Darwin Initiative for the Sustainable Use of Sea Cucumber in Egypt

Final Report 2006

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Darwin Initiative for the Survival of Species

Final Report

1. Darwin Project Information

Project Reference No.	162/10/027
Project title	Darwin Initiative for the Sustainable use of Sea Cucumber in Egypt
Country	Egypt
UK Contractor	Department of Biological Sciences, University of Hull
Partner Organisation (s)	Suez Canal University, Egyptian Environmental Affairs Agency, Red Sea Governorate,
Darwin Grant Value	£160, 700
Start/End date	October 2001 – October 2004 Extended to March 2005
Project website	None
Author(s), date	A.J.Lawrence

2. Project Background/Rationale

2.1 Project Background

The project was based in Egypt, with the lead partner based at the Suez Canal University in Ismailia. However, the study area encompassed the whole of the Egyptian Red Sea coastline.

At the time of the project, the commercial exploitation of marine invertebrates has been receiving increasing attention worldwide. Part of this expanding sector included the sea cucumber fishery. Unfortunately, this fishery, known as Beche-de-Mer or Trepang, has a history of over-exploitation and collapse. The pattern of the fishery is to mine out an area in a few years and then move on leaving behind a disrupted and impoverished environment. The fishery had mostly collapsed throughout the Indo Pacific with many species commercially extinct. Several countries had recently opened sea cucumber fisheries and experienced a 2-year cycle of expansion followed by rapid decline in export volume through over-fishing, highlighting the need for management of the fishery.

Egypt had opened a sea cucumber fishery in the late 1990s. However, this was done with no baseline data on the stocks or species available. The concern was that the fishery in Egypt would follow the pattern seen elsewhere in the world, with the collapse of the fishery occurring in a very short period. The aim was to gather the baseline data

necessary to recommend sustainable fishery yields before this could happen. In addition to developing a sustainable fishery, the project aimed to provide data to Egypt's National Biodiversity Unit for inclusion in its National Biodiversity Strategy.

The need for the work was initially identified when I met Dr Raouf Kilada at a marine symposium in 1997. We discussed both our interest in holothurians and the problems being encountered in the Red Sea. Further contact lead to the specific problems of over-exploitation of the holothurian fishery and the lack of expertise in the identification, surveying and mariculture of this group in the Red Sea.

Evidence of the commitment by local partners to the project objectives is evidence first by the support provided to the project by the Egyptian Environmental Affairs Agency. Second, by the fact that once the project had gained the support of the Darwin Initiative the Red Sea Governorate placed a ban on sea cucumber fishing until the stock assessment and management plan could be prepared. Project Summary

2.2 Project Objectives

The aim of this project was to develop a sustainable sea cucumber fishery along the Egyptian Red Sea coast before the resource became over-exploited. This will be achieved through the successful completion of 4 principal objectives:

- First, to complete a fishery stock assessment, including baseline data on sea cucumber population dynamics and prepare a fishery management plan.
- Second, to develop a pilot mariculture system for sea cucumber in the Red Sea, to restock depleted reef areas and evaluate the feasibility of the process as a direct source of sea cucumber/ income generation for small community based mariculture systems
- Third, to examine the potential of the main species of farmed sea cucumber as a source of bioactive substances of potential medical benefit
- Fourth, to develop and run training courses in stock assessment and mariculture to build capacity in Egypt to continue the work beyond the period of funding.

Outputs from the project were to include a species list and collection, a Monitoring and Management together with student theses exploring the potential medical use of sea cucumber. The full list of objectives, including outputs, activities and measurable indicators are shown in the original Project Logical Framework (Appendix 5).

2.3 Changes to Project Objectives

A number of the project objectives were changed or modified during the period of the project. For example, it became apparent very early on that the level of fishing that had taken place up to the start of the project had significantly depleted resources to the degree whereby it was no longer possible to work toward a strategy of developing a sustainable fishery but rather to examine the level of impact and status of the remaining stock. This was exacerbated by the continued, illegal, fishing of sea cucumber during the period of the project.

