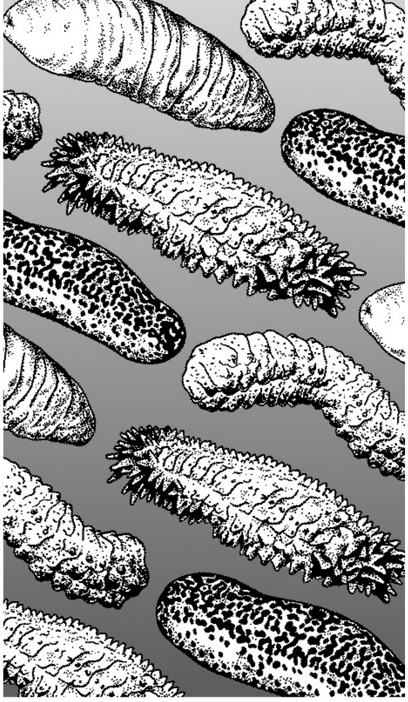

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Inside this issue

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Editorial

This issue principally includes seven original papers, the list of publications on sea cucumbers by Dr D.B. James and the abstracts about holothuroids presented during the 9th International Echinoderm Conference held in Hobart (Tasmania) in January.

The first paper is from S.W. Purcell et al. (p. 3). They complete the published results of Conand, Skewes and other authors in estimating the change in length and weight, during processing stages, of several tropical commercial species for which data were lacking.

A.D. Morgan reports that survival of larvae of *Australostichopus mollis* relies on the characteristics of the females laying the eggs and particularly on the number and size of the eggs that the females are able to lay (p. 7).

P. Purwati et al. show that for *Holothuria atra*, the natural fission plane may no longer be important in fission inducement and that it could be manipulated. In their induced fission experiments, the survival rate reached almost 100 per cent (p. 16).

T. Lavitra et al. detail the problems related to the farming of *H. scabra* in Madagascar and explain how to solve these problems or avoid them completely (p. 20). New parasites and predators are mentioned and the effects of a drop of salinity occurring after hurricanes are analysed.

M.H. Hassan exposes the stock assessment of holothuroid populations in the Red Sea waters of Saudi Arabia (p. 31). As has happened elsewhere, sea cucumber traders heavily exploited Saudi Arabia's Red Sea coastline for about five years. Consequently, a dangerous depletion in the fishery has resulted with populations of both *H. fuscogilva* and *H. scabra* completely destroyed.

In Madagascar, G. Robinson and B. Pascal expose how local communities, non-governmental organisations (NGOs) and private-sector stakeholders are working together to pioneer a form of village-based mariculture in which coastal communities raise hatchery-reared juvenile sea cucumbers in simple sea pens (p. 38).

X. Kun and H. Yang close the articles in presenting a quantitative analysis of the phagocytosis by amoebocytes in *Apostichopus japonicus* (p. 44).

In the 'Communications' section, M. de la Torre-Castro gives news about the work that her team is doing in Zanzibar (Tanzania) (p. 47), and Z. Ilias informs us about the first assays of grow-out of *Stichopus horrens* in Malaysia (p. 48).

At the 9th Echinoderm International Conference, 29 presentations (oral and posters) concerned holothuroids; their abstracts are presented here (p. 50). It is also a pleasure to publish here the list of the publications by Dr D.B. James, who devotes his professional life to sea cucumbers and to the methods used to rear them in aquaculture (p. 61).

As usual, this and all previous issues of the bulletin are available in PDF format on SPC's website at: http://www.spc.int/coastfish. And I'd like to attract your attention to the SPC Fisheries Digital Library, available on SPC's website (http://www.spc.int/mrd/fishlib.php). It gives access to electronic versions (in PDF format) of more than 7300 fisheries- and aquaculture-related documents (in English and French), produced by, for, or in collaboration with SPC. It includes a search engine that allows full-text searches as well as searches by title, author, and year. The search can be limited to one publication, such as this bulletin. For example, a search in full text of the words '*Holothuria* AND *fuscogilva*' limited to the *Beche-de-mer Information Bulletin* will give links to 106 articles, downloadable in PDF format.

Igor Eeckhaut

Changes in weight and length of sea cucumbers during conversion to processed beche-de-mer: Filling gaps for some exploited tropical species

Steven W. Purcell[1], Hugues Gossuin[1], Natacha S. Agudo[1]

Abstract

Converting the weights or lengths of sea cucumbers in processed forms (e.g., salted or dried) to their original (live) measurement is essential for standardising data from fishery-dependent surveys and exports. We estimated the proportionate change in length and weight, during processing stages, of several species for which published data were lacking. The wide variation among species in the percentage of weight lost during processing emphasises that conversions should be made on a species-by-species basis. *Stichopus hermanni* shrunk more in length and weight than any other species we studied. We present new estimates for *Actinopyga spinea*, *A. palauensis*, *A. echinites*, *Holothuria lessoni*, and *H. whitmaei*. These findings complement previous studies by filling some gaps in conversion factors for tropical species, and will allow for realistic conversions of data from fishery assessments and national exports.

Introduction

Reporting the weights of sea cucumbers for exports or studies using fishery-dependent surveys is fraught with difficulty and errors because they can be in various states of commercial processing at the time they are measured. Some studies have been conducted on various holothurian species to determine the average change in weight from whole, unprocessed animals to dried beche-de-mer. Conand (1989, 1990) presented a comprehensive table of percentage changes in seven species of commercially exploited sea cucumbers. Skewes et al. (2004) reviewed some additional literature on weight change in processing sea cucumbers, and conducted a further study of six species.

We recently conducted a multi-disciplinary programme in New Caledonia to evaluate wild stocks and current patterns of exploitation of sea cucumbers (Purcell et al. 2009). Animals sold or presented by fishers were in various forms of processing into beche-de-mer. On some occasions, the catch was just gutted; other times, the animals were gutted and salted; other times they were dried already. We needed to be able to convert the individual weights back to whole body weight in order to have a common unit for analyses and to allow comparisons with data from our field population surveys. The conversion factors given by Skewes et al. (2004) allowed us to convert weights of many species, but no published data existed for some of the conversion ratios we needed for other species. This article reports a short study from the programme, in which we estimated the average weight loss at each principal stage of processing for six species for which published data were lacking. It therefore complements the results and reviews by Conand (1989, 1990) and Skewes et al. (2004) by filling in some missing gaps for tropical commercial species.

Methods

The 70 samples for this study were obtained either by accompanying a fisher and using animals he collected, or by using animals we collected during a population survey near Île Ouen, New Caledonia. Once collected, the animals were drained on the deck of the boat for approximately 1 minute, and then the whole body was weighed to nearest 5 g using an electronic balance. The viscera were then removed, by cutting the animals as practised by the fishers. Tags were placed through the body wall of the animals; a plastic label was threaded through the anus with fishing line, and (for *A. palauensis* and *H. whitmaei*) a T-bar tag was also inserted through the body wall. The bodies were then weighed (i.e. gutted weight). Next, salt was added to the sea cucumbers (Fig. 1a) and they were left for 10 days (with two salt changes during that time), which is standard for the processor we worked with. Then each individual was weighed again. For *Stichopus hermanni*, animals were not salted by the fisher, since his practise was to place them immediately on ice (Fig. 1b) and boil them after returning to land. The sea cucumbers were boiled and dried to a hard product (beche-de-mer) and weighed again (Fig. 1c).

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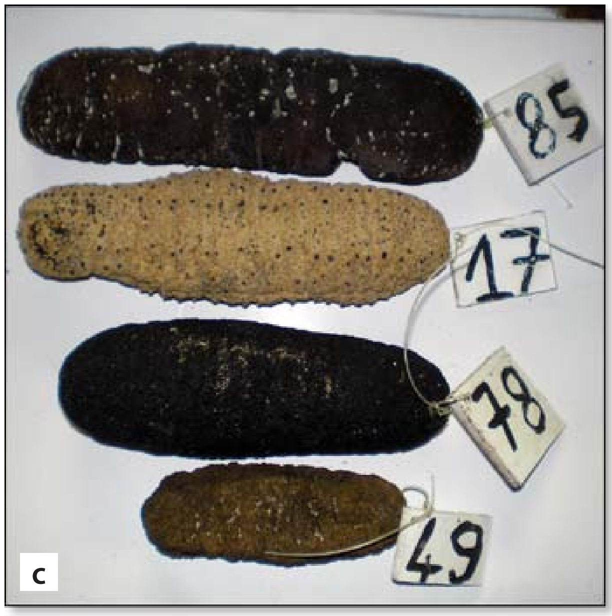

Figure 1. (a) Freshly caught sea cucumbers being salted in a tub, with plastic tags attached through their body wall. (b) Tagged *Stichopus herrmanni* on ice prior to landing and processing. (c) Some of the tagged sea cucumbers after being boiled and dried into well-processed beche-de-mer; from top to bottom: *H. lessoni*, *S. herrmanni*, *A. palauensis*, *A. echinites*. Photos: H. Gossuin

The species and number of individuals for which we calculated weight loss at each successive stage of processing are given in Table 1. Note that some tags were lost during the salting and boiling stages. The loss of tags resulted in fewer replicates for conversion factors of the later stages. Averages of proportionate changes in length and weight that we present are based on values of proportionate loss calculated for each individual separately.

Results

This study provided some reliable estimates of conversion ratios for the six species (Table 2). About 30–45 per cent of the body weight is lost in the initial stage of gutting animals. For species that were salted by the processor we worked with, the salting stage decreases the body weight by a further 12–17 per cent of the initial body weight. At that stage,

Table 1. Number of individuals of the six species measured at different stages of transformation into beche-de-mer.

Species	Number of individuals weighed (n)			
	Whole (fresh)	Gutted	Salted	Dried
Actinopyga echinites	15	15		14
Actinopyga spinea	15	15	13	9
Actinopyga palauensis	7	7	7	7
Holothuria lessoni	11	11	9	8
Holothuria whitmaei	10	10	10	10
Stichopus herrmanni	11			9

Holothuria lessoni, *H. whitmaei* and *Actinopyga palauensis* weighed about one-half of their initial weight. In contrast, *A. spinea* specimens experienced greater weight loss, weighing 38 per cent of their initial weight after salting.

The final boiling and drying stages greatly decrease body weight and length (Table 2). There is a large variation in the percentage reduction in weight of animals to the dried beche-de-mer stage, presumably due to differences in initial content of water in the body tissue of the animals. Dried *Holothuria whitmaei*, *Actinopyga echinites*, and *A. palauensis* averaged about 11–12 per cent of their initial whole body weights. Dried *Holothuria lessoni* averaged about 10 per cent of their initial body weights, while *Actinopyga spinea* averaged 7 per cent of their initial body weights. Of the species we studied here, *Stichopus herrmanni* decreased the most in length and lost the most weight during processing into beche-de-mer; it lost 96.7 per cent of its initial weight through processing to a dried form.

Discussion

As shown in similar studies, the proportion of weight lost through processing varied markedly among species. If export data of dried, or salted, weights of sea cucumbers are to be converted to fresh (landing) weight, it is far more accurate to convert data on a species-by-species basis than to use one approximate factor to convert weights of all species combined. Even within genera, we found large variations in conversion factors for weights. With the exception of *S. herrmanni*, a generality was that dried sea cucumbers were roughly one half the length of their original fresh bodies.

Our finding that dried *Stichopus herrmanni* represented just 3.3 per cent of their initial body weight concurs closely with 3.9 per cent stated by Preston (1990). While Conand (1989, 1990) reported that dried golden sandfish *Holothuria lessoni* (then known as *H. scabra* var. *versicolor*) averaged 6 per cent of their fresh weight, our result of 9.8 per cent is much higher. These findings stress that this species does not lose nearly as much weight during processing as sandfish *H. scabra* (dried weight just 5 per cent of fresh weight — Skewes et al. 2004), even though these species are closely related (Uthicke et al. 2005, Massin et al. 2009). Our results of percentage weight loss for *A. echinites* correspond closely with those of Conand (1989, 1990), but we estimated less weight loss of *H. whitmaei* to the dried form than studies reviewed by Skewes et al. (2004).

Our measurements on *A. spinea* and *A. palauensis* are the first reported for these species, to our

Table 2. Changes in mean body length and weight (± standard error [SE]), and their percentage of initial (whole, fresh) measurements, across the different stages of processing selected species of sea cucumbers into beche-de-mer. Stages: 1 = whole, fresh body; 2 = gutted, fresh; 3 = gutted and salted (after 10 days); 4 = boiled and dried.

		Body length (cm)			Body weight (g)			
	Processing stage:	1	3	4	1	2	3	4
Actinopyga echinites	Mean	19		8	334	231		35
	SE	±0.3		±0.2	±20	±14		±2
	%	100		42.1	100	69.2		10.5
Actinopyga spinea	Mean	27	21	13	1352	735	507	99
	SE	±1	±1	±1	±72	±39	±26	±11
	%	100	77.8	48.1	100	54.4	37.5	7.3
Holothuria lessoni	Mean	31	28	16	2256	1456	1187	221
	SE	±1	±1	±0.2	±80	±50	±32	±7
	%	100	90.3	51.6	100	64.5	52.6	9.8
Stichopus herrmanni	Mean	37		14	2658			88
	SE	±2		±0.3	±154			±5
	%	100		37.8	100			3.3
Holothuria whitmaei	Mean	25	27	15	1829	1174	968	213
	SE	±0.8	±0.9	±0.4	±104	±45	±35	±14
	%	100	108.3	59.9	100	64.2	52.9	11.6
Actinopyga palauensis	Mean	27	23	15	1416	985	740	165
	SE	±0.7	±2	±0.5	±86	±61	±44	±11
	%	100	85.9	53.8	100	69.6	52.3	11.7

knowledge. This study is the only one to date to provide estimates for converting gutted or salted *A. echinites* to the fresh (whole) or dried form. Likewise, we present the first estimates for obtaining fresh or dried weights or lengths from measurements of gutted or salted *H. lessoni* and salted *H. whitmaei*. These results were particularly important for standardising data from catch surveys in our overarching programme because the sea cucumbers were often salted, or sometimes just gutted, at the time of sale when we measured them.

We encourage readers to consult the comprehensive review by Skewes et al. (2004) and use 'recovery rates' of species from studies matching their localities closest. While Skewes et al. (2004) present regression relationships between weight measurements (e.g. live weight vs. dried weight, for each species), we chose not to do this in the current study. Our view was that as any of the measurements theoretically approach zero, so too will the (paired) measurement of the other processed form, so regression relationships should be forced through the origin (i.e. zero). This means that linear regressions we would perform would not have a constant, and the result is, effectively, just a single conversion factor that is multiplied by one measurement to achieve the other.

Acknowledgements

We are grateful to Wigrial Mouzin, a local fisher, who allowed us to take measurements of the sea cucumbers he collected. Our fieldwork was assisted by Bernard Fao, Bureau of Fisheries and Aquaculture, Province Sud. We also thank Warwick Nash, Daniel Taboga and staff of SPC for administrative support. This project was funded predominantly by ADECAL through the ZoNéCo programme in New Caledonia.

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The correlation of attributes of egg source with growth, shape, survival and development in larvae of the temperate sea cucumber *Australostichopus mollis*

Andrew David Morgan[1]

Abstract

The proportion of larvae completing the larval cycle and contributing to subsequent generations can be affected by earlier impacts on egg source or maternal origin. Consequently, during the larval cycle a suite of larval attributes that are impacted on exogenously may be correlated with attributes of egg source. For the temperate sea cucumber *Australostichopus mollis* egg size and egg numbers from four females were correlated with an index of larval viability, which was the proportion surviving, multiplied by the proportion in late auricularia. This survival viability index for larvae from each female was weakly associated with the mean number of hyaline spheres appearing in the folds of the ciliated band. Furthermore, for larvae from each female the ciliated band length to larval length ratio was correlated with the proportion of embryos with features characteristic of normal development, a measure of reproductive success. Although overall shape did not differ between larvae from different females the rate of change in larval shape did. However, growth and development did not differ between larvae from different females and were not correlated with egg numbers and egg size. Growth and development appeared decoupled from any effects of egg source and its association with reproductive success, the rate of change in shape characteristics, or the survival viability index. Larvae that appear competent as expressed in phenotype are not necessarily representative of attributes related to egg source.

Introduction

In aspidochirote sea cucumbers, relative to the number of gametes produced during spawning there is considerable variation in the numbers of larvae surviving the entire larval period and being competent to complete the larval cycle (James et al. 1994; Ito 1995; Ramofafia et al. 1995; Martinez and Richmond 1998; Battaglene et al. 1999; Mercier et al. 2000; Morgan 2000; Asha and Muthiah 2002; James 2004; Mercier et al. 2004; Pitt et al. 2004; Wang and Chen 2004; Sui 2004; Liu et al. 2004; Giraspy and Ivy 2005, 2006; Laximinarayana 2005; Morgan 2008a, b and c). This may occur because of the interaction between attributes related to fertilisation, reproductive success and maternal origin or egg source, and its subsequent impact on larval growth, shape, survival and development.

Variation in size of larvae may be related to the food source of parents and its impact on egg quality and subsequent growth during the larval cycle. In earlier studies egg size was correlated with larval size in *Strongylocentrotus droebachensis* and a number of other echinoderms (McEdward 1986). For instance, in *S. droebachensis* larger eggs have been correlated with animals from a food-rich site, resulting in larvae that increased in size (Bertram and Strathmann 1998). Conversely, in another study no relationship was found between egg size and food rations in captivity for *S. droebachensis* and no difference in larval size was observed (Meidel et al. 1999). The allocation of resources to reproduction does not necessarily correlate with changes in egg size (McEdward and Carson 1987) as individuals may produce more, smaller eggs.

Variation in the shape of larvae has also been related to egg size and implicated as reflecting differences in maternal origin. Variation of larval shape in echinoderms such as the sea urchins *Arbacia lixula* and *S. droebachensis* and the sea star *Pisaster ochraceus* has been related to maternal origin (George et al. 1990; Bertram and Strathmann 1998; George 1999; Meidel et al. 1999). However, for *S. droebachensis* variation in shape as a result of maternal origin was small compared to developmental plasticity in response to food availability (Bertram and Strathmann 1998). Changes in the shape of larvae in relation to maternal origin have also been related to differences in egg quality, either due to different diets in captivity or different habi-

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tats *in situ* (McEdward and Carson 1987; George et al. 1990; George 1999). However, egg size was not correlated with larval shape and changes in ciliated band length in *S. droebachensis* and a number of other echinoderms (McEdward 1986).

Variation in survival and the viability of larvae may reflect differences in maternal origin and reproductive success. Recent studies have determined that fertilisation history affects reproductive success or 'the fraction of a female's eggs that are fertilised' (Levitan 2005). For the sea urchin *Strongylocentrotus franciscanus* differences between sexes in the intensity of sexual selection were dependant on mate density. Despite multiple paternities the variance in reproductive success was lower in males and higher in females (Levitan 2005). In the sea urchins *Heliocidaris erythrogramma* and *Holopneustes purpurescens* mating order influenced the quantity and quality of offspring sired by competing males (Marshall et al. 2004). Differences in egg size between females and its relationship with fertilisation meant that fertilisation history influenced the size distribution of offspring. First spawning males produced larger fitter offspring.

Variation in the development of larvae has also been correlated with differences in egg size and related to differences in maternal origin. For instance, in *S. droebachensis* smaller eggs resulted in delayed development during early larval stages (Sinervo and McEdward 1988), while in another study on *S. droebachensis* larger eggs were correlated with larvae that had a shorter larval period even though development was not affected (Bertram and Strathmann 1998). In *S. droebachensis* the rate of metamorphosis was faster and larvae metamorphosed sooner when originating from parents fed an enriched diet even though there was no effect on growth rate or size at metamorphosis (Meidel et al. 1999).

Previous studies on the effects of egg source on larval growth, shape, survival and development and its implications for larval competency in sea cucumbers are limited. The period of time in captivity has been implicated in affecting egg quality in the sea cucumber *Holothuria scabra* because of a change in broodstock condition (Morgan 2000). Hatch rates and numbers of eggs spawned from *H. scabra* were reduced for broodstock kept in captivity for extended periods of time (Morgan 2000). However, subsequent effects on larval growth and development were not quantified. The size and shape of larvae from *Actinopyga echinites* has been found to differ between ditheothreitol-induced (DTT-induced) shedding of oocytes and resulting larvae, which were smaller, and ovary-induced shedding of oocytes and resulting larvae (Chen et al. 1991). DTT-induced larvae in the mid- to late auricularia larval stages were morphologically different, having an asymmetrical shape (Chen et al. 1991). Without determining the relationship between suites of larval attributes and egg source it is difficult to determine the contribution of parentage to the ability of larvae to complete the larval cycle.

The reproductive season of *Australostichopus mollis* occurs over a period of approximately four months, in summer from November to February (Sewell 1992; Archer 1996; Morgan 2008a). It is hypothesised that for individual females the quality of embryos is related to attributes of egg source such as egg numbers and egg size, which is then expressed as variation in larval phenotype. Changes in growth, shape, survival and development of larvae should relate to egg source and affect the ability of larvae to complete the larval cycle. Three questions were posed in this study: (i) Was there a relationship between egg source and the growth and shape of larvae? (ii) Were larval survival and development related to differences in egg source? (iii) Was there any relationship between the appearance of hyaline spheres prior to metamorphosis and egg source?

Methods

Larval source

Larvae of *Australostichopus mollis* were obtained from broodstock collected in the field once every two weeks and spawned in the hatchery (Morgan 2008a). Spawned eggs from four females were kept separate and fertilised with freshly spawned sperm mixed together from four males. Fertilised eggs were then rinsed and left in a 40 L tub of 1 µm filtered UV-sterilised seawater to hatch (Morgan 2008b). Attributes of egg source were measured and included broodstock weight, egg size from biopsies prior to spawning, numbers of eggs spawned, and the proportion of embryos developing with features characteristic of 'normal' development (see Morgan 2008b). Approximately 1000 ± 100 'normal' early auricularia larvae were counted out for each jar three days after fertilisation. Larvae were concentrated in a 60 mm mesh sieve for counting and used in experiments at an initial concentration of approximately 1 larva ml^{-1}. Larval age was subsequently referred to as time from first feeding.

Larval culture

A windscreen wiper motor was used in conjunction with a variable voltage transformer (1 to 12 volts) to power a paddle stirrer. Glass preserving jars containing 1 L of 1 µm filtered UV-sterilised seawater were used for experiments. Experiments were conducted in a light dark cycle (L:D) of 16:8 hours at a temperature of 20 ± 1°C. The diatom *Chaetoceros muelleri* was cultured in Gillard's F2 medium and during log phase growth fed to larvae at 2000 cells ml^{-1} day^{-1} once a day. There were three replicate jars for larvae from each female.

Every second day 90% of the seawater was siphoned out of each jar using a siphon hose and a 60 mm mesh cup to prevent larvae being extracted. Larvae were removed for morphological measurements by pipetting 5 to 10 mL of seawater containing 15 to 40 larvae into a petri dish. The remaining larvae contained in the seawater in the jars were then drained into a clean jar and 1 mm UV-sterilised seawater added to fill the jar up to 1 l.

Larval growth and development

Larval development was defined by the development of the left and right somatocoel and the axohydrocoel (Smiley 1986; Archer 1996; Morgan 2001; Sewell and McEuen 2002; Morgan 2008c). On days 1, 3, 7, 12, 18 and 22 from first feeding (three days post fertilisation) 10 larvae were measured for growth and development from the 15 to 40 removed from each jar. Measurements were made using a compound microscope and eyepiece micrometer at ´ 100 magnification. Total length, width, posterior length (post-oral hood length), posterior gut (posterior end to the front of the gut), lateral maximum (posterior end to maximum width), lateral depth (maximum depth of the posterior fold), mouth length, mouth width, gut length, gut width, left somatocoel length, and axohydrocoel length were measured and the number of hyaline spheres counted (see Morgan 2008c).

Data analysis

Changes in total length of larvae from each female over successive days were analysed for each experiment. A Tukeys test for a significant difference between means with the Kramer adjustment for multiple comparisons was used to compare means. A one-way analysis of variance was used for the total numbers of surviving larvae counted in each jar at the end of each experiment. Counts were corrected for removal of larvae for measurements. An index of larval viability was also calculated by multiplying the proportion surviving by the proportion in late auricularia. This gave a value for the proportion of larvae likely to complete the larval cycle.

