11	70.66	2.22	61.40
	Average	130.58	19.08	16.08	15.18	27.58	69.71	2.71	62.37
	SD	10.97	1.37	2.37	1.09	7.55	6.30	1.32	1.96

The highest average weight of *H. hawaiiensis* (745 g) was recorded in February and the lowest (323 g) in January. The highest average gut contents weight (68.97 g) was recorded in December, and the lowest (11.20 g) in July. December showed the highest average percentage (18.51%) of gut contents and July the lowest (1.50%). Individuals of *H. hawaiiensis* were much heavier than *H. atra* (Fig. 2), subsequently the amount of sediment they consumed was relatively higher (Table 3).

Medium-weight individuals of *H. atra* and *H. hawaiiensis* consume proportionally more sediments than light- and heavy-weight individuals. *H. atra* consume around one-third (and up to one-half) of their total weight; *H. hawaiiensis* around

22.74%. This could be due to growth and sex organ development (maturation), which require high amounts of energy. The highest average gravel percentage of *H. atra* (37.05%) was recorded in January, while the lowest percentages were recorded in November, December and February (less than 1%). Sand percentage showed the highest average 95.18% in July and the lowest 61.57% in January. Mud percentage varied between 0.19% in November and 25.88% in February, while the highest average (6.06%) was recorded in October. The average gravel percentage in *H. hawaiiensis* varied between 3.53% in October and 38.86% in December. The average sand percentage fluctuated between 60.12% in November and 98.13% in August, and mud between 0.36% in September and 7.59% in February.



**Figure 2.** Body weight frequency distribution of *H. atra*, *H. hawaiiensis* and *B. vitiensis*.

The gravel percentage in the gut contents of some individuals of *H. atra* and *H. hawaiiensis* reached about 75% of the total sediment content while in others it was less than 1%. The recorded gravel percentage of the animal guts reached about three times that of the benthic sediments. The sand content in the guts varied relative to the gravel percentage, while the mud percentage was highly variable: in most months, the mud percentage in the gut contents was slightly higher than in the benthic sediments; but in October and November, it increased abruptly to about 11 and 9 times more, respectively, than in the sediments; and in February, it was about 25 times more than in the sediments. This value is also found in light-weight individuals.

Light-weight individuals may not be able to consume the larger particles due to their thin tiny tentacles, or they may prefer sediment quality to quantity, and subsequently consume the finest organic-rich particles rather than coarse particles. This finding is supported by the TOM contents in *H. atra*, which may reach two times those of the benthic sediments.

TOM contents in *H. atra* recorded the highest value (99 mg g<sup>-1</sup>) with the highest mud content (25.88%) in February. TOM in *H. hawaiiensis* guts showed the highest average (95.72 mg g<sup>-1</sup>) in April and the lowest (42.90 mg g<sup>-1</sup>) in August (Tables 2 and 3).

Two observations were made with TOM contents in guts: the first is that the highest TOM content corresponded to the highest mud contents; the second is that the highest TOM averages were recorded in light- and heavy-weight individuals, rather than in medium-weight individuals. This means that the light- and heavy-weight individuals tended to consume the organic-rich sediments more than the other sediment types, while the

**Table 3.** Body weight, gut sediment percentages, reworking sediment load, and TOM content (mg g<sup>-1</sup>) for *Holothuria hawaiiensis*.

		Body weight (g)	Sed. weight (g)	Sed. (%)	Re. load (kg y <sup>-1</sup> )	Gravel (%)	Sand (%)	Mud (%)	TOM (mg g <sup>-1</sup> )
<b>Spring</b>	Apr.-003	573.50	21.24	3.58	16.90	15.34	82.11	2.55	95.72
	May.-003	455.56	16.03	4.64	12.76	7.90	57.36	21.42	85.50
	June.-003	510.00	43.67	8.68	34.75	21.37	77.43	1.20	51.55
	Average	513.02	26.98	5.63	21.47	14.87	72.30	8.39	77.59
	SD	48.20	11.99	2.20	9.54	5.51	10.73	9.23	18.88
<b>Summer</b>	July.-003	739.00	11.20	1.50	8.92	5.92	91.80	2.27	51.95
	Aug.-003	508.00	56.95	11.11	45.32	24.98	74.42	0.60	42.90
	Sep.-003	480.00	67.78	14.03	53.93	24.57	75.06	0.36	52.25
	Average	575.67	45.31	8.88	36.05	18.49	80.43	1.08	49.03
	SD	116.06	24.52	5.35	19.51	8.89	8.05	0.85	4.34
<b>Autumn</b>	Oct.-003	720.50	65.10	9.02	51.80	3.53	89.73	6.74	85.30
	Nov.-003	406.50	59.49	14.86	47.34	32.76	65.63	1.67	52.80
	Dec.-003	379.50	68.97	18.51	54.88	38.86	60.12	1.01	78.90
	Average	502.17	64.52	14.13	51.34	25.05	71.83	3.14	72.33
	SD	154.78	3.89	3.91	3.09	15.42	12.86	2.56	14.06
<b>Winter</b>	Jan.-004	323.00	47.42	14.94	37.73	26.78	72.19	1.02	63.00
	Feb.-004	745.00	39.14	5.33	31.15	17.12	75.29	7.59	52.60
	Mar.-004	346.50	50.01	14.91	39.79	14.66	78.53	6.82	50.60
	Average	471.50	45.52	11.72	36.22	19.52	75.34	5.14	55.40
	SD	193.63	4.63	4.52	3.69	5.23	2.59	2.93	5.44

medium-weight individuals may have consumed all particles to make up for their increased biogenic activities. Also, the higher TOM contents in the animals' guts, rather than in the benthic sediments, show that *H. atra* and *H. hawaiiensis* may absorb the rich biogenic film on top of the sea floor (the upper 5 mm of the sediment surface).

During the study period, some individuals were left in water basins at the Marine Biological Station at Hurlhada. These basins were connected to the sea and held sediments significantly covered with a bio-film of organic matter. With time, holothurian individuals seemed accustomed to the organic film and the seawater appeared to be clearer than in basins that did not contain holothurians. This observation indicates that holothurians may be able to feed on organic sources other than sediments. But, sediments, and especially coarse particles, may be required for other functions.

In the gut contents of *H. atra*, the coarse sediments group is the predominant category in the animals' guts in all seasons. In summer, the fine sediments group represented 26.60% and 20.39% in autumn, while it only represented 7.10% in winter and 9.23% in spring. The coarse sediments group is the predominant category in *H. hawaiiensis* guts while the fine sediments group recorded equal percentages in all seasons. Autumn recorded the highest average weight of the gut sediments (64.52 g), sediment and gravel percentages; 14.13% and 25.05%, respectively.

### *Bohadschia vitiensis*

*B. vitiensis* recorded the highest average body weight (863 g) among the studied species. It was recorded in June while the lowest average (372.50 g) was recorded in March (Table 4). The weight of the gut contents varied between 25.50 g in March and 56.22 g in August. Gut contents percentage, in relation to total weight, varied between 3.33% in June and 7.23% in March. Gravel recorded the highest average (23.83%) in April and the lowest (1.61%) in November. Mud percentage varied between 0.09% in May and 43.06% in February. Recorded gut content weights in *B. vitiensis* were higher than in *H. atra* and nearly subequal to the gut contents of *H. hawaiiensis*. Gut sediment percentages gradually increased as body weight decreased, indicating that the relatively light-weight individuals ingested proportionally much more sediment than did heavy-weight individuals. Gravel represented the main component of the sediments inside the animals' guts, especially for light-weight individuals. This means that the medium- and heavy-weight individuals ingested smaller organic-rich particles, rather than coarse ones. This finding is supported by the mud percentages and TOM contents. High TOM contents corresponded to the highest mud contents, which were generally observed inside the heavy-weight individuals' guts.

The highest average body weight (772.15 g) and the highest gut sediments average weight (45.93 g) were recorded in summer. The highest gut sedi-

**Table 4.** Body weight, gut sediment percentages, reworking sediment load, and TOM content (mg g<sup>-1</sup>) for *Bohadschia vitiensis*.

		Body weight (g)	Sed. weight (g)	Sed. (%)	Re. load (kg y <sup>-1</sup> )	Gravel (%)	Sand (%)	Mud (%)	TOM (mg g <sup>-1</sup> )
<b>Spring</b>	Apr.-003	496.90	25.49	5.29	20.28	23.83	75.54	0.63	72.60
	May.-003	672.00	42.55	6.19	33.86	16.57	82.17	1.27	58.15
	June.-003	863.00	29.25	3.33	23.27	5.69	91.14	3.17	73.20
	Average	677.30	32.43	4.94	25.80	15.36	82.95	1.69	67.98
	SD	149.51	7.32	1.20	5.83	7.45	6.39	1.08	6.96
<b>Summer</b>	July.-003	782.50	37.90	4.97	30.15	4.81	83.82	11.37	58.31
	Aug.-003	854.44	56.22	6.64	44.73	14.41	83.22	2.37	67.61
	Sep.-003	679.50	43.66	6.53	34.74	6.06	86.47	7.48	42.85
	Average	772.15	45.93	6.05	36.54	8.42	84.51	7.07	56.26
	SD	71.79	7.65	0.77	6.09	4.26	1.41	3.69	10.21
<b>Autumn</b>	Oct.-003	691.88	42.28	6.78	33.64	2.12	87.71	10.17	68.94
	Nov.-003	742.00	38.69	5.25	30.79	1.61	84.91	13.47	53.60
	Dec.-003	704.00	43.73	6.30	34.80	5.04	81.47	13.50	57.30
	Average	712.63	41.57	6.11	33.07	2.92	84.70	12.38	59.95
	SD	21.35	2.12	0.64	1.68	1.51	2.55	1.56	6.54
<b>Winter</b>	Jan.-004	697.00	38.49	5.53	30.62	5.66	81.04	13.30	58.00
	Feb.-004	628.50	39.14	6.13	31.15	5.32	68.57	26.10	49.00
	Mar.-004	372.50	25.50	7.23	20.29	11.47	75.32	13.21	55.13
	Average	566.00	34.38	6.30	27.35	7.48	74.98	17.54	54.04
	SD	139.65	6.28	0.70	5.00	2.82	5.10	6.06	3.75

ments average percentage (6.30%) and the highest mud average percentage (17.54%) were recorded in winter (Table 4).

### Feeding behaviour

The three holothurians consumed large amounts of benthic sediments but did not depend on them completely as their main food source. The measured TOM in the animals' guts and surrounding sediments illustrate that the TOM contents in the guts are much higher than those in the surrounding environment, and that the holothurians absorbed particulate materials from the water column and by sweeping the organic biofilm off the top 5 mm of sediment (as indicated by Moriarty 1982).

The highest average weight and the lowest sediment percentage average of *H. atra* were recorded in spring. During this season, the animals are mostly in the pre-maturation stage and are preparing for the spawning period in June and July. The highest sediment average weights were recorded in summer when the animals need sediments to help contraction and gamete expulsion. In autumn, *H. hawaiiensis* consume the highest amounts of sediments in order to help the sex organs to grow and spawn. The lowest average weights in winter indicate that the animals suffered from food shortage and/or were in their first stages of growth (see Fig. 5).

The seasonal variation in the body weights of *B. vitiensis* was relatively small. The spawning season

began in May and extended through to June, July and August. Late spring and early summer are the periods of maturation, spawning and post-spawning. The highest recorded TOM average content in spring corresponded to the increasing energy needs for maturation and spawning.

### Feeding selectivity of the deposit feeder holothurians

Particle selectivity is the selection of certain grain sizes or particles with higher organic content within a given sediment patch or microhabitat. In contrast, patch selectivity describes the choice of a mobile organism to feed on preferred sediment patches in a heterogeneous environment (Uthicke and Karez 1999). According to Trefz (1958), holothurians are able to choose the richest organic sediments. Diversity in tentacle structure and mode of feeding may allow a degree of selectivity, but studies of deepsea holothurians have shown no correlation between tentacle structure and gut contents (Wigham et al. 2003).

Yingst (1976) recorded that selection of grain size by holothurians is generally low. Uthicke and Karez (1999) concluded that *H. atra* and *H. edulis* exhibited no preference for any food type, but that *S. chloronotus* significantly selected sediments with the highest contents of microalgae. Miller et al. (2000) reported that the surface deposit feeders fed very selectively on particles settled on the sea floor. *Stichopus tremolus* feeds mainly on coarse particles



(Haukson 1979), and *H. scabra* tends to assimilate the coarsest particles more than the finest fractions (Basker 1994). *S. japonicus* may ingest select sediments and their faeces contain higher organic contents than benthic sediments (Michio et al. 2003). Dar (2004) recorded that there is a strong selective behaviour in the feeding habits of some holothurians in the Red Sea: *H. atra*, *B. marmorata* and *H. leucospilota* scavenge through coarse sediments much more than through medium or fine ones.

The medium-weight individuals were the most efficacious animals in selective operations. They mostly represented the pre-maturation stage while the medium-heavy- and heavy-weight animals represented adult stages. The adult animals tended to ingest the finer sediments that provide the essential needs for growth, gamete maturation and spawning, while in the pre-maturation stage, the animals tended to consume coarser sediments.

The authors think that the presence of coarse particles in gut contents may help in many essential operations such as: 1) the contraction process for moving downward and upward from one depth to another, in addition to horizontal motion (in many places, the animals' faecal pellets were observed as long rows along their motion path) from locations with little food availability to locations with rich organic matter, and from high temperature areas to lower temperature areas; 2) the digestion process; 3) *B. vitiensis* cuvierian tubule expulsion (when it is threatened); and, most importantly, 4) the expulsion of gametes during spawning.

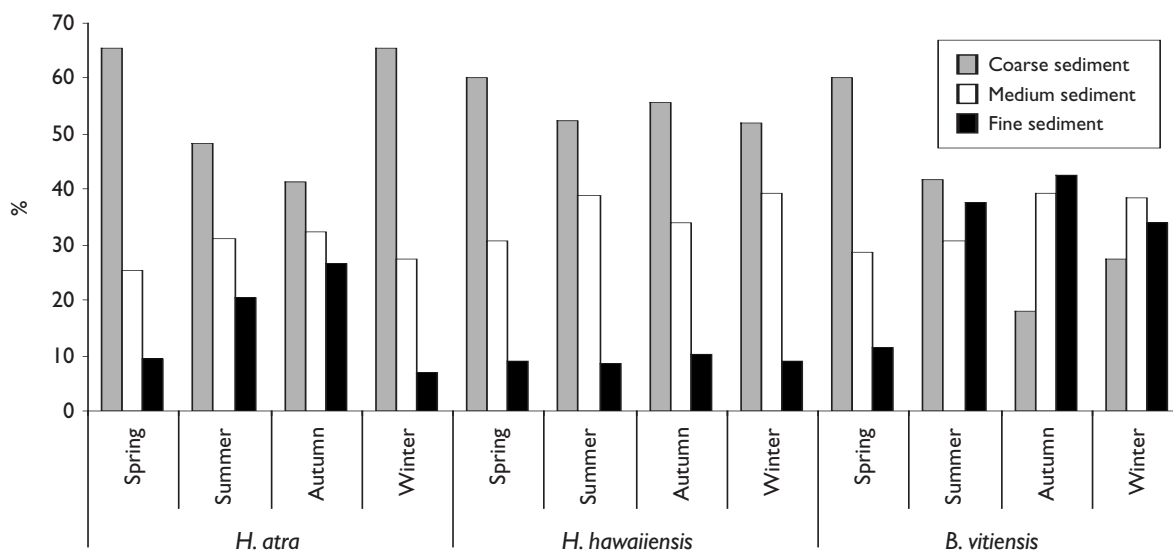
The selective feeding of holothurians throughout the seasons has been observed during this study

(Fig. 3). The animals alternatively select between the coarse and fine sediment groups in the different seasons according to their biogenic needs and food availability. The relatively high fine and particulate sediment contents inside the animal guts during the summer demonstrates that the animal requires enough amounts of sediments that help in biological activities (Fig. 3). *H. hawaiiensis* is considered one of the coarse sediment consumers.

There are definite variations in the feeding mechanism of holothurians throughout the different seasons corresponding with the animals' life stages (immature, pre-maturation and maturation stages).

### The ecological role of holothurians in sediment reworking

Holothurians are very important members of benthic communities as they can cause significant changes in the sea floor sediment composition. On tidal flats and sheltered coastal areas, dead algae and the organic remains of other benthos often cause anaerobic conditions, as, over time, sediments become rich in organic matter that decompose and cause dissolved oxygen depletion (Michio et al. 2003). Holothurians are active sediment "reworkers" that alter the bottom stability (Rhoads and Young 1971) by reducing volatile sulfide concentrations and increasing oxidation-reduction potential. Holothurians are also important recyclers of inorganic nutrients and are thus a part of the closed nutrient cycling (Uthicke and Karez 1999). *H. mexicana* and *Isostichopus dadiionotus* faecal pellets influence the nutrient cycle: the organic matter from decaying faecal pellets can be re-suspended by waves, currents and biota and help in relocating the particulate materials, rather than the dis-



**Figure 3.** Percentage of three different sediment sizes in *H. atra*, *H. hawaiiensis* and *B. vitiensis* gut contents.

solved nutrients (Conde et al. 1991). High densities of *H. arenicola* in shallow lagoons may significantly rework the top 3 cm of sediment in less than a month (Powell 1977). Populations of *H. atra* and *Stichopus chlorontus* can be dense enough to turn over the upper 5 mm sediments of the reef flat at least once a year (Uthicke 1999). Pawson (1966) recorded that in a small (1.7 km<sup>2</sup>) enclosed bay of Bermuda, *Stichopus* species passed between 500 and 1000 t of substrate through their intestines annually. Coulon and Jangoux (1993) reported that *H. tubulosa* only ingests the upper few millimetres of sediments. Kaufmann and Smith (1997) estimated that seven species of holothurian in the northeastern Pacific Ocean move over 100% of the sediment surface in about 400 days.

*H. atra* may ingest as much as 40 g of sediments containing 80–216 mg of organic matter per day and the sediments take about 11 h to pass through a 25-cm-long specimen (Trefz 1958). Klinger et al. (1993) recorded that the total sediment consumption by *H. atra* and *H. leucospilota* in the lagoon and reef flat of Horn Island was 3.93 and 12.76 g m<sup>2</sup> day<sup>-1</sup> respectively. Uthicke (1999) demonstrated that the average-sized individual of *H. atra* (125–129 g) consumed about 67 g day<sup>-1</sup> of sediment (dry weight). Rhoads and Young (1971) reported that *Molpadia oolitica* feeds selectively on the fine particle sediments and produces vertical sediment sorting, high sediment–water contact and topographical relief of the sea floor. Dar (2004) indicated that holothurians consume large amounts of the surface sediments throughout each feeding period: the annual reworked sediments by each individual of *B. marmorata*, *H. atra* and *H. leucospilota* were estimated at 45.78 kg yr<sup>-1</sup>, 28.72 kg yr<sup>-1</sup> and 21.23 kg yr<sup>-1</sup> respectively.

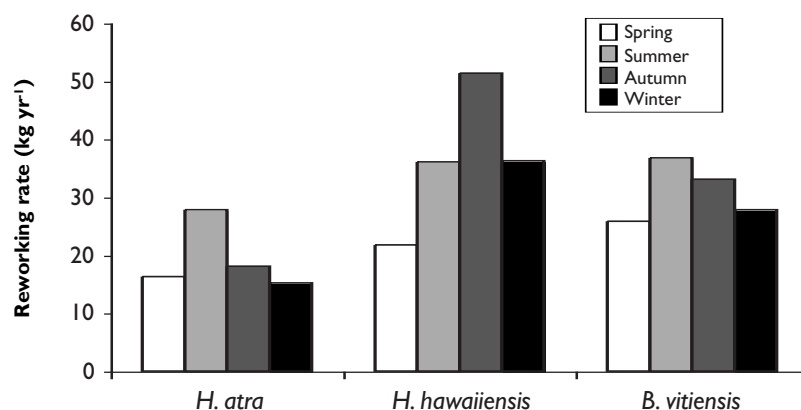
Month and season of highest recorded sediment-reworking effectiveness were July (46.36 kg yr<sup>-1</sup> ind.<sup>-1</sup>)

and summer (27.77 kg yr<sup>-1</sup> ind.<sup>-1</sup>) for *H. atra*; December (54.88 kg yr<sup>-1</sup> ind.<sup>-1</sup>) and autumn (51.34 kg yr<sup>-1</sup> ind.<sup>-1</sup>) for *H. hawaiiensis*; and August (44.73 kg yr<sup>-1</sup> ind.<sup>-1</sup>) and summer (36.71 kg yr<sup>-1</sup> ind.<sup>-1</sup>) for *B. vitiensis*. It was observed that the most active reworking operations take place from late spring to the end of autumn (Fig. 4). This period covers the three essential stages of the holothurian reproduction: maturation, spawning and post-spawning. It shows that the effective sediment reworking operations are increasing during the maturation and reproduction periods, as shown in Figures 5, 6 and 7, and as mentioned by Wiedemeyer (1992), who reported that the dry weight of daily reworked sediment was 46.5% and 45.2% of the drained body weight of *H. atra* individuals during spawning and post-spawning seasons.