Consequently, at the request of the EEAA, and with its support in the form of resources and man-power, the stock assessment was significantly expanded to assess the

level of over-fishing together with its impact. These changes were reported to the DI in the first year report.

In addition, due to the continued problems encountered in the mariculture aspect of the study, the scope of this aspect of the project was modified during the final year to specifically explore the environmental impact of sea cucumber over-fishing on habitat quality. This was driven partly by the need to generate a useful dataset for the student working on this aspect of the project that could be submitted as an MSc thesis. However, it was also, again, requested by the EEAA.

The project is best described under Articles 7, 8 and 12 of the CBD. It has promoted the identification and monitoring of components of biodiversity and research and training which has contributed and is contributing to the conservation and sustainable use of these components. It has also made recommendations that will aid the recovery of a group of threatened species. To a lesser extent it has also advanced aspects of ex-situ conservation (Article 9) through the work on mariculture, promoted the public understanding of these issues through the media (Article 13) and promoted the exchange of information on surveying programmes (Article 17) see Appendix 1.

2.4 Success and Accomplishments of the Project

The project has mostly been successful in meeting its objectives. It has produced a Management and Monitoring Report which outlines the current status of sea cucumber resources and makes recommendations to aid their recovery. It has identified a number of bioactive extracts which may be of future use. Students engaged in these components of the study have produced a Field Guide to aid identification of sea cucumber, the Management and Monitoring Report, and have submitted and successfully defended their Mphil and PhD theses.

The mariculture component of the project has been less successful. This was primarily due to the problem of not being able to collect enough broodstock of the most valued species, *Holothria scabra*. Mariculture of an alternative species was attempted and whilst some advance was made, no successful spawnings were achieved. However, the student engaged in this aspect of the project was re-directed to examine the impact of sea cucumber removal on habitat quality and submitted his MSc thesis on this subject. This has been passed, subject to minor corrections, which are currently being completed.

The recommendations of the Management and Monitoring Report have, to date, been partly adopted by the relevant government agencies and Red Sea Governorate, most notably through the continuance of the fishery ban whilst a monitoring project has been established through a follow up project to determine any recovery in sea cucumber stocks.

3. Scientific, Training, and Technical Assessment

3.1.1. Species Taxonomy, Survey and Stock Assessment

3.1.2. The Survey Team

Mr Mohammed Ismail Ahmed, Mr TarekTemraz, Dr Ashraf Ibrahim, Dr Mahmoud Hanafy, EEAA Rangers, Dr Andrew Lawrence,

3.1.3. Training

Mr Mohammed Ahmed was selected to undertake this aspect of the study as the top performing undergraduate student in the Department of Marine Science, Suez Canal University at the commencement of the study. He was registered for a 2-year MPhil at Hull University.

As part of his training at Hull, Mr Ahmed had to undertake specific Post-Graduate Training Modules (PGTM) to a minimum of 20 credits per year. The specific modules he completed were: Health and Safety (5 credits, assessed by submission of risk assessment for his field work) Numerical Methods and Statistical Analysis (15 credits, assessed by submission of hand calculated and SPSS generated work sheet, including review of the appropriate test to use and why) English for Academic Purposes (10 credits, assessed by submission of an essay and oral presentation) First Year Report (10 credit, assessed by an internal viva by two academic members of the Department) and Scientific Paper (10 credit, assessed by the successful publication of a peer reviewed scientific paper).

In addition, as an output from the project, and due to personnel changes in Egypt, Mr Ahmed completed a GIS and Remote Sensing PGTM at Hull University (20 credit, assessed by submission of a mini-project and examination).

In relation to his research, Mr Ahmed received training in holothurian taxonomy, including spicule preparation and scanning electron microscopy; survey methods and data analysis (including the use of fishery models). He also underwent taxonomic training at the Natural History Museum. This work formed part of his MPhil thesis.