Mixed model analysis of variance in SAS version 8 was used for morphometric variables measured for larvae in each experiment. Effects tested were FEMALE, FEMALE x DAY, REPLICATE within (FEMALE) and DAY x REPLICATE within (FEMALE). Ratios of morphometric variables were analysed simultaneously by reducing the 9 morphometric ratios to their principal components using principal component analysis (PCA) (see Morgan 2008c). Analysis of variance was performed on the first principal component.

Scale-free length ratios were used, as the first principal component in size dependant analysis represents an unknown number of allometrically related shape variables (James and McCulloch 1990; George 1999; Morgan 2008c). Log transformations of length ratio data did not significantly improve normality so the original data was adhered to. For the PCA of length ratios least squares (LS) means were used to compare differences between treatments across days in experiments.

The length of the ciliated band of larvae was calculated for larvae from each female. For each female, four larvae were photographed using a digital camera mounted on a compound microscope. Images were then analysed using SigmaScan Pro software (see Morgan 2008c). The ratio of ciliated band length to all length variables for larvae that were photographed was then compared between females using PCA analysis on day 12.

Discriminant function analysis was used to examine all morphometric variables simultaneously for length ratio and ciliated band length ratio data to determine variables that best reflected differences between larvae from different females. This is used to estimate randomly how efficiently larvae could be discriminated as having come from different females. For each experiment a discriminant function was calculated and each individual larva classified as coming from a particular female.

A log-linear model was used to quantify differences in the numbers of larvae in different stages of development between females. A three-way contingency table was constructed where treatments were FEMALE, DAY (1, 3, 7, 12, 18 and 22) and STAGE (early, mid-, late auricularia). Exclusion of the term 'DAY' in the model would mean accounting for only the summed differences in stages across females in experiments. No information on variation through time could be determined for each stage using this approach.

Results

Larval attributes and egg source

Females with a higher proportion of viable embryos ('normal'), having features characteristic of development, were correlated (0.50) on day 12 with larvae that had a longer ciliated band relative to total larval length. For females F1 and F2, 90% and 80% of embryos respectively were considered to have features characteristic of normal development compared to 65% for F3 and F4.

Larvae with a higher survival viability index were correlated with females that produced more eggs (0.64) and had a larger egg size (0.80). Females F1 and F2 produced 195,000 ± 40,100 and 88,000 ± 20,600 eggs respectively compared to 31,000 ± 1,900 and 62,000 ± 15,300 from F3 and F4 (mean ± SE). In F1 and F2 egg size was 120 ± 17 and 124 ± 23 µm respectively compared to 101 ± 17 and 111 ± 20 µm for F3 and F4 (mean ± SD). No difference in wet weight of broodstock existed for larvae from different females ($p > 0.05$).

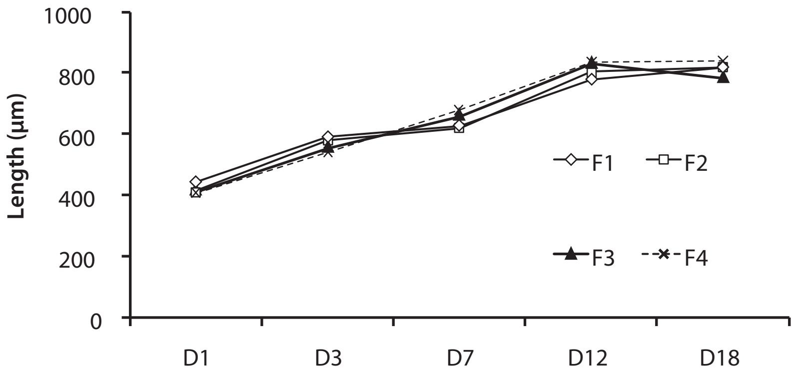

Figure 1. Growth in total length (µm; mean ± SE) of larvae from females where day = day 1 from first feeding (larval age = plus 3 days). F1, F2, F3 and F4 = female number. Larvae were fed 2000 cells ml^{-1} day^{-1} of the diatom *Chaetoceros muelleri*.

Larval growth

Larvae from all four females grew at a similar rate through to day 18, reaching a maximum of 841 ± 22 µm for F3 and a minimum of 781 ± 28 µm (mean ± SE) in F4 ($p > 0.05$; Fig. 1). Larvae on days 7, 12 and 18 were longer than larvae on day 3 and larvae on days 12 and 18 were longer than larvae on day 7 ($p < 0.01$; Tukey-Kramer).

Larval shape

Principal component 1 (PCA1) of length ratio variables accounted for 30% of variation in the data. Differences between females were attributed to the ratios of length to gut length and mouth length to mouth width in PCA1 and gut length to gut width and gut length to lateral maximum in PCA2. Discriminant function analysis showed there was only a 25% to 50% chance of correctly identifying a larva when chosen at random as belonging to any particular female. For PCA1 overall there was no difference in shape characteristics ($p > 0.01$; Fig. 2). However, the rate of change in shape differed as larvae from F1 and F3 tended to retain shorter more rounded guts and mouths for longer, relative to other larval structures as time progressed ($p < 0.01$; LS means). Larvae from F2 and F4 tended to have longer, narrower guts and mouths earlier and were wider and had more lateral folding in the ciliated band earlier.

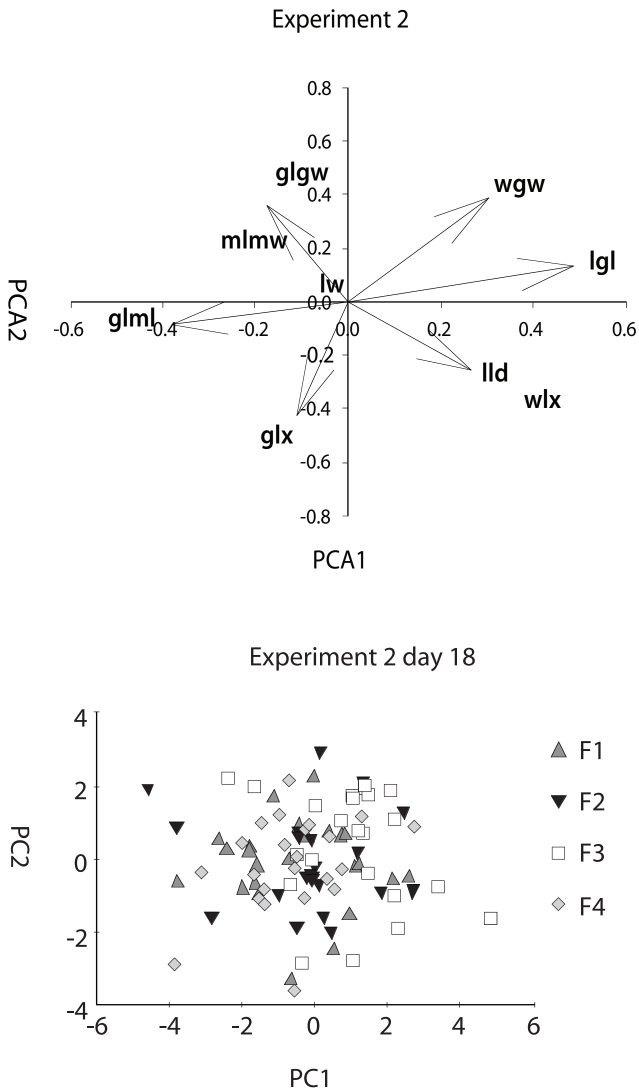

Figure 2. Principal components analysis (PCA) plot of length ratio variables. lw = length/width; glgw = gut length/gut width ; mlmw = mouth length/mouth width; lgl = length/gut length; wgw = width/gut width; glml = gut length/mouth length; glx = gut length/lateral max; wlx = width/lateral max; lld = length/lateral depth.

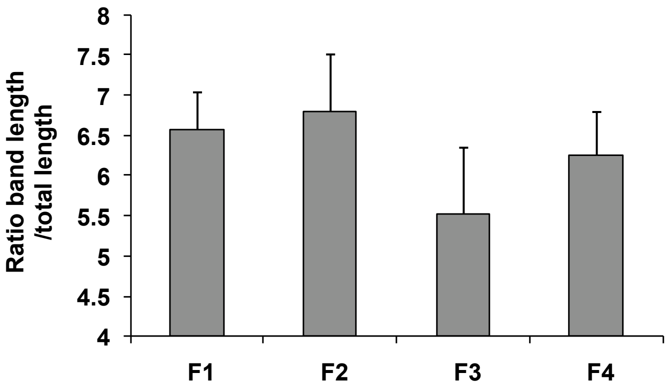

Figure 3. Ratio of ciliated band length to total length for larvae from females F1, F2, F3 and F4 on day 12 (mean ± SE; n = 4 larvae per female).

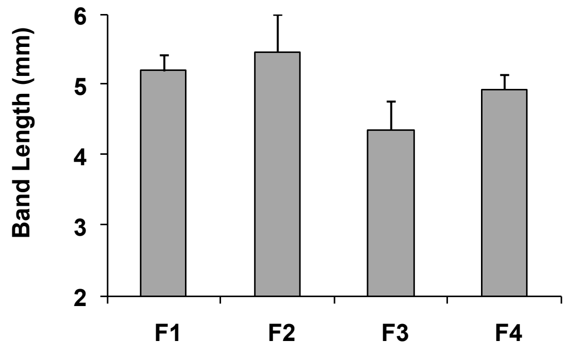

Figure 4. Ciliated band length for larvae from females F1, F2, F3 and F4 on day 12 (mean ± SE; n = 4 larvae per female).

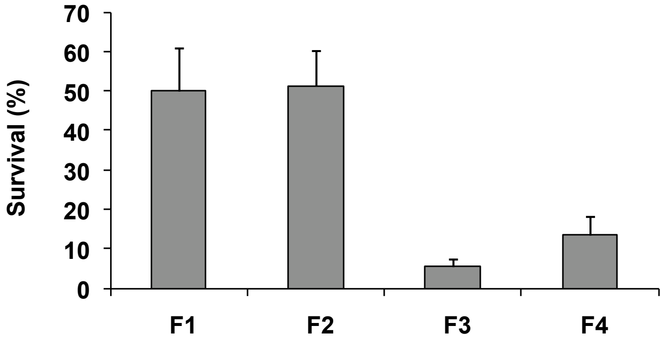

Figure 5. Survival (%) of larvae from females F1, F2, F3 and F4 (mean ± SE) fed 2000 cells ml^{-1} day^{-1} of the diatom *Chaetoceros muelleri*.

For ciliated band length ratios PCA1 accounted for 83% of variation between females. Most of the variation was explained by changes in band length to larval length and band length to larval width, similar to the weighting of variables contributing to treatment effects observed for length ratios. When all ciliated band length ratio variables were considered simultaneously there was only a 0% to 12.5% chance of incorrectly identifying larvae as belonging to a female other than that from which it originated. PCA1 of band length ratios differed between females ($p < 0.01$; LS means; Fig. 3). Overall, larvae from females F1 and F2 had a total band length of 5.2 ± 0.23 and 5.5 ± 0.54 respectively while larvae from F3 and F4 had a total band length of 4.4 ± 0.41 and 4.9 ± 0.21 (mean ± SE) respectively ($p < 0.01$; Fig. 4).

Survival and larval viability

The highest survival was recorded in larvae from females F1 and F2 at 50.0% ± 11% and 51.7% ± 9% (mean ± SE) respectively compared to 5.5% ± 2% and 13.5% ± 4.7% for F3 and F4 respectively ($p < 0.01$; Tukeys; Fig. 5). However, larvae less likely to reach late auricularia and successfully metamorphose may survive long periods of time in cultures. Survival was adjusted for numbers of larvae reaching late auricularia by multiplying the proportion surviving by the proportion in late auricularia to give an index of larval viability or larval success. For F1 and F2, 40% and 54% respectively were likely to complete metamorphosis compared to 3.6% and 11% for F3 and F4.

Development

Overall there was no difference in development between larvae from different females ($Chi^2 > 0.01$). The mid-auricularia stage appeared on day 7 and there was a relatively low proportion of mid-auricularia occurring from day 7 to day 12 (Fig. 6). A large number of remaining larvae were late auricularia by day 18 for all females. However, the rate of development differed between larvae from different females across days ($Chi^2 < 0.01$). Furthermore, for late auricularia the number of hyaline spheres in the folds of the ciliated band did not differ between females ($p > 0.05$). This was also reflected in the proportion of remaining larvae that were metamorphosing (Fig. 7). For larvae from females F3 and F4 the rate of transition to late auricularia over day 12 was faster and on day 22 a higher proportion was observed metamorphosing sooner ($p < 0.01$).

Discussion

Larval growth and shape

No correlation was found between growth and egg source for larvae from different females. Furthermore, differences in shape characteristics between larvae obtained from different females were limited. However, band length to larval length ratios were distinguished between larvae from different females. Ratios were also correlated with the proportion of embryos that had features characteristic of 'normal' development. The use of ciliated band length to length ratios to determine differences between larvae from different females and their relationship to early development and attributes of egg source may have some merit and requires further investigation.

Other studies have found inconsistencies in the relationship between egg size and larval shape. Starved larvae from small eggs of *Pisaster ochraceus* were wider than starved or fed larvae from large eggs, representing an increase in the length of the ciliated band (George 1999). Larvae of *Strongylocentrotus droebachensis* were larger in high food rations for eggs originating from low broodstock rations in captivity even though no difference in egg size was found (Meidel et al. 1999).

Alternatively, larvae from *S. droebachensis* originating from large eggs at a shallow food-rich site were larger in both high and low food rations but the trend was more evident in low food rations (Bertram and Strathmann 1998). However, both size and shape of larvae from *S. droebachensis*, *S. purpuratus*, *S. franciscanus*, *Dendraster excentricus*, *Heterocentrotus mammillatus*, *Colobocentrotus atratus* and *Tripneustes gratilla* were not correlated with egg size (McEdward 1986).

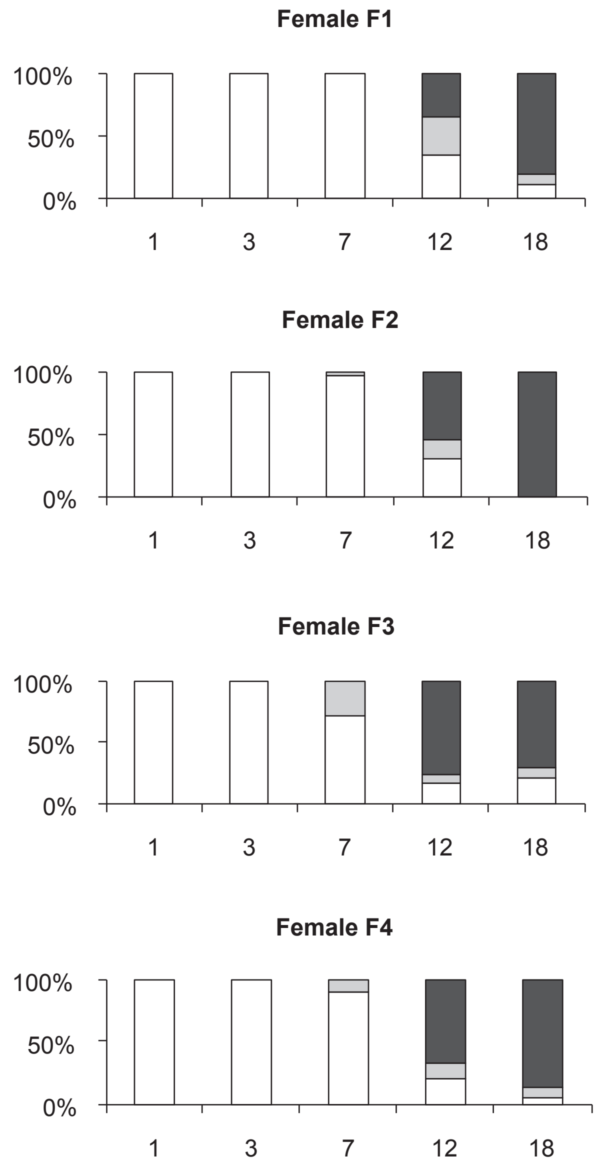

Figure 6. Summary of development of larvae from different females where day 1 = day 1 from first feeding (larval age = plus 3 days). X axis = days; Y axis = proportion of larvae in each stage (%). White = early; Grey = mid-; Black = late auricularia larval stage.

Survival and larval viability

The survival viability index used as a measure of larval competence was correlated with both egg size and egg numbers, distinguishing between larvae from different females. Effects of maternal nutrition on eggs and offspring are common even for animals that lack parental care and include performance and survival (Bertram and Strathmann 1998). Survival, measured as the ability to complete the larval cycle relative to the number of larvae in the late auricularia stage, was higher for females that produced more eggs with a larger egg size. Furthermore, this viability index was weakly associated with the mean number of spheres in the folds of the ciliated band prior to metamorphosis.

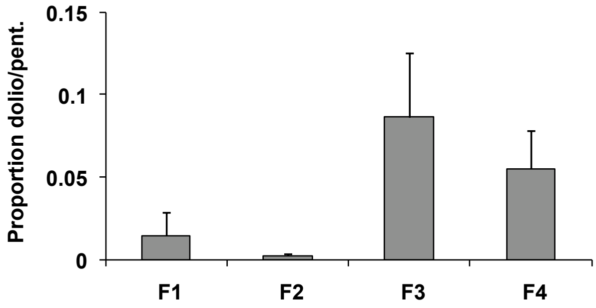

Figure 7. Proportion of surviving larvae on day 22 from females F1, F2, F3 and F4 that were either doliolaria or pentacula (mean ± SE).

Incorporation of a survival viability index in addition to measures of reproductive success (see Levitan 2005) is a way of determining downstream effects on the contribution of parentage (Marshall et al. 2004; Evans and Marshall 2005) to the fraction of eggs fertilised. A change in egg size has been correlated with larval viability in the sea urchins *Strongylocentrotus droebachensis* and *S. purpuratus* (Sinervo and McEdward 1988). When a decrease in egg size resulted in smaller larvae with simpler body forms, there was slower development through early feeding, and the effects of egg size were restricted to the early larval stages (Sinervo and McEdward 1988).

Larval development

No overall difference in development was observed for larvae from different females but the rate at which development proceeded differed. However, as with growth, development appeared decoupled from any effects of maternal origin and was not related to attributes of egg source. For example larvae from female F4 developed at a similar rate as those from F2 yet F4 had a significantly reduced survival viability index, numbers of eggs, and egg size. A direct relationship does not always exist between egg content and/or egg size and larval development as was found for the starfish *Solaster stimpsoni* and in other studies on the sea urchin *S. droebachensis* (McEdward and Carson 1987; Bertram and Strathmann 1998; Meidel et al. 1999). In high food rations for broodstock of *Strongylocentrotus droebachensis* the rate of larval metamorphosis was higher and larvae metamorphosed sooner (Meidel et al. 1999).

In another study the strong correlation of larval development of the seastar *Pisaster ochraceus* with maternal origin could only be distinguished during the middle phase of the larval cycle (George 1999). In the present study the rate of transition to late auricularia was reflected in a higher proportion metamorphosing sooner for larvae from different females. However, the appearance of hyaline spheres in larvae had limited correlation with attributes of egg source. Furthermore, there was no difference in numbers of hyaline spheres between larvae from different females.

Previous studies have determined that these hyaline spheres were not essential for metamorphosis as larvae have been observed settling and surviving subsequent development without them (Smiley 1986; McEuen and Chia 1991; Dautov and Kashenko 1995; Dautov 1997; Sewell and McEuen 2002). However, hyaline spheres may be needed to fuel metamorphosis by providing a reservoir to meet the structural requirements of morphogenic changes during metamorphosis, increasing the probability of survivorship post-settlement (see Sewell and McEuen 2002). Consequently, the rate of larval stage transition and the relationship between mean number of spheres and the survival viability index may act in synergy, reflecting differences in the contribution of egg source to the larval life cycle.

Conclusion

In conclusion early development in embryos appeared related to later differences in ciliated band length to larval length ratios for larvae from different females. Furthermore, the larval survival viability index appeared correlated with egg source, represented by egg numbers and egg size. However, attributes of egg source appeared decoupled from subsequent growth and development. A limited correlation between attributes of egg source and phenotype existed for larvae from different females. These attributes should be examined further to determine causal relationships.

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Shifting the natural fission plane of *Holothuria atra* (Aspidochirotida, Holothuroidea, Echinodermata)

Pradina Purwati[1], Sigit A.P. Dwiono[2], Lisa F. Indriana[2] and Varian Fahmi[2]

Abstract

Fission stimulation involves the fission plane as the division area. In natural fission of Aspidochirote and Dendrochirotida, only a single fission plane is present. This paper presents successful assays of shifting the natural fission plane. The experiment was conducted in July 2007, when two stimulated areas were applied on each of 20 individuals of *Holothuria atra*. New tentacles and new anal pores started to appear in week 7 after division in anterior group (A) and posterior group (P), respectively. The middle group (M), which did not carry both mouth and anal complex, regenerated slightly slower than the A and P groups. Eleven individuals of the M group developed both tentacles and anal pore in week 9, and the number increased subsequently. When the experiment was terminated in week 12, the survival rate reached 96.67%. In induced fission, the natural fission plane was unlikely to be a definitive area of body division; therefore, in such experiments, the great potential of regeneration was expressed more than the phenomenon of induced asexual reproduction.

Introduction

The great potential of regeneration in sea cucumbers is demonstrated through the phenomena of evisceration and asexual reproduction by fission. In evisceration, the sea cucumbers expulse most of their internal organs due to physiological change or in response to varied external factors. Within a certain period of time the eviscerated sea cucumbers regrow new internal organs and live normally. The process of regrowing internal organs occurs in asexual reproduction as well.

Naturally, asexual reproduction through self-division results in posterior and anterior parts. Each fissiparous population tends to have their own fission plane located in various places of the, e.g. one third of the body length, anteriorly, in *Holothuria leucospilota* (Purwati 2004); 44% of the body length, anteriorly, in *H. atra* (Chao, Chen et al. 1993); or at the mid-body in *H. parvula* and *H. surinamensis* (Crozier 1917; Kille 1942). Each part resulting from fission develops into intact animal.

Fission stimulation, in which holothurians are induced to divide, has gotten more attention in the last two decades. The technique is relevant for overfished areas in which the population size no longer supports successful fertilisation. Successful fission stimulation doubles the initial number of individuals through a low-cost and low-technology procedure. The method can be used by coastal communities (Purwati and Dwiono 2008).

Fission stimulation has been proven fruitful in both fissiparous and non-fissiparous holothurians. Among those reporting on this field are Reichenbach et al. (1996), Purwati and Dwiono (2007), Laxminarayana (2006) and Razek et al. (2007). The experiments conducted mainly imitate the asexual reproduction occurring in sea cucumber natural habitats, with introduction of a single fission plane. The individuals resulting from fission show high rates of survival and rebuild new tentacles and anal pores in 2–3 months. In the case of induced fission on non-fissiparous holothurians such as *H. arenicola* and *Bohadschia marmorata*, expression of a great potential of regeneration seems more relevant than asexual reproductive capacity.

Up to now, only a single fission plane per individual was considered in induced fissions, the fission plane being similar to that occurring naturally. Learning from induced fission on non-fissiparous species, we deduced that the natural fission plane in fissiparous holothurians may not be the only area of division and that it may therefore be possible to shift fission planes and to multiply them. Thus, one individual could be induced to divide on more than one plane in a single event. This would be a breakthrough in terms of physiology and reproduction, and it would also benefit population enhancement efforts.

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Material and methods

We have previously reported successful fission stimulation on *H. atra*, in which we doubled two groups of 10 and 30 individuals in two separate experiments with survival rates 85% and 100% respectively (Purwati and Dwiono 2005, 2007; Dwiono et al. in press).

To prove that the position of natural fission plane can be shifted, in July to September 2007 we stimulated 20 individuals of *H. atra* at two positions: at 1/3 and at 2/3 of the body length starting from the anterior. The initial body weight of the stimulated specimens was 35–143 g. Similar procedures of inducement and rearing as those described in Purwati and Dwiono (2005) were followed. A rubber pipe used as inlet air of bicycle wheels (available in most areas of Indonesia) was used to tie each holothurian's body tightly. Each part obtained — A (anterior, mouth part), P (posterior, anal part) and M (middle part, with no mouth and anus) — was about the same length. During stimulation, the holothurians were kept in buckets with fresh sea water and slow aeration. No food was added.