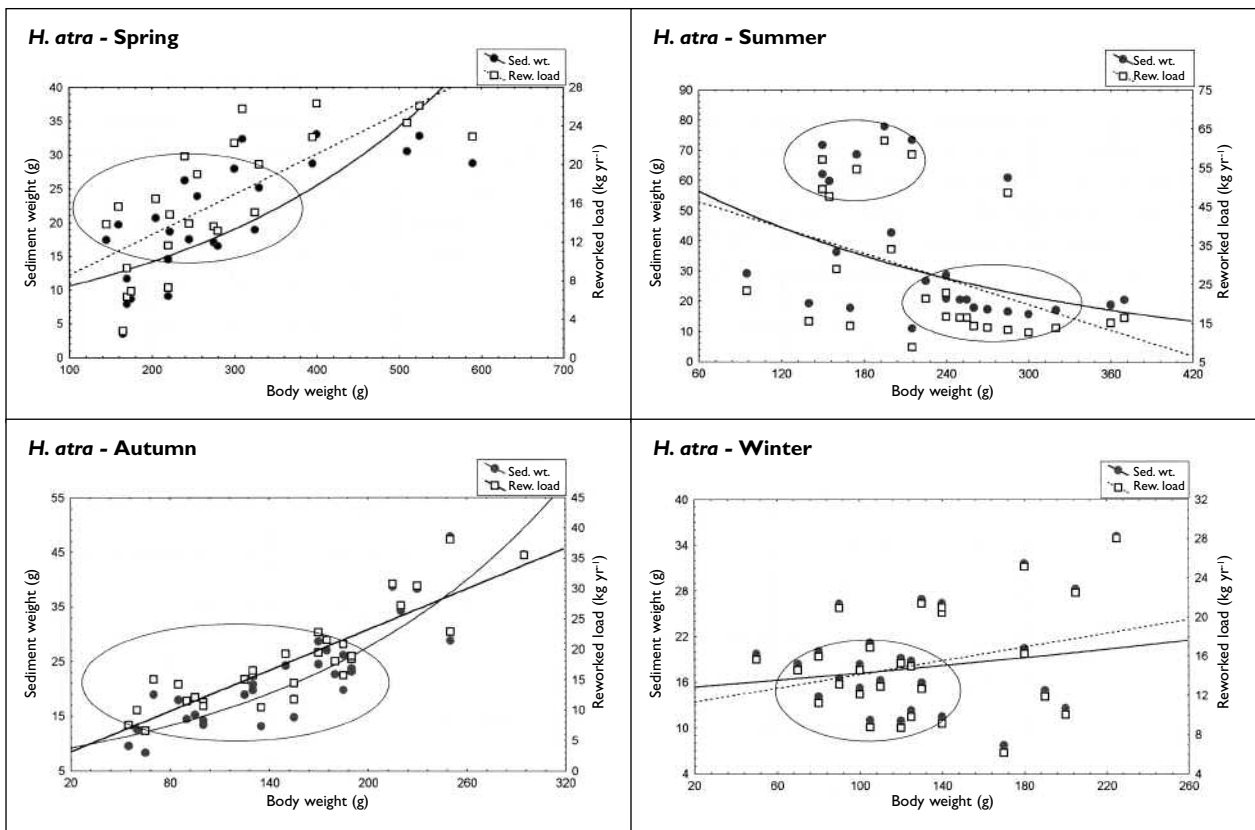
## Conclusion

*Holothuria atra*, *Holothuria hawaiiensis* and *Bohadschia vitiensis* are found in the shallow water and tidal habitats of the Red Sea. Feeding selectivity is a distinctive behaviour of these species. Sediments inside the animals' guts vary widely throughout the different seasons depending on the animal's weight, local conditions and the maturity status of the species. Proportionally, the medium-weight individuals are more able to swallow larger amounts of sediments than light- and heavy-weight individuals.

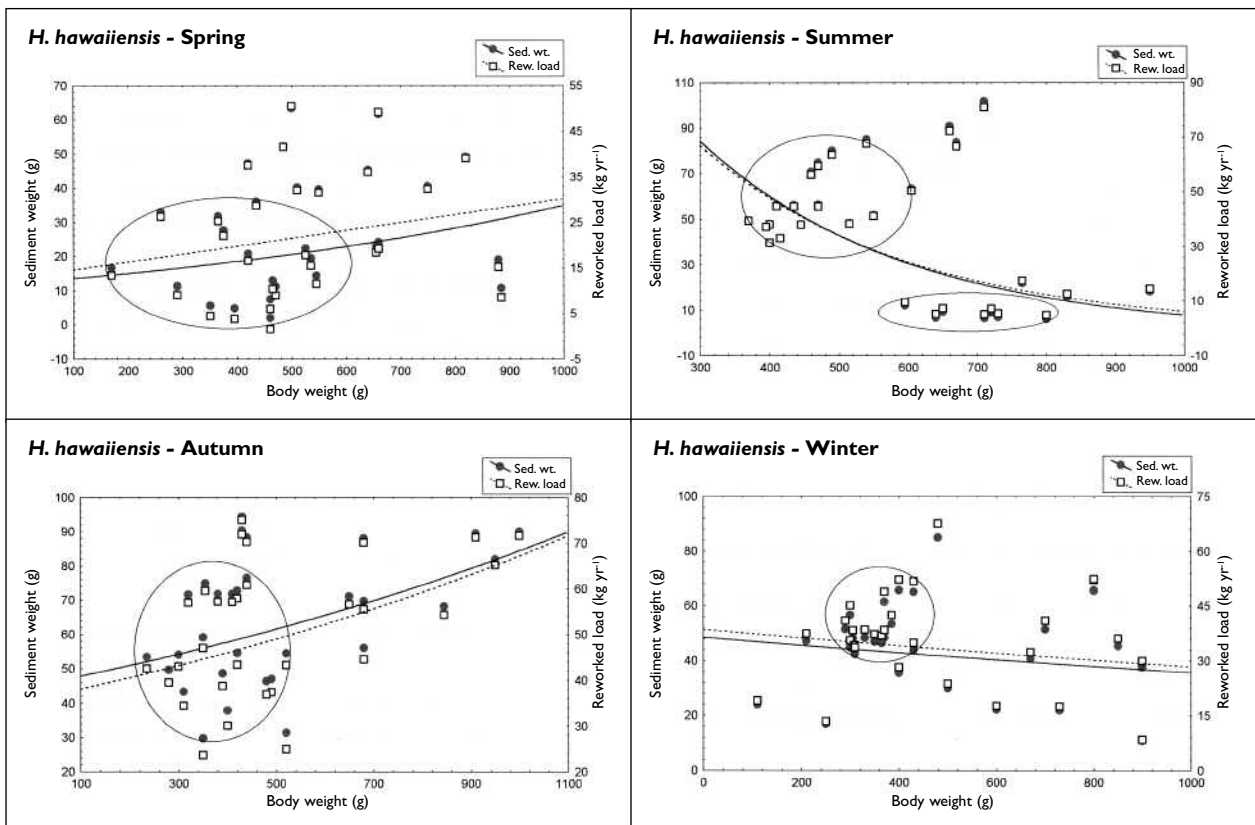
The light- and heavy-weight individuals tended to consume the rich organic sediments more than the other sediment types, while the medium-weight individuals consumed all sized particles in order to find the elements necessary for their biogenic activities. The coarse sediments may be required for some essential operations and not just as a food source: the contraction/motion process, which regulates the dynamics of deposit feeders, helps in the digestion process, and facilitates spawning.



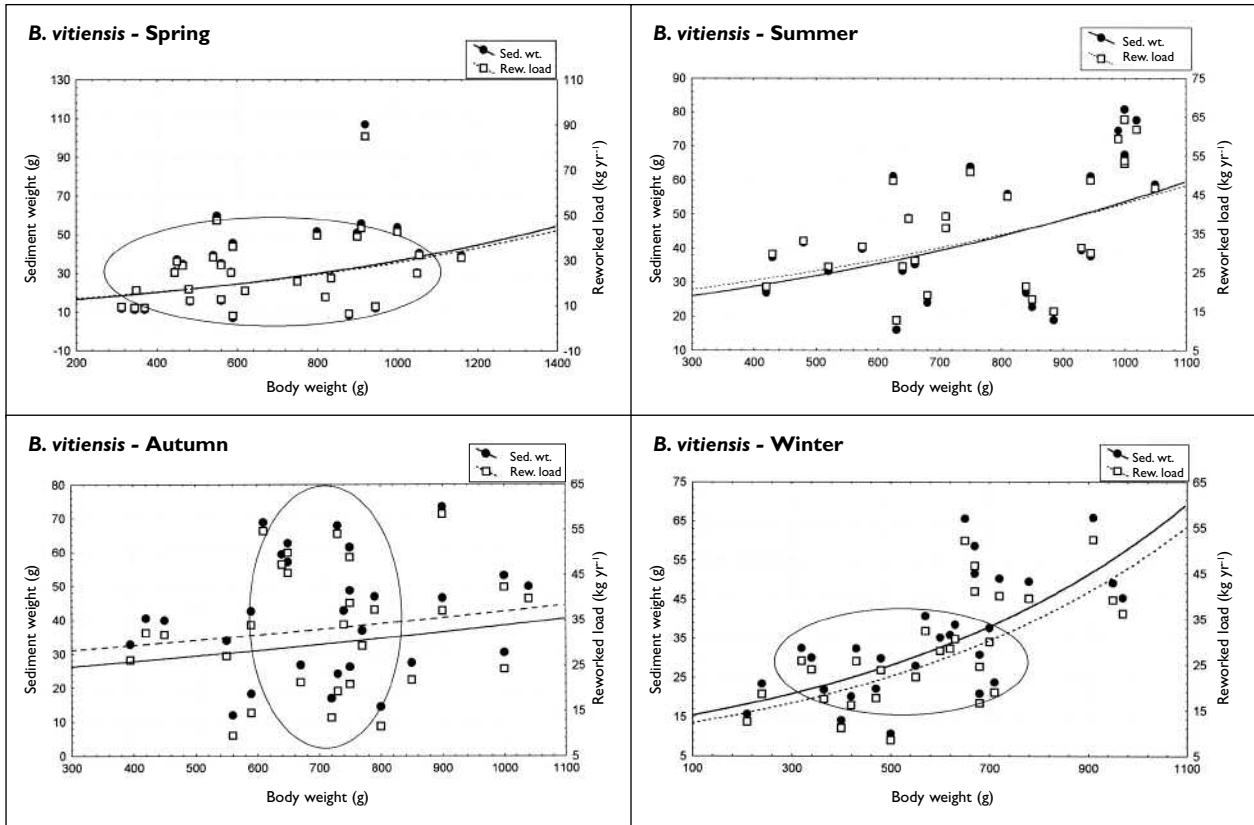
**Figure 4.** Sediment reworking rate of the three species in relation to seasons.



**Figure 5.** Relationship between body weight, sediment weight, and reworked loads in *H. atra* for the different seasons.



**Figure 6.** Relationship between body weight, sediment weight, and reworked loads in *H. hawaiiensis* for the different seasons.



**Figure 7.** Relationship between body weight, sediment weight, and reworked loads in *B. vitiensis* for the different seasons.

The feeding selectivity of the holothurians throughout the different seasons illustrated that the coarse sediments were the main component in the animals' guts during the different seasons, but there was a differential occurrence present between the coarse sediments and the fine sediments. The animals alternatively selected between the two categories in the different seasons according to their biogenic needs and food availability. The feeding behaviour and mechanism in the different seasons were related to the animals' sexual maturity stages.

The sediment reworking process increases as the amount of sediment consumed by the holothurian individuals increased. The volume of reworked sediment amounts was controlled by the number, size and the sexual maturity stage of the sea cucumbers and food availability as well as local conditions. The intensive sediment reworking by *H. atra*, *H. hawaiiensis* and *B. vitiensis* began in late spring and lasted until the end of autumn.

### Acknowledgement

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## Reproductive cycle of *Stichopus herrmanni* from Kish Island, Iran

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### Abstract

This study provides information on the reproductive biology of *Stichopus herrmanni*. The gonad morphology of *S. herrmanni* from Kish Island, Iran, is similar to that of other populations. Gonad colour is an unreliable characteristic for sex determination except at maturity. The sequence of gametogenesis events begins in late winter and continues until summer. The active stage of gametogenesis coincides with increasing photoperiod and temperature. Very little spawning activity out of season was noticed and a distinct peak spawning period was identified in summer. The average body length at first maturity was 310 mm and the average diameter of mature oocytes was 200  $\mu\text{m}$ . Relative and absolute fecundities were around  $8 \times 10^3$  oocytes.

### Introduction

Several studies have been conducted on the reproductive biology of different species of the genus *Stichopus*: *Stichopus tremulus* (Jespersen 1971), *Stichopus variegatus* (= *herrmanni*) (Conand 1993a), *Stichopus japonicus* and *Stichopus chloronotus* (Uthicke 1997; Conand et al. 2002). But no published work is available on the reproductive biology of sea cucumbers from Iran. This paper details some aspects of the sexual reproduction of the sea cucumber *Stichopus herrmanni* from Kish Island, Iran, in the Persian Gulf, which was studied over a 16-month period. The results presented were obtained from histological sectioned gonads and smears.

also where the majority of sampling (by local divers using scuba) took place over a 16-month period. Altogether, 220 specimens of *Stichopus herrmanni* were collected at various depths, mainly from the east coast where reefs are denser and where most sea cucumbers occur (Fig. 1).

### Macroscopic examination

In the laboratory, total length (TL), body wall wet weight (BW) and gonad wet weight (GW) were measured, and the gonads fixed in formalin (10%). Eviscerated weight (We) was also recorded. Frequency distributions and biometric relationships were determined. For each specimen, information

### Materials and methods

#### Sampling site

Kish Island lies between 26°29' N and 26°35' N, and 53°53' E and 54°4' E, some 18 km south of Iran's mainland. Kish — a coral island with an area of 90 km<sup>2</sup> and fringing reefs — is one of the most important recreational sites along the coast and is also a free trade zone. This situation puts considerable pressure on the marine environment, and has resulted in the destruction and disappearance of many corals and coral communities over the last 10 years. Today, most of what remains are a few scattered corals reefs located on the east coast of the island where the main recreational activities occur. This is

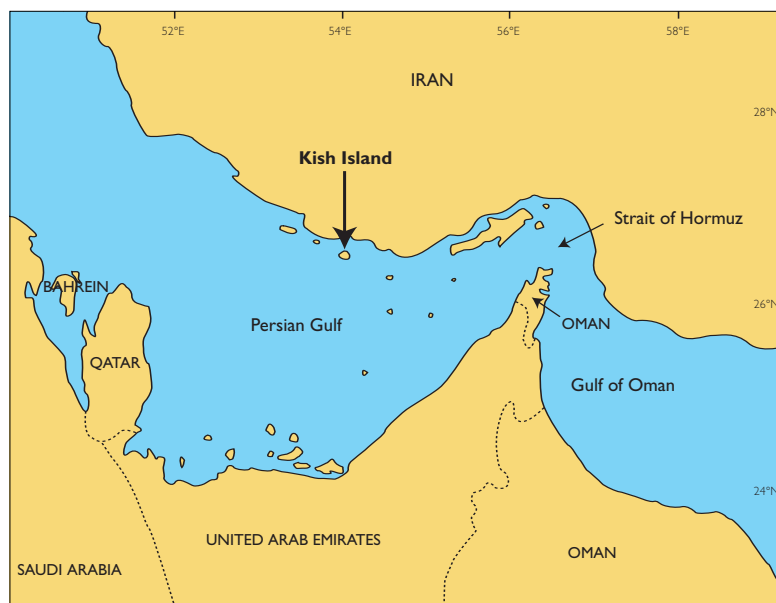


Figure 1. Kish Island, in the Persian Gulf.

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on gonadal development, sex and colour of gonadal tubules was noted. Gonadal development was estimated by taking a small piece of gonadal tubules, using a clean glass pipette and smearing the sample on a slide. Smears were examined under a compound Zeiss microscope at  $\times 400$  and/or  $\times 1600$ . Five stages were recognised: Stage 1: immature, Stage 2: growth, Stage 3: advanced growth, Stage 4: mature and spawning, and Stage 5: post spawning. A gonad index ( $GI = GW \times 100 / BW$ ) was calculated each month for males and females. Fecundity was measured from mature ovaries at Stage 4. A portion of gonad tubules were extracted, weighed and fixed in Gilson fluid — 100 ml of 60% alcohol + 880 ml of distilled water + 15 ml of 80% nitric acid + 20 ml of acetic acid, Hgcl — for five hours (Conand 1990). Then it was immersed in a saturated sodic acid EDTA, which was stirred with a magnetic shaker and filtrated through a riddle to isolate the oocytes, which were later counted. Absolute fecundity ( $Fa$ ) was calculated as  $Fa = n (GW/g)$ . Relative fecundity was calculated as  $Fr = Fa/Dw$  (Conand 1990).

### Histological examination of the gonads

Stichopodid gonads have two tufts of tubules located on either side of the mesentery on which saccules develop (Conand 1993b). Gonads were transferred to Bouin fixative for four weeks then dehydration was performed through a series of alcohol solutions at 30%, 50%, and 70%, allowing two hours between each change. Samples were then preserved in 70% alcohol. To prevent the loss of tubule contents during embedding, the tubule sections were cut well beyond the segment selected for sectioning (Hamel and Mercier 1996). For each individual, six 5- $\mu$ m sections were cut from tubules. The slides were stained with hematoxylin-eosin.

### Environmental factors

Continuous temperature measurements at the sampling site were made throughout our study and average monthly temperatures were calculated; day lengths were obtained from the Kish Airport (see Fig. 5 c and d).

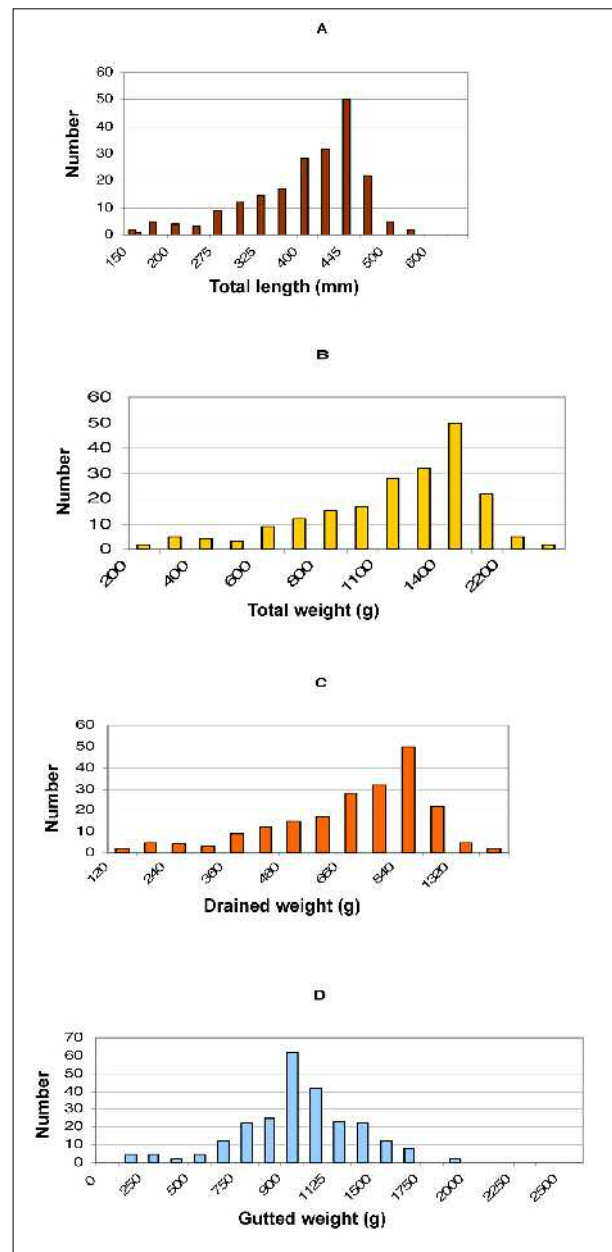
## Results

### Biometry

Total length, total weight, drained weight and gutted weight frequency distributions calculated from the monthly sampling are shown in Figure 2.

### Gonad morphology

The gonads of *Stichopus herrmanni* consist of two tufts of tubules on which saccules develop. The



**Figure 2. Biometry of *Stichopus herrmanni*. (a) total length, (b) total weight, (c) drained weight, and (d) gutted weight.**

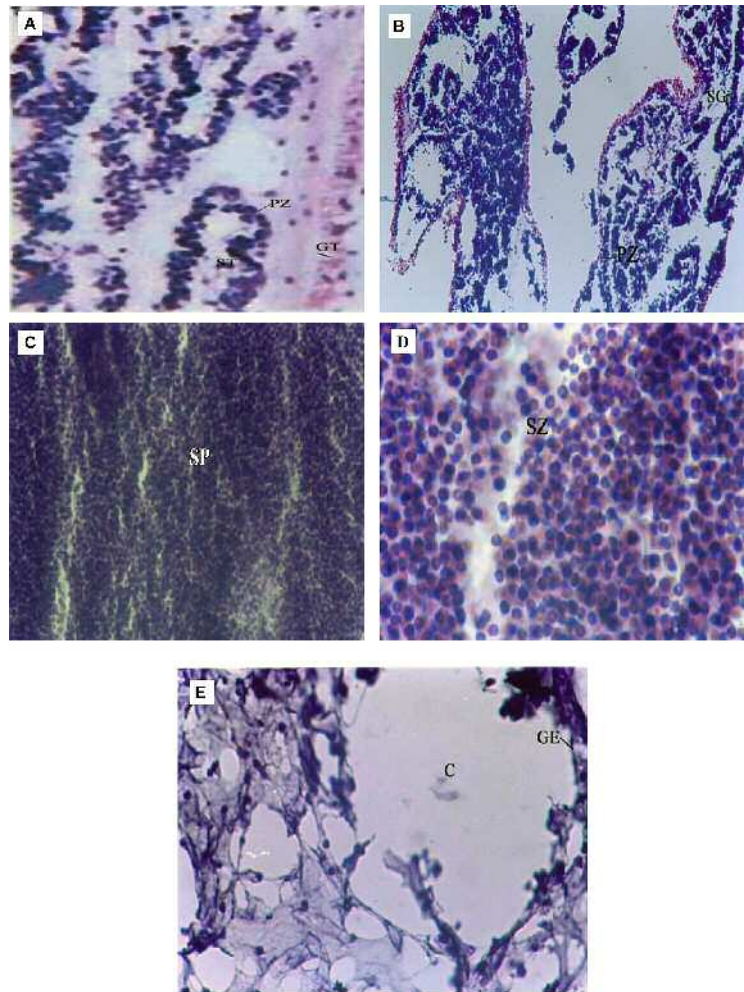
tubules join at a single gonoduct, which exits through a gonopore located between the feeding podia. The number of tubules is greater in males (z test,  $p > 0.01$ ).

### Spermatogenesis

Figure 3 shows the sequence of spermatogenesis events based on histological data from monthly preparations over a 16-month period:

- Stage 1 (immature) (Fig. 3a): The tubule wall is thick and contains small quantities of spermatozoa.