Mr Ahmed went on to train several EEAA Rangers in the survey method adopted for the study and the taxonomic identification of commercial species of sea cucumber.

3.1.4. Methodology

In this study the Red Sea was divided into 2 main sectors. The first sector is the Gulf of Aqaba running from Taba to Sharm El-Sheikh. The second sector is the Red Sea sector starting from north of Hurgada and moving south to the Shalatein (including inshore and offshore sites) . In both sectors priority was to cover all the habitat (coral reef, seagrass bed, mangrove swamps and sandy lagoons). A total of 160 sites were surveyed inshore and offshore. Of these 37 site were in the Gulf of Aqaba sector while 123 sites were in the Hurgada (Figure 1)

Sampling was carried out from July 2002 to January 2004. The survey employed modified rapid marine assessment techniques that have applied to bech-de-mer surveys in Torres Strait (Long et al., 1999; Skewes et al., 2000) and Moreton Bay (Skewes et al .,2002). Field work was undertaken by two teams of divers (all diving under the PADI safety regulations).

A Belt Transect method was used in the survey to determine the density and abundance of Holothuria at each site. The belt consisted of a 50 m line transect with a 5 m width (2.5m each side of the line) and was surveyed by two divers, one each side of the transect line.

On the reef flat three belt transects running parallel to the shoreline were surveyed by snorkelling or walking at low tide in order to determine the holothurians density and composition. On the reef edge, due to the topographic differences between sites, site remoteness, and diver safety issues, a protocol was developed in which a 150 m line

transect was run perpendicular from the reef edge to 150m distance or 35 m depth whichever came first. From this point belt transects, running parallel to the shoreline, were surveyed within 35-20m, 20-15m, 15-10m and 10-5m depth ranges and data on the density and composition of holothurians were recorded. All the sea cucumber within each transect were identified and counted.