After division, each of three parts (A, P and M) was kept in a bucket, then packed in a net and hung in the water column at about 7 m depth. This hanging method has proven to be effective (Dwiono unpublished report). The morphological appearance of the fission planes was observed weekly to monitor the reformation of tentacles and anal pores, in addition to the survival rate. Regeneration of the M individuals was observed at both fission planes. The experiment was terminated when most of the individuals redeveloped the anal pore and/or tentacles. By this time, feeding was necessary and rearing techniques had to be changed.

Regeneration intensity (R) was measured as follows (X indicates A, M or P individuals):

R(X)(expressed in %) = (number of regenerating individuals / total number of individuals) x 100%

Results

Failure occurred in the first week after fission, when one M individual did not survive. Among animals that survived, the wounds took three weeks to heal, during which the fission plane closed and showed principally a concave surface. Regeneration, which was characterised by a convex surface or a protrusion of the fission plane, started in week 3, 4 and 6 after division for groups A, P and M, respectively. Regeneration reached 100% in week 9 for A individuals and in week 7 for P individuals.

In A and P individuals, anal pore or tentacle reformation began after 7 to 9 weeks, while M individuals needed more time — between 8 and 11 weeks (Fig. 1). The recovery of the wound on the fission plane took longer in M individuals, while regeneration time was shorter.

New anal apertures in A individuals had not developed until week 7, concurrently with the appearance of new tentacles in P individuals. In week 8, one of M individuals regenerated anal pores, and another two grew tentacles. Among them, one individual had both new tentacles and an anal aperture.

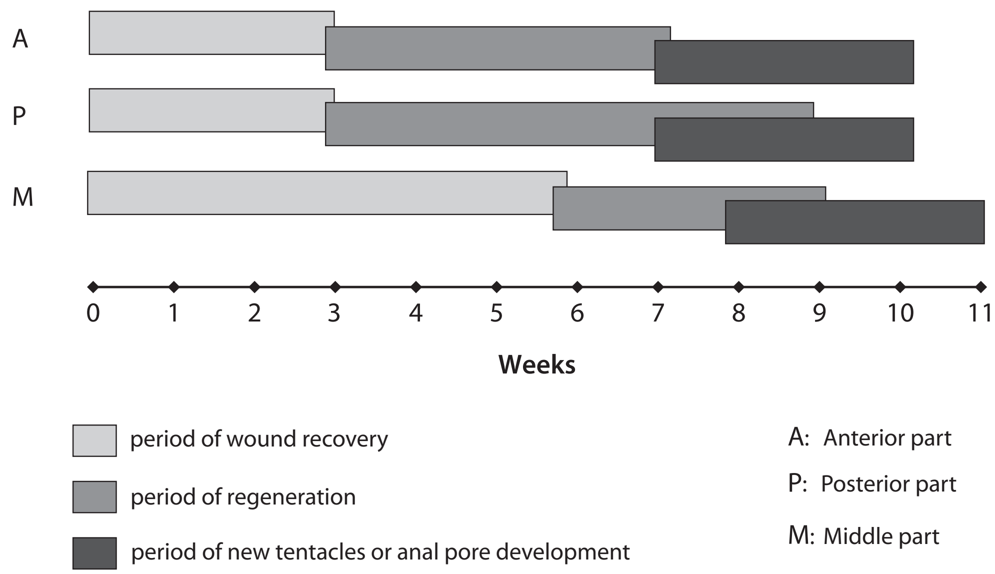

Figure 1. Regeneration intensity of A, P and M individuals.

Table 1. Time of appearance of new tentacles (t) and anal pore (a). The numbers in the table give the percentages of individuals.

Time (in weeks)	7		8		9		10		11	
	t	a	t	a	t	a	t	a	t	a
Individuals (%)										
Anterior		10		15		30		40		85
Posterior	5		35		65		95			
Middle			10	5	45	15	50	30	85	55

In week 9, another 10 M individuals reformed both tentacles and anal pores, and another 6 grew new tentacles (Table 1). It seemed that tentacles grew slightly earlier than the anal pore. When the experiment was terminated after week 11, 29 out of 30 M individuals originated from 10 parents survived; the overall survival rate was 96.67%.

Discussion

Although echinoderms are not at the base of the animal phylogeny with groups in which self-division or other asexual reproduction (budding etc.) is very common (e.g. Porifera, Cnidaria), they share some characters with these basal groups such as asexual reproduction (Carneveli 2006). And even though natural fission demonstrates that there exists a single fission plane with one specific position in each fissiparous species, stimulated fission shows that the position of the natural fission plane may not be important.

Success of reformation of external alimentary organs may not be separated from internal body tissues. Mesentaries are tissues that keep the position of the anterior alimentary organs (oesophagus, stomach and the anterior part of small intestine) and connect them to the middorsal interadius. Hyman (1955) and Bai (1994) reported that the dorsal mesentery is responsible in the development of the missing digestive organs of individuals resulting from fission. Accordingly, redevelopment of the alimentary duct would occur as long as the applied fission stimulation and rearing procedures do not cause any damage to the mesentary tissue.

Nature must have its own strategy in having one single fission plane in asexual reproduction. During the process, there must be a guarantee that each of the parts resulting from fission will develop into a normal individual afterward; consequently, the aim of reproduction in maintaining and enhancing the population is fulfilled. Survival at the individual level in asexual reproduction by fission is higher than in sexual reproduction as there is no critical larva stage, no partner needed and a minimised predatory pressure (Purwati 2002; Purwati 2004). When natural fission results in more than two individuals, the middle parts, which carry neither mouth nor anal complex, are probably the weakest in terms of regenerating body parts as they need more energy and time to redevelop both anterior and posterior complexes. Based on this assumption, the success of fission inducement should be measured through the rate of survival and the time required to regrow into a complete individual. The present experiment demonstrated good results and the redevelopment of middle parts was not much different from that of the anterior and posterior parts.

Anterior and posterior individuals recovered in 7 weeks, which was similar to the experiment results of Purwati and Dwiono (2007), and relatively earlier compared to the individuals in one tie stimulation by Dwiono et al. (2008). Dwiono et al. (2008) measured drained body weight in their weekly measurement and this process mayhave stressed the animals and delayed the development of the missing organs. In the current experiment, disturbance on individuals was minimised.

As a comparison, regeneration period in *H. parvula* (excluding gonad regrowth) has been reported to be 3 weeks (Kille 1942) and reports from other experiments on the same species state that posterior individuals regenerate faster than anterior ones. The individuals start to feed after 2 months (Emson and Mladenov 1987). In *Stichopus chloronotus* and *Thelenota ananas*, feeding began after 3 and 5 to 7 months, respectively (Reichenbach and Holloway 1995).

When natural fission of *H. atra* and *H. leucospilota* is monitored (Chao et al. 1993; Conand et al. 1997; Purwati 2004), posterior individuals are more likely to stay in their habitat than anterior individuals. It is difficult to determine whether they are being washed away, being prayed upon, or are dying. In stimulated fission, both anterior and posterior individuals survive in great number, which may indicate that the loss of anterior individuals from their natural habitat in natural fission is caused by external factors.

Conclusion

There is no significant difficulty in producing two or three individuals with a survival rate of 90% to 100%. Fission at the laboratory protects the individuals from predatory and extreme environmental changes. In the same time, our experiment showed that every fission product possesses similar capability to become a complete individual. The feasibility of producing four or more individuals is probably a question of energy capacity required to regrow the missing organs, mainly for the middle parts. Survival also depends on the capability of mesentery tissue to act as totipotent tissue. This experiment proved that (*i*) the natural fission plane may no longer be important in fission inducement and can be manipulated, (*ii*) high regeneration capacity may be expected to work on such situations rather than the asexual reproductive process, even in fissiparous species, (*iii*) the induced fission technique can potentially be applied to non-fissiparous holothurian species in order to increase individual numbers.

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Problems related to the farming of *Holothuria scabra* (Jaeger, 1833)

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Abstract

Different problems related to *Holothuria scabra* farming were observed at the sea cucumber aquaculture project Madagascar Holothurie S.A. (Toliara, Madagascar). In this paper, those problems are presented, their impact on sea cucumber production is characterised and solutions permitting to avoid or to minimise their impact are proposed. A drastic salinity drop occurred during low tides in one period of the year and after cyclones. This provoked behaviour troubles in the *H. scabra*: individuals stayed burrowed in sediment even at night, a period when they are usually at the surface. However, that did not affect their growth. When possible, we suggest choosing a site far from areas where salinity is apt to drop.

Isopods *Cymodoce sp*. infested sea cucumbers in outdoor ponds during the hot season, provoking a high mortality rate (average 8% week^{-1}) in cultivated *H. scabra*. The introduction of the carnivorous fish *Terapon jarbua* in the ponds proved successful in preventing this problem. The fish eat isopods and can eliminate them within 10 days. Infections induced by isopods totally disappeared within two weeks.

The crabs *Thalamita crenata* were abnormally abundant in the pens during some periods of the year. They are the most redoubtable sea cucumber predators in the region and may provoke the mortality of the entire stock within one month. The crabs attack principally the newly transferred juveniles. The adults whose weight was more than 250 g were never affected. The elimination of the crabs in the surrounding area is to be carried out before the transfer of the juveniles and a daily watch of the pens is necessary in order to limit the impact of predation on production.

Introduction

The control of biotic and abiotic parameters of the sea cucumber rearing sites is primordial for sea cucumber farming (Purcell 2004; Wang et al. 2004). Juveniles of *H. scabra* are cultivated first in outdoor ponds for two months until they reach the size of 6–7 cm. They are transferred afterward to pens built in sea grass beds (Battaglene 1999; Pitt and Duy 2004). Thus, several factors are to be considered in order to choose the best site for the holothurian growth, including the physico-chemical parameters and the presence of predators (Chen 2004; Pitt and Duy 2004). The predators could be crabs, shrimps, gastropods and fishes of the family Siganidae which attack preferably the young holothurians that have been newly transferred (Pitt and Duy 2004).

Recurrent controls should be carried out to identify all diseases or parasitism in order to avoid the introduction of harmful organisms to the natural stock (Eeckhaut et al. 2004; Purcell and Eeckhaut 2005). Identified parasites of sea cucumbers include bacteria, protozoa and several metazoa (Jangoux 1990; Eeckhaut et al. 2004). In some cases, they may provoke the death of diseased juveniles (Becker et al. 2004). However, as holothuriculture is relatively recent, pathogenic agents and their treatment are still widely unknown (Battaglene 1999; Xilin 2004). In Madagascar, a disease named skin ulceration was observed for the first time in September 2000 (Becker et al. 2004). The infection, which affects the integument of *H. scabra*, spreads very quickly in the ponds and may provoke the death of individuals within as little as three days after the appearance of the first symptoms. This disease results from infection by opportunistic bacteria. Nevertheless, the agent causing the ulcers was not identified (Becker et al. 2004). The research project Aqua-Lab/Belaza, which gave rise to the trade company Madagascar Holothurie S.A. in Toliara, has optimised the growth of *H. scabra* between 2004 and 2008. During this period, several problems related to biotic and abiotic parameters occurred and had to be solved. Three, in particular had an impact on the progress of the farming. They were: (*i*) a drastic salinity drop encountered during low tides at a certain period of the year and after cyclones, (*ii*) the abnormal abundance of isopod

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parasites in outdoor ponds during the hot season and (*iii*) the overabundance of crabs in the pens at a certain period of the year. This article presents those problems, characterises their impact on the sea cucumber production and proposes solutions permitting to avoid or to minimise their impact.

Material and methods

Salinity drop

The salinity was measured daily between July 2006 and December 2007 in the ponds (Fig. 1) and in the pens (Fig. 2). When an important salinity change was observed, all changes in behaviour, nycthemeral cycle and anatomy of cultivated *H. scabra* were recorded. Twice (once in the cool season and once in the hot season) the salinity dropped to 20‰ (2%). These salinity drops always occurred during the low tide and were due to a freshwater resurgence that ran out to the pens. In order to analyse the effects of the salinity drop on the anatomy of specimens, four juveniles (7 cm length and 15 g weight) were transferred for four weeks into a 15 l tank at a low salinity of 20‰ and compared to four juveniles maintained at a normal salinity of 35‰. Oxygenation of each tank was assured by a diffuser linked to a compressor. Seawater was changed every two days and juveniles were fed ground *Sargassum* daily. Juveniles were photographed and their symptoms characterised. Also, at two times, tropical cyclones provoked a salinity drop in the lagoon to 10‰. To study the effects of this drastic salinity drop on the survival of the cultivated *H. scabra*, specimens (8 cm length and 41 g weight) were placed in tanks (five specimens per tank) at a salinity of 10‰ for 1, 2, 3, 6, 12, 18 and 24 hours (35 specimens in total) after which salinity was brought back to 35‰. Those specimens were kept afterward in the same tanks for four weeks in order to evaluate the eventual effects of the salinity drop on their behaviour and their survival. Finally, the effects of the salinity drop on the growth of *H. scabra* were also studied. To do so, two ponds of 8 m^2 were used. Each pond was subdivided into 4 compartments of 2 m^2 each where 4 batches of 6 *H. scabra* of a respective average weight of 4 g, 24 g, 68 g and 117 g (48 specimens in total) were placed. Seawater (kept at 20‰ in one pond and 35‰ in the other) was changed weekly. Sediments (covering 10 cm depth) were unchanged during the experiment. This experiment lasted five weeks, after which specimens were weighed again and respective average weights calculated. The data analysis consisted of comparing the average weight of specimens placed at 20‰ with those kept at 35‰.

Study of parasites

In 2007, during the warm season, an abnormal abundance of crustacean isopods was observed in outdoor ponds. This provoked a disease among the cultivated juveniles. A daily survey was conducted for six weeks from when the disease first appeared. The stages of disease were characterised and specimens presenting those stages were counted and weighed. The isopods responsible were identified. The numbers of isopods and diseased *H. scabra* were counted. At the laboratory, a stomach dissection of five isopods was carried out to see what they fed on and to check particularly if spicules of *H. scabra* might be found.

Integument of normal and diseased *H. scabra* (at different stages of infection) was fixed adequately to be characterised in histology and on a scanning electron microscope (SEM) (see below). Two experiments were conducted in order to find solutions to eradicate this disease: (*i*) the transfer of diseased juveniles (n = 189) to a new pond containing freshly collected sediment that was free of isopods and (*ii*) addition of carnivorous fish *Terapon jarbua* (Forskall, 1775), of the family of Teraponidae (average weight:

Figure 1. Outdoor ponds for juveniles of *H. scabra* from 1 to 7 cm.

Figure 2. Pens installed in sea grass bed for *H. scabra* growth (from 7 cm to marketable size: 22 cm).

44 g; average length: 12 cm; n = 15), to a pond containing diseased juveniles (n = 114). Each experiment lasted three weeks and the evolution of the disease was recorded daily.

Study of predators

An abundance of crabs in the pens, accompanied by an excessive mortality of *H. scabra*, was observed at certain periods of the year. After the identification of the crab, an experiment was carried out in an outdoor pond (with three replicates). It consisted of putting different batches of *H. scabra* in the presence of crabs. A pond of 8 m long and 4 m width was subdivided into four compartments, each containing (*i*) 20 *H. scabra* of an average weight of 17 g (7 cm) with 5 crabs and without any food supply; (*ii*) 10 *H. scabra* of an average weight of 54 g (10 cm) with 5 crabs and without any food supply; (*iii*) 20 *H. scabra* of an average weight of 17 g (7 cm) with 5 crabs and with a food supply; and (*iv*) 10 *H. scabra* of an average weight of 54 g (10 cm) with 5 crabs and with a food supply. The pond contained sediments collected from the sea grass bed covering 10 cm of depth. The sea water was changed two times a week. The crabs were of an average weight of 51 g. Their food consisted of hermit crab cut in small pieces (3 cm long). The food was distributed daily at the end of afternoon. The control group consisted of 20 *H. scabra* (17 g) and 10 *H. scabra* (54 g) in ponds without any crabs. The ponds were daily observed during 30 days and dead juveniles were counted at each observation.

Histology and scanning electron microscopy

For histology analyses, samples were fixed for 48 hours in Bouin's fluid. Specimens were then dehydrated through a graded series of ethanol solutions (70%, 90% and 100%), placed in butanol (overnight at 60° C), embedded in paraplast, cut into 7 µm sections and stained with G orange aniline blue azocarmin. For SEM, specimens were fixed for 48 hours in Bouin's fluid (without acetic acid). They were then dehydrated through a graded series of ethanol solutions (50%, 70%, 90% and 100%), critical point dried, mounted on stubs, coated with gold and examined with a Jeol JSM-6100 electron microscope.

Results

Salinity drop

The salinity of the sea water observed near the pens varied over the year but also daily. The extremes observed in a year were 17‰ and 35‰. Specimens submitted to a low salinity of 20‰ or below changed their behaviour: they stayed burrowed into sediment even at night when they are usually on the surface. Their body swelled (Fig. 3 A and B). Placed in ponds at a normal salinity (35‰), diseased

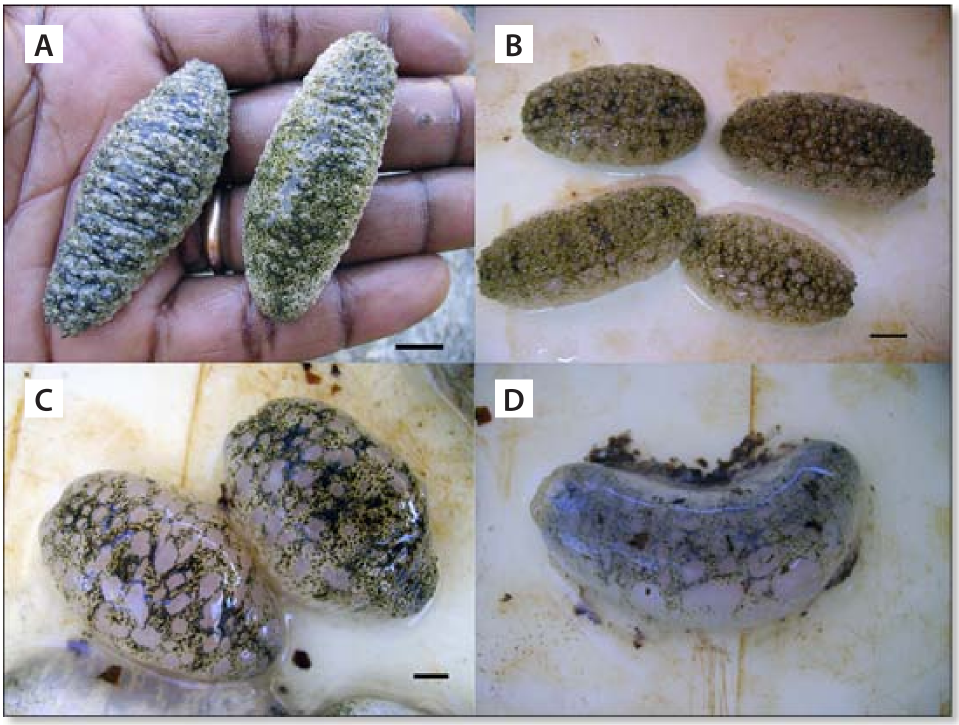

Figure 3. Juveniles of *H. scabra* placed in a tank at a low salinity (20‰). A: Juveniles at the beginning of the experiment (normal state); B: Juveniles under stress, the integument has become sluggish (stage 1); C: The integument is destroyed in different parts of the body wall (stage 2); D: The integument is totally destroyed (stage 3). Scale: bar = 1 cm.

Table 1. Variation of the average weight of *H. scabra* (n = 48 in total) after five weeks of farming in outdoor ponds at salinities of 20‰ and 35‰. The rearing density is 3 individuals m^{-2}. The statistical analysis compares final average sizes of specimens cultivated at 20‰ and 35‰.

Initial size	Final size (five weeks)		Statistical analysis
	Control (salinity: 35‰)	Experiment (salinity 20‰)	
3.80 +/- 1.49	28.04 ± 6.26	21.13 ± 8.67	$P_{T\ test} = 0.172$
23.84 +/- 10.39	60.64 ± 17.03	70.70 ± 16.11	$P_{T\ test} = 0.318$
67.79 +/- 5.06	128.53 ± 12.54	114.04 ± 22.64	$P_{U\ test} = 0.259$
116.85 +/- 28.39	180.82 ± 50.22	188.36 ± 50.46	$P_{T\ test} = 0.801$

specimens became normal within 96 hours. After 9 days at a salinity of 20‰ without the possibility to burrow into sediment, normal specimens (Fig. 3A) became weak, shrank in length, and developed a sluggish integument (Stage 1, Fig. 3 B). After 17 days, the epidermis was destroyed on various body parts (Stage 2, Fig. 3 C). Juveniles were eviscerated and their integument was strongly affected after 22 days (Stage 3, Fig. 3 D). They became translucent and died in the days that followed.

Table 1 shows the results of the experiment during which *H. scabra* of different sizes were kept on sediments at a low salinity of 20‰ during five weeks. During the experiment, they did not present any abnormal anatomic symptom. They stayed burrowed in the sediment even at night although their growth was not affected (Table 1). When *H. scabra* were placed in ponds at a very low salinity (10‰; equivalent to the conditions after a cyclone), they recovered from their stress if the exposure did not last more than 12 hours, after which they eviscerated (18 hours) or died (24 hours) (Table 2).

Table 2. Behaviour of *H. scabra* (n = 35 in total) at low salinity (10‰). Average weight and length of specimens: 41g, 8 cm.

Exposure time at low salinity of 10‰ (hours)	Specimen state
1	100% alive, normal
2	"
3	"
6	"
12	"
18	60% eviscerated, 40% dead
24	100% dead

Study of parasites

Epidemiology. The first signs of disease in the juveniles of *H. scabra* (7 cm, 15 g average) were observed in January 2007 in two outdoor ponds. In the first pond, there were 500 juveniles, of which 10% were infected. The second pond contained 480 juveniles, of which 8% were dead, 50% were highly infested and only 42% were healthy. The first symptoms of the disease always manifested near the cloacal opening: the integument became whitish over an area of few centimetres. This appeared afterward near the oral opening before covering the entire dorsal surface of the juvenile body wall. Once the whole surface was covered, juveniles became very weak, did not burrow into sediment anymore and died. We noted that the infection always appeared on the dorsal side of juveniles, never on the ventral side (Fig. 4). The same symptoms appeared again in February 2007 and reached three outdoor ponds: one pond for pre-growing and two ponds where larger individuals (> 6 cm long) were kept for growing. The two latter ponds contained respectively young sea cucumbers of 66 g (n = 82, of which 91% were still healthy) and 108 g (n = 114, which were all diseased). We note that this disease has never been observed in *H. scabra* cultivated in the pens set up in the sea grass bed.

Figure 4. Diseased juveniles of *H. scabra* (ulceration on dorsal side). Arrows indicate infected zone. Scale: bar = 1 cm.

Figure 5 (A, B, C) summarises the evolution of the disease in the affected ponds. On average, a mortality rate of 1% to 8% per week was recorded during the four weeks of the survey (Fig. 5 A). On the other hand no mortality was observed among the bigger specimens (Fig. 5 B and C). In general, whatever the specimen size, all specimens were totally infected and reached stage 3 in three to six weeks (Fig. 5 A–C).

Observed on SEM, the integument at the level of the wounds presented a disorderly structure (Fig. 6 B and C) compared to that from healthy areas (Fig. 6 A). The spicules were exposed in some areas (Fig. 6 D). In highly infected zones, the integument was totally destroyed and spicules were entirely exposed (Fig. 6 E and F). The observation of the wounds did not show any high concentrations of bacteria (Fig. 6 C and F).

The histological study showed that the integument of healthy juveniles of *H. scabra* measured 1 mm thick and included an epidermis and a cuticle (0.24 mm), a connective tissue layer (0.5 mm), a circular muscle layer (0.24 mm) and a coelomic epithelium (0.02 mm) (Fig. 6 G). In the infected zone, the cuticle and the skin were totally destroyed as well as the upper part of the connective tissue; only the coelomic epithelium was intact (Fig. 6 H).