- Stage 2 (growth) (Fig. 3b): The gonadal tubule wall is beginning to decrease in thickness and spermatogonia are abundant along the germinal epithelium. There is a layer of spermatocytes in the lumen of tubules.
- Stage 3 (advanced growth) (Fig. 3c): The tubule wall is thinner and the lumen is filled with spermatozoa.
- Stage 4 (mature and spawning) (Fig. 3d): The tubules are stretched and completely filled with spermatozoa and earlier spermatogenesis stages are absent.
- Stage 5 (post spawning) (Fig. 3e): In the sections, we observed an empty area along the length of tubules.



**Figure 3.** Light micrographs of testicular sections of the spermatogenic cycle of *Stichopus herrmanni*.

- Immature gonad showing the germinal epithelium (GE) and the proliferation zone (PZ);
- Growth stage showing the proliferation zone (PZ) and spermatogonia (SG);
- Advanced growth stage showing a gonadal tubule and an abundant of spermatozoa (SP);
- Mature stage showing a great numbers of spermatozoa (SZ) in the lumen;
- Post spawning stage showing the germinal epithelium (GE) and channels where sperm passed during spawning and atresia in the lumen of the tubules.

The nuclear chromatin staining is dark in spermatogonia, which are free from the tubule wall. Primary and secondary spermatocytes can be distinguished as the primary spermatocytes undergo cytoplasmic and nuclear growth producing a darker nucleus, and suggesting meiosis-prophase in the secondary spermatocytes.

### Oogenesis

The development of female gametes in *Stichopus herrmanni* is shown in Figure 4:

- Stage 1 (immature) (Fig. 4a): The gonadal tubule wall is very thick. The germinal epithelium has small oocytes.
- Stage 2 (growth) (Fig. 4b): The tubule wall is still very thick. Along the surface of the germinal epithelium, many small oocytes and some pre-vitellogenic oocytes are present.
- Stage 3 (advanced growth) (Fig. 4c): The tubule wall is thinner and the diameter of the tubules is increased. In the lumen of tubules, large pre-vitellogenic and vitellogenic oocytes are present.
- Stage 4 (mature and spawning) (Fig. 4d): The tubule wall is thin and filled with mature oocytes. Each oocyte contains a germinal vesicle; immature oocytes are absent.
- Stage 5 (post spawning) (Fig. 4e): The gonadal tubule wall is thin. Some residual oocytes are present. Empty areas are seen.

### Seasonal changes in gametogenesis

Tubules showed a seasonal pattern that is correlated with the gonad-index cycle (Fig. 5a and b). Following spawning, there is a period of inactivity until mid-March and a progressive increase in the frequency of the growth and advanced growth stages between May and June.

It can be seen in Figures 5 and 6 that early gametogenesis takes place during March and April,



Stage 2 occurs in May, Stage 3 in June, Stage 4 in July–August, and Stage 5 during the cold season.

During June and July, the gonads underwent a rapid transition to ripe and spawning stages. Stage 4 — throughout July and August — corresponded to the summer peak spawning and was followed by an abrupt decline in late August. In September, there was a noticeable decline in the percentage of *Stichopus herrmanni* in the spawning stages (Fig. 6 a and b).

Both indices give similar patterns to that obtained from histology and are clear enough to suggest that there is one peak spawning event in summer (July–August). Figure 5c shows the mean monthly seawater temperature. In December, January, February and March, the temperature ranges from 15–20°C. In April the temperature begins to rise, and goes above 30°C in August and drops in September.

To summarise, the reproductive cycle of *Stichopus herrmanni* is seasonal with one peak spawning period in summer (July–August), related to highest seawater temperature. The sexual stages studied from both histological preparations and from simple gonad smears showed similar annual patterns. The average diameter of mature oocytes is 200 µm. Absolute fecundity was measured at  $6\text{--}10 \times 10^3$  oocytes, and relative fecundity at  $8 \times 10^3$  oocytes per gram of drained weight.

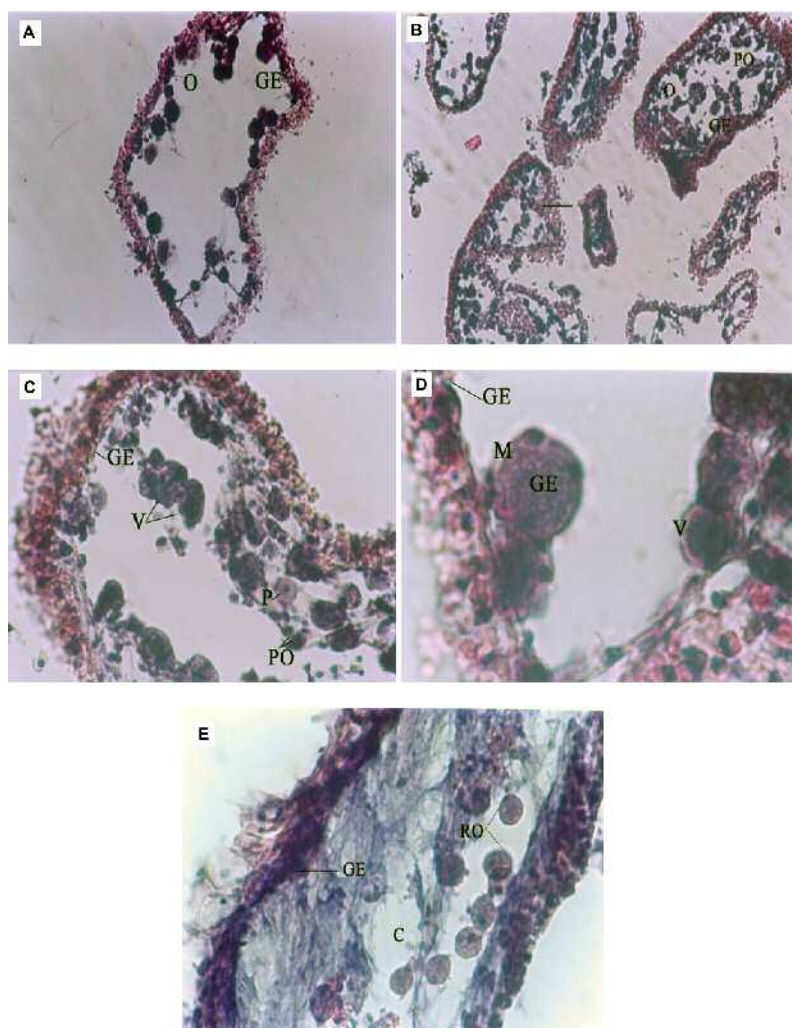
## Discussion

### Gonad colour

The gonad colour of the *Stichopus herrmanni* population studied was reddish brown in females and orange in males at the mature stages, whereas immature gonads were all cream coloured. These characteristics are similar to those of other populations studied, apart from the colour of mature female gonads in New Caledonia (Conand 1993a).

### Gametogenesis

A mature *Stichopus herrmanni* is identifiable by the size of its gonads and microscopically by examin-



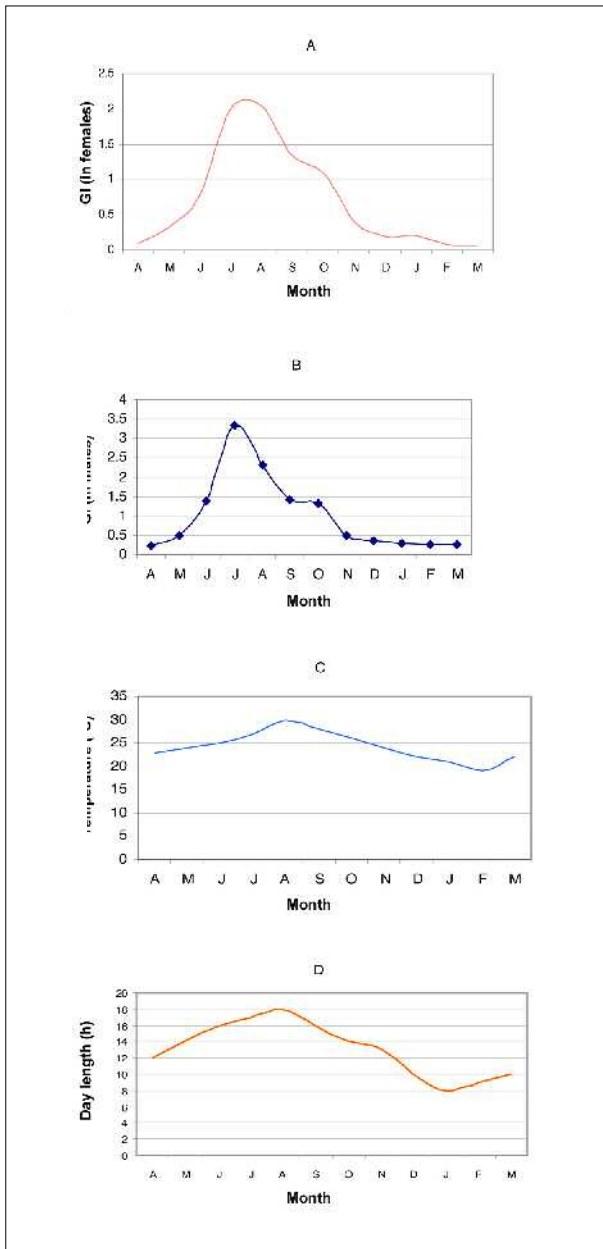
**Figure 4.** Light micrographs of ovarian sections showing the oogenic cycle of *Stichopus herrmanni*.

- Immature stage showing oogonia (O) and the germinal epithelium (GE);
- Growth stage showing oogonia (O) germinal epithelium (GE) and primary oocytes (PO);
- Advanced growth stage showing germinal epithelium (GE) and primary oocytes (PO) vitellogenic oocytes (V) and phagocytes (P);
- Mature stage showing large mature oocytes (M) containing germinal vesicle (GV) and vitellogenic oocytes (V);
- Post-spawning stage showing the germinal epithelium (GE) and residual oocytes (RO).

ing histological preparations of gonads. Several schemes of histological gonad indices in this study were found to be reliable.

### Reproductive cycle

Conand (1993b) studied *Stichopus variegatus* (= *herrmanni*) and found that in New Caledonia, in the southern hemisphere, maturing occurs from September to November when seawater tempera-



**Figure 5.** Monthly variations of (A) females gonadal index, (B) males gonadal index, (C) temperatures, and (D) day length.

tures increase, and spawning takes place during the warmer months of January and February.

In Iran, one distinct peak spawning period was identified in summer (July and August) and gametogenesis took place in spring, which corresponds to that of many tropical aspidochirotetes (Harriot 1985; Franklin 1980; Conand 1981, 1993a, b).

Our study lasted 16 months and covered all the variations of the sexual stages and the gonad index. We suggest that seawater temperature is the major trigger for the summer peak spawning. But,

the only notable environmental change in late March, when *S. herrmanni* gametogenesis begins, is the return to increasing photoperiod, as the sea temperature has not yet begun to rise. Therefore, we suspect that photoperiod may also play a role in gametogenesis. Experiments with sea urchins have shown a correlation between gametogenesis and photoperiod (Pearse et al. 1986).

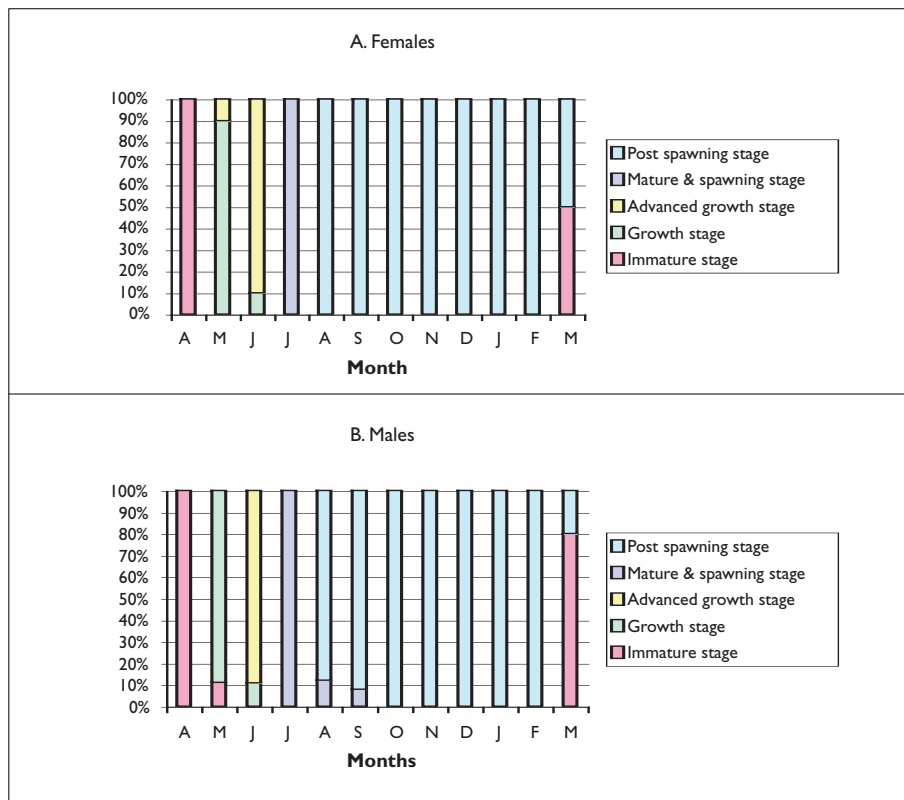
In *Stichopus herrmanni*, oogenesis begins in January with the production of stem cells in the gonadal tubules. Throughout the winter and spring these cells are transformed into oogonia and primary oocytes, and in summer large oocytes migrate into the lumen during maturation. Finally, these large oocytes are released during spawning in mid-summer. Our study also showed a spermatogenesis as long as the oogenesis, and beginning with the production of stem cells in the gonadal tubules in mid-winter. In late winter and spring, spermatogonia, spermatocytes and spermatids accumulate in the tubules. In summer, the production of spermatozoa increases, until spawning.

### Acknowledgments

I am greatly indebted to Dr Daneshian and Mr Shomalimoghadam for their help in collecting the samples. I wish to thank Prof Conand, Dr Hamel and Dr Pawson for their useful help.

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**Figure 6.** Reproductive cycle of *Stichopus herrmanni*.

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## Development of large-scale hatchery production techniques for the commercially important sea cucumber *Holothuria scabra* var. *versicolor* (Conand, 1986) in Queensland, Australia

Grisilda Ivy and Daniel Azari Beni Giraspy<sup>1</sup>

### Abstract

Overexploitation is an issue affecting sea cucumber fisheries worldwide. Improved management plans for existing sea cucumber populations and/or aquaculture of depleted stocks are considered indispensable to maintaining wild populations and sustainable fisheries. In this context, we evaluated the potential of the commercially important sea cucumber *Holothuria scabra versicolor* (golden sandfish) for mass culture in a hatchery. Adult *H. scabra versicolor* were collected from Hervey Bay (Queensland, Australia) waters by scuba diving and were induced to spawn by increasing the water temperature. More than 46 million eggs were produced from 18 females during 2004 and 2005. Larvae from 9 million eggs were reared to juvenile stage, with more than 300,000 juveniles produced during the 2004 and 2005 hatchery culture period. Juveniles reaching 3 to 5 cm in length three months after settlement are suitable for release in the wild. The present study shows that this species can be hatchery-reared on a large scale to restock depleted populations for sustainable harvesting. Data collected during the two-year trial period indicate that the survival rate increased considerably during the second year, following modifications made to culture techniques. Results obtained are quite promising and considering the market potential, industry value and technical feasibility, this species seems most suitable for stock enhancement.

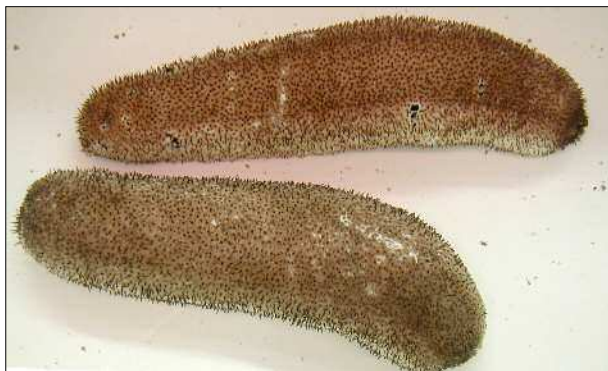
### Introduction

Increasing demand for beche-de-mer along with steady price increases have led to worldwide intensification of sea cucumber harvesting (Conand 2004). The sea cucumber *Holothuria scabra versicolor* (golden sandfish) is one of the most highly sought after species in Asia. *H. scabra* and *H. scabra versicolor* are distributed throughout the tropical Indian and Western Pacific Oceans and their occurrence was noted from Madagascar to the Solomon Islands and New Caledonia (Conand 1998a). Although *H. scabra* and *H. scabra versicolor* are both found over a large geographical range, they often inhabit dissimilar microhabitats, with *H. scabra versicolor* often found in deeper waters than *H. scabra* (Conand 1990). Despite the diverse disparity in its ecological distribution and biological characteristics, *H. scabra versicolor* is considered to be a variety of *H. scabra*, because of a lack of recognizable dissimilarity in spicule structure and internal anatomy (Conand 1998b). However, the speculation that *H. scabra versicolor* is a subspecies or new species (Conand 1990; Massin 1999) has yet to be looked at in more detail. Recent studies on allozyme and 16S mtDNA sequence analyses of *H. scabra* and *H. scabra versicolor* indicates that these two sea cucumbers are distinct but young biological and phylogenetic species (Uthicke et al. 2005).

*H. scabra versicolor* is a deposit-feeding sea cucumber (Fig. 1) commonly found burrowed in inner rubble reef flats and coastal lagoons, feeding on bottom deposits, rich in nutrients. There are three colour morphs associated with this sea cucumber: black, moderate black spots, and speckled (Conand 1990). Because of the high price and growing demand for sea cucumbers in Asian markets, golden sandfish is fished intensively like other commercial species. High-quality, golden sandfish beche-de-mer fetch more than USD 130 per kg on the export market. Although the processing method for golden sandfish is the same as that for sandfish, the processed product is golden in colour, very different from the grey and wrinkled appearance of sandfish beche-de-mer (Fig. 2).

The worldwide supply of high quality beche-de-mer will not be sufficient to meet the Asian market demand, unless a viable sea cucumber aquaculture develops to partially replace the steady decrease in wild stocks. Aquaculture studies on tropical sea cucumbers have been largely focused on the widespread commercial sea cucumber *H. scabra*. Few studies concern *H. scabra versicolor* (Conand 1990, 1993; Hamel et al. 2001), and little information is available on its biology, in particular, its early life stages. So far, to our knowledge, no attempt has ever been made to captively breed this

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**Figure 1.** Golden sandfish  
*Holothuria scabra* var. *versicolor*.



**Figure 2.** Processed *H. scabra*  
and *H. scabra* *versicolor*.

species, other than the preliminary trials carried out at Bluefin Seafoods sea cucumber hatchery, which produced 33,500 juveniles during a 2004 spawning (Giraspy and Ivy 2005).

Bluefin Seafoods Pty. Ltd., Hervey Bay, Queensland has received an innovation grant from the Federal Government of Australia to perfect the hatchery technology for mass production of sea cucumbers for restocking programmes. Under this programme, Bluefin Sea cucumber hatchery has released millions of hatchery produced *H. scabra* juveniles in the designated aquaculture areas of Hervey Bay during the past few years to accelerate the recovery of depleted populations and allow sustainable harvests. With the ongoing research on

other species of sea cucumbers, the hatchery is now capable of mass-producing *H. scabra* *versicolor*. Based on preliminary results obtained during 2004 spawnings, culture techniques have been refined and hatchery technology has been developed for this species. Our results demonstrate that culturing golden sandfish is quite feasible and that it could be used to contribute to the restoration of depleted natural populations and allow, in due course, a sustainable fishery.

## Materials and methods

### Broodstock collection

Golden sandfish, *H. scabra* *versicolor*, were collected from Hervey Bay, Queensland, Australia, by divers, between October and December 2004 and during the same months in 2005. They were immediately placed into small 44-L portable bins filled with fresh seawater. A maximum of three animals were placed in each bin equipped with a continuous aeration system. Upon arrival in the port, the broodstock were transported by road to the hatchery facility (Bluefin Seafoods sea cucumber hatchery, Hervey Bay, Queensland). They were then placed in 10,000-L flow-through tanks for a period of nearly 30 minutes before induced spawning was attempted.

### Induced spawning

Several spawning trials were carried out using different methods and combination of methods, such as thermal variations, a powerful jet of water on drying animals, addition of Algamac and grinded male gonads, to find out the ideal inducement method for spawning. Thermal stimulation was found to be the most successful and was used thereafter. For each spawning, 10 to 15 animals were gently cleaned and washed to remove sediments and other small organisms attached to their body, and placed in a 1000-L spawning tank. Seawater temperature in the tank was then raised by 3–5°C to induce spawning.

Once the spawning was over the animals were put back in the broodstock holding tanks with flow-through seawater. Enough sand mixed with sea-grass powder was added daily to these holding tanks to keep a layer of approximately 1-cm thickness on the bottom.

An egg count was made after each successful spawning from a 0.5-mL sample of water taken from the spawning tank, using a plankton counting chamber under a stereo-microscope. The egg size measurements were also taken using the microscope with an ocular micrometer. Eggs were

then collected from the spawning tanks using an 80- $\mu\text{m}$  sieve and washed for 10 minutes in 1- $\mu\text{m}$ -filtered UV sterilized seawater to remove excess sperm and dirt.

### Larval rearing

The larvae were cultured in 1000-L fiberglass larval rearing tanks with the temperature maintained between 26 and 27° C. During the larval rearing period the salinity ranged between 37.5 and 38 ppt, while pH remained at 8.2. The larval quality (presence of unsatisfactory shape, size and stages of maturity) and the mean larval size were checked regularly by examining samples of 40 larvae under a microscope, using an eye-piece graticule.

Larval diet consisted of *Rhodomonas salina*, *Chaetoceros calcitrans*, *C. mulleri*, *Tetraselmis chui*, *Isochrysis galbana* and *Pavlova lutheri* in different combinations at different stages. The microalgal feed density was gradually increased from 15,000 cells mL<sup>-1</sup> on day 3 to 35,000 cells mL<sup>-1</sup> on day 14. The higher microalgal feed density (35,000 cells mL<sup>-1</sup>) was maintained thereafter until larvae metamorphosed to the doliolaria stage. The larvae were fed two times a day and the food cell density was maintained at appropriate level at all times.