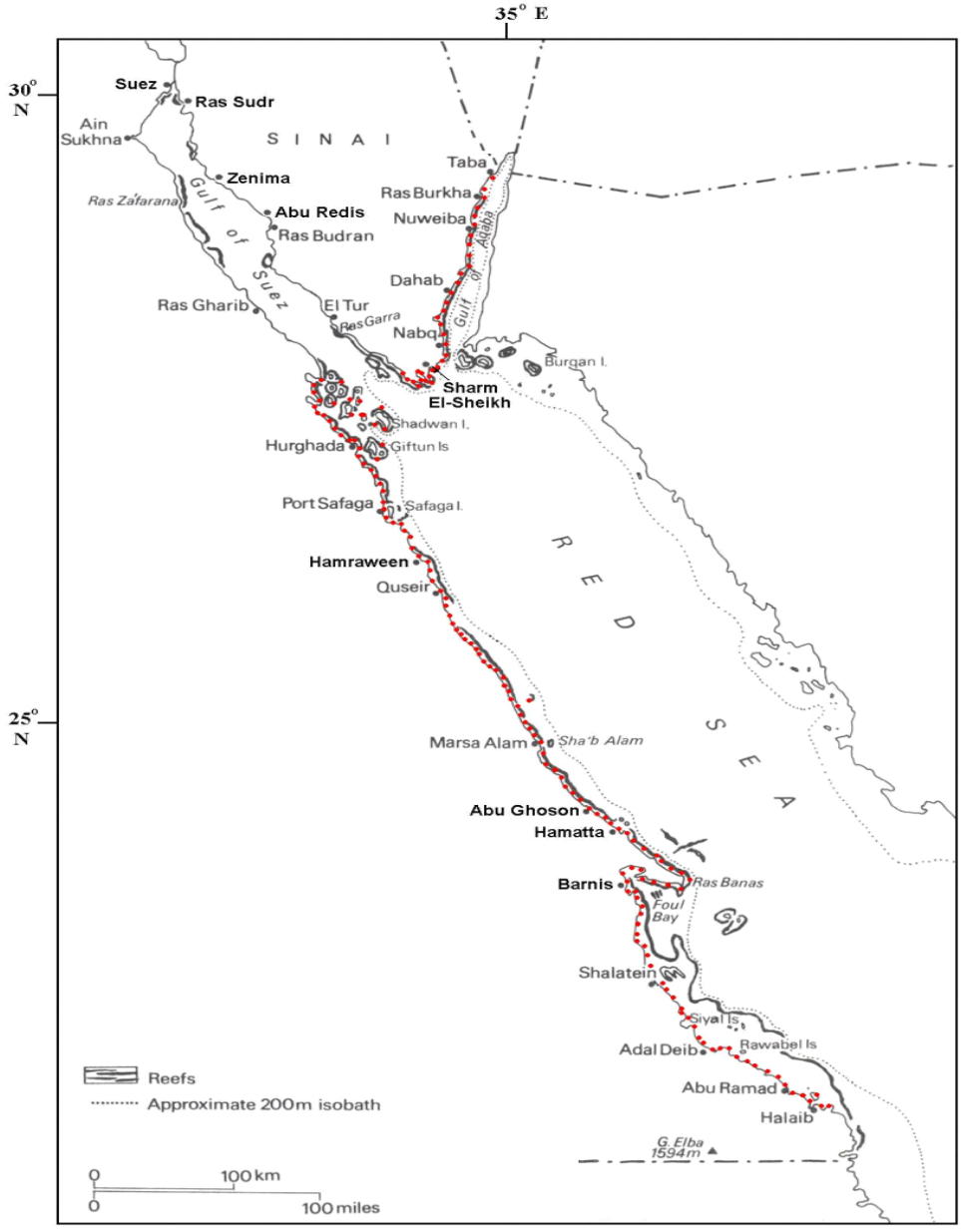


Figure 1: Map of the Red Sea Coast of Egypt. All of the sites surveyed during the study are shown in Red and are divided into two sectors, North and South.

3.1.5. Results

The following represent the significant findings from this aspect of the study.

Over 690 transects have been surveyed at 116 sites from Taba, on the border with Israel in the northern Gulf of Aquaba, to Shalatein in the southern region of the Red Sea on the border with Sudan. In Sharm El-Sheikh area 34 sites have been surveyed covering the whole coast of the Gulf of Aqaba whilst about 82 site have been surveyed in the northern portion of the Red Sea.

A total of 22 species have been identified (Table 1). Of these, 2 are described for the first time in Egyptian waters. Whilst none of the species so far identified are unique to the northern coastline of Egypt (Ras Mohammed to Taba), 10 species have only been found in the southern region (Hurgada to Shalatein) (Figure 6 and Table 1). In addition, seven species are predominantly collected as the commercial species. These can be divided into 3 groups based on market value. First class species include H. scabra (sandfish), H. fuscogilva (white teatfish) and H. nobilis (black teatfish). These are fished preferentially. Second class species include S.hermanni and S. horrens. Third class species include: A. mauritiana, H. atra, and Pearsonothuria graeffi.

Table 1. Species of sea cucumber identified through an Area Survey along the Red Sea coast and offshore islands of Egypt. Species described for the first time in Egyptian waters or important commercially are highlighted together with their value category and broad geographic range.

Species	**First record in Egypt**	**Commercially important and Grade**	**Range of Species (North & South Sectors)**
Actinopyga crassa		-	N&S
Actinopyga mauritiana	-	3rd Class	N&S
Pearsonothuria graeffei	-	3rd Class	South only
Bohadschia cousteaui	-	-	N&S
Bohadschia tenuissima	-	-	N&S
Bohadschia marmorata	-	-	N&S
Holothuria spinifera	-	-	N&S
Holothuria edulis	-		N&S
Holothuria atra	-	3rd Class	N&S
Holothuria pardalis	-	-	N&S
Holothuria leucospilota	-	-	N&S
Holothuria scabra	-	1st Class	N&S
Holothuria fuscogilva	Yes	1st Class	South only
Holothuria nobilis	-	1st Class	South only
Holothuria fuscocinerea	-	-	N&S
Holothuria albiventer	-	-	South only
Holothuria flavomaculata	Yes	-	South only
Holothuria sp	-	-	South only
Stichopus hermanni	-	2nd Class	N&S
Stichopus horrens	-	2nd Class	N&S
Synapta maculata	-	-	N&S
Synaptula reciprocans	-	-	South only