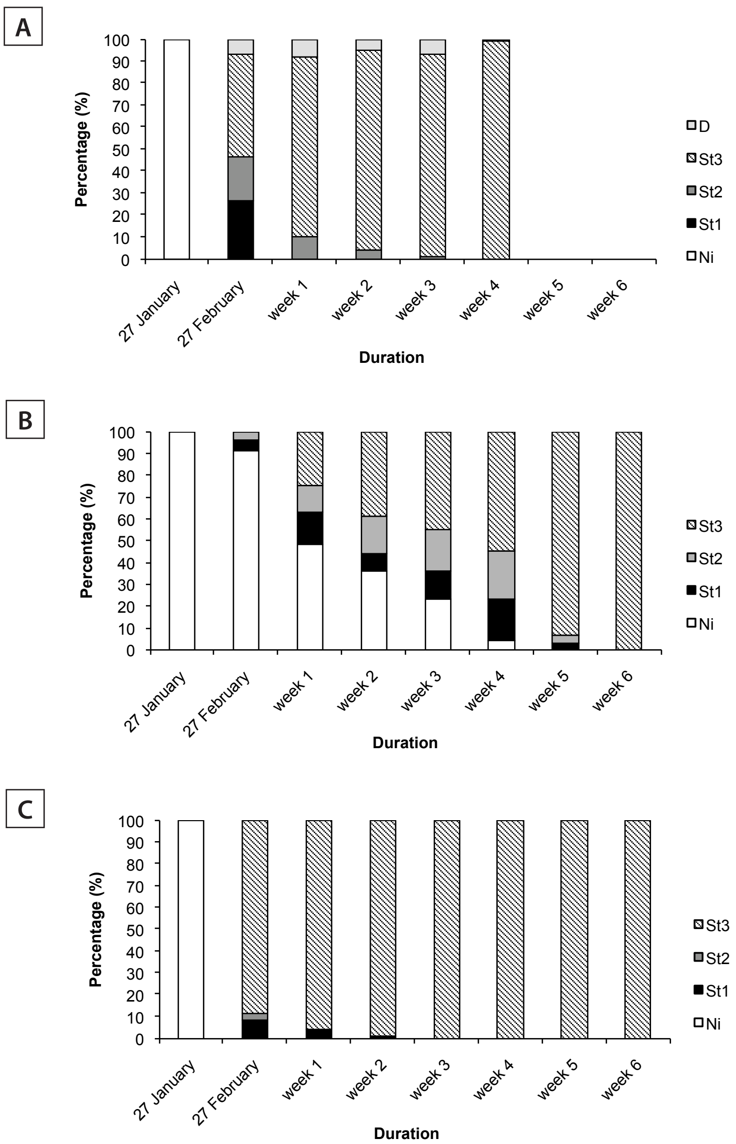

Figure 5. Evolution of the disease observed in outdoor ponds infested by isopod *Cymodoce* sp. The percentage expresses the number of specimens at different stages of the disease in relation to the total number of stock.

A: Average weight 15 g (n = 189); B: Average weight 66 g (n = 82); C: Average weight 108 g (n = 114).

NI: non-infested specimen; St1: cloacal opening infested; St2: cloacal and oral openings infested; St3: the entire body wall infested; D: dead specimen.

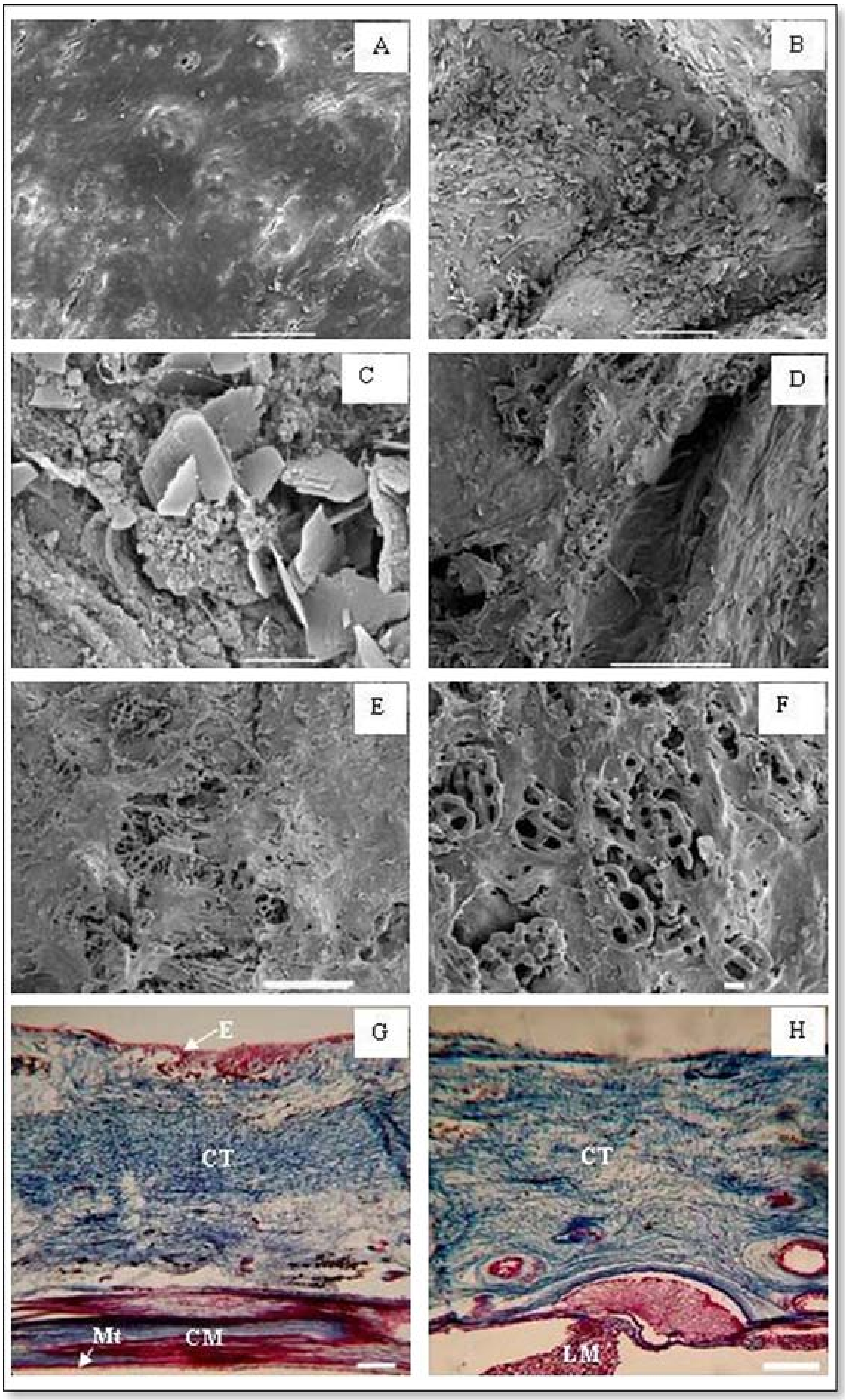

Figure 6. Integuments of *H. scabra* (A–F: observed on SEM; G–H: transversal section). A and G: Healthy integuments; B–F and H: Infected integuments. B: disorderly structure; C: disorderly structure (observed at 2000 x); D: spicules are exposed; E: spicules are totally uncovered. F: E observed at 500 x; H: cuticle and epidermis totally destroyed. E: epidermis; CM: circular muscle; LM: longitudinal muscle; Mt: mesothelium; CT: connective tissue layer. Scale: Bars = 100 µm for A, B, D, E, G and H; 10 µm for C and F.

Etiology

Field observations and laboratory experiments showed that the isopod *Cymodoce sp.* of the family of Sphaeromatidae (Order: Flabellifera) (Fig. 7) was responsible for the disease. They were observed in abundance on diseased *H. scabra* (15 to 30 isopods per juvenile, see Fig. 8), as well as on the substrate and in the water column. Outside of the period of the disease, we found only 10 isopods m^{-2} in the outdoor pond. On the other hand, during the period of the disease, we recorded 520 isopods m^{-2} on average. The density of the isopods was not uniform in the ponds: they were found in greater concentration near the sides close to the concrete wall. Stomach dissection of isopods showed presence in abundance of holothurian spicules.

Symptoms of diseased juveniles (n = 189) placed in ponds without isopods disappeared within the days following the transfer. Only two days after their transfer, the integument lesion closed up and juveniles became very active and began once again to follow the burrowing circadian cycle. After a week, 95% of juveniles were cured; after two weeks, they recovered totally from the disease (Fig. 9 A). The transfer of *T. jarbua* fish also provided very good results in curing the infection. The fish ate and eliminated the isopods in less than 10 days. After two weeks, the infection totally disappeared (Fig. 9B) as well as the isopods.

Study of predator

In 2007, an abnormal mortality of newly transferred juveniles of *H. scabra* in pens (in sea grass bed) was observed while the adults (average weight > 250 g) were never affected. A disappearance of 70% of 400 juveniles transferred in March 2007 was observed one month after the transfer; by the following month they had all disappeared. In August 2007, 800 juveniles disappeared one month after their transfer, followed by 500 more at the end of the same month. Only a few juveniles were found dead in the pens during the observation. Several day and night surveys helped to identify the causes of these abnormal disappearances. Crabs *Thalamita crenata* (Rüppell, 1830) (Fig. 10) of the Portunidae family were observed in abundance near and inside the pens. After several observations, we often found these

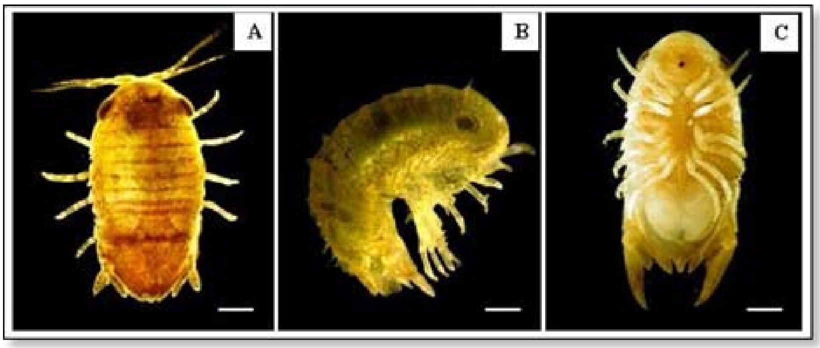

Figure 7. *Cymodoce* sp. A. Dorsal view; B: lateral view; and C: ventral view. Scale: Bar = 1 mm.

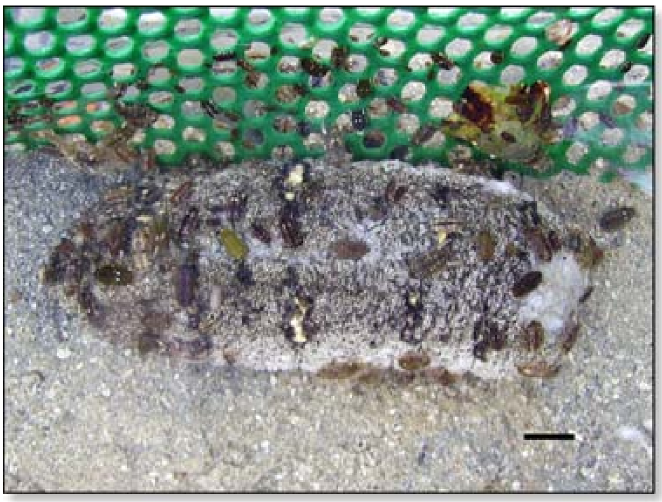

Figure 8. Young *H. scabra* covered by isopods. Scale: Bar = 1 cm

crabs eating the newly transferred juveniles of *H. scabra*. Experiments in outdoor ponds showed that the crabs did not eat *H. scabra* when they were fed daily. On the other hand, five crabs were enough to kill and eat 20 juveniles of an average weight of 17 g within five days and 10 juveniles of an average weight of 54 g within 10 days when they were kept in external ponds without any food supply (Table 3).

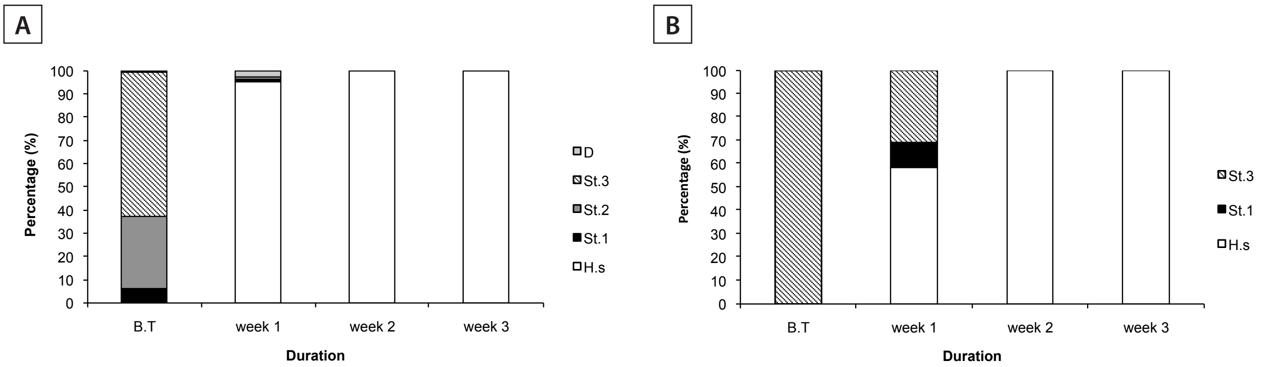

Figure 9. Evolution of the disease in farming ponds after the treatment. The numbers express the percentage of specimens at different stages of disease in relation to the total number of stock.

H.s: healthy specimen (or recovered from disease); St.1: infestation near the cloacal opening; St.2: cloacal and oral opening are infested; St.3: the entire body wall is infested; D: dead specimen; B.T: beginning of the treatment;

A: Treatment with newly collected sediments from the sea grass bed. Average size of *H. scabra*: 15 g (n = 189). B: Treatment with transfer of carnivorous fish *T. jarbua*. Average size of *H. scabra*: 108 g (n = 114).

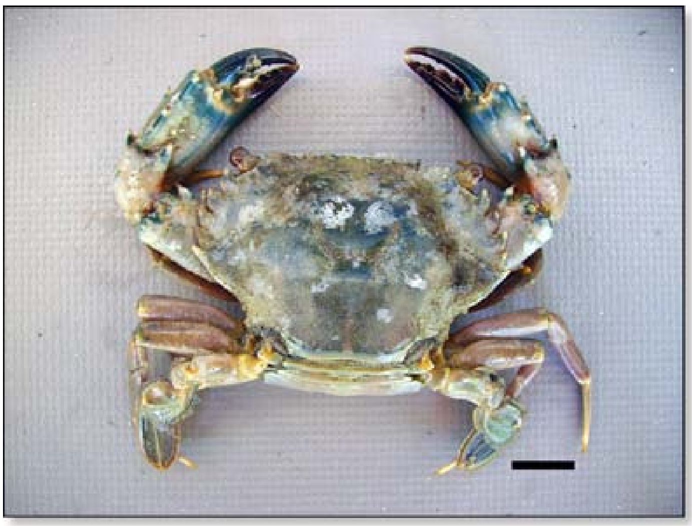

Figure 10. Dorsal view of crab *Thalamita crenata* (Rüppell, 1830). Scale: Bar = 1 cm.

Table 3. Average mortality (expressed in percentage) of *H. scabra* reared in outdoor ponds in presence of crabs *T. crenata*. Control: *H. scabra* farmed without crab. Batch A: 20 juveniles (average weight: 17 g; average length: 7 cm) + 5 crabs, without any food supply. Batch B: 10 adults (average weight: 54 g; average length: 10 cm) + 5 crabs, without any food supply.

	Starting	D1	D2	D 3	D4	D5	D6	D7	D8	D9	D10	D30
Control	0	0	0	0	0	0	0	0	0	0	0	0
Batch A	0	15	37.5	47.5	55	100	-	-	-	-	-	-
Batch B	0	0	0	0	5	5	5	55	65	80	100	-

Discussion

The rapid expansion of holothuriculture may favor the appearance of several diseases that could compromise the production of sea cucumbers on a large scale (Wang et al. 2004; Purcell and Eeckhaut 2005). The diseases could reach the larvae, juveniles, adults and even brood stock (Table 4). They are caused by various pathogenic agents such as bacteria (Morgan 2000; Becker et al. 2004; Eeckhaut et al. 2004; Wang et al. 2004), fungi (Wang et al. 2004), protozoa (Eeckhaut et al. 2004; Mercier et al. 2004), copepods (Wang et al. 2004), platyhelminthes (Eeckhaut et al. 2004; Wang et al. 2004), gastropods (Jangoux 1990), crabs (Jangoux 1990; Mohan and James 2005), isopods and viruses (Wang et al. 2007). Pathogenic agents and their treatment are still largely unknown (Xilin 2004). Within the diseases of sea cucumbers, skin ulceration is a widespread symptom (Table 4). It can be induced by various pathogenic agents and affect various holothurian species such as *A. japonicus* in China, *Isostichopus fuscus* in Ecuador and *H. scabra* in Australia, New Caledonia and Madagascar (Becker et al. 2004). Skin ulcerations are often accompanied by mucus secretions on the body, discoloration of the skin and behaviour changes (Purcell and Eeckhaut 2005). Other diseases without skin ulceration do not generally cause high mortality. Diseased specimens got thinner and became weak and lethargic. Some eviscerated in cases of severe infection, however, the integument do not present any suspicious lesion (Wang et al. 2004).

Besides pathogenic parasites, holothurians in farming are also victims of predators (Hamel et al. 2001). In the hatchery, copepods and ciliates are the most redoubtable predators of auricularia larvae (James et al. 1994). They may also attack newly metamorphosed juveniles (Tanaka 2000; Wang et al. 2004). Tectibranches (gastropods) and some amphipod species feed on pentactula larvae and newly metamorphosed holothurian juveniles (Mercier et al. 2000). In the natural environment, newly released juveniles are attacked and eaten by (*i*) different species of fishes (Hamel et al. 2001; Pitt and Duy 2004), (*ii*) crabs (Pitt and Duy 2004), (*iii*) shrimps (Pitt and Duy 2004) and (*iv*) sea stars (Hatanaka et al. 1994). This predation may lead to disappearance of the entire stock in a very short period of time (Mercier et al. 2000; Tanaka 2000).

In the natural environment, predators constitute one of main risks to be considered for sea cucumber aquaculture using pens. Careful observation of sites must be carried out in order to avoid them. In this work, the crab *T. crenata* was found to be the most redoubtable predator in the Toliara region. Aside from the control of biotic parameters, the choice of adequate sites for building sea pens is one of the key parameters for insuring the success of sea cucumber farming. The sites should be sea grass bed zones, protected from winds and waves, with tides such that sea cucumbers are not out of the water for too long. Sandy muddy substrates, rich in organic matter, are also a favorable habitat. Even *H. scabra* could support a low salinity of 20‰ (Mercier et al. 1999a, 1999b; Pitt and Duy 2004). It is preferable to have the farming site far away from estuaries and fresh water flow. The ideal salinity for sea cucumbers is between 28‰ and 31‰ (Chen 2004; Xilin 2004).

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Table 4. Diseases of cultivated sea cucumbers recorded between 2000 and 2007. The bar indicates that the responsible agent was not identified.

Diseases	Symptoms	Infected stages	Responsible agent	Country	References
I	Darkening of the body edges; diseased specimens undergo autolysis and the body completely disintegrates within two days.	Auricularia	Bacteria	China	Wang et al. 2004
II	The stomach and/or the intestine walls of larvae are atrophic.	Auricularia	Bacteria	China	Wang et al. 2004
			/	China	Liu et al. 2004
			Protozoa	Ecuador	Mercier et al. 2004
III	Presence of gas bubbles inside the body of the larvae, which results in anorexia.	Auricularia	/	China	Wang et al. 2004
IV	Diseased specimens present oedema near the peristome. The juveniles' tentacles cannot retract; they lose the ability to remain attached to available substrate. The juveniles may eviscerate. The body wall becomes covered of mucus; the epidermis disappears and the whole body can dissolve with autolysing process.	Juveniles	Bacteria	China	Wang et al. 2004
			Virus	China	Wang et al. 2007
V	The wound appears near the cloacal orifice and extends over the whole body surface. The integument becomes whitish and spicules may be exposed. The highly infested specimens become weak and died in the days that followed. The disease is highly contagious and expands quickly in farmed populations.	Juveniles	Copepods	China	Wang et al. 2004
		Juveniles	Bacteria	Madagascar	Becker et al. 2004; Eeckhaut et al. 2004
		Juveniles	/	Solomon Islands	Hamel et al. 2001
		Juveniles	/	/	Purcell and Eeckhaut 2005
		Juveniles and adults	Bacteria	China	Wang et al. 2004
		Broodstock	Bacteria	Australia	Morgan 2000
		Juveniles and adults	Isopods	Madagascar	Present work
VI	Infected individuals are weak and anorexic. The body becomes stiff and is covered in excessive mucus. As the infection progresses, the entire viscera is usually expelled and eventually the infected specimen dies.	Juveniles and adults	Platyhelminth	China	Wang et al. 2004
VII	The papillae of diseased specimens become white. The body wall appears bluish white with the development of the infection. The body wall becomes thinner and the affected individuals develop oedema.	Juveniles and adults	Fungi	China	Wang et al. 2004
VIII	The protozoa live in the digestive tract and in the respiratory trees of holothuroids and may provoke internal wounds. The infected animals tend to be weak and sluggish. The body usually shows no conspicuous lesions, however the intestine, respiratory tree, etc. can be eviscerated in severe infections.	Juveniles and adults	Protozoan	China	Wang et al. 2004
			Protozoan	/	Eeckhaut et al. 2004

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Stock assessment of holothuroid populations in the Red Sea waters of Saudi Arabia

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Abstract

The present work estimates the stocks of sea cucumbers at sites in the Red Sea — for which there is currently a lack of reliable data. The survey was carried out at 18 sites located in three areas (Al-Wajh, Thowal and Farasan Islands) along the Red Sea coast of Saudi Arabia. The major findings revealed that all sites surveyed were subjected to heavy overfishing that led to high depletion of sea cucumber stocks.

Twelve species were recorded during this survey, with the highest diversity recorded at Farasan Islands (eight species), followed by the Thowal area (four species) and the Al-Wajh area (three species). The species included three high value species (*Holothuria fuscogilva*, *H. nobilis* and *H. scabra*), five medium value species (*Actinopyga echinites*, *A. mauritiana*, *Holothuria atra*, *H. fuscopunctata* and *Stichopus variegatus*), three low value species (*Bohadschia vitiensis*, *Pearsonothuria graeffei* and *Holothuria edulis*) and one non-commercial species (*Holothuria leucospilota*). Population structure investigation showed high depletion in populations of *H. nobilis*, while populations of both *H. fuscogilva* and *H. scabra* were completely destroyed. Only *H. atra* showed a healthy population.

Introduction

The Red Sea has a unique environment with a wide range of habitats and outstanding biodiversity, which give it great scientific and ecological importance (Head 1987). Saudi Arabia's Red Sea coast extends for about 2,000 km (nearly all the eastern seaboard of the Red Sea) and many islands exist offshore that have flourishing ecosystems and rich species communities. Holothurians are one of the most important members of the Red Sea ecosystem and influence the structure and functioning of coral reef ecosystems (Bakus 1973), contributing significantly to the food chain and to the modification of the substrate (Lawrence 1975). In addition, sea cucumbers have a high economic value due to their high market demand and high prices (Holland 1994).

The environmental parameters in the marine habitats of Saudi Arabia's Red Sea coast are suitable for the existence of sea cucumbers (Price 1982, 1983). The availability of food, suitable substrata (Roberts 1979; Mercier et al. 1999), a high variety of niches (Clark and Rowe 1971; Guille and Ribes 1981), suitable depths (Preston and Lokani 1990; Lokani et al. 1996) and the small number of natural enemies (Hasan 2003, 2005) favour the existence of sea cucumbers. The trend of overfishing of sea cucumber that started in 1999 resulted in overexploitation of the resource, causing their stocks to deplete (Hasan 2008). This situation not only caused the decrease in species density, but also created the possibility of the disappearance of some species from the marine habitat, especially those with high commercial value.

Due to the overfishing of Saudi Arabia's sea cucumbers and the apparent depletion of natural stocks, the Ministry of Agriculture of Saudi Arabia banned sea cucumber fishing. The Ministry of Agriculture requested a full survey for all sea cucumber fauna and the stock status prior to the reopening of the fishery. In a previous paper (Hasan 2008), I reviewed the status of fisheries and proposed a management plan for Saudi Arabian sea cucumbers. The current study describes a two-month survey that has been made to evaluate the natural stocks of sea cucumbers in Saudi Arabia's Red Sea.