Larvae were collected with sieves every two days and washed for 10 minutes before being transferred to new tanks with filtered and temperature-controlled seawater. On day 17, when they reached the non-feeding doliolaria stage, the larvae were transferred to tanks with different settlement cues such as seagrass extract, seaweed extract, Algamac 2000, Algamac Protein Plus, dead algae, benthic diatoms (*Nitzschia* sp. and *Navicula* sp.) and spirulina, and the flow-through system was maintained. The corrugated settlement plates were covered with settlement cues to facilitate pentacula attachment

at metamorphosis. The settled juveniles were initially fed with Algamac 2000, Algamac Protein Plus, seagrass extract, seagrass powder, seaweed extract and seaweed powder. Once they attained an average length of 10-mm, they were fed with fine sand mixed with the above components. The growth rate was monitored for each food type.

### Results

The sea cucumbers responded well to thermal induction and showed pre-spawning behaviours such as twisting and crowding in corners of spawning tanks (Fig. 3). More than 75% of our spawning trials were successful with males, while females shed their eggs in less than 35% of the attempts. Males responded to the heat stress induction first, raising their anterior end and swaying while releasing sperm. The swaying movement was less vigorous than that of the common sandfish *H. scabra*. After 30 to 90 minutes, females responded by lifting their anterior end and remaining erect for a few minutes before expelling mature oocytes in powerful intermittent jets (Fig. 4). Males stayed upright and continuously spawned for more than an hour in most occasions, while females spent less than 15 minutes in the erect position before releasing their eggs, and went back down afterwards.

The total number of eggs produced from seven females during six spawning events of 2004 was 14.23 million, among which only 3 million were used for larval rearing. During the 2005 spawning season, eleven females produced, in nine successful spawning events, 32.76 million fertilised eggs, among which only 6 million were used for larval rearing trials.

The developmental kinetics of *H. scabra versicolor* larvae at 26–27° C is given in Table 1. The mature



**Figure 3.** Spawning behaviour of *H. scabra versicolor*.



**Figure 4 .** Spawning male and female *H. scabra versicolor* in the spawning tank.

eggs of *H. scabra versicolor* were spherical and visible to the naked eye with a mean size of  $205.36 \pm 17.54 \mu\text{m}$  ( $n = 40$ ). The auricularia larvae constituted the first feeding stage and they began to appear nearly 48 hours after fertilisation. The larvae are transparent and they feed well during their pelagic phase. The newly hatched early auricularia larvae were  $409.48 \pm 11.5 \mu\text{m}$  in length. Auricularia larvae developed rapidly, reaching the middle auricularia stage on day 8 and the late auricularia stage around day 14. The middle auricularia measured  $954.72 \pm 12.23 \mu\text{m}$  in length.

During this progressive growth the larvae accumulated hyaline spheres in their body. After 13 to 15 days they reached the late auricularia stage with a maximum size of 1.25 to 1.31 mm. The late auricularia is transformed to the non-feeding doliolaria stage on the day 17, with a mean size of  $853.82 \pm 7.74$ . The doliolaria metamorphosed to creeping

stage pentacula on the day 19, and the pentacula larvae possessed five well-developed primary tentacle and a single ventroposterior podium. The pentacula develop tentacles and tube feet and form the juvenile with more distinct spicules. The survival and development of larvae up to the pentacula stage is shown in Figure 5.

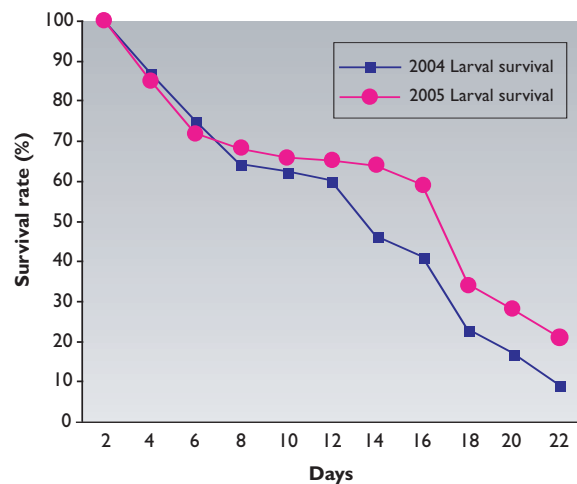
The culture success based on the survival rate of fertilized eggs across the different larval stages increased markedly between 2004 and 2005 (Table 2). The larval development, settlement and juvenile growth were asynchronous, as different stages and sizes of larvae and juveniles could be seen at the same time in a batch. The overall survival at the juvenile stage was 1.12% in 2004 and 4.53% in 2005. The larval settlement experiments showed that the juveniles attached to the settlement plates or to the tank base and fed on the biological film that had developed.

The best larval settlement was obtained with a mixture of *Nitzschia* sp. and *Navicula* sp., followed by the single use *Navicula* sp., *Nitzschia* sp., Algamac 2000 and Algamac Protein Plus (Fig. 6). The first settled juveniles were clearly visible on the settlement substrate after 25 days of culture and measured 1–1.5 mm in length. Wide variation in growth was

**Table 1.** The developmental kinetic based on the observations of four spawnings of *H. scabra versicolor* during 2005.<sup>a</sup>

Time for fertilization	Stage	Remark
0	Fertilized egg	Size: $205.36 \pm 17.54 \mu\text{m}$
40 min	1st cleavage	2 cells
2 h 10 min	2nd cleavage	4 cells
3 h 40 min	3rd cleavage	8 cells
9 h	Blastula	Rotary motion
2 d	Late gastrula	Gastrula – auricularia transition
3 d 12 h	Early auricularia	Pre and post oral lobes formation
8 d	Mid auricularia	Lateral processes extension
14 d	Late auricularia	Distinct hyaline spheres
17 d	Doliolaria	Five ciliary rings
19 d	Pentacula	Disappearance of ciliary bands and benthic life
22 d	Early juvenile	Feeding benthic diatom and detritus.

a. Larvae were considered to have reached a developmental stage when more than 50% of the larvae had accomplished the specified stage.

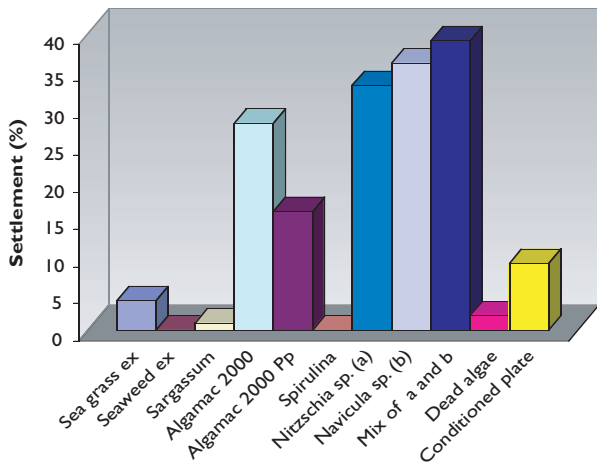


**Figure 5.** Survival rate of *H. scabra versicolor* larvae.

**Table 2.** Details of 2004 and 2005 spawning trials and respective larval survival obtained

Year	No. of spawning	Eggs ( $10^6$ )	Hatch (%)	Middle auricularia (%)	Late auricularia (%)	Doliolaria (%)	Days to settle
2004	6	14.23	87	64	46	32	21
2005	9	32.76	93	68	64	46	19

noticed among the juveniles in all batches. After six weeks, more than 45% of the juveniles reach 15 mm in length. The juveniles reached 20–25 mm within eight weeks. But, after metamorphosis, the juveniles took three months to reach an overall average length of 30 mm under optimum stocking density and good feeding conditions.



**Figure 6.** Settlement of *H. scabra versicolor* larvae in different settlement regimes.

## Discussion

The high demand for beche-de-mer on the Asian market and the systematic overexploitation of wild populations support the call for sea cucumber farming. Aquaculture is a potential alternative source for the market and can also sustain wild harvest fisheries. Over the past decade, efforts to develop hatchery techniques for the culture of commercially important sea cucumber species have increased significantly (James et al. 1994; James 1996a; Ramofafia et al. 1995; Ito 1995; Asha and Muthiah 2002; Lovatelli et al. 2004; Giraspy and Ivy 2005). Several hatchery and grow-out projects have also been undertaken by International agencies with the aim of restocking commercially valuable sea cucumber species.

Among the several commercially important sea cucumbers, spawning and larval rearing in captivity has been successfully achieved only in a few species. In Japan, *Apostichopus japonicus* juvenile production was started nearly 70 years ago (Inaba 1937) and the juveniles were reared in captive conditions (Imai et al. 1950). Later, this species was successfully cultured in China (Shuxu and Gengeheo 1981; Li 1987). Among the tropical holothurians, *H. scabra* is a high-value species and considered as one of the best aquaculture candidates (Battaglène

1999, 2000; Battaglène and Bell 1999). This species has been successfully mass-produced in India (James et al. 1988; James 1996b), Madagascar (Jangoux et al. 2001), Vietnam (Pitt and Duy 2004), and Australia (Giraspy and Ivy 2005). Currently the WorldFish Center project is developing optimal releasing strategies for hatchery-produced *H. scabra* juveniles in New Caledonia (Purcell et al. 2002). But hatchery-raised juveniles of *H. scabra versicolor* have been produced here for the first time and no previous studies exist to compare with.

Thermal stress is a well-known practice used to stimulate spawning in sea cucumbers (James et al. 1988; Morgan 2000; Battaglène et al. 1999, 2002; Giraspy and Ivy 2005). The stress on sea cucumbers associated with collection and transportation, powerful water jets on drying individuals (James et al. 1994, 1996) and the addition of dried algae (*Schizochytrium* sp.) (Battaglène et al. 2002) also induced spawning. In all our spawning trials, males spawned first, releasing sperm for more than 30 minutes. Females reacted later with the release of eggs that lasted for less than a minute in most cases. This concurs with other observations showing that, generally, male sea cucumbers spawn first and are easier to induce to spawn (Battaglène et al. 2002), and that females are stimulated by the presence of sperm in the water column (James et al. 1994a). A rare observation of *H. scabra versicolor* spawning in the wild was recorded by Desurmont (2005) in New Caledonian waters, three days before full moon and just before high tide.

The larval cycle of the golden sandfish is similar to most aspidochirote holothurians with early, mid and late auricularia, and subsequent metamorphosis to the non-feeding doliolaria stage before settlement. However, the length of the larval cycle and other larval characteristics differ from other species, even from *H. scabra*. The larvae of *H. scabra versicolor* took 17 days to reach the non-feeding doliolaria stage; *H. scabra* (James et al. 1988), *H. spinifera* (Asha and Muthiah 2002) and *Actinophyga echinites* (Chen and Chian 1990) all take less than 15 days to reach this doliolaria stage, but *H. atra* takes 20 days.

In the present trials, *H. scabra versicolor* larvae were fed with *Rhodomonas salina*, *Chaetoceros calcitrans*, *C. mulleri*, *Tetraselmis chui*, *Isochrysis galbana* and *Paolova lutheri* at different proportions at different developmental stages. In a previous investigation Battaglène et al. (1999) fed *H. scabra* larvae with microalgal species such as *Rhodomonas salina*, *Chaetoceros muelleri*, *C. calcitrans*, *P. salinai* and *Tetraselmis chuii*. While James (2004) used mixed cultures of *Chaetoceros* sp. and *Isochrysis galbana* in his experiments on *H. scabra*.



A stocking level of 0.75 larvae mL<sup>-1</sup> is found to be suitable for *H. scabra versicolor*. James (1996) suggested stocking levels of 0.5–1 egg mL<sup>-1</sup> in 800-L tanks with light to moderate aeration. Battaglione and Bell (1999) indicated the suitable larval density as 1 mL<sup>-1</sup>, which is slightly higher than that used in the present investigation.

In the present study, the larval growth and survival rate was very good at an algal concentration of 4 × 10<sup>4</sup> cells mL<sup>-1</sup>. The optimal concentration of algae for larval ontogenesis of *S. japonicus*, *H. scabra*, *H. atra* and *H. spinifera* was between 2 and 3 × 10<sup>4</sup> cells mL<sup>-1</sup> (James et al. 1994; Ramofafia et al. 1995; Asha and Muthiah 2002). Archer (1996) in his experiments on *S. mollis* found that the continued presence of high algal concentrations (above 6 × 10<sup>3</sup> cells mL<sup>-1</sup>) in the larval culture reduced the ingestion rate of the algae. The optimal growth and good survival rate of *H. scabra versicolor* larvae at comparatively higher algal concentration may be because of their large stomach size when compared with *H. scabra*.

The higher mortality rates were noticed during the larval metamorphosis and settlement stages. Battaglione (1999) in his experiments with *H. scabra* noticed up to 35% mortality from survival to settlement and highest mortality occurred at first feeding and settlement. Settlement cues in sea cucumber culture play two important roles: as a biological signal for the induction of larval metamorphosis, and as a suitable food for settled juveniles. In the present investigation a higher settlement rate was observed in mixed periphytic diatom followed by Algamac Protein Plus.

Algamac is a potential settlement cue and food for settled pentaculæ of *H. scabra* (Battaglione 1999). Asha and Muthiah (2002) later observed that Algamac and periphytic diatoms acted as good settlement cues in their experiment with *H. spinifera*. The present results with *H. scabra versicolor* show that the hatchery culture techniques improved significantly over time and also in subsequent batches (1.12% during 2004 and 4.53% during 2005). This improved survival and settlement rate of juveniles during 2005 spawnings were made possible by improved larval rearing protocol, high standards of algal culture, and changes made to post-settlement conditions in the hatchery.

The growth rate of *H. scabra versicolor* juveniles observed in this study is higher than that of *H. scabra*. Battaglione et al. (1999) observed wide variations in growth in their experiments with early-stage juveniles of *H. scabra*. Growth variations among cultured sea cucumbers and also in wild caught juveniles are very common (Ito 1995; James 1996). But there are no previous studies on golden sandfish

culture growth to compare with our juvenile growth results. However, consistency in survival and growth rates of juveniles within several batches of cultures indicates that the present growth rate is good for the hatchery production.

### Acknowledgement

We thank Prof Conand for her excellent scientific suggestions for and assistance with this paper.

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# Seasonal abundance of sea cucumber larvae at Toliara Great Reef, Madagascar

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## Abstract

The abundance of echinoderm larvae, in particular sea cucumber larvae, was assessed in the lagoon of Toliara Great Reef in southwest Madagascar from December 2000 to May 2002. More than 9000 echinoderm larvae were collected, 33% of which came from sea cucumbers. Mean larval density varied greatly depending on the season. Sea cucumber larvae were most abundant during the hot season (November to April) with an average of 77 larvae per 350 m<sup>3</sup> as compared to 1 larva per 350 m<sup>3</sup> during the cool season (May to October). Sea cucumber larvae were found in large numbers at the time temperatures dropped, accounting for 50% of the total number of echinoderm larvae observed. Sea cucumber larvae were not found in the plankton from June to October. Three different families of sea cucumbers were observed: Holothuriidae, Stichopodidae and Synaptidae. Holothuriidae were the most numerous, accounting for 86% of all sea cucumber larvae collected.

## Introduction

In the early part of 20<sup>th</sup> century, almost all studies on echinoderm larvae focused on species found in Europe and North America (Mortensen 1921, 1931, 1937, 1938). Recently though, studies have investigated the larval development of tropical species (Byrne and Selvakumaraswamy 2002; Emler et al. 2002; McEdouard et al. 2002; Sewell and McEuen 2002). In Madagascar, only the larvae of a few species have been studied (Rasolofonirina 2004; Vaïtilingon 2004). At this time, no analysis of the diversity and seasonal abundance of echinoderm larvae in the Mozambique Canal has been made. The study described in this paper is the first to concentrate on echinoderm larvae found on the continental slope of southwest Madagascar. The 18-month study was carried out in the lagoon of Toliara Great Reef, and was designed to analyse the abundance of both sea cucumber and echinoderm larvae in that period.

## Materials and methods

Plankton was collected from Toliara Great Reef (23°21'S and 43°40'E) from December 2000 to May 2002. Ocean water samples were taken once a week from the surface waters (0.5 m depth) using a pelagic net with a 150-µm mesh and a 35-cm opening. The net was pulled behind a boat at the end of a 10-m-long nylon rope for 13 minutes. The distance covered was about 900 m and the volume of seawater filtered for each plankton haul was 350 m<sup>3</sup>.

All plankton hauls were carried out at the same time of day (17:00) to reduce time-related fluctuations in physical or biological parameters.

After each haul, the sample was taken to the laboratory where it was set in formalin (10%) and seawater. Larvae were sorted and counted using a binocular microscope, and the contents of each sample were analysed. Four times a month, seawater temperature and salinity readings were taken at the same time as the plankton hauls. The different larval stages were determined and the taxa identified in line with the descriptions of Mortensen (1921, 1931, 1937, 1938), Byrne and Selvakumaraswamy (2002), Emler et al. (2002), McEdouard and al. (2002), Sewell and McEuen (2002), and Rasolofonirina (2004).

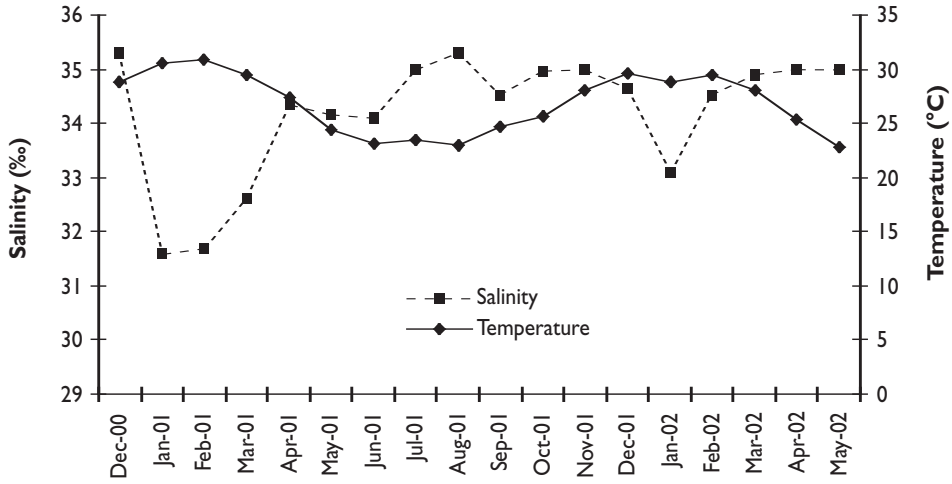
## Results and discussion

Lagoon water temperatures and salinity showed some slight variations over the course of the year (Fig. 1). Two separate seasons could be noted in southwest Madagascar: a hot season (November to April) and a cool season (May to October), during which times mean ocean water temperatures were, respectively, above or below 26°C. Salinity was generally about 34‰, except during the rainy season (mainly January), when it decreased to about 31.5‰.

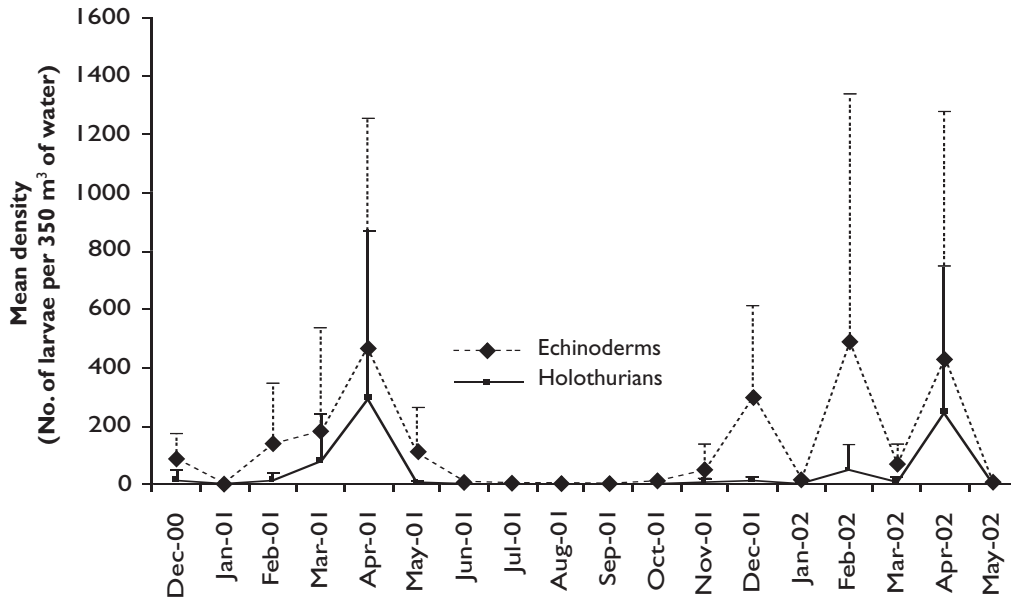
Table 1 shows the total number of larvae from the various echinoderm categories observed during the study period along with the respective percentages. No crinoid larvae were observed. Sea cucumber, echinid and brittle star larvae were found in almost identical proportions in Toliara Bay and accounted for, respectively, 33, 35 and 31% of the total number of echinoderm larvae collected. There were some starfish larvae but their numbers were low compared with the other three categories mentioned (1% of the total number).

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**Figure 1.** Seasonal variations in mean lagoon water temperatures and salinity at Toliara Great Reef.



<i>Holothuriidae</i> larvae	++		+	+++	+++							+	+	+	+++	+	+++	
<i>Stichopodidae</i> larvae			+	++									+	+		+	+	
<i>Synaptidae</i> larvae	+		+	++	+	+	+	+				+	+		+	+	+	
Unidentified larvae	+		++	+++	+++				+	+	++	+	+	+++	+	++		

+: less than 10 larvae per haul (350 m<sup>3</sup>); ++: between 10 and 20 larvae; +++: more than 20 larvae

**Figure 2.** Mean monthly echinoderm (all categories combined) and holothurian larvae densities.

A total of 2954 sea cucumber larvae were collected, 222 of which could not be identified (i.e. 8% of the total number collected). The unidentified larvae mainly consisted of very young specimens for which the characteristics needed for identification were not yet visible. Holothuriidae larvae were, by far, the most numerous (86% of all sea cucumber larvae), followed by Synaptidae (5%) and Stichopodidae (1%).