The distribution of each species has also been examined in relation to depth and habitat type. Results from this are shown in figures 2, 3, 4 and 5. These highlight the fundamental importance of sea grass beds to most of the main commercial species from each class. The two exceptions being *H. nobilis* and *P. graeffei* which have only been found on coral substrate. In addition, each of the commercial species have been mostly found in the depth range of 5-10m. The main exception to this is *H. fuscogilva* which was predominantly found beyond 30m depth and *H. nobilis* and *H. atra* which was mostly found on the reef flat.

Table 2. Abundance of Holothurian species in Hectares in north (Gulf of Aqaba) and South (Hurghada) of the Egyptian Red Sea

	Gulf of Aqaba	in total area of 590520ha	Hurgada	in total area of 2362080ha
A. crassa	8	393680	30	5905200
A.mauritiana	70	3444700	204	40155360
Pearsonothuria graeffei	4	196840	19.6	3858064
B. cousteaui	0	0	19.2	3779328
B.tenussima	0	0	16.2	3188808
B.marmorata	92	4527320	69.2	13621328
H. atra	950	46749500	1002.4	197312416
H. leucospilota	794	39072740	816	160621440
H. fuscogilva	4	196840	8	1574720
H. nobilis	2.2	108262	6.4	1259776
Stichopus hermanni	28	1377880	46	9054640

Cluster analysis and PCA were used to investigate whether there was any similarity in distribution between different species of sea cucumber according to both depth and habitat. Some species were found to share the same habitat and depth profiles, while others have a unique habitat preferences or unique depth profiles both cluster

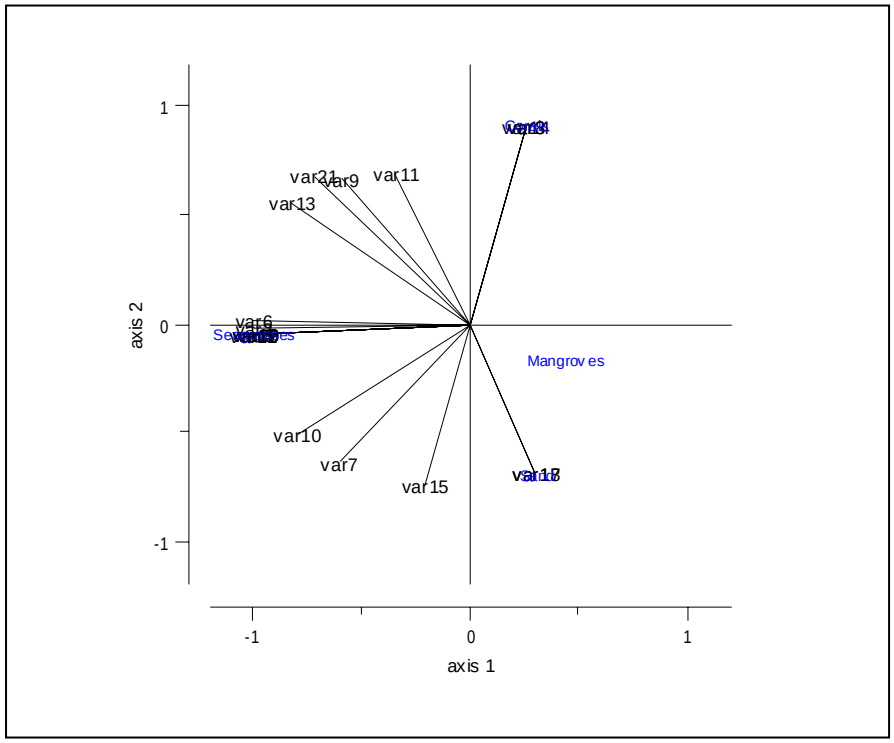
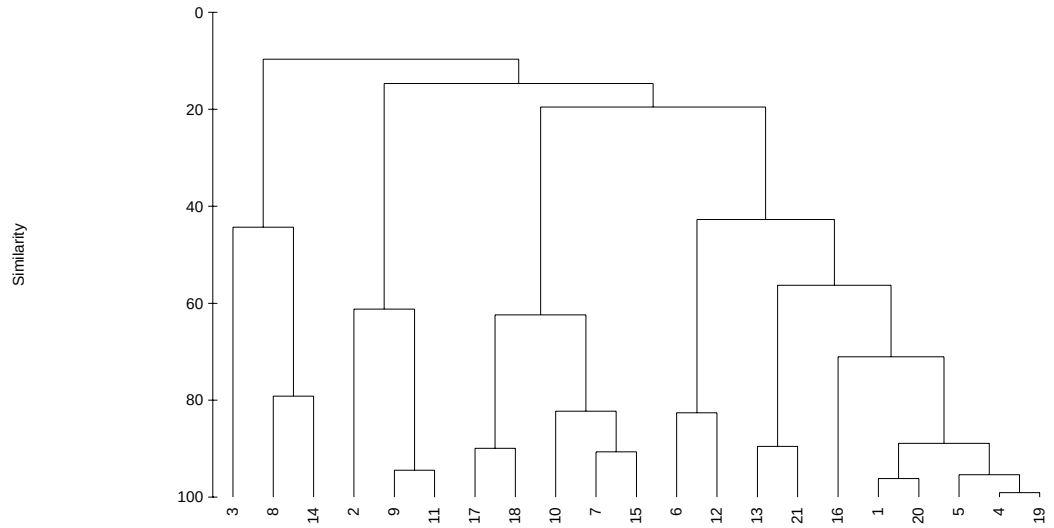