Materials and methods

Study sites

The sea cucumber stocks were surveyed at 18 offshore sites along Saudi Arabia's Red Sea coast from April to May 2006. Three areas along the coast were surveyed: the Al-Wajh area, the Thowal area and the Farasan Islands. The three areas have been extensively detailed in Hasan (2008). At each of these areas a number of sites were considered. In Al-Wajh,

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five sites were explored: North Al-Ewandia (underwater reef), Kotaa Al-Ewandia (shallow reef), Shaab Al-Jaziera (shallow reef), Braim Island and Kaad Al-Jazeira. In Thowal, three sites were surveyed: Bousti (underwater island), Tallah reef and Abou Koussa reef. The Farasan Islands are a group of islands 50 km offshore opposite the Jazan area at the southern borders of Saudi Arabia. Ten sites were surveyed there: Umm El-Hagar (submerged reef), Umm El-Raak (submerged reef), Bagel (submerged reef), Al-Hayla, Al-Shabeen, Umm Al-Madah, Gadeefa (underwater island), Al-Hacece, Al-Sharaa and Abou Atteque.

Sea cucumber population assessment methods

Estimation of sea cucumber populations at the surveyed sites was carried out using underwater visual transects. Direct visual assessment is the method conventionally used and is effective for the direct enumeration of specimens of epifauna (Lokani et al. 1996). At each site, transects were made covering the different depths, zones and habitats. The length of each transect was about 150 m. Between five and nine replicates were made at each zone and/or depth. Along each transect 10 quadrats of 10 m x 10 m (100 m^2) were made. The shallow areas were surveyed by snorkelling and the deep areas were surveyed by scuba diving.

Population estimates and distribution

The population density of different sea cucumber species inside each quadrat was counted and expressed as number of individuals per 100 m^2. At each quadrat the different biotopes of the reef and type of substrate were described in terms of percentage of sand, rubble, pavement, seagrass, algae, rocks, and dead and live corals.

The total population was estimated for each species for all the surveyed sites. The following formula was used to estimate the species population:

$$T = X * N$$

where:

T = total population
X = mean number per transect
N = number of transects that fit into the total area (N = total area/transect area).

The total area of each site was calculated based on distances measured by using a boat with a fixed speed and the formula:

$$D = S * T$$

where D is the distance, S is the boat speed and T is the time.

Estimation of standing stocks

Biomass was calculated by collecting the different sea cucumber species from representative quadrates and weighing them, after which they were returned them to the same site. Weight was measured after gently drying the animals with a cloth and leaving them out of the water for five minutes. The standing stocks were calculated by the formula:

$$SS = M.wt. * TA / QA$$

Where SS is the standing stocks, M.wt. is mean weight at the representative quadrats, TA is the total area and QA is the quadrat area.

Results

Sea cucumber faunal composition

A total of 12 species were recorded in the three surveyed areas. The species composition included three high value species: *Holothuria fuscogilva*, *H. nobilis* and *H. scabra*; five medium value species: *Actinopyga echinites*, *A. mauritiana*, *Holothuria atra*, *H. fuscopunctata* and *Stichopus variegatus*; three low value species: *Bohadschia vitiensis*, *Pearsonothuria graeffei* and *Holothuria edulis*; and one non-commercial species (*Holothuria leucospilota*) (Table 1). Of the 18 sites surveyed, 5 sites had no records of sea cucumber (North El-Ewandia, Al-Hayla, Al-Shabeen, Umm-El-Madah and Al-Sharaa). *Holothuria atra* was the most common species (recorded at nine sites), followed by *Holothuria nobilis* (recorded at three sites), while the rest of the species recorded were recorded at only one or two sites.

The surveyed sites differ in the diversity and density of species recorded. It was evident that all sites have low diversity of sea cucumber species. The study revealed that of the surveyed sites, 27.78 per cent did not have any sea cucumber species, 27.78 per cent recorded only one species, 38.89 per cent recorded two species and only 5.56 per cent recorded six species.

Species density and habitat distribution

In the Al-Wajh area, sea cucumbers were recorded at four of the five surveyed sites at low densities and diversities. Only one high value species was recorded in the area (*Holothuria nobilis*; recorded at Shaab Al-Jaziera), and was found at low densities that ranged between 0.1 and 0.2 individuals 100 m^{-2} (Table 2A). The medium value species *Holothuria atra* was recorded at three of the five surveyed sites (Shaab Al-Jaziera, Braim Island and Kaad El-Zawrak Island). The highest densities were recorded in sandy habitats (ranging between 4.3 individuals 100 m^{-2} at Shaab Al-Jaziera and 8.4

Table 1. Sea cucumber species composition at the surveyed sites (present/absent)

Species	Al-Wajh area				Thowal area			Farasan Islands					
	Kotaa Al-Ewandia	Shaab Al-Jaziera	Baim Island	Kaad El-Za-wrak Island	Bousti	Tallah	Abou Koussa	Umm El-Hagar	Umm El-Raak	Bagel	Gadeefa	Al-Hacece	Abou Atteque
Actinopyga echinites									+		+		
A. mauritiana							+						
Bohadschia vitiensis											+	+	
P. graeffei	+												
Holothuria atra		+	+	+		+	+	+	+	+	+		
H. edulis											+		
H. scabra										+	+		
H. fuscogilva					+								
H. nobilis		+			+	+							
H. fuscopunctata												+	
H. leucospilota													+
Stichopus variegatus											+		

individuals 100 m^{-2} at Braim Island), while the lowest densities of *H. atra* were recorded in dead coral habitats. The species were not recorded in areas with live coral habitats.

In the Thowal area four sea cucumber species were recorded at the three sites. Two high value species — *Holothuria fuscogilva* (only at Bousti), and *Holothuria nobilis* (at Bousti and Tallah) — were recorded in rocky and dead coral habitats at low densities (Table 2B). The medium value species *H. atra* was recorded at high densities at Tallah in both sandy (12.3 individuals 100 m^{-2}) and rocky areas (2.4 individuals 100 m^{-2}). Low densities were present at Abou Koussa (0.2 individuals 100 m^{-2}), in both sandy and rocky habitats. *Actinopyga mauritiana*, another medium value species, was recorded at very low densities (0.01 individuals 100 m^{-2}) on corals at only one site (Abou Koussa).

In the Farasan Islands, the high value species *Holothuria scabra* was recorded only on sandy substrates but at low densities at Bagel (0.1 individuals 100 m^{-2}) and Gadeefa (0.3 individuals 100 m^{-2}). The medium value species were also present at low densities. Of them, *H. atra* was found at the highest densities. *H. edulis*, another medium value species, was found only in dead coral (0.1 individuals 100 m^{-2}) and coral habitats (0.2 individuals 100 m^{-2}) at Gadeefa. Two other medium value species were found in the Farsan Islands: *Actinopyga echinites*, recorded only at Umm El-Raak (0.1 individuals 100 m^{-2} in both rocky and dead coral habitats), and *Stichopus variegatus*, recorded only in sandy habitat at Gadeefa. The non-commercial species *Holothuria leucospilota* was recorded at high densities at the Abou Atteque site in sandy habitats (7.8 individuals 100 m^{-2}) and rocky habitats (3.9 individuals 100 m^{-2}) (Table 2C).

Stock assessment

The population size recorded for *Holothuria scabra* was 35 individuals at Gadeefa and only 8 at Bagel. *Holothuria fuscogilva* is another high value species that is important in the catch composition of Saudi Arabia's sea cucumber fishery. There were only 10 individuals at Bousti. *Holothuria nobilis* was recorded at three sites: 14 individuals at Shaab Al-Jaziera, 7 individuals at Bousti and 18 individuals at Tallah reef. The only species that showed higher number was the medium value *Holothuria atra*, of which a population of 1567 was recorded at Tallah reef with signs of population recovery after three years of closure. It was also found at Shaab Al-Jaziera (87 individuals), Braim Island (189 individuals), Kaad El-Zaurak Island (112 individuals), Bagel (65 individuals) and Gadeefa (92 individuals).

The total biomass (in wet weight) was little more than half a tonne (Table 3). The majority of the biomass consisted of *Holothuria atra*, of which a total of 450.5 kg was recorded. The high value species were recorded at negligible levels at all surveyed sites. A

Table 2A. Species density of sea cucumber (individuals 100 m^{-2}) at different habitats at the surveyed sites in the Al-Wajh area (data expressed as means).

	Bousti			Tallah			Abou Koussa		
Species	Rocks	Dead corals	Live corals	Sand	Rocks	Dead corals	Sand	Seagrass	Live corals
Holothuria atra	0	0	0	12.3	2.4	1.2	0.2	0.2	0
Holothuria nobilis	0.1	0.1	0	0	0.2	0.1	0	0	0
Holothuria fuscogilva	0	0.2	0.01	0	0	0	0	0	0
Actinopyga mauritiana	0	0	0	0	0	0	0	0	0.01

Table 2B. Species density of sea cucumber (individuals 100 m^{-2}) in different habitats at the surveyed sites in the Thowal area.

	Kotaa Al-Ewandia	Shaab Al-Jaziera			Braim Island			Kaad El-Zawrak Island	
Species	Live corals	Sand	Rocks	Dead corals	Sand	Seagrass	Dead corals	Sand	Rocks
Holothuria atra	0	4.3	1.1	0.9	8.4	4.2	0.3	5.6	1.2
Holothuria nobilis	0	0.01	0.2	0	0	0	0	0	0
Pearsonothuria graeffei	0.01	0	0	0	0	0	0	0	0

Table 2C. Average regional variation in species density of sea cucumber (individuals100 m^{-2}) in different habitats at some representative sites in the Farasan Islands area.

	Umm El-Hagar		Umm El-Raak		Bagel		Gadeefa			Al-Hacece		Abou Atteque	
Species	Sand	Rocks	Sand	Dead corals	Sand	Rocks	Sand	Dead corals	Live corals	Sand	Live corals	Sand	Rocks
Actinopyga echinites	0	0	0.1	0.1	0	0	0	0	0	0	0	0	0
Bohadschia vitiensis	0	0	0	0	0	0	0.2	0	0	0.2	0.1	0	0
Holothuria atra	0.4	0.2	0.2	0.1	0.5	0.2	4.6	2	0	0	0	0	0
H. edulis	0	0	0	0	0	0	0	0.1	0.2	0	0	0	0
H. fuscopunctata	0	0	0	0	0	0	0	0	0	0.1	0.1	0	0
H. scabra	0	0	0	0	0.1	0	0.3	0	0	0	0	0	0
H. leucospilota	0	0	0	0	0	0	0	0	0	0	0	7.8	3.9
Stichopus variegatus	0	0	0	0	0	0	0.3	0	0	0	0	0	0

total biomass of 32.5 kg was recorded for *Holothuria scabra*, with 26.85 kg recorded at Gadeefa and 5.65 kg at Bagel. The situation was not better for *Holothuria nobilis,* of which a total biomass of 24.95 kg was recorded. *Holothuria fuscogilva* was recorded only at Bousti, with a biomass of 6.5 kg (Table 4).

The status of sea cucumber stocks is given in Table 4. Tallah recorded the highest biomass (347.45 kg wet weight), but most of the sea cucumbers are of poor value. Sea cucumbers at Bousti are high value species, but the biomass is very low.

Discussion

The major finding of this study is that there has been heavy overfishing in all the surveyed sites. It is evident that illegal fishing is continuing, especially in the southern and northern sections of the coastline. In spite of satisfactory environmental conditions that would enable sea cucumber species to grow, the species diversity is low and the density of the high value species is even lower. It is probable that the natural parameters are not enough for maintaining the Saudi stocks of sea cucumbers.

Table 3. Standing stocks (biomass expressed in kg) of different sea cucumber species at the surveyed sites.

Site	*Actinopyga echinites*	*A. mauritiana*	*Bohadschia vitiensis*	*Pearsonothuria graeffei*	*Holothuria atra*	*H. edulis*	*H. leucospilota*	*H. fuscogilva*	*H. nobilis*	*H. fuscopunctata*	*H. scabra*	*Stichopus variegatus*
Kotaa Al-Ewandia	-	-	-	0.5	-	-	-	-	-	-	-	-
Shaab Al-Jaziera	-	-	-	-	18.50	-	-	-	9.25	-	-	-
Braim Island	-	-	-	-	36.75	-	-	-	-	-	-	-
Kaad El-Zaurak Island	-	-	-	-	21.10	-	-	-	-	-	-	-
Bousti	-	-	-	-	-	-	-	6.5	2.75	-	-	-
Tallah reef	-	-	-	-	334.50	-	-	-	12.95	-	-	-
Abou Koussa reef Umm El-Hagar	-	0.65	-	-	0.85	-	-	-	-	-	-	-
Umm El-Raak	-	-	-	-	2.10	-	-	-	-	-	-	-
Bagel	2.3	-	-	-	1.45	-	-	-	-	-	-	-
Gadeefa	-	-	-	-	13.65	-	-	-	-	-	5.65	-
Al-Hacece	2.7	-	4.1	-	21.60	1.25	-	-	-	-	26.85	21.35
Abou Atteque	-	-	4.8	-	-	-	-	-	-	1.35	-	-
	-	-	-	-	-	-	189.75	-	-	-	-	-

Table 4. Total abundance, total wet weight and proportion of total high commercial value species (*Holothuria scabra, H. fuscogilva* and *H. nobilis*) by site.

Site	Total abundance	Weight (kg)	% high value species (no.)	% high value species (wt.)
Kotaa Al-Ewandia	3	0.500	0.00	0.00
Shaab Al-Jaziera	101	27.750	16.09	33.33
Braim Island	189	36.750	0.00	0.00
Kaad El-Zaurak Island	112	21.100	0.00	0.00
Bousti	17	9.250	100.00	100.00
Tallah reef	1585	347.450	1.15	3.73
Abou Koussa reef	8	1.500	0.00	0.00
Umm El-Hagar	19	2.100	0.00	0.00
Umm El-Raak	13	3.750	0.00	0.00
Bagel	73	19.300	12.31	29.27
Gadeefa	186	77.850	18.82	34.49
Al-Hacece	22	6.150	0.00	0.00
Abou Atteque	451	189.750	0.00	0.00

The population density of sea cucumber species differed widely by habitat (Hasan and Hasan 2004), species behaviour (Hammond 1982; Young and Chia 1982) and level of exploitation (Conand 1990, 1998, 2004). The population densities of the high value species *Holothuria scabra*, *H. nobilis* and *H. fuscogilva* were very low in the surveyed sites. The same species showed much higher densities in Papua New Guinea in 1981 (0.29 to 0.37 individuals m^{-2}) (Shelly 1981). In 1979, Lawrence recorded very high abundance of *A. mauritiana* in Guam (12 individuals m^{-2}). The density of *H. scabra* recorded in Saudi Arabia's Red Sea is similar to the reduced densities measured in other places: e.g. in Indonesia *H. scabra* density ranged from 0.0025 to 0.39 individuals m^{-2} (Darsono et al. 1998) and in Papua New Guinea it was 0.01 to 0.02 individuals m^{-2} (Lokani et al. 1996).

The biomass of all the commercial species of sea cucumbers at all the surveyed sites was low and was probably not sufficient to maintain a population or sustain a reliable fishery. Moreover, the quantity of high value species (*Holothuria fuscogilva, H. nobilis* and *H. scabra*) was very low, indicating heavy depletion. The quantity of medium and low value species on all reefs at the studied sites was also low. The currently targeted species, *H. atra*, was severely depleted at all sites except for Tallah reef and Braim Island, which showed population recovery and relatively high densities. The biomass of *H. atra* at Tallah reef was low due to the high percentage of small animals.

Harvesting of sea cucumbers in Saudi Arabia started in 1999 and was banned in 2004. The recovery of overfished sea cucumber stocks is a long process, taking several years (Purcell et al. 2002). Reduction of population density by overfishing may render remaining individuals incapable of successful reproduction. It is now apparent that depleted stocks of high value sea cucumber species at the surveyed sites may take decades to recover. The absence of effective control, surveillance and enforcement of regulations has resulted in widespread illegal fishing of sea cucumber leading to depletion of the resource. There is little awareness of the benefits that may be gained from an effective sea cucumber fishery managed by stakeholders in this sector and this is a critical cause of the overexploitation and depletion of sea cucumber. More effective training opportunities for managers of fisheries, scientists, shore protection personnel, environmental protection officers, cooperative staff and fishers are required.

The authorities must make appropriate rules and regulations to maintain sea cucumber resources. These rules and regulations should spell out the proper procedures for harvesting. Also, the recording of reliable and accurate data on the sea cucumber fauna of Saudi Arabia's Red Sea is required. These requirements should be implemented within a management plan.

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From hatchery to community – Madagascar's first village-based holothurian mariculture programme

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Abstract

In the region of Toliara (Madagascar), a novel partnership has emerged between local communities, non-governmental organisations (NGOs) and private sector stakeholders. The collaboration is working to pioneer a form of village-based mariculture in which hatchery-reared juvenile sea cucumbers are raised by coastal communities in simple sea pens. The present paper describes how partners are working to demonstrate the social and economic viability of a new model for alternative livelihood creation amongst coastal people in this impoverished region.

Introduction

The sea cucumber fishery plays an important role in the economy of Madagascar. In 2002 exports were valued at an estimated USD 3.1 million, representing about 2 per cent of the total export value of all marine resources (Rasolofonirina 2007). In the Toliara region (southwest of Madagascar), the fishery is a traditional activity (Rasolofonirina et al. 2004) that is very actively pursued (Rasolofonirina and Conand 1998) by coastal communities. For Vezo fishers who inhabit this region, the collection of sea cucumbers has become an integral component of local livelihoods and provides the primary source of income for a significant proportion of the population (McVean et al. 2005). The simple fishing techniques used, such as harvesting on foot or free-diving, require minimal material investment and ensure the equal participation of men, women and children (Pascal 2008).

Since the early 1990s, fisheries scientists and local communities in southwest Madagascar have witnessed a marked decline in the abundance of holothurians. Current signs of overexploitation include declining quality, a decrease in product size, the use of illegal material for harvesting (216 diving tanks were seized in 2002), the strong competition between collectors (Conand et al. 1998), the exploitation of fishing areas out of Malagasy waters (Rasolofonirina et al. 2004) and the collection of juveniles (Conand et al. 1997; Rasolofonirina 2007). This overexploitation of the resource is associated with a transition from a traditional or family-based fishery to an artisanal, semi-industrial fishery, driven by the increased international demand for trepang (beche-de-mer) and the scarcity of sea cucumbers in shallow waters (Rasolofonirina et al. 2004).

Overexploitation of holothurians has potentially serious adverse socio-economic and ecological consequences. At a community level the increasing scarcity of a high value export product would lead to increased poverty and instability in village communities that have gradually concentrated their activities around the exploitation of trepang (Rasolofonirina et al. 2004). From an ecological viewpoint, sea cucumbers are major components for sustaining coastal ecosystems in tropical areas, as ecosystems engineers that increase the structural complexity of the habitat and as macro-detritivores that consume various organic detritus (Coleman and William 2002; Rasolofonirina et al. 2004).

It is within this regional context that a novel partnership has emerged between local communities, non-governmental organisations (NGOs) and private sector stakeholders. The collaboration is working to pioneer a form of village-based mariculture in which hatchery-reared juvenile sea cucumbers are raised by coastal communities in simple sea pens. Partners are working to demonstrate the social and economic viability of a new model for alternative livelihood creation amongst coastal people in this impoverished region. If successful the project will mark an important transition from exploitation to husbandry of a critical marine resource. In the long term this initiative will also support the regeneration of natural populations.

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Development of holothurian aquaculture in Madagascar

The complex nature of holothurian fisheries makes the implementation of fisheries management measures difficult due to the numerous levels of the supply chain that require regulation. In Madagascar, holothurian fisheries management is exacerbated by inappropriate regulations, weak enforcement and inadequate collection and management of statistics (Rasolofonirina 2007). For this reason, sea cucumber aquaculture is currently considered to be the best solution to manage exploitation (Lavitra 2008). *Holothuria scabra*, also known as the sandfish, is one of the most economically valuable sea cucumber species. It is heavily exploited throughout the Indo-Pacific region for marketing in Asia (Rasolofonirina et al. 2005). It is a suitable species for aquaculture as it has a larval development that can be controlled and is tolerant of a range of environmental conditions (Hamel et al. 2001).

Madagascar was one of the first countries in the world to pioneer the hatchery technology to rear *H. scabra* and remains the only country in the Western Indian Ocean capable of culturing them. The project originated in Madagascar in 1999 with the launch of a sea cucumber mariculture project (Jangoux et al. 2001) funded by the Belgian University Cooperation for Development (Coopération Universitaire pour le Développement — CUD) and the government of Madagascar (Eeckhaut et al. 2008). The project linked scientists from two Belgian universities (Université de Mons-Hainaut and Université Libre de Bruxelles) and the Institut Halieutique et des Sciences Marines (IHSM) in Toliara. Over two main phases between 1999 and 2007 the project successfully developed the technology and the facilities (hatchery, nursery site and sea pens) to produce juvenile sandfish and grow them to commercial size.

In March 2008, the project evolved from its experimental roots into the commercial domain with the creation of Madagascar Holothurie SA (MHSA), the first private company based on sea cucumber aquaculture in Madagascar (Eeckhaut et al. 2008). The company was formed to incorporate representatives from the Belgian universities, the IHSM and Copefrito SA, a private fisheries collection and export company based in Toliara. The diverse actors within the new company provide the company with expertise in beche-de-mer production and export. The main objective of MHSA is to scale up the production of the current sea cucumber hatchery at IHSM and the nursery site at Belaza to commercial levels and produce 200,000 juveniles per annum over the next five years (Eeckhaut et al. 2008). MHSA identified the main bottleneck in the production of holothurians on a commercial scale as the limited space in artificial ponds. However, recognising the potential of the vast intertidal seagrass beds along Madagascar's southwest coast, coupled with the pressing need to provide viable alternative livelihoods for fishing communities, the company opted to collaborate with local NGOs to grow out sea cucumbers through village-based mariculture.

Launch of a village-based mariculture program

Two local NGOs, conservation group Blue Ventures (http://www.blueventures.org) and Trans'Mad-Développement (http://www.transmad.org), working in partnership with MHSA, have recently received support from ReCoMaP (Regional Programme for the Sustainable Management of the Coastal Zones of the Indian Ocean Countries) to develop holothurian mariculture as an alternative livelihood for communities in the region. During the two-year project, their role is to support and accompany the development of approximately 50 mariculture units along Madagascar's southwest coast. ReCoMaP funding will enable the NGOs to provide partner communities with the financial and logistical support to build and stock locally-owned pens to rear juvenile sea cucumbers purchased from MHSA to commercial size. By the end of the project, the objective is to establish the model and support mechanisms to enable the replication of this activity along the coast of Madagascar, involving new villages and community beneficiaries.

The two NGOs are operating in geographically distinct areas to ensure maximum spatial coverage for the project. Trans'Mad-Développement already runs projects in the marine domain in the Toliara region, including the establishment of a regional salt depot for iodisation (Co.Re.SEL) and the creation of a specialist marine college. For the pilot mariculture trials, the NGO has selected villages in the vicinity of Toliara, including Fiharenamasay and Andrevo-Bas to the north and Sarodrano to the south (Fig. 1). Selection criteria for villages have been based primarily on the availability of suitable grow-out sites for *H. scabra* in close proximity to the village.