**Table 1.** Number and percentage of echinoderm larvae collected at the Toliara Great Reef.

Taxa	No.	Percentage
Holothuroidea	2954	32.4
Holothuriidae	2547	86
Stichopodidae	32	1
Synaptidae	153	5
Unidentified	222	8
Echinoidea	3209	35.2
Ophiuroidea	2822	31.0
Asteroidea	123	1.4
<b>Total</b>	<b>9108</b>	<b>100</b>

Figure 2 gives the seasonal abundances of echinoderm and sea cucumber larvae. Echinoderm larvae were found almost always in significant numbers from November to May, whereas their mean density was nearly zero outside that time period (maximum 15 larvae per 350 m<sup>3</sup> of sea water). Density peaks were observed in April 2001 (467 larvae per 350 m<sup>3</sup> of sea water) and in February and April 2002 (respective means of 488 and 429 larvae per 350 m<sup>3</sup> of sea water).

Seasonal abundances of sea cucumber larvae roughly followed the profile for echinoderm larvae (Fig. 2). Mean larval density reached a very high peak in April with 295 larvae per 350 m<sup>3</sup> of sea water in 2001 and 248 larvae per 350 m<sup>3</sup> of seawater in 2002. These peaks coincided with the drop in mean ocean water temperatures between March and April (a decrease of about 3°C).

A comparison of the mean number of larvae throughout the hot period (228 echinoderm larvae on average per plankton haul, 34% of which were sea cucumbers) and the cool period (19 echinoderm larvae, 5% of which were sea cucumbers) clearly shows that larval abundance was greatest during the six-month hot period.

Holothuriidae larvae were generally found in the water column from November to April, Stichopodidae from November to March, and Synaptidae from November to July. Nevertheless, larval abun-

dance was low for all three families in January because in January 2001, no sea cucumber larvae were found, and in January 2002, only a few Holothuroidea larvae were recorded.

Table 2 shows the numbers and percentages of the various stages of sea cucumber larvae collected. Most larvae were auricularia (94%), generally in an early phase (i.e. the larval body did yet display all the lateral projections). The other larvae were metamorphosing or were doliolaria larvae. A few specimens were embryos in the process of transforming into auricularia larvae.

**Table 2.** Number and percentage of different stage holothurian (Holothuroidea) larvae.

Stage	No.	Percentage
Embryo	131	4.43
Early auricularia	2226	75.36
Late auricularia	545	18.45
Metamorphosing	35	1.18
Doliolaria	17	0.58
<b>Total</b>	<b>2954</b>	<b>100</b>

All the results indicated that the hot season is the period when echinoderm larvae, particularly sea cucumber larvae, are abundant, especially at the end of that period. This agrees with the results given in scientific literature about the reproductive cycle of tropical sea cucumbers (Conand 1989; Mara et al. 1997; Rasolofonirina 2004). In the Toliara region, the reproductive cycles of just a few sea cucumbers are known (Mara et al. 1998; Rasolofonirina 1997; Rasolofonirina et al. 2005). Those sea cucumbers have annual reproductive cycles. Mature specimens can be found throughout the year but they are much more numerous in late summer. Egg laying in tropical sea cucumbers is generally linked to an increase in seawater temperatures and is spread out over a fairly long period (Hyman 1955). This hot period corresponds to an abundance of phytoplankton in the environment — phytoplankton that serves directly as food for echinoderm larvae. The low density of larvae during the cool season can be explained by the fact that most larvae cannot withstand the drop in seawater temperatures and/or the lack of food (decrease in phytoplankton biomass).

Metamorphosing sea cucumber larvae and postlarvae were only found occasionally in the plankton. The scarcity of these larvae in the water column can be explained by the increase in their weight and the regression of their cilia. This scarcity was probably also due to the brief length of the metamorphosis process, which generally does not last more than an

hour (Hyman 1955), and to the high level of larval mortality that characterises this phase.

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## A new approach for measuring *Holothuria mexicana* and *Isostichopus badionotus* for stock assessments

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### Abstract

Body length and wet and dry weight measurements of *Holothuria mexicana* and *Isostichopus badionotus* collected in shallow water (< 4 m) habitats were assessed. A new approach for measuring length consisted of submerging specimens in cold (~ 8° C) seawater, where they reached maximum contraction in less than 10 seconds. After about a minute, individuals were measured and weighed and then released into their habitat. Our method was compared with that of Yingst (1982), who proposed hand-rubbing specimens until maximum contraction was achieved, in order to measure contracted length. Although there were no significant differences between the two methods ( $t = 1.65$ ), the new approach resulted in trustworthy body measurements and was adequate for rapid assessment of size distribution in the field. It also diminished the time that individuals were removed from their habitat, and the disturbance caused by handling-related factors. Regression equations correlating biometric characteristics for both species were determined and can be applied to estimate biomass and stock assessment in field studies that do not require sacrificing sea cucumbers.

### Introduction

Obtaining accurate body measurements of soft-bodied animals is a difficult task that can lead to biased data. In many invertebrates, weight is often a function of the cube of the length, thus linear measurement errors are magnified when determining weights (Crisp 1990). This situation is evident in holothurians due to their high contractibility (Pérez-Ruzafa and Marcos-Diego 1985; Conand 1990, 1993a), difficulties in distinguishing contracted adults from elongated juveniles (Laboy-Nieves 1997), and the sparse information on their biohistory and behaviour (Cutress 1996).

The sediment feeding sea cucumbers *Holothuria mexicana* and *Isostichopus badionotus* are among the largest and most common holothurians inhabiting shallow waters in the Caribbean Sea. They coexist as conspicuous settlers on seagrass beds, muddy bottoms in mangrove impoundments, and channels and sandy substrates next to coral reefs (Laboy-Nieves 1997; Guzman and Guevara 2002). Their distribution range comprises the circumtropical coasts of the Atlantic (Hendler et al. 1995).

Holothurians have been harvested for centuries in the Indo-Pacific region (Conand 2004; Muthiga and Conand 2006), and there is evidence of population declines and some local extinctions (Samyn et al. 2005). Since the depletion of sea cucumber harvesting grounds in Asia, fishing efforts have focused on the Americas. Uncontrolled exploitation of holothurians peaked during the infamous "Pepino War" at the Galápagos Islands, which drew world-

wide attention in 1994–1995 (Conde 1996; Toral-Granda and Martínez 2004). In Mexico, *Isostichopus fuscus* was harvested for more than 10 years before being banned because of the alleged endangerment of the species (Fuente-Betancourt et al. 2001). In Panama, uncontrolled harvesting resulted in over-exploitation of the species (Guzman and Guevara 2002). Holothurians — including commercially valuable species — have important functions in nutrient recycling, which increases the productivity of coral reef ecosystems. Removal of holothurians through fishing may reduce the overall productivity of affected coral reefs (Uthicke et al. 2004).

Although a number of authors have reported body length measurements of *H. mexicana* and *I. badionotus*, the available information is mainly restricted to average values. Only Laboy-Nieves (1997) and Guzman and Guevara (2002) have presented a relationship between length and weight of these species that could be useful for field monitoring and stock assessment. However, the authors' main concern is the variety of methods employed for measuring tropical sea cucumbers. This situation presents several dilemmas: 1) it is difficult to establish comparisons, 2) most methods require the mechanical disturbance of specimens, 3) relaxing sea cucumbers after immersion in a  $\text{KMnO}_4$  solution is very time-consuming, and 4) measuring length underwater may be prone to errors. The aim of this paper is to: 1) provide a simple and cost-effective method for field stock assessment by measuring contracted length of these species, and 2) determine the relationship between length and weight to establish regression equations among those variables.

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## Methodology

One hundred individuals from each species were randomly collected by hand during daytime from shallow water (< 4 m) habitats in Morrocoy National Park, Venezuela (10°52'N, 68°16'W, average water temperature around 29° C, Laboy-Nieves 1997). A field balance and calliper were used to measure wet weight and contracted length, respectively. Length was determined by modifying the method described by Yingst (1982). Instead of hand-rubbing specimens to attain maximum contraction (Yingst 1982), length was measured from the mouth to the anal podia through the lateral aspect, after immersing specimens in a bucket of cold seawater and ice. The dorsal aspect was not considered because *H. mexicana* hosts a complex epibiota and other adhered materials (*I. badionotus* did not exhibit epibiota). The ventral ambulacra were not considered because both species increase their arc after attaining maximum contraction. An assay to determine which water temperature triggers the fastest contraction of sea cucumbers was performed, rendering body contractions after about five seconds for water 10° C or less. Both methods were compared.

Specimens were placed in a submerged bucket close to the boat, then immersed individually into the bucket containing cold seawater. Contracted length was measured. Once measured, the echinoderms were released into their habitat. Thirty specimens were sacrificed in order to estimate dry weight after removing visceral tissues and epibiotic material, and oven dried at 80° C for two days. Gutted weight was not considered because specimens were initially sampled for an ongoing study on nutrient content and trace element bioaccumulation. Regression equations were determined by correlating these measurements.

## Results

*Holothuria mexicana* and *Isostichopus badionotus* exhibited significant differences in body size. Nearly 88.8% of *H. mexicana* (wet weight) corresponded to water and fecal material; for *I. badionotus* this value was 95.6%. Table 1 shows the descriptive statistics of the contracted length and wet weight for both species. It was found that the length and weight of *H. mexicana* was 172.1% and 273.7% higher than that of *I. badionotus*, respectively (Table 1). Most specimens (74%) of *H. mexicana* exhibited a contracted length between 20 and 30 cm, while for *I. badionotus*, 77% of the values were between 10 and 20 cm (Fig. 1). Adult individuals ( $x > 6.5$  cm, Cutress 1996) accounted for about 95% of the specimens examined. The only juveniles collected were *I. badionotus*.

All specimens achieved maximum contraction in less than 10 seconds after submersion in cold water. Smaller individuals shrank faster than larger ones. It took nearly one minute to weigh and measure the length of each individual. Once measured, the holothurians were released into their habitat where they resumed normal activities after about 10 minutes. Some individuals (seven *H. mexicana* and four *I. badionotus*) spawned after been reintroduced into warm water.

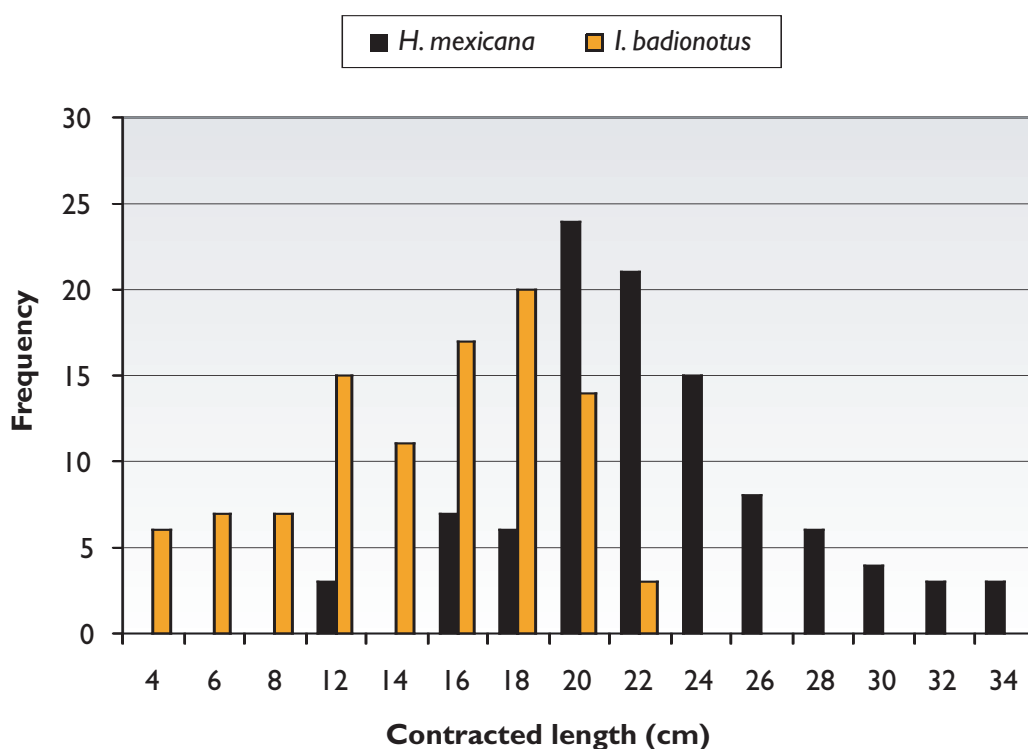
Table 2 shows the regression equations and correlation coefficients for biometric variables for *H. mexicana* and *I. badionotus*. Contracted length and wet weight were significantly correlated in both species. It was observed that contracted length was significantly and directly proportional to wet and dry weight for *H. mexicana* and to wet weight for *I. badionotus*. In the case of wet and dry weight, only *H. mexicana* exhibited significant differences.

There were no significant differences ( $t_s = 1.65$ ,  $p = 0.121$ ) after comparing the contracted lengths using Yingst's method and the procedure used by the authors of this paper. Yingst's method requires hand-rubbing individuals until the contracted length is achieved, however, handling can damage the soft epidermis of *I. badionotus*, and is followed by evisceration in some individuals. For *H. mexicana*, hand-rubbing resulted in the removal of epibiota and other marine debris fragments attached to the skin. For the handler, Yingst's method sometimes resulted in minor abrasions and small injuries, because of the hard materials (shells, pebbles, coral fragments) attached to the body of *H. mexicana*. Our method for immersing specimens in cold water was less harmful than Yingst's, because animals were not exposed to handling-related disturbances except for the collection from the substrate.

## Discussion

The lack of a standardised method for measuring the length of holothurians, and thus their biomass as required for modelling and stock assessment, presents difficulties for comparing data and following variations. Although each author aims at the most objective data (Pérez-Ruzafa and Marcos-Diego 1985), the variety of methods, and relative complexity of some of them, defeats the attempts of normalisation. Conand (1989), cited by Dalzell et al. (1996), faced several problems in determining linear size measurements of sea cucumbers because of their plasticity. She further preferred using the ratio of wet gonads to drained body weight (Conand 1993a) or weight to express size frequency (Conand 1995). Conand and Byrne (1993) acknowledged that the diversity of techniques used to sur-





**Figure 1.** Contracted length distribution of *Holothuria mexicana* and *Isostichopus badionotus* (ANOVA  $F = 260.37^{**}$ ).

**Table 1.** Contracted length (cm) and wet weight (g) for *Holothuria mexicana* and *Isostichopus badionotus*.

	<i>Holothuria mexicana</i>		<i>Isostichopus badionotus</i>	
	Length	Weight	Length	Weight
Mean	23.4	561.9	13.61	204.8
(stand. dev.)	(4.64)	(212.3)	(4.26)	(62.3)
Range	12.2–5.6	163–1205	4.1–21.3	79–355

**Table 2.** Regression equations for the contracted length (CL), wet weight (WW) and dry weight (DW) of *Holothuria mexicana* and *Isostichopus badionotus*. Significant values ( $0.01 < P < 0.05$ ) are indicated by an asterisk ( $n = 30$ , except for contracted length and wet weight:  $n = 100$ ).

	<i>Holothuria mexicana</i>	<i>Isostichopus badionotus</i>
Contracted length and wet weight	$CL = 11.514 + 0.021 \times WW$ $r = 0.97^{**}$	$WW = 27.505 + 13.034 \times CL$ $r = 0.89^{**}$
Contracted length and dry weight	$CL = -70.610 + 5.724 \times DW$ $r = 0.76^*$	$DW = 4.226 + 0.371 \times CL$ $r = 0.24$
Wet weight and dry weight	$DW = -15.613 + 0.143 \times WW$ $r = 0.84^{**}$	$DW = 1.630 + 0.037 \times WW$ $r = 0.54^*$

vey holothurians limits data comparisons and that adequate statistics should be standardised. Like the authors of this paper, Conand (1993b) established biometric paired relationships between total length and total wet weight, but did not specify the method employed to measure total length.

In the particular case of *Holothuria mexicana* and *Isostichopus badionotus*, Sloan (1979) employed biomass instead of linear measurements. Hammond (1982) measured the average elongated length, Yingst (1982) preferred contracted length after hand-rubbing specimens, Sambrano (1987) anaesthetised specimens to further measure maximum elongation, and Guzman and Guevara (2002) submersed sea cucumbers in magnesium permanganate and waited about five minutes to measure elongation.

Yingst's (1982) original method provides a handy approach for rapid monitoring in the field. Although there were no significant differences in the results obtained by our study, our new approach has some advantages over Yingst's because it is simpler, faster, and harmless to the animals and the handler. Only a bucket with cold water is needed for the contraction of specimens, which shrank immediately after immersion. These factors allow for sampling or processing more specimens when time and resources are limited.

With regards to the methods used by Sambrano (1987) and Guzman and Guevara (2002), our method is an improvement, because it does not require exposing sea cucumbers to chemicals. And, unlike Hammond's method (1982), our procedure reduces the time for determining measurements because there is no need to wait for specimens to achieve an average elongated length. Measuring specimens at normal (~ 29° C) sea temperature in tropical areas is difficult because individuals may eviscerate upon handling, show erratic contraction and elongation, and increase their respiration rate (pers. observ.). One benefit of our method is that immersing individuals in cold water triggers an immediate contraction that consequently stops cloacal respiration and provokes the halting of body movements in less than 10 seconds. Therefore, the handling and measuring of specimens is faster and does not require the use of gloves or chemicals to rub or tranquilise individuals. Besides, the typical epibiota of *H. mexicana* was not mechanically altered; nor was the fragile epidermis of *I. badionotus* broken, as happened with Yingst's method, while rubbing the animals. Our method is an improvement for the data collector, because it diminishes the risk of contact with eviscerated materials and fluids, and injuries from rubbing species covered with hard debris, such as broken

shells. This method could also be used for harvesting sea cucumbers when there are legal live size limitations.

Bruckner (2005) emphasised that minimum sizes should be based on the size at first sexual maturity. This is advantageous for managing an export fishery, because enforcement can be done at the marketplace. The disadvantage of this approach is that rejected undersized animals are already dead, and represent a loss to the reproductive capacity of the stock as well as an economic loss to fishermen (Richmond 1996). Another disadvantage of using minimum sizes is that this method does not guarantee that the maximum sustainable yield will be harvested, and it does not predict how many sea cucumbers will be harvested. In fisheries managed solely by minimum size, the initial quantities harvested will be large, as all the individuals larger than the minimum size will be subject to harvest. Over time, the largest individuals will become scarce, and the annual fishery will depend on how many animals grow to legal size.

Australia, Papua New Guinea, Fiji and Tonga have minimum size restrictions for the harvesting of sea cucumbers, but their rules are based either on the live or dried length, which technically could be inconsistent. Bruckner (2005) stated that minimum size requires a significant amount of data and the primary burden is placed on fishermen, who must determine if each specimen collected meets the minimum established size requirement, a difficult task when live sea cucumber length is so dependant of the animal behaviour (contracted or relaxed). The method described here (measuring contracted length) could be a practical approach for standardisation of length measurements. Further studies should be conducted to compare contracted length with sexual maturity.

The low size variation found in this study shows that populations of *Holothuria mexicana* and *Isostichopus badionotus* are largely composed of individuals of similar sizes. It has been reported that juveniles inhabit deeper waters or occupy other habitats in shallow waters (Cutress 1996). This could occur because of interspecific and intraspecific competition, predation and availability of food (Laboy-Nieves 1997), marked environmental heterogeneity (Laboy-Nieves et al. 2001), and different physiological responses to pollution (Laboy-Nieves and Conde 2001). For logistical reasons, all specimens were collected during daytime, a period when juveniles may not be active or exhibit cryptic behaviour (Cutress 1996).

The contracted length of each species was significantly correlated to its wet and dried weight. Al-

though there are specific differences in the strength of the relationships of these variables, regression equations can be applied to estimate or predict biomass in studies that do not require sacrificing these animals. However, it is crucial to consider biology as well as statistical significance, because no statistics can relieve our responsibility for the biological conclusions drawn from biometric data.

The fact that all individuals resumed activities around 10 minutes after being released may imply that the 5 second thermal shock did not alter their health. The very few spawnings that were observed after reintroducing the sea cucumbers into warm water is consistent with the thermal stimulation effect reported by Mosher (1982), Baskar (2004) and Laxminarayana (2005).

Members of the Stichopodidae family have been commercially overexploited in South America (Powell and Gibbs 1995). Furtive extraction of *I. badiotus* was observed by the senior author and has been reported by Conde (1996). Therefore, human factors might introduce a bias in field observations of natural populations of any other commercially valuable holothurians. In eastern Venezuela, *I. badiotus*, was the object of supervised exploitation for a brief period, but fishing rights were discontinued due to the scarcity of information relevant to management, such as body size and weight distributions (Buitrago and Boada 1996). These examples illustrate the urgent need to evaluate stocks to assess the sustainability of sea cucumber fisheries.

To promote more reliable data and further establish size classes or categories, the above factors as well as other that could affect body size, for instance gonads development (Conand 1993a), or temperature and dissolved oxygen (Laboy-Nieves 1997), ought to be considered. Meanwhile, the method herein described can prove to be a valuable tool for fast stock assessment of shallow water sea cucumbers. Stock size and indications of stock status are two useful parameters on which to base robust management strategies. These parameters can then be used to indicate future catch levels that allow for sustainable development of the sea cucumber fishery. Management regimes for aquatic species vary in complexity from stock assessment models relying on extensive catch and monitoring data, to the application of relatively simple measures such as closed areas and minimum size limits. Depending on the nature of the resource, an effective management regime may not necessarily require the most extensive and complex measures to be applied to support a sustainable fishery. There is, however, a fine balance between allowing a harvest at levels that are probably sustainable and being precautionary enough to ensure the survival of the species is not at risk.