Figure 2 The output for principal component analysis, clustering the sea cucumber species of the Egyptian Red Sea coast according to habitat preferences. *var1- A.crassa, var 2- A.mauritiana, var 3- P. graeffei, var4- B.cousteaui, var5- B.tenussima, var6-B.marmorata, var7- H.spinifera, var 8- H.edulis, var9- H.atra, var10- H.paradilis, var11- H.leucospilota, var12- H.scabra, var13- H.fuscogilva, var14- H.nobilis, var15- H.fuscocinerea, var16- H.albiventer, var17- H.flavomaculata, var18- H.sp, var19- S. herrmanni, var20- S. horrens, and var21- Synapta macul* analysis and PCA results (Figure 2, 3, 4 and 5) were supported by the abundance data collected on each site on both habitat and depth preferences for each sea cucumber species under the study.



	3	8	14	2	9	11	17	18	10	7	15	6	12	13	21	16	1	20	5	4	19
Coral	0.9	0.1	0.1	1.5	17	16	0	0	0	0	0	0.2	0.1	0.1	0.1	0	o.5	0	0	0	0
Sand	0	0	0	3	30	40	0.2	0.3	0.2	0.1	0.2	1.4	1.1	0	0	0	0	0	0	0	0
seagrass	0	0	0	82	34	23	0	0	0.1	0.3	0.3	3.4	7.9	0.4	0.2	0.3	1.5	1.3	0.8	0.9	1

Figure 3 Output for the Cluster analysis of habitat preferences for sea cucumber species on the Red Sea, *1- A.crassa, 2- A.mauritiana, 3- P. graeffei, 4- B.cousteaui, 5- B.tenussima, 6- B.marmorata, 7- H.spinifera, 8- H.edulis, 9- H.atra, 10- H.paradilis, 11- H.leucospilota, 12- H.scabra, 13- H.fuscogilva, 14- H.nobilis, 15- H.fuscocinerea, 16- H.albiventer, 17- H.flavomaculata, 18- H.sp, 19- S. herrmanni, 20- S. horrens, and 21- Synapta maculate*. Inserted table indicate mean density of animals collected from different site / habitat.