Blue Ventures is based in the remote village of Andavadoaka, some 200 km north of Toliara. It works primarily in partnership with Velondriake (http://www.livewiththesea.org), a community association meaning 'to live with the sea' that unites 24 coastal villages grouped into three constituent geographic regions. Together they manage a network of community-run marine and coastal protected areas that were established in 2006 to protect over 800 km^2 of marine and coastal resources. In 2007, Blue Ventures commenced pilot studies to demonstrate the feasibility of holothurian mariculture in collaboration with IHSM, Copefrito, the Women's Association of Andavadoaka and the village of Ambolimoke. In

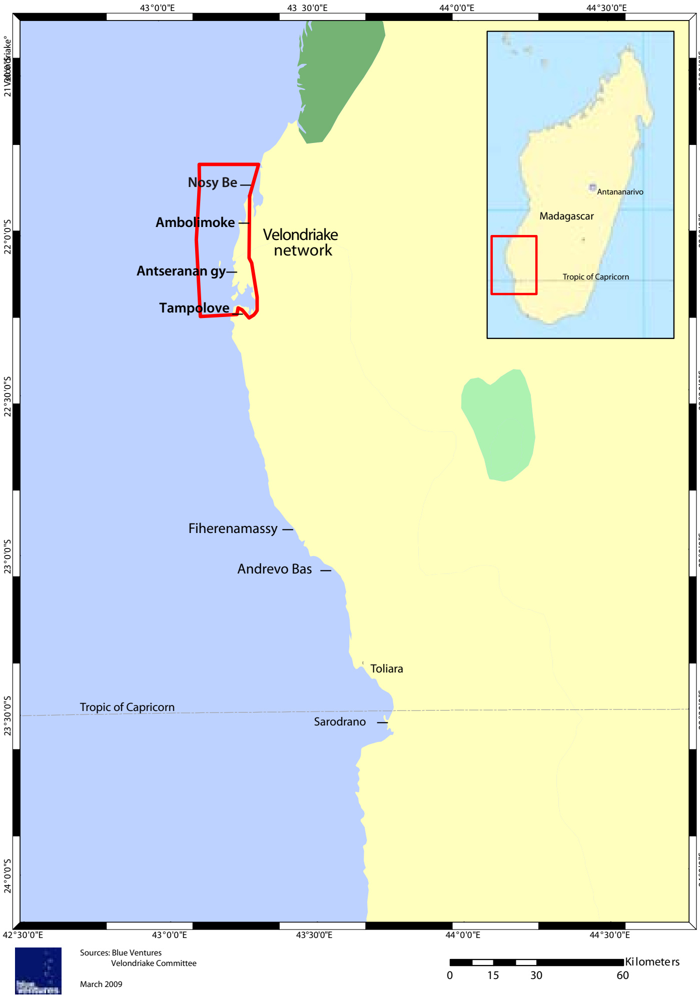

Figure 1. Locations of the pilot mariculture trials by Blue Ventures and Trans'Mad-Développement in southwest Madagascar. (Image: B. Pascal, Trans'Mad-Développement)

this new project with MHSA, holothurian mariculture will continue with the Women's Association and the villagers of Ambolimoke and will be expanded to the villages of Tampolove and Nosy Bé.

Technical and financial solutions

A number of different social models of ownership are being tested in the project including working with family lineages, associations, nuclear families and groups of families. A written agreement that specifies the conditions and responsibilities of each party is drawn up between each group and the NGO. The project aims to fund and obtain the start-up materials for a total of four pens per group and provide training and supervision to villagers during pen construction.

The pens are constructed from locally-available materials comprising nylon fishing net with a 10 mm^2 mesh, wooden stakes, and 2 mm and 4 mm rope (Figs 2 and 3). The two-meter-wide net is doubled and iron rebar is stitched into the base of the net, which is buried 25 cm deep in the sediment to prevent escape of juveniles.

The farming system has been designed to follow best-practice methods for aquaculture and simultaneously generate substantial revenues for the farmers. Different pen sizes of 12.5 m x 12.5 m and 15 m x 15 m are being tested, stocked with 300 and 450 sea cucumbers respectively. As sea cucumber growth is density dependent it is recommended that stocking densities should not exceed 2 individuals m^{-2} to allow for optimal growth (Lavitra 2008).

With financial support and technical guidance from partner NGOs, the farmers are able to buy batches of juveniles on credit from Madagascar Holothurie to rear in their pens (Figs 4–7). Once the sea cucumbers reach a commercial size of 300–350 g the adults are re-sold live to Madagascar Holothurie with the cost of the juveniles deducted. As the grow-out cycle of *H. scabra* is estimated to be 12 months, stocking and harvesting of the four pens will be spread throughout the year at three-month intervals.

This quarterly cycle of stocking and harvesting is intended to spread both the risks associated with the exploitation and the income generated from harvest evenly throughout the year. This strategy is also aimed at helping farmers understand the need for re-investment in order to pursue the activity in the long term once financial support has ended, when farmers will need to meet the costs of purchasing juveniles and maintaining pens independently of direct NGO assistance.

Although sea cucumber farming is a relatively straightforward activity requiring minimal labour and no input of additional feed, there are a number of regular supervision activities that need to be undertaken to assure maintenance of pens and security of the stock. Loss of individuals through theft currently poses a major risk to the project. Sandfish are a high value sea cucumber species and there are currently numerous beche-de-mer traders operating in the region. Locally, top-grade sandfish are valued at USD 2.5–3 per individual (Lavitra 2008); therefore for people in Madagascar, where 60 per cent of the population has an income level below USD 1 per day, poaching — even on a small scale — is a tempting prospect. For this reason, farmers are obliged to undertake nightly surveillance in order

Figure 2. Wild sea cucumbers drying in a Vezo village. (Image: B. Pascal, Trans'Mad-Développement)

Figure 3. The sea cucumber nursery at Belaza, southwest Madagascar. (Image: B. Pascal, Trans'Mad-Développement)

Figure 4. Hatchery-reared juvenile *H. scabra.* (Image: G. Robinson, Blue Ventures)

Figure 5. The Women's Association of Andavadoaka building their first pen. (Image: G. Cripps, Blue Ventures)

Figure 6. Commercial size *H. scabra* reared by the Women's Association. (Image: G. Cripps, Blue Ventures)

Figure 7. Families in Ambolimoke preparing nets and building their pens. (Image: G. Robinson, Blue Ventures)

to guarantee the integrity of their stock over the entire grow-out cycle. In addition, the pens require regular attention and maintenance, including the removal of predators such as crabs (*Thalamita crenata*) and sea stars (*Culcita* spp.), checking net integrity and removing net fouling to ensure adequate water exchange. However compared to the challenge of ensuring nightly surveillance these tasks are a small consideration. Monthly monitoring of growth rates is carried out at night during spring tides with the assistance of NGO workers to allow better understanding and evaluation of the factors affecting growth and mortality.

Conclusion

This new model for initiating community-based holothurian aquaculture in southwest Madagascar provides a novel approach to developing new alternative livelihoods for Vezo communities in the region. The project clearly has enormous potential for revenue generation for local communities and MHSA, however the initiative currently faces diverse challenges spanning a range of social, economic and biological issues.

Given the minimum 12-month grow-out time required to accrue the first economic benefits of this activity, it is necessary for families to continue their former daily working and economic activities in parallel with this mariculture initiative. As such the success of the project will rely on finding a means of effectively integrating this activity alongside current livelihoods in order that aquaculture tasks are not abandoned or neglected. It should be recognised however that this new venture is not intended to replace fishing, which remains critical to the subsistence needs of families. Rather, objectives for holothurian mariculture in the region are to provide a complementary activity, intended to provide communities with a supplementary source of income and to decrease the economic incentive of fishing.

In the long term it is hoped that the effective initiation of holothurian mariculture will slow the local overexploitation of wild stocks. This may come about as a result of income generated from mariculture activity reducing the need to exploit sea cucumbers as a cash commodity, as well as by farmed adults supplying recruits needed to replenish local fisheries. In southwest Madagascar, stocks of *H. scabra* are severely overexploited. Depletion of breeding adults may have already diminished the reproductive success of wild populations beyond the threshold needed for natural recovery. Studies from elsewhere in the Indo-Pacific region have postulated that the threshold for this so-called 'allele effect' for tropical sea cucumbers may be between 10 and 50 individuals ha^{-1} depending on species and location (Bell et al. 2008). Research on the abundance and distribution of exploited holothurians carried out on Toliara's Grand Récif barrier reef in 1997 showed the average biomass of *H. scabra* in seagrass beds to be 3.774 kg ha^{-1}. These data indicate that even 10 years ago, wild stocks of sandfish were close to the threshold for the allele effect. As sandfish reach sexual maturity at a size of 150–200 g (Conand 1990; Hamel et al. 2001), the majority of sea cucumbers reared in pens will have the opportunity to reproduce before they are harvested at a size of 300–350 g. The pens therefore constitute a 'spawning biomass' of sandfish. The planned creation of 50 mariculture units along Madagascar's southwest coast will establish a network of protected spawning aggregations which may be of sufficient size and density to allow for successful reproduction and fertilisation of the species, thus providing a means of supporting recruitment for severely overexploited wild populations of *H. scabra*.

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Phagocytosis by amoebocytes in *Apostichopus japonicus*

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Abstract

The present study is a quantitative analysis of phagocytosis by amoebocytes of *Apostichopus japonicus*. Zymosan A was used as a marker. It was mixed *in vitro* with the coelomocytes and the rate of phagocytosis was analysed by counting cells under a light microscope. The phagocytic rates (expressed as percentages) of ingested zymosan A by amoebocytes were quantified when the marker was placed in contact with amoebocytes at different temperatures (4, 10, 15, 20 and 30°C) and for different periods of incubation (15, 45 and 60 min). It was found that the phagocytic activity varied in relation with the temperature and the exposure time. Amoebocytes are highly efficient at cleansing zymosan A.

Introduction

Most of the immune responses are performed by phagocytes (reviewed by Gross et al. 1999), and phagocytes may also function in the cellular encapsulation of foreign materials and microbes through clotting, in which the phagocytes intermesh with each other (Hillier and Vacquier, 2003). Characterisation of the coelomocytes of *A. japonicus* has been demonstrated directly by using a light microscope and an electron microscope (Eliseikina and Magarlamov 2002; Xing et al. 2008). Six types of coelomocytes were identified: lymphocytes, morula cells, amoebocytes, crystal cells, fusiform cells and vibratile cells (Xing et al. 2008). In echinoderms, there have been some quantitative analyses of phagocytes. Beck and Habicht (1993) demonstrated that *in vitro* phagocytosis in the sea star *Asterias forbesi* was positively correlated with incubation time. Xing and Chia (1998) used a flow cytometric method to study the quantitative characteristics of amoebocytes in *Holothuria leucospilota*; phagocytosis was abundant and positively correlated with the bead/cell ratio. The goal of the present study was to quantify the rate of phagocytosis of *A. japonicus* amoebocytes using zymosan A.

Materials and methods

Coelomic fluid collection

Healthy sea cucumbers *Apostichopus japonicus* (body length: 10 to 15 cm; weight: 100 to 150 g) were obtained from aquatic farms in Qingdao, Shandong Province, China. The sea cucumbers were transferred to the laboratory in a 500 L PVC tank supplied with 10°C sand-filtered and aerated seawater, which was renewed daily. All animals were acclimatised for at least four weeks prior to the experiments. About 5 to 10 mL of coelomic fluid (CF) were drawn from the right lateral side of the body (Santiago-Cardona et al. 2003) using a 25 gauge needle, and diluted with an equal volume of artificial seawater (ASW, pH 7.4); EDTA fixative (6 x 10^{-3} M EDTA, 0.01 M phosphate buffered saline [PBS], ASW, pH 6.0) (Noble 1970) was used as a disaggregating agent. The CF was stored in sterile 5 mL centrifuge tubes at 4°C.

Phagocytosis of zymosan A

The CF was placed in sterile 1.5 mL centrifuge tubes and then centrifuged at 2000 r min^{-1} for 10 min at ambient temperature. Zymosan A (BSA, Sigma — Aldrich, Steinheim, Germany) was then added to the extracted serum two hours prior to the phagocytosis assays at a concentration of 0.1 g mL^{-1} at ambient temperature. In order to study the phagocytosis of the zymosan A, 0.2 mL of filtered CF diluted with the sterile ASW (proportion 1:1) was extracted and mixed with the diluted activated zymosan A. The assays were performed at 4, 10, 15, 20 and 30°C for 15, 30 and 60 min in sterile centrifuge tubes. Subsequently, the percentage of phagocytising cells was estimated after observing 100 cells randomly in Opton image system at magnifications of 400x.

Results

Amoebocyte morphology

The amoebocytes possessed various pseudopodia radiated from the cytoplasm. All amoebocytes possessed petaloid or filiform pseudopodia (Figs. 1

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and 2) that radiated in different directions from the central endoplasmic mass of the cells. Amoebocytes were capable of clotting with filiform pseudopodia (Fig. 2).

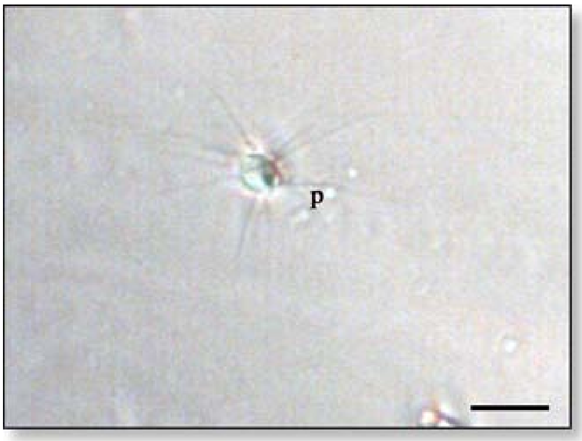

Figure 1. Light micrograph showing petaloid amoebocytes; p: pseudopodia (bar = 10 µm).

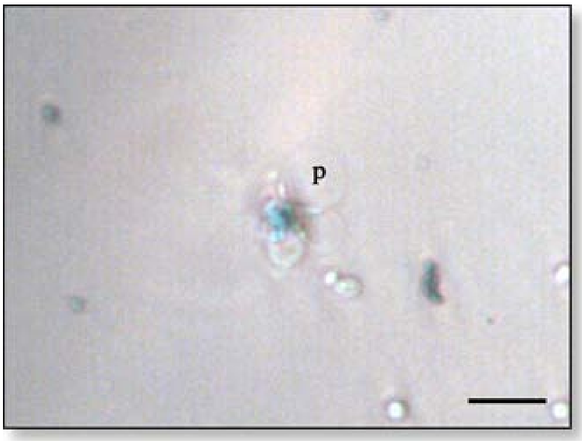

Figure 2. Light micrograph showing filiform amoebocytes; p: pseudopodia (bar = 10 µm).

Phagocytosis of zymosan A

Light microscopy revealed that amoebocytes showed a phagocytic response to the zymosan A (Fig. 3) when incubated in suspension. Amoebocytes were shown to be actively phagocytic. The results suggested that the phagocytic ability of amoebocytes was high: phagocytising cells represent from 30 to 70% of the counted cells. The relationship between the phagocytising ability and temperature was consistent (Fig. 4). The phagocytising ability was efficient when zymosan A was added and soon after the CF was extracted from the sea cucumber; after 1 hour the coelomocytes had clotted at 30°C.

Figure 3. Light micrograph showing phagocytic responses to the zymosan A; arrowhead: zymosan A (bar = 5 µm)

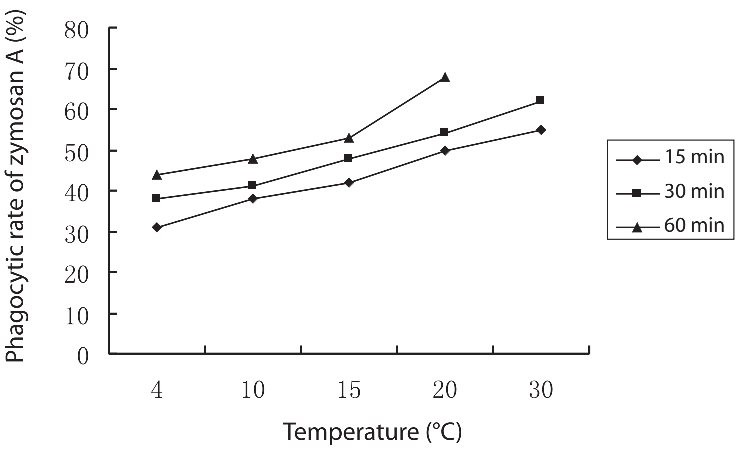

Figure 4. Quantitative analysis of phagocytic rate of zymosan A by amoebocytes in *Apostichopus japonicus*.

Discussion

Of the six types of coelomocytes of *A. japonicus,* the amoebocytes were the second most abundant cells, the first being the lymphocytes (Xing et al. 2008). Amoebocytes can take up dyes and other particles from the gut contents, and filiform amoebocytes participate in wound healing and clotting. On the whole, amoebocytes carry out echinoderm cellular immunity as they are capable of phagocytosis and nutrition and are active agents in the clotting reaction. Amoebocytes are recognised as containing heterogeneous materials of various sizes. These cells frequently have plenty of pseudopodia, and amoebocytes loading with pseudopodia often clump together. In the holothurian species studied, as well as in other echinoderms, the pseudopodia of the amoebocytes were petaloid and filiform (Edds 1993). The amoebocytes present the morphological changes that occur prior to and during cell aggregation. Careful observations of the fresh preparations reveal that all amoebocytes possess ectoplasmic pseudopodia that radiate in different directions from the central perinuclear cytoplasm of the cell. Petaloid-shaped phagocytes were capable of direct transformation into amoebocytes with filiform pseudopodia, but the transformation of filiform pseudopodia into petaloid pseudopodia has never been observed (Hetzel 1963). Amoebocytes were sensitive to induced stress. Amoebocytes also undergo a stress-induced petaloid-filopodial transition in response to UV-B radiation (Matranga et al. 2006). The changes of the petaloid pseudopodia into filiform forms were accelerated and the cell aggregation was apparent after the cells were mixed with zymosan A *in vitro*.

The quantitative study of phagocytosis showed that there is a consistent relationship between phagocytising ability and temperature. Both of the petaloid and filiform amoebocytes reacted; after phagocytosis the amoebocytes transformed from petaloid form to filiform form, other types of coelomocytes showed limited phagocytising ability. The amoebocytes showed the ability to agglutinate *in vitro*. In fresh preparations the petaloid pseudopodia appear to collapse and a redistribution of the cytoplasm produces branching filiform pseudopodia of considerable length, often intermeshing with the filiform pseudopodia of other amoebocytes. As the temperature increased and time elapsed, the amoebocytes clotted with morula cells and lymphocytes, which would reduce the effectiveness of phagocytosis.

Acknowledgements

This research was supported by the National Natural Science Foundation of China (grant no. 40576073) and the National Key Technology Research and Development Program (grant no. 2006BAD09A02/09). Prof. Conand provided suggestions and assistance with this paper; we are grateful for her help and information.

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Communications...

Abstract of a degree project thesis sent by Hanna Nilsson

Management strategies in the sea cucumber fishery in Zanzibar, Tanzania

Source: Degree project thesis.

Supervision: Dr. Maricela de la Torre-Castro and Prof. Nils Kautsky, Dept. of Systems Ecology, Stockholm University, Sweden

Sea cucumbers are considered key organisms for functioning ecosystems since they maintain essential ecological functions such as nutrient recycling and bioturbation. They also provide an important source of income for coastal communities. There is a long history of commercial demand, harvest and export of sea cucumbers from the Western Indian Ocean (WIO) to China and other Asian countries. At present, the demand remains high and signs of overexploitation are emerging. However there is little knowledge about sea cucumber biology and ecology and about their socio-economic importance as well as the management practices in the WIO region. To increase the knowledge about these aspects, a three-year project was initiated to study sea cucumber management in Zanzibar, Tanzania. The aim was to investigate the current formal and informal management strategies and to suggest improvements to enhance the sustainability of the sea cucumber fishery on the island. The information was gathered using semi-structured interviews held with resource users and government officials and from acts and policy documents. Formal sea cucumber management in Zanzibar was found to be weak and insufficient. Regulations and license control are highly unclear. No statistical data are gathered on fresh sea cucumber catches; data are gathered only for dried product before export. In addition, no stock assessment is regularly done and no formal monitoring is carried out. A minimum size limit of 10 cm for fresh sea cucumbers exists, but this regulation is not properly enforced. In addition, the informal management strategies seem to be insufficient and cannot maintain the sustainability of the activity. However, a willingness to maintain sustainable harvest levels was detected among both resource users and government officials. This is of great importance for successful management. In light of this willingness, management suggestions were developed based on ideas provided by the resource users and managers. The suggestions are rooted in the actual management organisation and structure in Zanzibar and are strengthened by studies from resource management literature. The proposed ideas may function as an initial step to establish a more comprehensive sea cucumber management plan in Zanzibar and will contribute to the regional studies in WIO.

Abstract of a degree project thesis sent by Caroline Raymond

The structure of the sea cucumber production chain and resource use in Zanzibar, Tanzania

Source: Degree project thesis.

Supervision: Dr. Maricela de la Torre-Castro and Prof. Nils Kautsky, Dept. of Systems Ecology, Stockholm University, Sweden

In the Western Indian Ocean (WIO), sea cucumber fisheries are considered to be an important income source for several coastal communities. However, due to high demand, sea cucumbers suffer from worldwide overexploitation and reduction in populations. This also occurs in the WIO area. The general objective of this study is to map the structure, actors and flows of the sea cucumber fishery in Zanzibar (Unguja Island). The study is based on 100 interviews with actors involved in sea cucumber fishery activities. The results show that the structure of the sea cucumber production chain consist of collectors (fishers), processors (middlemen) and traders (exporters). There are three collection techniques: walking by the shoreline, snorkelling and diving. Often, specialised processors work as intermediaries, acquiring the catch from collectors and selling it to traders who in turn do further processing before exporting to the world market (most commonly to Hong Kong). Both women and men are involved in collection, although women collect only by the shoreline. The collection activity is estimated to involve nearly 800 specialised sea cucumber collectors, and even more people are involved occasionally. However, the average income per day varies considerably between collector groups — a diver earns over TZS 30,000 per collection day, which is ten times more than a shoreline collector. The sea cucumber activity is estimated to generate an accumulated value of TZS 872,000,000 (USD 665,000) yearly for the specialised collectors. Furthermore, 20 species of sea cucumbers are of commercial value in Zanzibar; nearly all are collected by each of the three collector groups. However, the trend is for snorkelers and divers to collect the most valuable species in much higher quantity. The exported amount of beche-de-mer for the year 2007 was estimated to be 56 tonnes. The result indicates that collection of sea cucumbers may be a way to contribute to livelihoods. However, the sustainability of the activity should be carefully addressed.

Communication from Maricela de la Torre-Castro

As part of the project 'Sea cucumbers, a poorly understood but important coastal resource: National and regional analyses to improve management in the Western Indian Ocean (WIO)' (2006–2008), financed by the Marine Science for Management (MASMA) programme, we investigated the sea cucumber fishery system in Zanzibar. This part of the project covered the management, structural and economic issues of the sea cucumber fishery system and constitutes the first systematic study performed in Zanzibar. The main results are presented in the abstract section of this number. We will continue with stock and biodiversity assessments as well as comparative work to analyse the different management systems in the countries of WIO.

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Communication from Zaidnuddin Ilias

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Observation of the first grow out activities with *Stichopus horrens* juveniles in Malaysia

Stichopus horrens is an important commercialised sea cucumber in Malaysia. It is one of the main ingredients in local traditional medicines. The industry based on *S. horrens* trade supports a large number of individuals and promotes one of the islands (Langkawi) as a centre for production of sea cucumber medicine. Extracts from this species were used widely and known to have antifungal and antibacterial components. Local pharmaceutical companies have also become involved in researching compounds from the sea cucumber extracts.

Due to the increasing use and collection of sea cucumbers, the Department of Fisheries Malaysia started ecological and aquaculture research on this species. Research on aquaculture for this species started as early as 1994 at the Fisheries Research Institute of Malaysia, but recently great progress has been achieved. Two subsequent hatchery spawnings occurred in late 2007 and mid-2008 at the National Prawn Fry Production and Research Centre (NAPFRE), giving us the opportunity to attempt to grow the juveniles. Grow out in the hatchery was very slow, taking 30–40 days for the juveniles to reach approximately 4 cm in length. A trial in a sea cage in November 2008 was more successful. Growth to the same size took only half the number of days with a survival rate of 80 per cent. Further study is being carried out to determine the stocking density and possibility of holding the juveniles until they reach maturity.