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## Probiotics and sea cucumber farming

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### Abstract

With the development of sea cucumber farming, new diseases are expected to appear in the near future. To prevent aquaculture-related diseases, antibiotics are generally used in large quantities although their unlimited application results in the appearance of new virulent pathogens. Probiotic technology is rapidly becoming popular due to its effectiveness against pathogenic microorganisms. This work emphasises the use of probiotics against pathogens in the sea cucumber farming industry.

### Introduction

Sea cucumbers are the most commonly consumed echinoderms and have been eaten since ancient times. Although holothurian fishing has a long history, sea cucumber aquaculture has only developed in recent decades. It is now well established in many countries such as Australia, China, the Galápagos Islands, Indonesia, Japan, Malaysia, and the Philippines (see Conand 2004 for review). The raw products were consumed within the producing countries, while processed products were exported mainly to China, Hong Kong and Taiwan (Conand 2004; Vannuccini 2004).

In addition to their good flavour, sea cucumbers are also eaten for their medicinal value. They are commonly used for treating weakness, impotence, constipation and frequent urination (Hamel and Mercier 2004). They are also remarkably rich in vitamins, trace elements, and polysaccharides (chondroitin sulfate), which reduce arthritis pain and inhibit viral activities, and saponin glycosides that inhibit cancer activities (Hamel and Mercier 2004).

The rapid increase in market demand has resulted in the overexploitation of natural sea cucumber populations all over the world. As a result, sustainable industries with modern hatchery techniques have been established in several countries. Because of the low oxygen consumption of sea cucumbers, some industries have accomplished financially profitable polyculture farming (shrimp and sea cucumber) without extra aeration or frequent water exchange (Hamel and Mercier 2004). The quick spread of intensified farming has led to an increase of various pathogenic diseases, which have become a major limiting factor in the industry. A lack of information regarding diseases and preventive measures makes it difficult to ascertain the expected profit of the industry if faced with a disease outbreak.

Holothurians are easily infected by parasites, including protozoans, bacteria and metazoans

(Becker et al. 2003; Eeckhaut et al. 2004). The high density of animals in hatchery tanks and ponds facilitates the spread of pathogens, and the aquatic environment — with regular applications of protein-rich feed — is ideal for culturing pathogenic and non-pathogenic microorganisms. Even when pathogenic bacteria or viruses are not present, farmers use antibiotics as prophylactics in large quantities. This has led to an increase in bacteria that are resistant to multiple antibiotics and to an increase in more virulent pathogens. Therefore, the use of beneficial bacteria that would reduce pathogenic bacterial development by a competitive process is a better solution than antibiotic applications. This work discusses the possibility of using probiotics as an “eco-friendly”, biological method of disease control in sea cucumber farming.

### Diseases and associated microorganisms of sea cucumbers

Since sea cucumber farming is a relatively new industry, the emergence of disease outbreaks is not well understood in contrast to diseases observed in other economically important aquaculture industries. However, the “Advances in sea cucumber aquaculture and management” workshop, held in China in 2003, pointed out several diseases that appear worldwide and within different species.

Very few studies have been carried out to identify the causal agents of sea cucumber diseases. Zhan and Yu (1993) identified parasitic sporozoans. Most of these parasites were seen in the hemal system and in the gut of sea cucumbers.

To identify microbial diseases, Sun and Chen (1989) carried out studies on Ling Shan Island (China) and isolated 11 genera of bacteria, from the foregut, hindgut, coelomic fluid and the integument of *Stichopus japonicus*. The bacteria belonged to the following genera: *Vibrio*, *Pseudomonas*, *Neisseria*, *Acinetobacter*, *Flavobacterium*, *Arthrobacter*, *Micrococcus*, *Xanthomonas*, *Corynebacterium*, *Caulobacter*, and *Alcaligenes*. Four genera of yeast — *Torulop-*

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*sis*, *Rhotorula*, *Cryptococcus* and *Debaryomyces* — were also found in sea cucumbers and *Achromobacter* and *Bacillus* were the dominant genera in muddy environments (Sun and Chen 1989). *Bacillus* strains are commonly found in mud but a few of them are found in *Stichopus japonicus* species (Sun and Chen 1989).

At the aqualab hatchery in Madagascar, a highly contagious skin ulceration disease appeared on juvenile *Holothuria scabra* and spread quickly (Becker et al 2003). This disease has also been reported in *Aspistichopus japonicus* in China, in *Isostichopus fuscus* in Ecuador, and in *H. scabra* in Australia and New Caledonia (Becker et al. 2003). The results of this study indicate that a combination of events and agents, including bacteria, are required to induce the disease. *Vibrio* species (close to *V. harvey* and *V. alginolyticus*), bacteroid species and “alpha” proteobacteria were identified from the diseased animals.

Due to the availability of sea cucumbers in China, studies are frequently carried out to improve management and to control diseases. Wang et al. (2004) illustrated several non-reported diseases of *Aspistichopus japonicus*, including syndromes of rotting edges, stomach ulcerations in auricularia stages, and autolysis of young juveniles that were caused by bacterial agents. Skin ulcerations, epidermal erosion, and body oedema were triggered by various pathogens including bacteria, fungi and parasites during outdoor cultivation. The authors noted that in 2002–2004 the high mortality rate caused great economic loss, which was due to three epidemic diseases called “the syndrome of rotting edges”, “the syndrome of off-plate” and “the syndrome of skin erosion”. All were caused by *Vibrio* species and the results concluded that vibriosis is the main disease in farmed sea cucumbers. The authors recommended the use of antibiotics as preventive measures. *Vibrio* grow attached to algae and many reach high population densities after being ingested with algae and then expelled from digestive tubes with lysed algae in faecal pellets; they are gut bacteria in aquatic animals, including zooplanktons (Sun and Chen 1989).

### **Efficacy of probiotics in disease management**

A probiotic can be defined as a cultured product or live microbial feed supplement, which beneficially affects the host by improving its intestinal (microbial) balance (Fuller et al 1989). Verschuere et al. (2000) expanded the definition to include a live microbial adjunct that has a beneficial effect on the host by modifying its associated or ambient microbial community, by ensuring improved use of the feed or enhancing its nutritional value, and by improving the quality of its ambient environment.

With successful evidence from past decades, Irianto and Austin (2002) revealed that microalgae (tetraselmis), yeasts (*Debaryomyces*, *Phaffa* and *Saccharomyces*), gram-positive bacteria (*Carnobacterium*, *Enterococcus*, *Lactobacillus*, *Lactococcus*, *Micrococcus*, *Streptococcus* and *Weissella*) and gram-negative bacteria (*Aeromonas*, *Alteromonas*, *Photobacterium*, *Pseudomonas* and *Vibrio*) and immuno-stimulants (polysaccharides, glycans) can be used as successful probiotics. Unfortunately, no research has been conducted to investigate the application of probiotics to sea cucumber farming.

The humic substances found in bottom sediments, sea and lake waters, soils, peat and coals, resulting from the decomposition of organic matter, particularly dead plants, consist of a mixture of complex macromolecules with polymeric phenolic structures (Flaig et al. 1975; Kupryszewski et al. 2001). The antimicrobial characteristics of 12 preparations of humic substances isolated from seawater, marine bottom sediments and lake water were examined by Kupryszewski et al. (2001). The focused humic substances, humic acids and fulvic acids exhibited varied anti-microbial activities that can be used successfully in disease control. Verschuere et al. (2000) reported that the use of antibiotics does not allow microbial control and may result in an unfavourable change in microbiota. The establishment of a normal gut microbiota may be regarded as complementary to the establishment of the digestive system and under normal conditions serves as a barrier against invading pathogens.

Recently, Gorski et al. (2003) observed that bacteriophage bind to animal cells, including lymphocytes, and perhaps exert immuno-modulatory actions. It is reported that bacteriophage has a dramatic effect on infection, resulting in a 98% reduction in bacterial titre. These bacteriophages could be used as a simple vaccination strategy involving oral delivery of phage tablets or liquid suspensions (Gorski et al. 2003). Verschuere et al. (2000) reported that most probiotics proposed as bio-control measures are lactic acid bacteria (e.g. *Lactobacillus*, *Carnobacterium*), *Vibrio* (e.g. *V. alginolyticus*), *Bacillus* and *Pseudomonas*. A variety of *Vibrio* phages are prevalent in the Gulf of Mexico (Kellogg et al 1995). Moebus and Nattkemper (1983) revealed that 362–366 phage-sensitive bacteria isolated from the Atlantic belonged to the family Vibrionaceae and that 280 of them were *Vibrio* species.

The activities of microorganisms as probiotics in aquaculture or as biological control agents were described by Verschuere et al. (2000) and Irianto and Austin (2002). In particular, the use of probiotics in shrimp farming is highly effective and used worldwide. Ackermann (2003) illustrated that most pathogenic bacteria can be controlled by using their host phage (Table 1).

**Table 1.** Host range of major phage group (Ackermann 2003)

Phage group	Bacterial group or genus
Caudovirales	Eubacteria, Euryarchaeota
Microviridae	Enterobacteria, Bdellovibrio, Chlamydia, Spiroplasma
Cotocoviridae	Alteromonas
Tectiviridae	(a) Enterics, Acinetobacter, Pseudomonas, Thermus, Vibrio (b) Bacillus, Alicyclobacillus
Leviviridae	Enterics, Acinetobacter, Caulobacter, Pseudomonas,
Cystoviridae	Pseudomonas
Inoviridae	
(a) Inovirus	Enterics, Pseudomonas, Thermus, Vibrio, Xanthomonas
(b) Plectrovirus	Acholeplasma
Plasmaviridae	Acholeplasma
Lipothrixviridae	Crenarchaeota: Acidians, Sulfolobus, Thermoproteus
Rudiviridae	Crenarchaeota: Sulfolobus
Fuselloviridae	(a) Crenarchaeota: Aciaians, Sulfolobus (b) Euryarchaeota: Methanococcus, Pyrococcus

Tovar et al. (2002) pointed out that some strains of yeast produce polyamines, which enable the yeast to adhere to intestinal mucus. In this study it was found that the yeast *Debaryomyces hansenii* HF1 (DH), isolated from fish guts, has the ability to secrete polyamines in significant amounts.

Villasin and Pomory (2000) developed a method to extract substances with antibacterial activities from sea cucumbers. A methanol acetone extract from the body wall of the sea cucumber *P. parvimensis* had antibacterial properties against two species of bacteria (*Bacillus subtilis* and *Enterococci coli*). Ridzwan et al. (1995), tested extracts from the sea cucumber *H. atra*, *H. scabra* and *Bohadshia argus* against seven species of bacteria and found that lipid and methanol extracts had no inhibitory activity, though a phosphate buffered saline extract did have inhibitory activity. Because sea cucumbers lack a well-developed immune system and can ingest pathogenic bacteria together with food, some form of active antibacterial substances must be present in the body for defence (Ridzwan et al. 1995).

## Discussion

Because oceans cover three-quarters of the planet, marine phages are probably the most prevalent life forms. Approximately 2100 bacteriophages were listed in 1981, and this amount increases annually by about 100 for a present total of more than 4000. Several bacteriophages have been intensively investigated at the molecular level and the interaction between these bacteriophages and their hosts under laboratory conditions is well understood (Frank and Russell 2000). The majority of pathogenic bacteria associated with

sea cucumbers are common pathogenic species and possible bacteriophages have been investigated. Further research should concentrate on controlling sea cucumber diseases by using probiotics. The use of disinfectants and antimicrobial drugs has had limited success in the prevention or cure of bacterial diseases because of their ability to produce resistant genes and transfer genes from generation to generation. However, production and use of probiotics in successful disease control depends on an awareness of the relationship between particular species and strains of bacteria.

Management of water quality in an appropriate manner enhances the health of cultured animals. Disin-

fection methods normally used in wastewater treatment on land-based fish farms are ultraviolet radiation, ozonation and chlorination. Because effluent from fish farms normally consists of suspended solids, organic matter and different strains of harmful microorganisms, it is essential to focus on disinfection of effluent and pond environments to ensure they are free of pathogenic microorganisms, thus avoiding frequent disease outbreaks. Bomo et al. (2003) reported on the use of low-cost infiltration systems, such as sand filters, as an alternative to environmentally hazardous chemicals for the disinfection of fish-farm wastewater.

Some associated microorganisms present in sea cucumbers exhibited the ability to secrete chitinase (Sun and Chen 1989) and some other polysaccharides. Chitin is a natural polymer that exhibits antibacterial activities, and more attention should be paid to using natural substances as disease control agents. Many other extracts of sea cucumbers indicate antimicrobial activities that can be used in disease control methods.

There is evidence that diseases occurring in this new industry can be controlled in an eco-friendly manner to avoid outbreaks and develop antibiotic-resistant pathogens in the future. The authors of this review have isolated several marine bacteria and yeast species associated with sea cucumbers; two strains of yeasts were taxonomically identified as *Yarrowia lipolytica* and *Candida tropicalis*, which are capable of producing phytase. Identified yeast strains were deposited at the National Center for Biotechnological Information (NCBI), USA, under the accession numbers DQ 438177 and DQ 515959, respectively. The authors are conducting further research on these microorganisms.

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## Fact sheets and identification guide for commercial sea cucumber species

M. Verónica Toral-Granda<sup>1</sup>

The sustainable harvesting and conservation of sea cucumbers has been the subject of debate in various fora in recent years (e.g. ASCAM and CITES workshops). Such discussions have led to the identification of a need to develop a comprehensive guide to help enforcement officials and researchers. Although the original idea for this FAO publication was a simple identification guide for major commercial sea cucumber species, it was decided that this guide would also be the perfect opportunity to gather available scientific information on the biology, ecology, marketing and processing activities, as well as photos and descriptions of commercial species at different processing levels.

Jointly, with Alessandro Lovatelli from FAO, a two-page questionnaire has been developed, asking users about information on the topics mentioned above. The format allows users to fill in information using a computer, with the questionnaire then returned to me via email or regular mail. The information would then be later collated into a book to be published by FAO, under direct collaboration with the Charles Darwin Foundation. The questionnaire has already been distributed by email to fellow colleagues working on sea cucumbers (i.e. scientists, managers). However, if you feel you can contribute to this project but have not been contacted, please let me know and I will email you a questionnaire.

A short description of the information required for each species is included in Box 1, and respondents can contact me if they have any questions. Even species for which there is little or no information will be included in the guide, using the best available data. All contributions will be duly acknowledged in the book and a complimentary copy of the book sent for personal use.

So far, 43 species have been identified, based on the work of Prof Chantal Conand and further work carried out during the CITES workshop held in Kuala Lumpur in March 2004. This list of species (Table 1) is not final, however, and species can be amended, deleted and changed. This is where fellow colleagues conducting sea cucumber research will have a very important role, as I would appreciate any input you may have on a particular species. If you know of any other species that should be in-

cluded, fill in and return the questionnaire. If you know of other species, but are not able to contribute all of the information required, send me the contact details of an expert on that species so that I can contact them for the necessary information.

Besides specific information for each species, photos are also necessary of 1) the live animal, 2) the processed animal (in different forms), and 3) the calcareous spicules.

The procedure for obtaining spicules is rather simple and can be done in a few hours by following the steps below.

- Cut a slice (1 cm<sup>2</sup> x 1 mm thickness) from the dorsal area.
- Place the sample in a small test tube with 3 mL of commercial bleach (NaOCl) and label it for further identification.
- Leave the sample sit for approximately 30 min or until the body wall has dissolved and the spicules have settled on the bottom as fine white sediment.
- Using a pipette, transfer the fine white sediment onto a microscope slide, cover it with a cover slip, and examine using a microscope with 100 x magnification.
- Organise the spicules on your microscope slide and take a photograph. Please make sure that the picture size is *at least* 12 cm x 9 cm in 350 dpi resolution (or 1600 x 1200 pixels).

To avoid mistakes and deliver the highest quality product as possible, FAO asked Drs Jean-Francois Hamel, Annie Mercier, Sven Uthicke, Steve Purcell and Prof Chantal Conand to be part of a scientific committee that will oversee this work and amend errors as needed.

Some of you were present at the FAO-ASCAM workshop (Dalian, China, October 2003), some at the CITES workshop (Kuala Lumpur, Malaysia, March 2004), and some of you are regular contributors to the SPC *Beche-de-Mer Information Bulletin*, thus your feedback and help is important. Also, any suggestions are greatly appreciated. I kindly ask for your support, which will yield an invaluable tool for the well-being of commercial sea cucumbers species.

1. Charles Darwin Foundation. Email: [vtoral@fcdarwin.org.ec](mailto:vtoral@fcdarwin.org.ec)

Our very ambitious goal is to have all the information for this guide ready by the end of August 2006; therefore, I ask you to please fill in your question-

naire as soon as possible, so that all of the relevant information can be collated and submitted to FAO. I look forward to your help.

### Box 1: Description of the information required

1. **Scientific name:** The genus and species of your species in trade, including who named the species.
2. **English name:** The English name of your species in trade. If unavailable, provide the best translation of the local name.
3. **Local name:** The name under which your species in trade is known locally. Identify the language in which it is named (i.e. Pepino de mar, Spanish).
4. **Prepared/compiled by:** Your name as a contributor to this book. Further details on your affiliation and contact details will be provided at the end of the card.
5. **Family:** Taxonomic family to which your species in trade belongs to (e.g. Holothuriidae, Stichopodidae, Cucumaridae)
6. **Origin:** The region or area from where the information for your species in trade is provided. Please include country and continent.
7. **FAO region:** Code number of the FAO region where the information for your species in trade is provided.
8. **Photo:** Photograph of live specimen of your species in trade in the card. If possible, in its natural environment. Include name of photographer.
9. **Live appearance:** Written description of your live species in trade. Include colour, texture and general appearance.
10. **Average fresh weight (g):**
11. **Average fresh length (cm):**
12. **Growth:**
13. **Distribution range:** Geographical range where your species in trade is found.
14. **Habitat:** Include preferred substratum and depth range.
15. **Reproductive biology:** Information on size of maturity (SOM), or size at which 50% of the population is reproductive. Please specify if size corresponds to weight or length, and to fresh, gutted or dry specimens; reproductive season if annual, bi annual, continuous; and when in the year (months) reproduction occurs. Mean fecundity estimates, average number of eggs per gonad, larval development if lecithotrophic or planktotrophic, average egg diameter in microns (mm).
16. **Type of fishery:** (i) **Subsistence** fishery is when the product caught is consumed directly by the families of the fishers, rather than being bought by middle-(wo)men and sold at the next larger market; or, "a fishery where the fish caught are shared and consumed directly by the families and kin of the fishers rather than being bought by middle-(wo)men and sold at the next larger market";<sup>a</sup> (ii) **Artisanal** fisheries involve skilled but non-industrialised operators; typically, a small-scale, decentralized operation; normally subsistence fisheries although sometimes the catch may be sold. Usually fishing trips are short and inshore and fishing vessels are small;<sup>b</sup> or, "traditional fisheries involving fishing households (as opposed to commercial companies), using relatively small amount of capital and energy, relatively small fishing vessels (if any), making short fishing trips, close to shore, mainly for local consumption. Artisanal fisheries can be

a. <http://www.fao.org/glossary>

b. <http://www.fishbase.org/Glossary>

subsistence or commercial fisheries, providing for local consumption or export. They are sometimes referred to as small-scale fisheries;<sup>a</sup> (iii) **Semi-industrial** fisheries are those when some sort of mechanization of the fishing method occurs, the fishing vessels are bigger and have a bigger storing capacity; and, (iv) **Industrial** fisheries include bigger vessels with more storing capacity and non-artisanal fishing techniques. The produce is sold.

17. **To the author's knowledge, the population status is:** This represents the author's knowledge on the health of the population of your species in trade.
18. **Main market:** This is where most of the species are exported to, as a start of the chain of trade, if any.
19. **Main use:** The main human use for your species in trade. If "other", please specify. If needed, provide further information in # 30.
20. **Management of the fishery (if marked, please specify):** This will provide information on the different regulations used to control a sea cucumber fishery. Whenever you mark one, please specify.
21. **Fishing methods/fishing gear used:** This could be by means of scuba diving, hookah (an air compressor on the surface, which provides air by means of a hose) diving, hand collecting, weighted hooks, etc.
22. **Domestic consumption:** Please identify if your species in trade is also consumed locally. Please provide quantitative values (e.g. 30% of the total catch is consumed domestically).
23. **Processing technique:** Explain how you obtained your final product (i.e. beche-de-mer, gamat oil, viscera, etc).
24. **Conversion factors:** This refers to the weight change from live animal to brine, or live animal to dry.
25. **Photo of processed animal:** Photograph of processed specimen of your species in trade. Include name of photographer.
26. **Processed appearance:** Written description of what your live species in trade looks like. Include colour, texture and general appearance.
27. **Local price (in USD):**
28. **Photo of spicules:** Photograph of spicules specimen of your species in trade. Include name of photographer.
29. **Spicule description:** Specify the types of spicules found in the dorsal body wall of your species.
30. **Other useful information:** Please feel free to add any information you think would be valuable for this guide. Expand on any item number.

**References:** Please include references in the above items as this is a scientific guide. Provide full reference of the work cited. This will be included at the end of the guide.

**Author contact details:** Please specify your contact details, as this will be included at the end of the guide as a quick reference on sea cucumber experts and collaborators to the guide.

**Authorisation:** In order to publish this information, FAO requires your authorisation for the use of the information and photos provided in your card. The authorisation included in this guide is intended only for this guide. If you are not the photographer for the illustration, please provide contact details of the photographer, and I will contact them directly.

**Photo quality:** Please make sure that picture size is at least 12 cm x 9 cm in 350 dpi resolution (or 1600 x 1200 pixels). Please save each photo with the species name, region and author (for example: LIVE-I.fuscus-Galapagos-VTorai.jpg), and send them as a .jpg files. Include a scale in cm.