From the cluster analysis and PCA (Figure 2 and 3). *P.graeffei* (3), was clustered with both *H.edulis* (8) and *H.nobilis* (14) because they were found only on coral reef habitat while (8) and (14) were clustered together because of the similarity in density on coral habitat. The same was seen with *A.mauritiana* (2), *H.atra* (9) and *H. leucospilota* (11) which were clustered together because all of them were found in all habitat types while again (9) and (11) were clustered together because they share high density in all habitats. *H.flavomaculata* (17), *H.*sp (18), *H.paradilis*(10), *H.spinifera* (7) and *H.fuscocinerea* (15) were clustered together because they were found on sandy habitat. The rest of the species were mainly found on sea grass habitat so they are clustered together.

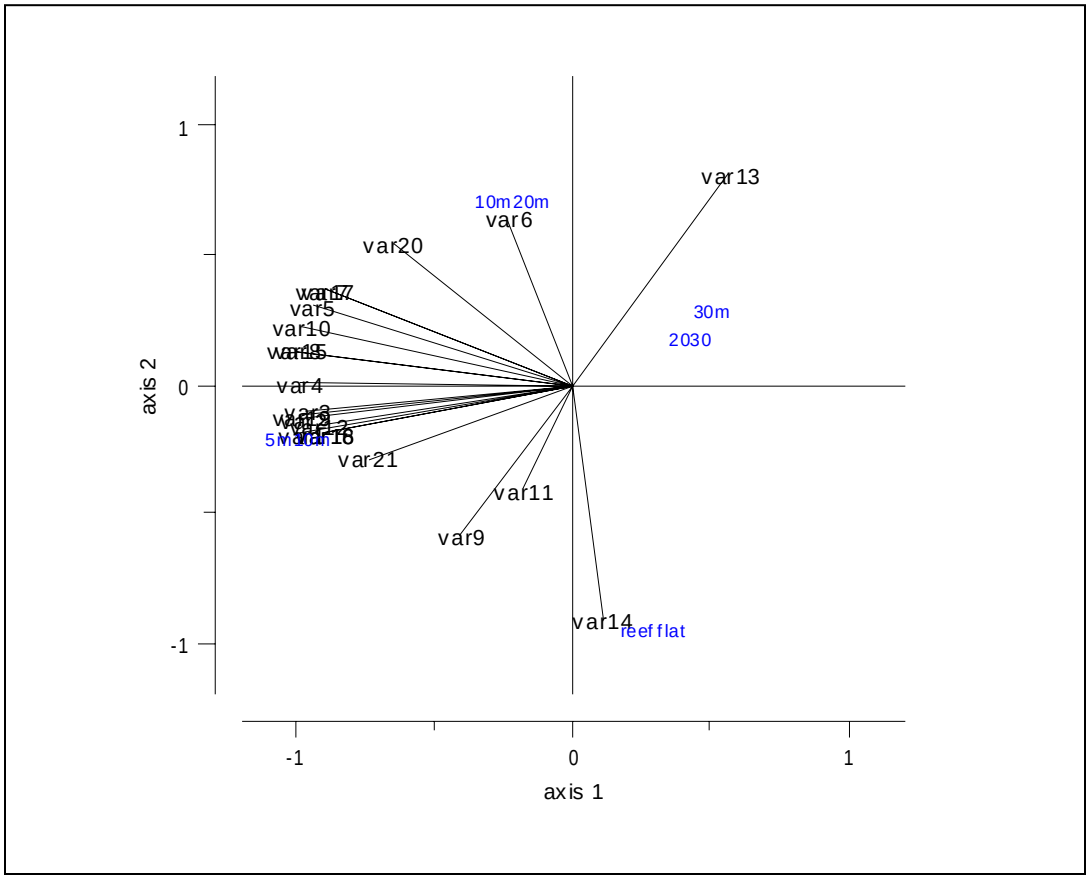
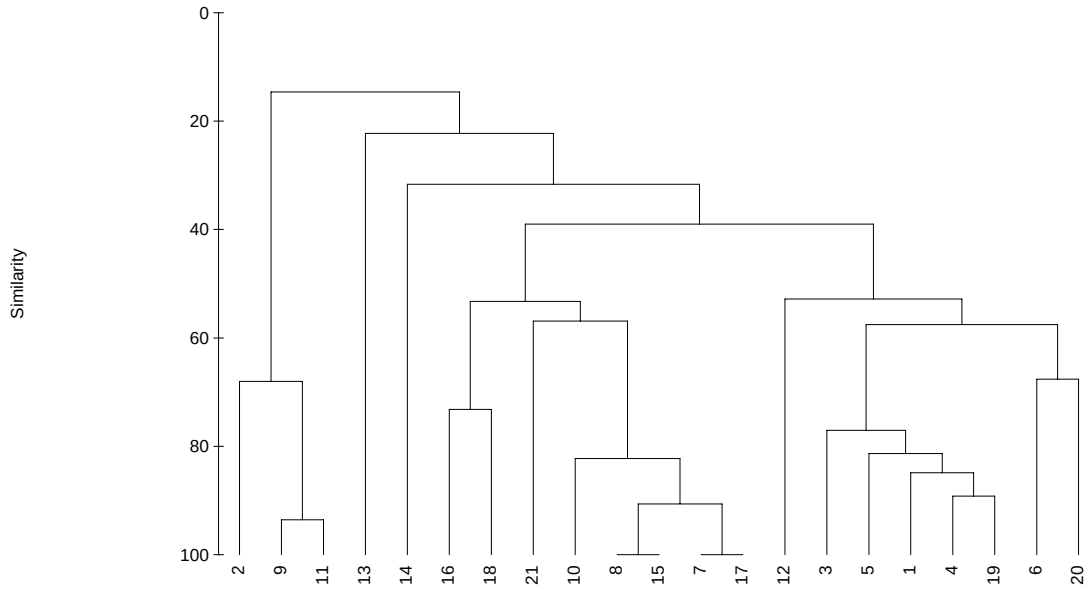