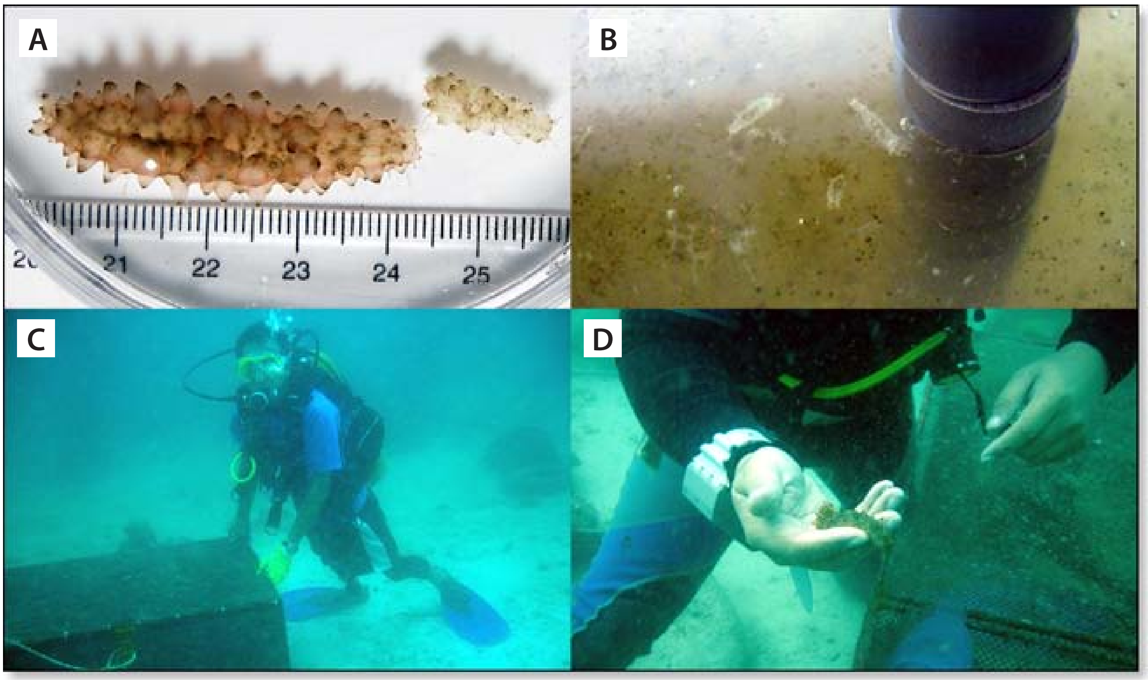

Figure 1. Juveniles reared in hatchery (A); early juveniles (B); diver with experimental cage (C); and juveniles after 20 days in cages (D)

Various news communicated by Poo Sze

Commercial harvest begins Monday for sea cucumbers

Published October 05, 2008, Ketchikan Daily News, http://www.ketchikandailynews.com

KETCHIKAN, Alaska — Divers are getting ready for the beginning of the commercial sea cucumber season in southeast Alaska with the lowest quota in several years.

The commercial season starts Monday morning.

Sea cucumbers are also known as sea slugs. They're echinoderms with an elongated body and leathery skin. They're a popular food in Asia. The Alaska Department of Fish and Game set a guideline harvest of just more than 1.1 million pounds for 18 harvest areas.

That's down about 19 per cent from last season and is 24 per cent lower than for the 2005–2006 season. The reason for the drop in the sea cucumber population isn't known. Biologist Marc Pritchett says there's an assumption that it's connected to sea otter predation.

Nukualofa, Tonga

Source: http://www.matangitonga.to/article/tonganews/economy/tonga_sea_cucumber_220908.shtml

After 11 years of closure, Tonga has lifted its ban on the fishing of sea cucumbers but only for six months per year, confirmed the Deputy Director of the Ministry of Fisheries 'Ulunga Fa'anunu today.

The ministry has awarded nine local companies licenses to fish for sea cucumbers in Tongatapu, Vavau and Haapai, and they will be mainly for export to Asian countries such as Hong Kong.

He said the Tonga Cabinet passed a new Fisheries Management (Conservation) regulation in July 2008 lifting the ban on sea cucumber fishing between April and September but keeping the ban for the remaining six months from October 1 to March 31 during the sea cucumber reproduction season.

In 1997 Tonga banned the fishing of sea cucumbers because it was overexploited; this action to protect the species has allowed for the stock to rebuild over the years.

Fa'anunu said that the current status of the Tongan economy was taken into account in the decision to re-open the industry. This activity may help the economy in terms of exports, while the fishery remains closed for part of the year to ensure its sustainability.

Mauritius to control sea cucumber fishing

Port-Louis — 27/08/2008

Port-Louis, Mauritius — The Mauritian Ministry of Agroprocessing and Fisheries will soon enact laws to control sea cucumber fishing, an official source told PANA here. An official from the ministry said on Tuesday evening that the decision was aimed at ensuring a sustainable exploitation of the marine resource.

'Under the regulations, anyone wishing to fish sea cucumbers will have to apply for a written authorisation from the Ministry of Agroprocessing,' he explained.

He added that fishing of sea cucumbers, commonly known as *bambara*, would be banned from 1–31 March each year to enable reproduction of the species.

Sea cucumber is a popular Chinese delicacy named for its cucumber-like shape and believed to be an aphrodisiac. The sea cucumber has an elongated body and leathery skin and is found on the sea floor worldwide.

Feasibility of Pacific oyster and California sea cucumber polyculture

Research team: Chris Pearce Fisheries and Oceans Canada (DFO), Debbie Paltzat University of British Columbia (UBC), Penny Barnes Center for Shellfish Research (CSR), Scott McKinley (UBC). For information contact Chris Pearce, PearceC@pac.dfo-mpo.gc.ca. Submitted by DFO Acquaculture Collaborative Research and Development Program (ACRDP).

Growth and production of California sea cucumbers (*Parastichopus californicus*), co-cultured with suspended Pacific oysters (*Crassostrea gigas*), were investigated in a 12-month study conducted at two deep-water, suspended oyster culture sites in British Columbia. Rates of oyster biodeposition (faeces and pseudofaeces), and the utilisation of this particulate material as a food source by *P. californicus*, were also examined.

Peaks in sedimentation rates (93.6 g dry weight m^{-2} day^{-1}) through 8.5 m of water depth were observed in April and July 2004. At the two study sites, maximum mean fluxes of total organic carbon in sediment traps at 8.5 m depth occurred in July 2004 and amounted to 3,123 and 4,150 mg dry weight C m^{-2} day^{-1}. Maximum mean fluxes of total nitrogen at the two sites were 633 and 441 mg dry weight N m^{-2} day^{-1} and occurred in July and November 2004, respectively. Mean C/N ratios of particulate material in the sediment trap samples collected at the two sites ranged between 5.93 and 8.39 and may be classified as being of high nutritional value.

Sea cucumbers grown in trays at both sites successfully utilised biodeposits from the cultured oysters and showed a mean weight increase of 42.9 g in approximately 12 months (average growth rates for both sites ranged from 0.061 to 0.158 g day^{-1}). Overall growth was affected by the absence of visceral organs and the cessation of feeding activity in the November 2004 sampling period. Mean values for organic content were significantly higher in the foregut of the sea cucumbers (233.0 mg g dry $sediment^{-1}$) than in the sediment (64.3 mg g dry $sediment^{-1}$) or in the hindgut (142.8 mg g dry $sediment^{-1}$), showing both active selection of organic material from the sediments and digestion/assimilation of these organics in the gut. Organic material deposited in the trays was assimilated by *P. californicus* with an average efficiency of 48.4 per cent.

The successful utilisation of the naturally-available biodeposits from the cultured oysters by sea cucumbers suggest the feasibility of developing a commercial-scale co-culture system that would both reduce the amount of organic deposition underneath shellfish farms and produce a secondary cash crop.

Abstracts and new publications...

Abstracts of papers presented at the 13th Echinoderm International Conference, University of Tasmania in Hobart, Australia

The fossil record, diversity and evolution of holothurians (Echinodermata)

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The palaeobiology and evolutionary history of holothurians are relatively poorly understood. Currently, there are only 800 fossil holothurian paraspecies and species, ranging from the Early Palaeozoic to the Quaternary, in comparison to more than 1,450 recent species. In part, this is due to their disjunct endoskeleton with ossicles and calcareous ring elements, which are released following decomposition of the surrounding tissue. However, under favourable conditions, holothurian body fossils may be preserved in various fossil lagerstaetten. The evolution and diversification of sea cucumbers have been reviewed and reinterpreted, including new records of Early Palaeozoic (Ordovician and Silurian) and Mesozoic (Jurassic and Cretaceous) material (calcareous rings and ossicles). Holothurians have a Phanerozoic history extending back more than 466 million years, ranging from the early Middle Ordovician to the present time. The maximum level of morphological diversification appears to have been reached in the Mesozoic, but this is in all likelihood due to a lack of research in other geological strata. A revised hypothesis of higher-level relationships within the Holothuroidea is presented. A more detailed interpretation of the fossil record requires a better understanding of the skeletal morphology of modern holothurian families, e.g. three-dimensional characters, stereom structure etc. of calcareous ring elements and body wall ossicles. There is also recent diversity that is hidden because detailed investigations are missing or sparse.

Morphological and molecular systematic data of elasipodid species (Echinodermata: Holothuroidea) from New Zealand's International Polar Year — Census of Antarctic Marine Life (IPY-CAML) 2008 NZ survey of the Ross Sea and Scott and Admiralty seamounts, Antarctica

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An extensive biodiversity survey of the Ross Sea and Scott/Admiralty seamounts was carried out by New Zealand in February/March 2008. Biological sampling using bottom trawls and coarse and fine mesh epibenthic sleds collected 900 holothuroid specimens. These specimens have been identified using traditional morphological systematics. Some molecular systematic analysis using the cytochrome oxidase-1 (CO1) gene has been completed by Gustav Paulay at the University of Florida. A total of 20 species of elasipodids were identified from the survey. The biogeographical relationships of these species are discussed in relation to the known ranges of Antarctic elasipodid biogeography. Distribution and depth records have been extended. Some new species or variations of known species were found. Five of the elasipodid species — *Pannychia sp.* cf. *moseleyi*, *Benthodytes sanguinolenta*, *Laetmogone wyvillethomsoni*, *Peniagone affinis* and *Peniagone wiltoni* — are discussed in relation to circumpolar distribution and occurrence north of the Antarctic Convergence. There is some degree of congruence between morphological systematic data and molecular phylogentics, as well as evidence of sister species north and south of the Antarctic Convergence. Genetic data thus suggest a review of some morphological systematic conclusions.

Holothurian settlement in two protected reefs at Cozumel, Mexico

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We analysed the intensity of settlement of holothurians in two reefs from the Arrecifes de Cozumel marine park using a series of suspended collector systems (Witham type). The collectors were composed of squares of Astroturf mat (50 x 50 cm) attached to plastic frames supported by buoys and weights to keep them straight. Three collectors were deployed at each site in two reefs (Palancar and Tormentos) (total 10 sites) from May 2007 to June 2008. A total of 191 individuals of *Holothuria arenicola* and 114 of *Euapta lappa* were counted during the study period. The smallest specimen belonged to *H. arenicola* (0.9 cm) and the largest (10 cm) to *E. lappa*, both found in November 2007 at Tormentos reef. The size of youngsters was not statistically different between months or between reefs. There were differences in settlement amongst reefs but not between months, suggesting that reproduction may be continuous throughout the year. Palancar showed the lowest number of recruits. These differences may be explained by the strong currents that influence Palancar reef and by the lack of hard substrate; this reef is located at southern boundary of the park and is dominated by sea grass and high levels of turbidity, associated with anthropogenic activities. Tormentos reef is located in the north of the park and is dominated by coral reef and characterised by clear waters. We concluded that both species settled most of the year and that settlement seemed to be strongly influenced by currents and water conditions.

Epifauna associated with the sea cucumber *Holothuria mexicana* in Puerto Rico

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The epifauna of two populations of the shallow water sea cucumber *Holothuria mexicana*, inhabiting sea grass beds in Guilligan Island y Caribe Keys (Puerto Rico), were analysed. We found that all individuals are covered by a whitish mucilage, probably secreted by them or by microorganisms, to which organic debris (shells, vegetative litter, and coral fragments) and nonorganic debris (gravels, sand, and plastic and metallic fragments) adhere. The epidermis of 122 specimens was gently scrubbed *in situ* with a spatula for approximately 15 seconds while washing the animal to collect the epidermic material. Individuals were returned to their habitat. The collected material was preserved in a 75 per cent ethanol solution. In the lab, samples were filtered with a set of meshes to separate the debris. Using a stereo microscope the debris was separated and organisms were collected and identified. The liquid sample was examined with a compound microscope to identify smaller organisms. A photographic inventory was developed. We found that 100 per cent of samples contained macro- and micro-invertebrates, which were classified as polychaetes (39.0 per cent), mollusks (29.5 per cent), crustaceans (isopods, copepods, ostracods, stomatopods, amphipods, decapods) (28.0 per cent) and others (zoanthids, ophiuroids, porifers) (3.5 per cent). Statistical analyses revealed that the presence of mollusks and crustaceans was significantly different in the two localities (ANOVA F = 3.097* and 12.542**, respectively). We do not know if the existence of this epifauna occurs at random or is a byproduct of commensalisms or symbiosis, or if the mucilage provides a food or shelter to them. This sea cucumber species secretes holothurin A and B, a toxin for many potential predators. These preliminary findings constitute baseline data for future projects aiming to assess the ecological niche of *Holothuria mexicana* and its epifauna.

Aspidochirote sea cucumber diversity and status of stocks in the Bunaken National Marine Park (BNMP), North Sulawesi, Indonesia

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The species richness of aspidochirote sea cucumbers associated with reefs of the BNMP is very high, suggesting, along with data for other faunal groups, that this archipelagic region, including other neighbouring islands at the tip of North Sulawesi, may constitute a diversity 'hot spot' within the Indo-Malay zone of maximum marine biodiversity. For some forms, abundances are high but the animals are patchily distributed compared to other areas in the Indo-Pacific, a phenomenon which may reflect localised concentration of settling recruits in areas of eddy currents. Yet in general, abundances of commercial sea cucumber species are low. Prior to initial designation of this area as a National Marine Park in 1991 and the commencement of protection measures, the reefs and reef slopes around the Bunaken Islands were heavily fished for commercial sea cucumber species. No reliable data exist for this fishery but recent preliminary findings of sea cucumber surveys indicate that over the last two decades recovery of populations has not occurred.

Molecular phylogeny of symbiotic pearlfishes

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Carapid fishes are certainly among the most remarkable organisms living in symbiosis with echinoderms. These fishes, known as pearlfishes, belong to the family of Ophidiiform, which is divided in two sub-families, the Pyramodontinae and the Carapinae. Symbiotic pearlfishes belong to the latter sub-family. They are found inside bivalves, ascidians, asteroids and holothuroids. Their behaviour and morphology are extremely adapted to their symbiotic way of life. Numerous aspects of the biology of these fishes have been recently elucidated, though not from an evolutionary perspective. We present here the first molecular phylogeny of the symbiotic pearlfishes, including *Echiodon*, *Onuxodon*, *Carapus* and *Encheliophis* genera. The phylogenetic relationships of 20 specimens from eight species coming from the Mediterranean, the Indian Ocean and the Pacific Ocean have been estimated. Five mitochondrial fragments (3,645 base pairs) have been sequenced and the phylogenetic trees were obtained via Maximum Parsimony, Maximum Likelihood and Bayesian analyses. The analyses suggest the paraphyly of the *Carapus* group in regard with the monophyletic *Encheliophis*. It also suggested that *C. boraborensis* is the sister group of all the other sequenced pearlfishes and that *C. bermudensis* and *C. acus* are grouped within a clade. Various characters have been mapped on the trees to estimate the history of these symbiotic fishes.

Mitochondrial markers reveal many species complexes and non-monophyly in aspidochirotid holothurians

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Despite their striking presence on coral reefs and the multi-million dollar industry targeting them, aspidochirotid holothurians have received relatively little taxonomic attention. Heavy reliance on ossicles has led to a confused taxonomy and masks substantial cryptic diversity. As part of a revisionary effort on holothurians, we are sequencing multiple specimens of available species to test for species limits and construct phylogenetic hypotheses. We are using a bottom-up approach, sequencing fast-evolving mitochondrial markers (16S and COI) first, as these are mostly informative at lower taxonomic levels. We have unraveled many species complexes and identified several undescribed species. For instance, our analyses revealed that the circumtropical 'species' *Holothuria impatiens* consists of a dozen reciprocally monophyletic, well-defined, evolutionary significant units (ESUs). Broad overlap in the range of some, in combination with recent divergence, indicates the rapid evolution of reproductive isolating barriers among these ESUs. Such rapid evolution to sympatric coexistence is also found in several other species complexes we identified, and contrasts with most other marine invertebrates. At a higher taxonomic level, preliminary results show that non-monophyly of currently recognised taxa is prevalent. Stichopodids emerge from paraphyletic synallactids. While holothuriids appear monophyletic, *Holothuria* is not, as several subgenera in that genus are deeply divergent. We present the latest phylogenetic hypotheses, which further our understanding of speciation and trait evolution in sea cucumbers.

Some molecular phylogenetic data for holothuroid species from Antarctica, Australia and New Zealand (Echinodermata: Holothuroidea)

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In collaboration with Gustav Paulay in the University of Florida, DNA phylogenetic data (CO1 gene) are becoming available for holothuroids from Antarctica, New Zealand and Australia. Data for some dendrochirotids and molpadiids and synallactids are reported. There is some good congruence for traditional morphological systematic conclusions and molecular phylogenetic data. These data also indicate a need for review of some morphological systematic conclusions in terms of synonymies and the existence of cryptic species. For example CO1 gene data indicate: *Molpadia musculus* Risso appears not to be cosmopolitan, but comprises at least four discrete species; the Antarctic *Psolus arnaudi* Cherbonnier and *Psolus cherbonnieri* Carriol and Féral appear to be junior synonyms of *Psolus dubiosus* Ludwig and Heding; specimens of *Molpadiodemas morbillus* O'Loughlin and Ahearn from the South Sandwich Trench (5452 m), New Zealand (2500 m) and southeast Australia (1993 m) show no genetic variations.

Diversity and distribution of holothuroid species south of the Antarctic Convergence (Echinodermata: Holothuroidea)

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A morphological systematic overview of current knowledge of the diversity and distribution of holothuroid echinoderm species from all depths south of the Antarctic Convergence is presented. There are 155 species, with 38 undescribed. Species occurrences south of the Convergence at Bouvet Island, Heard and Kerguelen Islands, Prydz Bay, Ross Sea, Bellingshausen Sea, Antarctic Peninsula and Weddell Sea, and north of the Convergence in the Magellanic region, are compared. Based on morphological systematics there is typically a circumpolar distribution south of the Convergence. However, most holothuroid species on the Heard/Kerguelen Plateau do not show a circumpolar distribution. The Convergence is a significant barrier to gene flow north from the Antarctic Ocean.

Observations of reproductive strategies for some dendrochirotid holothuroid species (Echinodermata: Holothuroidea: Dendrochirotida)

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Some recently observed reproductive strategies by dendrochirotid holothuroid species are reported and illustrated: fissiparity by *Cucuvitrum rowei* O'Loughlin and O'Hara, from southeast Australia; evidence of intra-coelomic brood fissiparity in *Staurothyone inconspicua* (Bell), from southeast Australia; intra-coelomic brood protection by an undescribed species of *Parathyonidium* Heding, from Antarctica; evidence of intra-coelomic brood auto-ingestion by *Neoamphicyclus materiae* O'Loughlin, from southeast Australia; brood protection in a longitudinal dorsal invaginated marsupium in small specimens of *Cladodactyla crocea* (Lesson), from the Falkland Islands. An analysis is presented of brood protection in interradial anterior marsupia by species of the *Cucumaria georgiana* (Lampert) group (including *Cucumaria acuta* Massin and *Cucumaria attenuata* Vaney), *Microchoerus splendidus* Gutt, *Psolidiella mollis* (Ludwig and Heding) and *Psolus charcoti* Vaney, all from Antarctica. The report and illustration of anterior interradial marsupial brood protection by *Psolus koehleri* Vaney, from Antarctica, is rejected.

Quantitative analysis of morphological characters in Stichopodidae (Holothuroidea, Aspidochirotida)

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Gross morphology and ossicles from the body wall provide the basic characters used in holothurian taxonomy. However, the plasticity of soft tissue morphology in both living and preserved specimens has focused taxonomic discrimination on the calcareous ossicles. Differentiation between ossicles is typically based on size and qualitative description of form. Variation in ossicle form exists, even within named forms (e.g. button, rosette, tack) within a single individual, and can challenge comparison of study specimens with published descriptions. While ossicles have been recorded from tissues other than the body wall, they have only been irregularly applied to taxonomy. By evaluating ossicles from tissues other than the body wall within the Stichopodidae and by applying statistical analysis (regression and multi-variate analysis) to ossicle size and presence-absence observations, this study makes available additional quantitative characters for differentiating between holothurian taxa.

Molecular diversity and body distribution of saponins in the sea cucumber *Holothuria forskali*

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Sea cucumbers contain triterpene glycoside toxins called saponins. We investigated the complex saponin mixture extracted from the common Mediterranean species *Holothuria forskali*. Two different body components were analysed separately: the body wall (which protects the animal and is moreover the most important organ in terms of weight) and the Cuvierian tubules (a defensive organ that can be expelled on predators in response to an attack). Mass spectrometry (MALDI-MS and MALDIMS/MS) was used to detect saponins and describe their molecular structures. As isomers were found in the Cuvierian tubules, a preliminary chromatographic separation (LC-MS and LC-MS/MS) was performed to identify each saponin separately. A quantitative study was also conducted to compare the amount of toxin in both body components. Twelve saponins were detected in the body wall and 26 in the Cuvierian tubules. All the saponins from the body wall are also present in the Cuvierian tubules but the latter also contain 14 specific saponins. The presence of isomeric saponins complicated elucidation of the structure of the whole set of toxins but 16 saponins have been characterised through their fragmentation pattern. Among these, three had already been reported in the literature as holothurinosides A and C, and desholothurin A. Molecular structures have been suggested for the 13 others which, in the present work, have been provisionally named holothurinosides E, F, G, H, I, A1, C1, E1, F1, G1, H1 and I1 and desholothurin A1.

Vertical sediment displacement caused by *Holothuria scabra* using fluorescent tracers

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This study investigated vertical sediment movement (mixing) caused by feeding and burying behaviours of the sea cucumber *Holothuria scabra* (sandfish). Animals were kept in aquaria with constant flow-through filtered seawater for six weeks. The entire sediment matrix (12 cm) was marked with three different coloured tracer particles. A sampling grid was established and sediment cores were randomly extracted after 1, 10 and 56 days. Cores were later cut in 2 cm sections and tracers were counted in subsamples from each section under a fluorescent microscope. Results were used to calculate instantaneous mixing rates of the surface layer as well as general sediment displacement throughout the entire sediment matrix. The study showed that sandfish did not affect sediment deeper than about 6 cm. After 56 days approximately 14 per cent of surface sediment was displaced to deeper layers with an instantaneous mixing rate of 0.25 per cent d^{-1}, meaning sandfish (at a natural density of 0.48 individuals m^{-2}) can displace the top 2 cm every 400 days. Furthermore, bioturbation figures were extrapolated to the sea cucumber population in Moreton Bay, showing that 33.4 tonnes of sediment would be displaced by the sandfish population (3.6 million) in the bay each day under summer conditions. Sandfish turned over sediment by a combination of two mechanisms: smearing (diffusion) and upward conveyor belt movements. Thus, based on existing functional groups of bioturbators, a new mode is suggested: the 'conveyor diffusors'. Holothurians have an important ecological role within subtropical seagrass habitats; however, they are quantitatively only half as effective as other important bioturbators such as polychaetes.

Daily and seasonal patterns in behaviour of the commercially important sea cucumber *Holothuria scabra*

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This study monitored and modelled long-term daily and seasonal patterns in behaviour of adult sea cucumber *Holothuria scabra* in Moreton Bay, Australia. Animals were kept in outdoor tanks for two years and behaviour was recorded each month for a 24-hour period by means of time-lapse video. Behaviour was classified into eight categories and a series of nested conditional, binomial models (generalised linear models) were applied to describe the probabilities of key behaviours occurring. Active behaviours, such as feeding and searching, were negatively correlated to water temperature and were approximately five times more common during summer (approximately16 hours day^{-1}) than during winter (approximately 3 hours day^{-1}). Animals were less likely to bury themselves during summer (December–February), with at least one month

where they did not bury themselves at all. There was an 80 per cent probability of animals being inactive during the early hours of the morning (around 5:00 a.m.), irrespective of the time of year; and a 50 per cent probability of animals being fully buried during mid-winter (July/August), irrespective of the time of day. Searching behaviour showed a bimodal pattern, where animals spent more time searching during autumn and spring (approximately 2 hours day^{-1}) than during summer (approximately1 hour day^{-1}) or winter (approximately 20 minutes day^{-1}). Describing patterns in holothurian behaviour, especially producing a probability matrix of active behaviour and burying frequencies, is crucial for designing sustainable fisheries management strategies and aquaculture projects. The key findings of this study provide information about optimal timeframes to conduct population surveys, and can be applied to the ecosystem function of tropical holothurians overall.