**Table 1.** Main commercial species with their commercial value, conservation concern and common name(s).

#	Species <sup>a</sup>	Family	Commercial value	Conservation concern <sup>b</sup>	Common name(s)
1	<i>Athyonidium chilensis</i>	Cucumariidae		3	Pepino de mar
2	<i>Cucumaria frondosa</i>	Cucumariidae		3	Pumpkins; Orange footed cucumber
3	<i>Pattalus mollis</i>	Cucumariidae		5	Pepino de mar
4	<i>Actinopyga lecanora</i>	Holothuriidae	Medium	3	Stonefish
5	<i>A. agassizi</i>	Holothuriidae		4	
6	<i>A. echinites</i>	Holothuriidae	Low	2	Deep-water redfish
7	<i>A. mauritiana</i>	Holothuriidae	Medium	2	Surf redfish
8	<i>A. miliaris</i> <sup>a</sup>	Holothuriidae	Medium	2	Blackfish; Hairy blackfish
9	<i>A. palauensis</i>	Holothuriidae	Medium	4	
10	<i>Bohadschia argus</i>	Holothuriidae	Low	3	Tigerfish; Leopardfish
11	<i>B. atra</i>	Holothuriidae	Low	5	
12	<i>B. marmorata vitiensis</i> <sup>a</sup>	Holothuriidae	Low	4	
13	<i>B. similis</i>	Holothuriidae	Low	4	Chalkfish; Brownspeckled sandfish
14	<i>B. subrubra</i>	Holothuriidae	Low	5	
15	<i>B. vitiensis</i> <sup>a</sup>	Holothuriidae	Low	4	Brown sandfish
16	<i>Holothuria arenicola</i>	Holothuriidae	Low	5	
17	<i>H. (Halodeima) atra</i>	Holothuriidae	Low	4	Lollyfish
18	<i>H. cinerascens</i>	Holothuridae	Low	5	
19	<i>H. coluber</i>	Holothuridae	Low	4	Snakefish
20	<i>H. edulis</i>	Holothuridae	Low	4	Pinkfish
21	<i>H. fuscogilva</i> <sup>a</sup>	Holothuriidae	High	1	White teatfish
22	<i>H. fuscopunctata</i>	Holothuriidae	Low	3	Elephant trunkfish
23	<i>H. impatiens</i>	Holothuriidae	Low	5	
24	<i>H. leucospilota</i>	Holothuriidae	Low	4	
25	<i>H. mexicana</i>	Holothuriidae		4	Donkey dung
26	<i>H. nobilis</i> <sup>a</sup>	Holothuriidae	Medium	1	Black teatfish
27	<i>H. scabra</i>	Holothuriidae	High	1	Sandfish
28	<i>H. scabra versicolor</i> <sup>a</sup>	Holothuriidae	High	2	Golden sandfish
29	<i>H. whitmaei</i>	Holothuriidae			Black teatfish
30	<i>Pearsonothuria graeffei</i>	Holothuriidae	Low	4	Flowerfish; Blackspotted sea cucumber
31	<i>Astichopus multifidus</i>	Stichopodidae		4	
32	<i>Isostichopus badionotus</i>	Stichopodidae		3	
33	<i>I. fuscus</i>	Stichopodidae	Medium	1	Brown sea cucumber
34	<i>Parastichopus californicus</i>	Stichopodidae		3	Giant red sea cucumber
35	<i>P. parvimensis</i>	Stichopodidae		4	Warty sea cucumber
36	<i>Stichopus mollis</i>	Stichopodidae		5	Pepino de mar
37	<i>S. (Apostichopus) japonicus</i>	Stichopodidae	High	4	
38	<i>S. chloronotus</i>	Stichopodidae	Medium	2	Greenfish
39	<i>S. hermannii (S. variegatus)</i> <sup>a</sup>	Stichopodidae	Medium	2	Curryfish
40	<i>S. horrens</i>	Stichopodidae	Medium	2	Warty sea cucumber; Selenka's sea cucumber
41	<i>Thelenota anax</i>	Stichopodidae	Medium	3	Amberfish
42	<i>T. ananas</i>	Stichopodidae	High	1	Prickly redfish
43	<i>T. rubralineata</i>	Stichopodidae	Low	4	

a. Species taxonomy to be revised

b. Conservation concern: 1 = high concern, 2 = concern in certain countries of its range, 3 = potential for future concern as harvest increases, 4 = no concern, and 5 = minor species of little commercial importance. Modified from Bruckner (2006).

## Observation of natural spawning of *Holothuria tubulosa*

Observer: Horst Moosleitner

Dates (moon phase):

- August 1972 (moon phase unknown)
- 18–19 August 1994 (3–4 days before full moon)
- 9–10 July 1997 (5–6 days after new moon)
- 11 July 2003 (2 days before full moon)
- 18 July 2003 (5 days after full moon)

Observation time: always from 16:00–17:00

Location: Mediterranean, Aegean Sea around peninsula of Chalkidike

Depth: 3–10 m

Tide: probably not relevant (less than half a meter)

### Description

Spawning of *Holothuria tubulosa* in the Mediterranean is well known, but most observations have been published in popular magazines or books (Erhardt and Moosleitner 1995; Moosleitner 1974; Riedl 1963; Weinberg 1992), and not in scientific journals. Riedl (1963) was perhaps the first person to record that *Holothuria* spp. are capable of raising two thirds of their body in order to release sperm and eggs.

I first observed the spawning of *H. tubulosa* in 1967 and published an article in *Submarine Magazine* (1974). Since that time, I have often seen this species spawning, and noted some of these observations when I took photographs (see below).

*Holothuria tubulosa* is the most common species of sea cucumber in the Mediterranean, living on soft bottoms sometimes in dense populations. Spawning occurs in the summer months (observed in July, August and September) in the afternoon from about 16:00 to 18:00, sometimes until sundown. Observations have shown that spawning starts with a few isolated specimens, followed by many others. So it is proposed that some males begin spawning, and their sperm includes informative substances that push other males and females to participate in the spawning.

A relationship between spawning events and moon phases is not clearly established in the above data but there seems to be a preference for spawning close to a full moon (within 5–6 days before or after).

Only once were some single specimens of *Holothuria polii* (clearly recognizable by the white ended podia and dorsal papillae) seen spawning at the same time as *H. tubulosa* (no exact data noted).

It should also be noted that some fish (*Chromis chromis* and *Coris julis*) were observed feeding on the spawn (see photo).

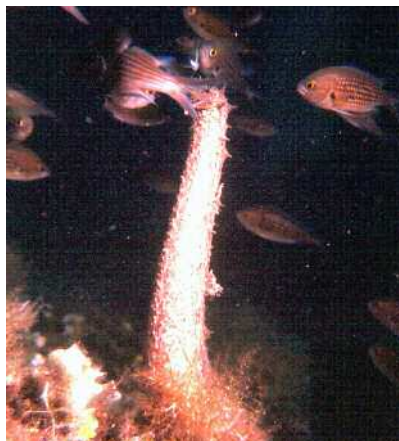
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Images: Horst Moosleitner



*Holothuria tubulosa* spawning.



A group of *Chromis chromis* feeding on spawn of *H. tubulosa*.



*Holothuria polii* spawning.

## Observation of natural spawning of *Bohadschia vitiensis*

Observer: Sylvie Gaudron<sup>1</sup>

Date: 19 February 2006

Observation time: 15:30–18:15

Location: Lagoon St Gilles-les-Bains, La Saline, Trou d'Eau, Réunion Island, Indian Ocean

Depth: 0.5–1 m back reef

Tide: ebb tide

Moon phase: 6 days after full moon

### Description

Observations of natural spawning of *Bohadschia vitiensis* have been described in past issues of this bulletin (Desurmont 2005, 2006, New Caledonia; Durville 1996; and Rard 2004, Reunion Island) and in the reviews of McEuen (1988) and Conand (1989).

This spawning event occurred during daytime after a tropical storm, when there was an increase in fresh-water runoff into the lagoon. The first observation was made in the mid-afternoon (15:30) where one specimen was observed in the typical upright spawning position. As soon as the observer went close to the specimen it laid down on the soft bottom. One hour later, 20 additional *B. vitiensis* were observed, covered with a thin layer of sediment, within a 100 m<sup>2</sup> area around the specimen. At 16:45 two other specimens were observed in upright positions, and one was releasing a thin layer of white sperm by its swollen gonopore. This individual did not sway. The same specimen was subsequently observed releasing sperm while laying down. The observation was conducted for another hour and a half, but no more spawning was observed. Several authors have described these two positions for spawning (either upright or laying flat) on substratum in *Bohadshia* spp. (e.g. McEuen 1988).

Interestingly, Rard (2004) observed spawning of two individuals of *Bohadschia vitiensis* in an upright position in the same location at the end of April 2004 at 17:00, as did Durville (1996) on 15 February 1995.

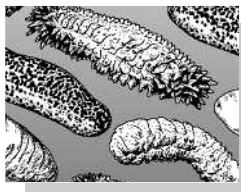
Was this spontaneous spawning a response to an environmental stress due to the tropical storm? Only more data about the natural spawning of sea cucumbers will answer this question.

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*Bohadschia vitiensis* spawning .



## Abstracts & publications

beche-de-mer

### Publication

#### **Restocking and stock enhancement of marine invertebrate fisheries**

*J.D. Bell, P.C. Rothlisberg P.C, J.L. Munro, N.R. Loneragan, W.J. Nash, R.D. Ward and N.L. Andrew*

**Source:** *Advances in Marine Biology* 49, 370 p.

Many of the world's fisheries are in trouble — they no longer yield the catches, and potential profits, they once did. The main problems are overfishing and destruction of important habitats. Overfishing means catching too many of the spawning adult fish, and too many of the juvenile fish before they become adults. The habitats that support fisheries have been damaged by pollution and other careless use of coastal land. Destructive fishing methods like trawling and blast fishing have also changed fish habitats so that they now support fewer fish.

Governments are responding to the fisheries crisis by raising awareness of the problems, and by legislating to restore and protect fisheries habitats and reduce fishing effort. But they are also intrigued by the possibility of using the recent gains in aquaculture technology to speed up the restoration of stocks.

Through aquaculture, it is now possible to produce juveniles of many marine species in hatcheries. Release of cultured juveniles is an appealing way to increase the stock because people can see fish going into the water and conclude that there will be more fish to catch.

But hatchery releases can be a two-edged sword. It can be very expensive to produce juveniles in hatcheries, which can mean that not enough juveniles are released to make a real difference to the stock. Bad hatchery practices can also produce "unfit" juveniles that have a different genetic make-up, and transfer diseases, to wild fish. Another potential downside is that fishermen may not worry so much about overfishing if they think that a restocking program will repair any damage that they do. As a result, many believe that money spent on hatchery releases would be better spent on other types of management, such as better policing of catch limits, minimum legal sizes and closed areas.

The counterpoints are that some fisheries have been so badly overfished that it will take decades for them to recover to the point where people can harvest the animals again. In such cases, releasing cultured juveniles could "fast-track" the build-up of the spawning adults needed to replenish the fishery. Releasing cultured juveniles for this purpose is called "restocking".

In other cases, although there are enough spawning adults some areas of good habitat still fail to produce many fish even when fishing is well-regulated. This can occur, for example, because the tiny juveniles are swept away by currents as they migrate to nursery habitats, such as mangroves and seagrasses. When this happens, there will be few fish to harvest once they grow up. Putting juveniles into a habitat where they sometimes fail to arrive naturally is called "stock enhancement".

This book summarises what has been learned about restocking and stock enhancement for valuable shellfish (clams, scallops, abalone, conch, trochus, shrimps, lobsters, sea urchins and sea cucumbers). The research that has been done to overcome the disadvantages of restocking and stock enhancement is weighed carefully. Clear guidance is then given to managers about the steps to follow to decide whether use of hatchery-reared juveniles can add value to the other methods they use to restore fisheries.

For ordering information please refer to:

[http://www.elsevier.com/wps/find/bookdescription.cws\\_home/706346/description#description](http://www.elsevier.com/wps/find/bookdescription.cws_home/706346/description#description)

## Summary

### The restocking of sea cucumbers in the Pacific Islands

S.C. Battaglene and J.D. Bell

**Source:** p. 109–128. In: Bartley D.M. and Leber, K.M. (eds.). Marine ranching. FAO Fisheries Technical Paper. No. 429. Rome, FAO. 213 p. (2004).

Restocking with hatchery-reared juveniles has great potential to help Pacific Islands break the long-term “boom and bust” cycle of the beche-de-mer fishery. The main benefit of restocking is that it provides a way to “fast-track” the restoration of stocks to the point where they can be managed for sustainable yields.

There are four steps to delivering the benefits promised by restocking: (1) development of methods for the mass-production of juveniles, (2) learning how to release the cultured sea cucumbers in the wild so that they survive in high proportions, (3) protecting the released sea cucumbers until stocks are replenished and (4) managing the restored stocks to obtain sustainable harvests of first-grade beche-de-mer (i.e., large individuals). The same technology used for restocking can then be applied to supplement the yields of restored stocks by releasing cultured juveniles in stock enhancement programmes to overcome recruitment limitation. This would allow the production of sea cucumbers to approach the carrying capacity of the habitat.

Although the way to restore and manage the fishery for sea cucumbers in the Pacific is now apparent, only some of the technology and procedures needed to achieve these goals are currently in place. Furthermore, the research has been confined largely to just one species, sandfish (*Holothuria scabra*). The major challenges remaining for restocking of sandfish are: learning how to scale-up the rearing techniques to produce the hundreds of thousands of juveniles needed to have an impact on the abundance of stocks; developing effective strategies for releasing cultured juveniles in the wild; and assessing the economic viability of restocking. In all three cases, however, there is cause for optimism. If research underway to investigate the potential for co-culture of sandfish and shrimp is successful, the mass-production of juvenile sandfish may be possible as a by-crop of shrimp farming. Otherwise, it should be possible to mass-produce sandfish in dedicated ponds, albeit at greater cost. There is no obvious impediment to the development of release strategies, nor are there any apparent reasons why releases cannot be done in a responsible way. For example, guidelines are in place for the management of broodstock to maintain genetic diversity, the risk of introducing diseases to conspecifics and other species appears to be low, and the fact that sandfish are low in the food chain means that there should be little or no impact on other fisheries species. Preliminary estimates also indicate that restocking should be economically viable in terms of returns to artisanal fishermen, although we hasten to point out that such an evaluation cannot be done thoroughly until the necessary research has been completed.

The key to harnessing the potential benefits of restocking sandfish is support from regional/international research organizations, fishing communities, governments and development agencies. The regional/international research organizations need to complete the development of cost-effective methods for restocking sandfish so that this tool is ready for application throughout the Pacific. The fishing communities need to be prepared to forego catches until the stocks are rebuilt, and then to comply with the advice and/or regulations designed to ensure that yields of high value can be obtained each year.

Governments must provide the framework for this process by implementing a moratorium on the export of sandfish until the stocks recover, applying a total export quota based on sustainable yields when the fishery is reopened and ensuring that there is compliance with these laws. Finally, as most governments in the Pacific do not have the financial or human resources to produce the very large numbers of juveniles required to replenish stocks, support is needed from development agencies for restocking programmes. They could also assist by contributing to the costs of developing the measures needed for sustainable management of the restored fishery.

Overall, the prospects for restocking sandfish look particularly promising and every effort should be made to bring this potential management tool to the attention of fishing communities, governments and development agencies so that they stand ready to support and implement restocking programmes as soon as the full suite of methods required becomes available.



## Abstracts

### **Restocking the sea cucumber *Holothuria scabra*: Sizing no-take zones through individual-based movement modelling**

S.W. Purcell and D.S. Kirby

Source: Fisheries Research 80:53–61 (2006)

The valuable sea cucumber *Holothuria scabra*, known as “sandfish”, has potential for restocking. However, there is little information available to determine the size of the no-take zones (NTZs) needed to protect the released animals so that they can form nucleus breeding populations. To do this, we measured short-term movement paths of released juvenile (1–105 g) and wild adult (130–690 g) sandfish in a seagrass bed in New Caledonia. We then developed an individual-based model (IBM) to predict long-term dispersal of sandfish released as juveniles (1–16 g) at 1 individual m<sup>-2</sup> within a 1-ha area, drawing on distributions of speed and directionality and the relationship between speed and animal weight from field data. Movement was non-random at the sampling scale used, since animals tended to turn <90° at each 2-h time step. We examined high- and low-growth scenarios by applying 50% and 25% of the modelled growth rates of sandfish held in earthen ponds (where they are known to grow faster). The dispersal of released sandfish was predicted to be limited in the first 2 years, then markedly faster thereafter. After 10 years, 6–12% of surviving animals were predicted to remain in the original 1-ha release site. To protect surviving sandfish as nucleus breeding populations for 10 years, and accepting 10% spillover, square NTZs would need to be 19–40 ha. The findings are useful for the management of restocking and pre-defining the size of sites for recapture surveys. Our model allows user-specified values for future releases and should be applicable for other sedentary marine invertebrates where basic data on movement and growth are available.

### **Efficacy of chemical and physical tags for large-scale release of an exploited holothurian**

S.W. Purcell, B.F. Blockmans and W.J. Nash

Source: Journal of Experimental Marine Biology and Ecology 334:283–293 (2006)

Hatchery-produced juveniles need to be distinguished from wild conspecifics in order to evaluate the success of restocking experiments and stock enhancement. The commercially valuable sea cucumber *Holothuria scabra*, or “sandfish”, has potential for stock enhancement but cheap, long-lasting tags have not yet been developed. We evaluated five non-genetic tags against several criteria: cost, ease of application, retention rate, and ease of detection. In a 1-month trial, T-bar tags and visible elastomer implants proved unsuitable on hatchery-produced sandfish juveniles. Coded-wire tags and two fluorochromes, tetracycline and calcein, showed more promise, so retention rates of these tags were tested on juveniles held in an earthen seawater pond for 1 year. Sixty per cent of juveniles retained at least one of two coded-wire tags for 1 year. Under UV illumination, spicules from the body wall of sandfish immersed in solutions of tetracycline are yellow, whereas calcein-stained spicules are green. From months 1 to 12 after immersion, the average percentage of stained spicules declined from 40% to 8% with tetracycline and 54% to 5% with calcein. After 1 year stained spicules were detected in all immersed animals, i.e. mark retention was 100%. Stained spicules from the ventral body wall of juveniles were generally in greater proportions, and fluoresced brighter, than those from the dorsal body wall. Spicule density exceeded 7000 spicules per mm<sup>2</sup> of body wall in juveniles of 2–13 g, and decreased with sandfish weight. Fluorescence provides a simple diagnostic tool for identifying marked individuals and the sampling is non-destructive. Several mm<sup>2</sup> of outer body wall is ample for tag identification. Immersion-staining with fluorochromes is preferred to coded-wire tags and provided a cheap and reliable batch-mark for distinguishing released and wild sandfish.

### **Experimental evaluation of co-culture of juvenile sea cucumbers, *Holothuria scabra* (Jaeger), with juvenile blue shrimp, *Litopenaeus stylirostris* (Stimpson)**

S.W. Purcell, J. Patrois and N. Fraisse

Source: Aquaculture Research 37:515–522 (2006)

The co-culture of juvenile sea cucumber *Holothuria scabra* (Jaeger), or “sandfish”, with juvenile blue shrimp *Litopenaeus stylirostris* (Stimpson) was tested by growing groups in co-culture and monoculture for 3 weeks

in tanks with enriched sand substratum. Feed was supplied on trays, accessible only to shrimp. Survival of shrimp and sandfish was high in all treatments (73–100%). Growth of shrimp did not differ between monoculture and co-culture, but sandfish grew significantly slower in co-culture ( $P = 0.03$ ), although their sand burying and surface foraging were apparently unaffected by shrimp ( $P = 0.76$ ). However, shrimp increased the levels of total ammonia-N in tanks, which related inversely with sandfish growth ( $P = 0.04$ ). Conversely, sandfish did not appear to lower the water quality for shrimp culture. While sandfish bioturbate sediments and eat organic deposits, the juveniles did not significantly reduce the organic content of sand in tanks. Co-culturing juveniles of the two species in earthen ponds appears feasible, with no detriment to shrimp production, presenting a cost-effective method for growing sandfish to larger sizes for restocking. These findings underpin further studies to test the viability of commercial co-culture of sandfish with blue shrimp at later stages in the production cycle of shrimp.

**Effect of invasive tagging on the activity of *Holothuria whitmaei* [Echinodermata: Holothuroidea]: A suitable mark-recapture method for short-term field studies of holothurian behaviour**

G. Shiell

Source: Marine and Freshwater Behaviour and Physiology 39(2):153–162 (2006)

Although mark-recapture techniques are commonly used to obtain ecological data, tagging of soft-bodied holothurians has proven difficult, and the degree to which invasive tags may confound the behaviour of holothurians remains unclear. In this study, diurnal patterns of activity were monitored in the commercial sea cucumber *Holothuria whitmaei* (black teatfish) after marking the tegument superficially with a number measuring 30 x 20 mm (to a depth of  $\leq 2$  mm). Rates of activity in marked specimens increased significantly in the initial hours following marking, and remained higher than those of unmarked control animals for up to three days following the procedure. These results suggest (a) that behaviour in holothurians may initially be compromised by marking the tegument and (b) that improved measures of activity may be obtained following a suitable recovery period. This is a useful finding given the need for improved knowledge of commercial holothurian biology, including diurnal and seasonal patterns of behaviour.

**The influence of diets containing dried bivalve feces and/or powdered algae on growth and energy distribution in sea cucumber *Apostichopus japonicus* (Selenka) (Echinodermata: Holothuroidea)**

X. Yuan, H. Yang, Y. Zhou, Y. Mao, T. Zhang and Y. Liu

Source: Aquaculture 256(1–4):457–467. (2006).

In recent years, bivalve feces and powdered algae have been used as the food sources of holothurians in China. In this study, growth and energy budget for sea cucumber *Apostichopus japonicus* (Selenka) with initial wet body weights of  $32.5 \pm 1.0$  g (mean  $\pm$  SE,  $n = 45$ ) when fed with five different granule diets containing dried bivalve feces and/or powdered algae in water temperature 13.2–19.8°C and salinity 30–32 ppt were quantified in order to investigate how diets influence growth and energy distribution and to find out the proper diet for land-based intensive culture of this species. Results showed that diets affected the food ingestion, feces production, food conversion efficiency and apparent digestive ratios, hence the growth and energy budget. Sea cucumbers fed with dried feces of bivalve showed poorer energy absorption, assimilation and growth than individuals fed with other four diets; this could be because feces-drying process removed much of the benefits. Dried bivalve feces alone, therefore, were not a suitable diet for sea cucumbers in intensive cultivation. The mixed diets of feces and powdered algae showed promising results for cultivation of sub-adult *Apostichopus japonicus*, while animals fed with powdered algae alone, could not obtain the best growth. According to SGR of tested animals, a formula of 75% feces and 25% powdered algae is the best diet for culture of this species. Extruded diets were used in the present experiment to overcome shortcomings of the traditional powdered feeds, however, it seems a conflict exists between drying bivalve feces to form extruded diets and feeding sea cucumbers with fresh feces, which contain beneficial bacteria. Compared with other echinoderms, in holothurians the energy deposited in growth is lower and the energy loss in feces accounts for the majority of the ingested energy. Such detailed information could be helpful in further development of more appropriate diets for culture of holothurians.

### Effects of body size and water temperature on food consumption and growth in the sea cucumber *Apostichopus japonicus* (Selenka) with special reference to aestivation

H. Yang, X. Yuan, Y. Zhou, Y. Mao, T. Zhang and Y. Liu

Source: Aquaculture Research 36(11):1085–1092 (2005)

To investigate the effects of body size and water temperature on feeding and growth in the sea cucumber *Apostichopus japonicus* (Selenka), the maximum rate of food consumption in terms of energy ( $C_{maxE}$ ; J day<sup>-1</sup>) and the specific growth rate in terms of energy (SGRe; % day<sup>-1</sup>) in animals of three body sizes (mean ± SE) — large (134.0 ± 3.5 g), medium (73.6 ± 2.2 g) and small (36.5 ± 1.2 g) — were determined at water temperatures of 10, 15, 20, 25 and 30° C. Maximum rate of food consumption in terms of energy increased and SGRe decreased with increasing body weight at 10, 15 and 20° C. This trend, however, was not apparent at 25 and 30° C, which could be influenced by aestivation. High water temperatures (above 20° C) were disadvantageous to feeding and growth of this animal; SGRe of *A. japonicus* during aestivation was negative. The optimum temperatures for food consumption and for growth were similar and were between 14 and 15° C, and body size seemed to have a slight effect on the optimal temperature for food consumption or growth. Because aestivation of *A. japonicus* was temperature dependent, the present paper also documented the threshold temperatures to aestivation as indicated by feeding cessation. Deduced from daily food consumption of individuals, the threshold temperature to aestivation for large and medium animals (73.3–139.3 g) was 24.5–25.5° C, while that for small animals (28.9–40.7 g) it was between 25.5 and 30.5° C. These values are higher than previous reports; differences in sign of aestivation, experimental condition and dwelling district of test animals could be the reasons.

### Feeding and growth on bivalve biodeposits by the deposit feeder *Stichopus japonicus* Selenka (Echinodermata: Holothuroidea) co-cultured in lantern nets

Y. Zhou, H. Yang, S. Liu, X. Yuan, Y. Mao, Y. Liu, X. Xu and F. Zhang

Source: Aquaculture 256(1–4):510–520. (2006).

Suspension aquaculture of filter-feeding bivalves has been developing rapidly in coastal waters in the world, especially in China. Previous studies have demonstrated that dense populations of filter-feeding bivalves in shallow water can produce a large amount of faeces and pseudofaeces (biodeposits) that may lead to negative impacts on the benthic environment. To determine whether the deposit feeder *Stichopus* (*Apostichopus*) *japonicus* Selenka can feed on bivalve biodeposits and whether the sea cucumber can be co-cultured with bivalves in suspended lantern nets, three experiments were conducted, two in tanks in the laboratory and one in the field. In a 3-month flow-through experiment, results showed that sea cucumbers grew well with specific growth rate (SGR) reaching 1.38% d<sup>-1</sup>, when cultured in the bottom of tanks (10 m<sup>3</sup> water volume) where scallops were cultured in suspension in lantern nets. Moreover, results of another laboratory experiment demonstrated that sea cucumbers could survive well on bivalve biodeposits, with a feeding rate of 1.82 ± 0.13 g dry biodeposits ind<sup>-1</sup> d<sup>-1</sup>, absorption efficiency of organic matter in biodeposits of 17.2% ± 5.5%, and average SGR of 1.60% d<sup>-1</sup>. Our longer-term field experiments in two coastal bays (Sishili Bay and Jiaozhou Bay, northern China) showed that *S. japonicus* co-cultured with bivalves also grew well at growth rates (0.09–0.31 g wet weight ind<sup>-1</sup> d<sup>-1</sup>) depending on individual size. The results suggest that bivalve lantern nets can provide a good habitat for sea cucumbers; and the co-culture of bivalve molluscs with sea cucumbers may provide an additional valuable crop with no additional inputs.

### Role of microtubules and centrosomes in the eccentric relocation of the germinal vesicle upon meiosis reinitiation in sea-cucumber oocytes

A. Miyazaki, K.H. Kato and S. Nemoto

Source: Developmental Biology 280(1):237–47 (2005)

In the oocytes of many animals, the germinal vesicle (GV) relocates from the center to the periphery of the oocyte upon meiosis reinitiation, which is a prerequisite to the formation of meiotic spindles beneath the cell surface in order for meiosis to succeed. In the present study, we have investigated nuclear positioning using sea-cucumber oocytes. Upon meiosis reinitiation, the GV relocates to the cell periphery beneath a surface protuberance. After GV breakdown, polar bodies were extruded from the top of the protuberance, which we therefore called the animal pole process. The GV relocation was inhibited by nocodazole but not by cytochalasin. Immunofluorescent staining and electron microscopy of microtubular arrays revealed that:

(i) in immature oocytes, two centrosomes were situated beneath the animal pole process far apart from the GV, anchoring to the cortex via astral microtubules; (ii) upon meiosis reinitiation, microtubular bundles were newly formed between the centrosomes and the GV; and (iii) the microtubular bundles became short as GV migration proceeded. These observations suggest that microtubules and centrosomes participate in GV relocation. A very large mass of annulate lamellae, having a 20-microm diameter, was found in the vegetal pole of the oocytes.

### **Isolation and characterization of twenty microsatellite loci in Japanese sea cucumber (*Stichopus japonicus*)**

M. Kanno, Q. Li and A. Kijima

Source: Marine Biotechnology 7(3):179–183 (2005)

Twenty microsatellite markers were first developed from the Japanese sea cucumber *Stichopus japonicus* using an enrichment protocol. Of the 20 microsatellite loci, 19 loci were polymorphic in the population examined. At these polymorphic loci, the number of alleles per locus varied from 2 to 15, and the observed heterozygosities ranged from 0.03 to 0.97, which is considerably higher than those previously found for allozymes. The high variability of the microsatellite markers identified in this study will make them excellent tools for genetic analyses of *S. japonicus*.

### **A review of pygal-furrowed Synallactidae (Echinodermata:Holothuroidea), with new species from the Antarctic, Atlantic and Pacific oceans**

M. O'Loughlin and C. Ahearn

Source: Memoirs of Museum Victoria 62(2):147–179.

<http://www.museum.vic.gov.au/memoirs/index.asp>

We review the genera and species of pygal-furrowed Synallactidae and describe new species from the collections of the United States National Museum of Natural History (Smithsonian Institution). *Meseres* Ludwig is reviewed and placed incertae sedis. *Molpadiodemias* Heding and *Pseudostichopus* Théel are rediagnosed. *Platystichopus* Heding is declared a junior synonym of *Molpadiodemias*. *Filithuria* Koehler and Vaney and *Peristichopus* Djakonov are declared junior synonyms of *Pseudostichopus*. Lectotypes are designated for: *Meseres involutus* Sluiter, *M. macdonaldi* Ludwig, *M. peripatus* Sluiter, *Pseudostichopus globigerinae* Hérouard, *P. mollis* Théel, *P. pustulosus* Sluiter, *P. trachus* Sluiter, *P. villosus* Théel. A neotype is designated for *Pseudostichopus nudus* Ohshima.

Except for the type species, *Meseres macdonaldi* Ludwig, and *Meseres (?) torvus* (Théel), all species previously referred to *Meseres* are reassigned to *Molpadiodemias* or *Pseudostichopus*. *Meseres (?) torvus* (Théel) is retained in its original combination. Eleven new synallactid species are described: *Molpadiodemias constrictus*, *M. crinitus*, *M. epibiotus*, *M. helios*, *M. morbillus*, *M. neovillosus*, *M. pediculus*, *M. porphyrus*, *M. translucens*, *M. ustulatus* and *Pseudostichopus tuberosus*. *Molpadiodemias constrictus* is fissiparous. *Pseudostichopus villosus* var. *violaceus* Théel is raised to species status; *Pseudostichopus alatus* Imaoka, *P. trachus* Sluiter and *P. nudus* Ohshima are junior synonyms of *Pseudostichopus mollis* Théel; *Pseudostichopus globigerinae* Hérouard and *P. dilatorbis* Imaoka are junior synonyms of *Meseres* (= *Molpadiodemias*) *involutus* Sluiter; *Pseudostichopus propinquus* Fisher and *P. lapidus* Hérouard are junior synonyms of *Meseres* (= *Pseudostichopus*) *peripatus* Sluiter; and *Pseudostichopus arenosus* Ohshima and *P. molpadioides* Ohshima are junior synonyms of *Meseres* (= *Pseudostichopus*) *hyalegerus* Sluiter.

New combinations are: *Molpadiodemias atlanticus* (Perrier), *M. depressus* (Hérouard), *M. involutus* (Sluiter), *M. pustulosus* (Sluiter), *M. villosus* (Théel), *Pseudostichopus elegans* (Koehler and Vaney), *P. hyalegerus* (Sluiter), *P. papillatus* (Djakonov), *P. peripatus* (Sluiter) and *P. spiculiferus* (O'Loughlin). Other species discussed are: *Molpadiodemias violaceus* (Théel), *Pseudostichopus aemulatus* Solís-Marín and Billett, *P. echinatus* Thandar, *P. mollis* Théel, *P. occultatus* Marenzeller, and *P. profundus* Djakonov. A key is provided for the species of *Molpadiodemias* and *Pseudostichopus*. Tables are provided of genera and subgenera with current systematic status, and species with original and current combinations.

**A new Indo-West Pacific species of *Actinopyga* (Holothuroidea:Aspidochirotida: Holothuriidae)**

Y. Samyn, D. Vandenspiegel and C. Massin

Source: Magnolia Press Zootaxa 1138:53–68 (2006) and <http://www.mapress.com/zootaxa/>

*Actinopyga* is one of the five genera commonly recognised in the family Holothuriidae. This small genus has sixteen species currently considered valid. The present paper describes a new Indo-West Pacific species, *Actinopyga caerulea*, of which the most striking character is its bluish coloration. The ossicle assemblage of the new species resembles mostly that of *A. bannwarthi* Panning, 1944 and *A. flammea* Cherbonnier, 1979.

**New host and geographical distribution for the pearlfish *Carapus mourlani* (Carapidae) with a discussion on its biology**

E. Parmentier, A. Mercier and J.-F. Hamel

Source: Copeia 1:122–128 (2006)

Specimens of the pearlfish *Carapus mourlani* (Carapidae) were observed for the first time in association with the sea cucumber *Isostichopus fuscus* (Holothuroidea: Echinodermata) along the coast of Ecuador. Out of 4345 sea cucumbers collected from various depths between 5 and 60 m, 12 harbored a pearlfish either in the coelomic cavity, the respiratory tree or the digestive tract, yielding a prevalence of ca. 0.28%. The presence of *C. mourlani* appeared to be detrimental to the holothurian host in some cases. Side effects resulting from coelomic cavity infections included less advanced gonad maturity (reduced gonadal tubule diameter and length, lower ratio of mature oocytes) and a significant proportion of necrotic and shriveled gonadal tubules, devoid of gametes. Aside from discussing this evidence, the present paper briefly describes the biology of the pearlfish, its relationship with the host and its daily activity cycle.

**Note on the association between *Plakobranthus ocellatus* (Mollusca, Gastropoda, Opisthobranchia) and *Holothuria atra* (Echinodermata, Holothuroidea)**

A. Mercier and J.-F. Hamel

Source: Les Cahiers de Biologie Marine 46:399–402 (2005)

This paper presents evidence of a commensal association between an opisthobranch (*Plakobranthus ocellatus*) and a sea cucumber (*Holothuria atra*). Field observations revealed that occurrences of *P. ocellatus* specimens and egg filaments were much more frequent on the body wall of *H. atra* than on that of two other holothurian species living in the same habitat. Roughly 95% of *H. atra* were colonized by *P. ocellatus* during the night, which corresponds to the active period of *H. atra* and the resting period of *P. ocellatus*. The proportion decreased to ca. 21% in the daytime when the sea cucumbers are resting and most opisthobranchs are foraging. Laboratory trials confirmed the daily pattern of association and the fact that *P. ocellatus* lay their eggs on the body wall of *H. atra*. Furthermore, multiple-choice experiments showed that ca. 71% of *P. ocellatus* favoured *H. atra*, whereas only ca. 9% colonised other species of sea cucumber. Because *H. atra* is known to exude toxic chemicals that deter several species of invertebrates and fishes, the opisthobranch may use the sea cucumber as a refuge against predators and as a secure spawning ground.

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

S.W. Purcell, B.F. Blockmans, N.N.S. Agudo

Source: Aquaculture 251:238–244 (2006)

**Holothurians role in the marine sediments reworking processes**

M. Dar

Source: Sedimentology of Egypt 12:173–183 (2004)

**Synallactes laguardai, a new species of sea cucumber from South Africa (Echinodermata: Holothuroidea: Aspidochirotida: Synallactidae)**

F.A. Solís-Marín.

Source: Proceedings of the Biological Society of Washington 118(3):570–575 (2005).

**A simple enzymatic method for examining calcite ossicles of Echinodermata**

C.G. Tiago, A.D. Brites, and G.Y. Kawauchi

Source: Journal of Microscopy 218(3):240–246 (2005)

**Regional sea cucumber project: Proceedings of the start-up workshop, Mombasa, Kenya, 26–29 January 2006**

N. Muthiga and C. Conand (eds)

Source: <http://www.wiomsa.org/default.asp?groupid=2004121144037123>

**A few recent publications on echinoderms in Spanish**

**Los equinodermos de las islas Lobos de Afuera (Lambayeque, Perú)**

Y. Hooker, F.A. Solís Marín and M. Lellish.

Source: Revista Peruana de Biología 12(1):77–82 (2005)

**Catálogo de los equinodermos (Echinodermata: Crinoidea, Echinoidea, Holothuroidea) nerítico-bentónicos del Archipiélago Cubano**

R. Valle García, F.A. Solís-Marín, M. Abreu Pérez, A. Laguarda-Figueras y A. Durán-González

Source: Revista de Biología Tropical 53 (Supl. 3):9–28 (2005)

**Equinodermos (Echinodermata) de la zona marina económica exclusiva del Golfo de México**

A. Durán-González, A. Laguarda-Figueras, F.A. Solís-Marín, B.E. Buitrón Sánchez, C.A. Gust y J. Torres-Vega

Source: Revista de Biología Tropical, 53 (Supl. 3):53–68 (2005)

**Equinoideos (Echinodermata: Echinoidea) del Golfo de México**

A. Laguarda-Figueras, A.I. Gutiérrez-Castro, F.A. Solís-Marín, A. Durán-González y J. Torres-Vega

Source: Revista de Biología Tropical 53 (Supl. 3):69–108 (2005)

**Equinodermos (Echinodermata) del Caribe Mexicano**

A. Laguarda-Figueras, F.A. Solís-Marín, A. de La Luz Durán-González, C. Ahearn, B. Estela Buitrón Sánchez y J. Torres-Vega

Source: Revista de Biología Tropical, 53 (Supl. 3):109–122 (2005)

**Equinodermos (Echinodermata) del Golfo de California, México**

F.A. Solís-Marín, A. Laguarda-Figueras, A. de La Luz Durán-González, C. Ahearn Gust y J. Torres Vega

Source: Revista de Biología Tropical 53 (Supl. 3):123–137 (2005)

## PhD and Masters theses

### **Habitats and macroinvertebrate fauna of the reef-top of Rarotonga, Cook Islands: implications for fisheries and conservation management**

D.J. Drumm

**Source:** PhD Department of Marine Science. University of Otago, Dunedin, New Zealand (282 + xvii p.) (2004)

Throughout the Pacific, many species of echinoderms and molluscs have cultural value and are harvested extensively in subsistence fisheries. Many of these species are sedentary and often associated with distinct reef-top habitats. Despite the significance of reef habitats and their fauna for fisheries, and biodiversity etc., little information has been available on the distribution of habitats and their influence on the reef-top fauna in the Cook Islands. This thesis developed a novel approach to assess the status of the shallow-water reef-tops of Rarotonga, Cook Islands, to provide critical information to fisheries and conservation managers. The approach used remote sensing (aerial photography with ground truthing) to map the spatial arrangement and extent of the entire reef-top habitats accurately, and historical wind data and coastline shape to determine the windward and leeward sides of the island. The benthic habitat maps and degree of wind exposure were used to design and undertake a stratified sampling programme to assess the distribution and abundance of the epibenthic macroinvertebrate fauna of the reef-top. I quantified the distribution and abundance of the epibenthic macroinvertebrates and how they varied with habitat, assessed the effectiveness of a traditional *ra'ui* (marine protected area) for conserving stocks of *Trochus niloticus* and other invertebrates, and investigated the reproductive biology and impacts of traditional gonad harvesting on *Holothuria leucospilota*.

There were four major habitat types (rubble/rock, sand/coral matrix, algal rim and sand) identified, the most extensive being rubble/rock (45%) and sand/coral matrix (35%). The degree of exposure to winds was found to correlate with the reef development and habitat distribution.

The assemblage composition of each major habitat type differed significantly from every other habitat. The rubble/rock habitat had the greatest substratum heterogeneity and structural complexity, and the highest number of species and individuals. The overall abundance of the fauna was dominated by holothurians (68%) and echinoids (30%), while *Trochus niloticus* and *Tridacna maxima* accounted for the remaining 2% of the total invertebrate assemblage. Clear habitat partitioning was also found for adult and juvenile *Trochus niloticus* and *Tridacna maxima*.

In the traditional fishery for *Holothuria leucospilota*, the mature gonads of males are harvested by making an incision in the body wall of the animal, removing the gonads and then returning the animal to the reef to allow regeneration. Monthly collections of *H. leucospilota* were used to describe the reproductive biology of this species. Gametogenesis and spawning were synchronous between the sexes and spawning occurred annually during summer, when water temperature and photoperiod were at their highest. Although the incision in the body wall and gonad removal had no impact on the survival of *H. leucospilota* in experimental cages, their body weight, and general sheltering and feeding behaviours were affected. Gonads took at least 41 days to start regenerating, suggesting a considerable delay in the spawning of fished individuals.

In 1998, five Rarotongan communities re-introduced the traditional *ra'ui* system of resource management, prohibiting all fishing and gathering from their reefs. The performance of the Nikao *ra'ui*, which had been put in place to allow trochus stocks to increase, was investigated. Comparisons of macroinvertebrate assemblage composition and species density were made between three fishing treatments, i.e. fished areas adjacent to the *ra'ui*, within the *ra'ui* after two years of protection, and in the *ra'ui* after it had been lifted for three weeks to allow a commercial trochus harvest. Analysis of variance on the count data for the twelve most abundant species, and non-metric multi-dimensional scaling indicated that there were no differences in the microhabitat or the invertebrate assemblage composition between the three fishing treatments. However, there were significant differences between the rubble/rock and sand/coral matrix habitat types. The results on the effectiveness of the Nikao *ra'ui* are equivocal, due to the small sample size and the variability between samples, which was highlighted by the wide confidence intervals.

This study highlights the importance of habitat to the macroinvertebrate fauna of the reef-top and the need for accurate habitat maps to increase the cost-effectiveness of future resource surveys, to provide information to management, and for the design of marine protected areas. The mapping and survey methods must

be reliable and repeatable in terms of the limitations of time, and the availability of expertise, funding and resources. The results provide important information for fisheries and conservation managers of Rarotonga and other Pacific Islands to better design rigorous sampling programmes for monitoring the status of reef-top resources, and for evaluating and planning marine protected areas.

### **Matter and energy transfers in soft-bottom sediments of an anthropogenic reef complex (La Réunion Island, Indian Ocean)**

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Source: PhD La Reunion University 6/06/2006

Coral reefs are very productive ecosystems. Soft-sediments generally play a role in storing place, in transforming and in producing of matter, and therefore could play a key role in reefs. The goal of this study was to evaluate the role of soft-bottoms compartment during the transfer of matter and energy in the reefs of Reunion Island. Three main lines of research were developed: the physicochemical characterisation of studied sites, the metabolism of soft-bottoms and, the characterisation of biological component by the study of macrofauna and holothurians. A limited disturbance was detected by physicochemical data although locally enrichments of freshwater by nutrients were measured. At the reef scale, the soft-sediments were found heterotrophic (net production = 31.6, respiration  $R = 109.6$ , gross production  $P_g = 77.9$  mmolC  $m^{-2} d^{-1}$  et  $P_g/R = 0.7$ ). Opposite to the two holothurians (*Holothuria atra* and *H. leucospilota*), macrofauna did not constitute a major group in the food web (134 taxa, with a mean of 32.86 ind.  $0.2 m^{-2}$  et 0.059 g (dry weight)  $0.2 m^{-2}$ ) compared to echinoderm biomass that could reach 7.92 g (dry weight)  $m^{-2}$ . At the station scale, spatial heterogeneity of metabolism was in agreement with the physicochemical characteristics of environment reflecting the double influence of ocean and land. The most productive and heterotrophic stations were located in the back-reef where the oceanic influence was the lowest. Finally, macrofauna was under hydrodynamic influence although holothurians are growing in the most productive area. The soft-bottoms played a key role in filtrating organic matter provided from the back-reef. This mechanism was deeply influenced by the high hydrodynamism, which modulated the loss of mater and energy of the reef. These losses were however limited by the action of holothurians (high density 3 ind.  $m^{-2}$ ), which store organic matter such as biomass and probably enhanced local production. To conclude soft-bottoms were responsible of recycling, which was reinforced by holothurians that limited the loss of matter and energy due to hydrodynamism.

### **Reproduction sexuée de *Holothuria leucospilota* à La Réunion**

S. Kohler (supervised by C. Conand and S. Gaudron)

Source: Mémoire de Maîtrise de biologie des populations et écosystèmes. Université de La Réunion. 25 p. + annexes (2006)

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