Concurring evidence of rapid sea cucumber overfishing in the Sultanate of Oman

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A fishery for *Holothuria scabra* recently developed in a small area of the eastern coast of the Sultanate of Oman. The area covered by the fishery is limited to a single shallow embayment covering only 320 km^2 in Mahout Bay and involves approximately 400 fishers, around 50 per cent of whom are women. The 2004–2005 fishing season (October to May) was the first officially on record. However, anecdotal evidence suggests a low level of exploitation since the early 1970s, although catch and export data for this period are unavailable. Average size of individuals collected in 2005 varied between 170 and 200 mm in length. The total biomass at the time was estimated at 1500 t (fresh weight). The following year at least 14.5 t of processed *H. scabra* were exported to the United Arab Emirates, corresponding to approximately 145 t or around 10 per cent of the recorded biomass. Interviews with fishermen and traders revealed that the CPUE for sandfish was around 100 individuals hour^{-1} in 2005. However, the CPUE had declined to 10–20 individuals hour^{-1} by 2007, indicating significant pressure on the resource. Over the same timeframe, the value of an average sized *H. scabra*, which was OMR 0.1 (USD 0.25) in 2005, increased to OMR 1.5 (USD 3.75) and is still climbing. Concomitantly fishers have begun targeting the less valuable *H. atra* in large numbers. This species commands market prices of OMR 0.2 (USD 0.5). Finally, an examination of the processed specimens for sale showed the presence of a significant number of very small individuals (<5 cm processed, corresponding to around 10 cm live length). This concurring evidence suggests a rapid decline of the population of *H. scabra* in Mahout Bay while indicating building pressure on *H. atra*. Accordingly, the Ministry of Fisheries Wealth has commenced a number of projects aimed at monitoring the sea cucumber fishery with the ultimate objective of providing a regulatory framework to ensure the sustainability of the resource. Projects also include an evaluation of enhancement and ranching techniques.

Gametogenic synchronicity with respect to time, depth and location in three deep-sea echinoderms

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This study examined the prevalence of inter-individual synchrony in the gamete synthesis of three deep-sea echinoderms, *Phormosoma placenta* (Echinoidea), *Zygothuria lactea* (Holothuroidea) and *Hippasteria phrygiana* (Asteroidea) collected along the continental slope off the coast of Newfoundland and Labrador (eastern Canada). Analysis of gonad development using histology and gonad indices revealed diverse degrees of asynchrony at the scales examined (within trawls, between trawls over similar or different periods, as well as between depths and locations over the same period). Annual and seasonal patterns were therefore largely masked by heterogeneousness in most samples. These data suggest that determination of so called 'continuous' reproductive cycles in many deep-sea species may in fact reflect sampling inadequacies inherent to most deep-sea studies. Assessment of true reproductive patterns and periodicities may require much tighter collection designs as these species are likely to rely on fine-scale cohesion and inter-individual exchanges (i.e. aggregation, chemical communication) to synchronise their breeding activities.

Evaluation of tagging techniques for *Australostichopus* (*Stichopus*) *mollis* (Hutton, 1872) for potential ranching studies

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The sea cucumber *Stichopus mollis* is a common benthic resident in New Zealand coastal waters and is often found in abundance under mussel farms. Since *S. mollis* has potential as a lucrative export commodity for Asian markets, ranching cultured sea cucumbers under farms presents an attractive investment opportunity. The first step in assessing the potential for ranching is to determine whether cultured sea cucumbers once placed under a farm remain in residence for later harvesting. To determine residence times under farms we applied a release-recapture design, which required the effective tagging of sea cucumbers. Because of the nature of their body wall, holothurians are not easy to tag, so we investigated a number of different tagging methods to find the one with the least potential for tag loss. Of the six methods trialled in the laboratory (freeze branding, micro-sand blasting, oxy-tetracycline, pit-tagging, T-bars and visible implant fluorescent elastomer [VIFE]), T-tags and VIFE had the best results after four weeks in tanks (87–93% tag retention). These two methods were then selected to assess whether tag retention was the same in the field as in the laboratory. Fifteen tagged sea cucumbers were placed in each of four tanks with either sand or mussel shell substrate in the laboratory. Cage experiments in the field with 15 tagged sea cucumbers each ran simultaneously on either sand or mussel shell substrate. Different substrata were tested because we hypothesised that substrate has an influence on sea cucumber movement, which in turn influences tag retention. Results showed that sea cucumbers in the field cages lost tags much faster than those in the laboratory tanks. Further, the rougher mussel shell substrate appeared to cause slightly higher tag loss for both tag types. The implication of these results for our release-recapture design to assess residence times of sea cucumbers under mussel farms is discussed.

Development of the sea cucumber *Holothuria leucospilota*

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Development of the tropical sea cucumber *Holothuria leucospilota* was studied from November to December 2007. Five adult individuals were collected from the northern part of Okinawa Island and maintained in an aerated aquarium. Spawning occurred spontaneously without any stimulation. The fertilised eggs reached the blastula stage 7 hours after fertilisation at 25°C. The early gastrula stage was reached at 20 hours. Hatching occurred at 22 hours. Early auricularia were formed after 48 hours. The diatom *Chaetoceros gracillis* was added every two days during the auricularia stage. By 6 days, the larvae reached the mid-auricularia stage with pronounced pre-oral and post-oral lobes, several pairs of lateral processes, and an ossicle at the posterior end. The auricularia continued to grow during the next two weeks. At this stage, they began to accumulate hyaline spheres at the tip of the posterior projection and lateral processes. The late-auricularia stage was reached at 19 to 21 days; animals in that stage had left and right stomatocoels, as well as a hydrocoel. The transition period from late-auricularia to dolioraria, which was marked by total resorption of all lateral processes, lasted several days. The larvae reached the barrel-shaped dolioraria stage at 23 to 25 days. To induce metamorphosis, the doriolaria were transferred to a Petri dish covered with a biological film. By 28 days, the doliolaria transformed into early pentactula possessing five primary tentacles and a single podium. Like other congeneric species, *H. leucospilota* has planktotrophic development through the auricularia, doliolaria and pentactula larval stages.

Intracellular pathways involved in oocyte maturation induced by dithiothreitol (DTT) and by a new maturation inducing substance (MIS) in sea cucumbers

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In sea cucumbers, oocyte maturation begins in ovaries but is stopped in prophase I of meiosis. In natural conditions, the blockage is removed during the laying by an unknown mechanism. When oocytes are

taken by dissection, the meiotic release can only be induced by an artificial inductor: dithiothreitol (DTT). We recently discovered a new maturation inducing substance (MIS) and compared its effects to DTT. DTT induces the maturation of 91% of sea cucumber oocytes but the rate of fertilisation never exceeds 40%. The new MIS induces the maturation of more than 90% of oocytes and all are fertilisable. To identify the intracellular pathway mediated by DTT and the new MIS, oocytes of sea cucumbers were incubated in the presence or absence of DTT or MIS after being treated with various modulators of cAMP, an important regulator of hormone-induced maturation in general. The use of these modulators (forskolin, isobutylmethylxanthine [IBMX] and hypoxanthine) in presence of DTT shows a decrease in cAMP during meiotic release. On the other hand, results strongly suggest that the way the new MIS acts is cAMP-independent. Whatever the maturation inducer used (either DTT or MIS), oocyte maturation always requires the synthesis of new proteins and the activation of protein serine/threonine kinase.

Sea cucumbers of Australia

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The Australian sea cucumber fauna comprises about 15 families, 69 genera and 211 species. Research was conducted at the National Museum of Natural History, Smithsonian Institution. The museum collection includes about 68 species of holothurians, each of which we studied. We began with bibliographic research to start getting familiar with Australian sea cucumbers. Then each specimen was photographed using a digital camera, and the ossicles were observed under a microscope and photographed. Using a scanning electron microscope, it was possible to obtain more detailed and precise photographs of ossicles from seven species. Data obtained from each specimen included the region, type of substrate, depth, etc. This work could be the foundation for a future taxonomic catalogue of sea cucumbers of Australia, with their respective description, photographs of specimens and ossicles, distribution, habitat, etc.

Taxonomic revision of the genus *Euapta* (Holothuroidea: Synaptidae)

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Integrative approaches help clarify many previously difficult problems in taxonomy. The sea cucumber family Synaptidae has many such problems, because it is relatively character poor and different researchers have often not agreed which characters are informative about species limits. Synaptids lack tube feet, respiratory trees, and enlarged haemal system, and they have thin, simple body walls and only a few types of ossicles. Three large-bodied genera are common on reefs: *Synapta*, *Euapta*, and *Opheodesoma*. Six nominal species of synaptids have been assigned to *Euapta*, three from the West Atlantic: *E. lappa*, *E. polii*, and *E. tobagoensis*, and three from the Indo-west Pacific: *E. godeffroyi*, *E. magna*, and *E. tahitensis*. Heding (1928), a notorious splitter, recognised all (five — *E. tahitensis* was described subsequently) and described two of these species. However most authors (Fisher, Clark, Deichmann, Rowe, Massin, Samyn, Pawson) over the past century have recognised only one in each biogeographic region: *E. lappa* and *E. godeffroyi*. We encountered two color morphs of *Euapta* in the Marshall Islands, which raised the issue of whether the current view of a single Pacific species is accurate, and led us to reflect on the differentiation of these and related forms. We show that there are at least two sympatric Indo-west Pacific species of *Eupta*, recognisable by color, ossicle, and DNA sequence characters. We also evaluated the genetic status of the East Pacific population and the divergence of *Euapta* across the Isthmus of Panama. Sequence data also support the recognition of *Synapta*, *Euapta*, and *Opheodesoma* as distinct, deeply divergent, monophyletic genera.

Conserved role of ARIS (acrosome reaction-inducing substance) in the echinodermata

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In many species acrosome reaction (AR) is a key process in fertilisation. Sperms specifically approach homologous eggs and undergo an exocytosis of the acrosomal vesicle in their head. This is followed by morphological change; this exocytosis enables sperm to pass through the egg coat by chemical and physical processes. In the starfish *Asterias amurensis*, the AR inducer has been purified from the egg coat and is a large proteoglycan-like molecule known as ARIS (AR-inducing substance). The part of its sugar chain part called Fr.1 is responsible for this activity; however its protein components are still unknown. In this study we make clear that ARIS consists of three dependent glycoproteins modified by Fr.1, named ARIS1, ARIS2 and ARIS3. These three ARIS proteins have similar structures and two of them conserved novel domains. The egg coats of other starfishes, *Distolasteras nippon* (Forcipulatida) or *Asterina pectinifera* (Asteroidea) share the ARIS protein structure as *Dn*ARIS1, *Dn*ARIS2, *Dn*ARIS3 and *Ap*ARIS1, *Ap*ARIS2, *Ap*ARIS3 respectively. Thereby the BLAST search results of ARISs were highly similar to those obtained with DY635177 and DY625100 in the sea cucumber *Apostichopus japonicas*. In the sea lily *Oxycomanthus japonica* we suggested the presence of ARIS-like glycoproteins in the egg coat, although a similar sequence for ARIS couldn't be detected in the sea urchins. Moreover, among Cephalochordata *Branchiostoma floridae*, BW770556, BW773889 and BW706662 are similar to ARIS1, ARIS2 and ARIS3 respectively. As in ARIS molecules, the protein is conserved in starfishes, sea cucumbers, sea lilies and Amphioxus; the ARIS protein may serve a significant role in the acrosome reaction evolutionally.

Characterisation and quantification of the intradigestive bacterial microflora in *Holothuria scabra* (Jaeger, 1833) (Holothuroidea), major macrodeposit feeder of recifal intertidal zones

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For a long time, sea cucumbers were considered nonselective deposit feeders, but recent research suggests that they absorb nutrients from only part of the sediment. The sediment is a complex environment, a mix of mineral material and living or dead organic fragments (i.e. microorganisms like bacteria, fungi, protists and meiofauna). The aims of the present study are to characterise and quantify the intestinal microbial community in *H. scabra* and that of the substrate on which it lives. To investigate the intradigestive microflora three methods were used: *(i)* gut bacterial cultures followed by 16S rDNA sequencing; *(ii)* denaturing gradient gel electrophoresis (DGGE) and *(iii)* cloning. The bacterial count was obtained by DAPI coloration (4',6-diamino-2-phenylindole) and the size of the substrate particles was analysed by sieving. The results suggest that *H. scabra* does not select particles by size. However, the number of bacteria is high in the foregut of the animal, indicating that bacteria are either selected from the substrate or cultured in the foregut. It is suggested that *H. scabra* is a nonselective deposit feeder (i.e. it ingests all particles of the sediment without selection) but that the nutrients absorbed by the sea cucumber come mostly from the living fraction of the sediment.

A nearly articulated aspidochirote holothurian from the Late Cretaceous (Santonian) of England

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With the exception of the Maastrichtian, only scarce data exist on fossil holothurians from the Upper Cretaceous, especially the Santonian (84 million years ago). Until now, only two records have been cited worldwide: one from the Munsterland area, Germany, and one from the Montsec area, Spain; both referred to the Apodida and Dendrochirotida. Here I report the discovery of a nearly articulated holothuroid specimen from the 'Upper Chalk' (Santonian; BMNH E 48764) of England (unfortunately the exact locality is unknown). The unique specimen shows two different types of ossicles: (A) tables and (B) 'buttons', demonstrating aspidochirote affinities, very likely to the family Holothuriidae. Concerning the morphology of the buttons, the new find shares characteristics with tube feet and papillae ossicles of *Holothuria (Mertensiothuria)*, *H. (Microthele)*, and *H. (Semperothuria)* and *H. (Theelothuria)*. According to the decomposition of the surrounding tissue after the death of the animal, only a part of the former body skeleton is preserved; the anterior part of the skeleton (calcareous ring) is missing. Due to the well-preserved body-wall ossicles and the formation of the ossicles of the new find, I can exclude digestion and excretion of the sea cucumber by any predator. After comparison with modern members of the Holothuriidae family, we can speculate that the body of our new Santonian species was probably also cylindrical, elongate and scattered with numerous podia over the entire body, dorsally as papillae and ventrally as cylindrical tube feet. A wider distribution

of the new genus / species can also be assumed, because comparable long button-shaped sclerites were also known from Early and Late Maastrichtian chalk sediments of the islands of Møn (Denmark) and Rugen (northeast Germany) as well as the Baltic Sea. Considering how important aspidochirote sea cucumbers are today (nearly 1/4 of all known modern species), their fossil record is meagre and poorly understood. The phylogeny of the family Holothuriidae was recently investigated by Kerr et al. and Samyn et al., unfortunately without detailed consideration to the fossil record. The origin and early diversification of the Holothuriidae can be located in the Late Palaeozoic. Some of the diversity of the Mesozoic is still hidden as detailed investigations are missing or sparse.

Unusual holothurians (Echinodermata) from the Late Ordovician of Sweden

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Compared to other modern echinoderms, the early evolutionary history of sea cucumbers is poorly understood. In part, this is due to their disjunct endoskeleton with ossicles and calcareous ring elements, which are released following decomposition of the surrounding tissue. Newly sampled öjlemyr flints from the northwestern part of Gotland, Sweden, yield well-preserved echinozoan echinoderms, including holothurian ossicles. The studied material is Ashgill (upper Pirgu stage; 447 million years ago) in age and reveals the presence of several new or poorly known taxa of elasipodid, aspidochirote, and apodid holothurians or stem group representatives. The minute ossicles, around 70–400 µm in length / diameter, exhibit an impressive morphology with arms, spires, perforations, teeth etc. The new material differs from previously described Palaeozoic and Mesozoic material and most of the modern material by its unusual symmetry. This study shows that the Holothuroidea diversified significantly through Late Ordovician times.

Glacial persistence of North Atlantic populations of *Cucumaria frondosa* (Holothuroidea: Echinodermata)

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In the upper latitudes of the Northern Hemisphere shallow water invertebrates were vastly impacted by glaciations during the Last Glacial Maximum (LGM) in the Late Pleistocene. Many marine taxa show a genetic signature of extirpation in the Western Atlantic followed by recolonisation by Eastern Atlantic populations after glaciers receded. *Cucumaria frondosa*, the most abundant and widely distributed holothuroid in the North Atlantic, was used as a model to test whether its present phylogenetic structure reflects a history of persistence or extirpation and recolonisation with respect to Pleistocene glaciations. Mitochondrial DNA (mtDNA) was extracted and sequenced from a total of 334 specimens collected from 20 locations (7 to 5900 km apart) throughout the North Atlantic. Distribution of shared haplotypes indicated groups sampled were part of a large panmictic population with high gene flow between regions. In contrast, exact test for population differentiation was overall significant ($p<0.001$) and showed differentiation of several pairs of populations irrespective of their geographic distance, signifying genetic patchiness at the local level. High connectivity between regions and estimates that the approximate time of expansion was the Late Pleistocene suggested persistence through the LGM. Haplotype diversity indicated the western Atlantic was recolonised by populations residing in ice-free areas east of Newfoundland and Labrador. Long larval times (ca. 6 weeks) and ability to tolerate arctic conditions enabled *C. frondosa* to survive on the fringes of glaciated regions and colonise areas as glaciers receded.

Resistance of pearlfishes to saponins

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Carapid fishes, known as pearlfishes, are anguilliformes fishes that enter and live in some invertebrates, especially in some echinoderms such as holothuroids and seastars. However, these echinoderms contain a strong concentration of saponins, a secondary metabolite which acts as a predator deterrent. These saponins, based on a triterpene glycosides structure, show a wide range of biological activities including haemolytic damage and ichthyotoxicity. The present work aims at analysing the effects of saponins on the gills of fishes including pearlfishes. Saponins were extracted from the sea cucumber *Bohadschia argus* (Tuléar, Madagascar) which is a natural host of some pearlfishes. Five carapid species (*Carapus acus*, *C. boraborensis*, *C. homei*, *C. mourlani* and *Encheliophis gracilis*) and five coral reef fish species (*Amphiprion akallopisos*, *Dascyllus aruanus*, *D. flavicaudus*, *D. trimaculatus*, *Neoniphon sammara*) were investigated. Animals were kept in tanks during two hours and exposed to various concentrations of saponin. At the end of each test, a sample of gill was taken and fixed in order to realise histological and scanning electron microscope analyses. Coral reef fishes exposed to saponin concentrations of 0.2–0.5 $\mu l\ ml^{-1}$ died within two hours and exhibited pathological alterations of gill filaments. The pearlfishes proved to be resistant and had no gill damage.

Genetic barcoding of commercial beche-de-mer species

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A recent review conducted by the Food and Agriculture Organization of the United Nations (FAO) identified 47 species of holothurians used for bêche-de-mer production. With three exceptions from the dendrochirotides, all bêche-de-mer species are aspidochirotides. Identifying many of these species is difficult. To improve this situation, we conducted a review of available genetic information and obtained additional data to test if genetic barcoding can be used as a tool for bêche-de-mer species identification. We focussed on the mitochondrial COI gene, as have other barcoding projects. Although some genetic information was available for about 50 per cent of all bêche-de-mer species, sufficient information and within-species replication was only available for 6 species. We obtained 96 new COI sequences, extending the existing database to cover most common bêche-de-mer species. COI unambiguously identified bêche-de-mer species in most cases and therefore provides excellent genetic barcodes. However, since this marker did not work for two of the most valuable species, development of additional methods will be required. In addition to species identification in adults, COI sequences were useful in juvenile identification, and sequences demonstrated that large (deep) and small (shallow) specimens of *Holothuria atra* belong to the same species. Our study has also demonstrated that further genetic and taxonomic work in this group is essential: work conducted here provided evidence for at least three further species that are currently undescribed (e.g. one *Bohadschia* species), or species that are likely to constitute separate species in the Indian and Pacific Oceans (e.g. *H. fuscogilva*).

Saponin diversity and body distribution in five tropical species of sea cucumbers from the family Holothuriidae

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Sea cucumbers lack structural defences because of their reduced skeleton. To face predation holothuroids contain feeding deterrent molecules — the saponins — in their body wall and viscera. Saponins are secondary metabolites based structurally on a triterpene glycoside structure. The aim of this study is to analyse and compare the saponin mixtures of five sea cucumber species from the Indian Ocean: *Actinopyga echinites*, *Bohadschia subrubra*, *Holothuria atra*, *H. leucospilota* and *Pearsonothuria graffei*. Mass spectrometry (MALDI-MS and MALDI-MS/MS) was used to detect saponins and describe their molecular structures. LC-MS and LC-MS/MS were also used to separate saponins and, in some case, to identify isomers. Two different body components were analysed separately — the body wall (which protects the animal and is moreover the largest organ) and the Cuvierian tubules (a defensive organ that can be expelled on predators in response to an attack) — for all species except *H. atra*, which lacks Cuvierian tubules. Holothuriid saponins are usually classified into two categories: non-sulfated in the genus *Bohadschia* and sulfated in all other genera. Our results do not completely corroborate this distribution. Indeed, although *B. subrubra* has only non-sulfated saponins and *A. echinites* and *H. atra* have only sulfated saponins, *H. leucospilota* and *P. graffei* contain both saponin types. The number of saponins in a mixture varies between species but also between the body components of the same species. For each species, some saponins are common to both body components while others are specific to one organ.

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Conferences & symposiums...

6th WIOMSA Scientific Symposium, August 2009

The Western Indian Ocean Marine Science Association (WIOMSA), l'Université de La Réunion, l'Institut de Recherche pour le Développement (IRD) and l'Institut Français pour la Recherche et l'Exploitation de la Mer (IFREMER) have the pleasure to announce the Sixth WIOMSA Scientific Symposium, which will be held on Réunion Island in August 2009.

Dates:
Monday 24 August 2009 to Saturday 29 August 2009.
The week is divided into the following:

- Scientific Symposium — 24 to 27 August 2009. This component will include: keynote presentations, oral and poster presentations as well as special sessions and roundtable discussions. The special sessions and roundtable discussions are scheduled for the afternoon of 27 August 2009.
- The Fourth WIOMSA General Assembly — 28 August 2009.
- Excursions and tours to different places of interest — 29 August 2009.

Venue:
L'Université de la Réunion in Saint Denis, Réunion Island.

Symposium objectives:
The symposium will bring together practitioners, academics, researchers as well as students, to share knowledge, experience and solutions to the challenges experienced in our coastal and marine environment. The specific objectives of the symposium are to:

- Present current knowledge on disciplines related to the theme of the symposium;
- Provide a forum for discussion and exchange of information and experiences on coastal and marine science issues in the western Indian Ocean region;
- Promote interaction among social and natural scientists in order to strengthen multi- and trans-disciplinary research for sustainable management of the coastal and marine environment; and
- Identify gaps and priority research areas for improved management of the coastal and marine environment of the western Indian Ocean region.

Contributions from all relevant scientific disciplines are welcome at this multidisciplinary symposium.

Symposium theme:
This year's symposium theme is 'The millennium challenge: How marine science and management meet development goals'

Themes:
All interested authors are invited to submit abstracts on the proposed themes listed below. Authors may also submit abstracts on other topics relevant to the wider field of coastal and marine science. The main themes include but are not limited to:

- Global warming and acidification of the ocean;
- Biodiversity and diversification processes (including biology and reproduction of marine organisms, connectivity of marine populations and community resilience and biologging for migratory species);
- Functional ecology of coastal ecosystems such as estuaries, coral reefs, rocky shores, mangroves, etc.;

- Fisheries: trends, challenges and sustainability;
- Ecosystem assessment and monitoring: roles of bioindicators, chemical tracers and models;
- Physical and geological processes in coastal and marine environments;
- Economic/social/environmental/cultural opportunities and challenges of economic activities in coastal and marine environments
- Effectiveness of the conservation initiatives and governance systems;
- Ecology and conservation of endangered marine species (turtles, mammals, sharks, seabirds); and
- Marine organisms and bioactive natural substances.

Experts are invited to consider organising a session in any area related to these broader topics. A number of prominent scientists have been invited to attend and present keynote presentations on topical issues. Some of them have already confirmed their participation :

i) Terry Done — How coral reefs respond to global change?
ii) Joshua Cinner — Overcoming overfishing: perspectives from the WIO and around the globe
iii) Francis Marsac — Fisheries trends: WIO perspective

For more details, please visit WIOMSA website at: http://www.wiomsa.org, or contact the Executive Secretary:

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The full meeting announcement and second call for abstracts is available, in PDF format, at:

http://www.wiomsa.org/filearchive/2/2779/SECOND ANNOUCEMENT AND CALL FOR ABSTRACTS-Final.pdf

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