Figure 4 The output for principal component analysis clustering the sea cucumber species of the Red Sea according to depth preferences. *var1- A.crassa, var 2- A.mauritiana, var 3- P. graeffei, var4- B.cousteaui, var5- B.tenussima, var6-B.marmorata, var7- H.spinifera, var 8- H.edulis, var9- H.atra, var10- H.paradilis, var11- H.leucospilota, var12- H.scabra, var13- H.fuscogilva, var14- H.nobilis, var15- H.fuscocinerea, var16- H.albiventer, var17- H.flavomaculata, var18- H.sp, var19- S. herrmanni, var20- S. horrens, and var21- Synapta maculate.*

The cluster analysis of distribution with depth and PCA result (Figure 4 and 5) indicate that *A.mauritiana* (2), *H.atra* (9) and *H. leucospilota* (11) were clustered together because they were found in a wide range of depths. *H. fuscogilve* (13) was separated from the other species because it was only found in quiet deeper water. While *H. nobilis* (14) was also separated because it was found only in very shallow water on the reef top. The rest of the species were clustered together because they were found in the same depth range.



	2	9	11	13	14	16	18	21	10	8	15	7	17	12	3	5	1	4	19	6	20
Reef flat	3.4	20	19	0	0.1	0	0	0.1	0	0	0	0	0	0.1	0.1	0	0.1	0	0.1	0.1	0
5-10m	75.2	26	13	0	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	2.1	0.5	0.6	0.8	0.8	0.4	1.3
10-20m	49.2	80	93	0.7	0	0	0	0.2	0.2	0	0.1	0.1	0.1	0.2	0	1.2	0.8	0.7	0.6	11	7.5
20-30m	12	0	0	3	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0
>30m	0	0	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Figure 5 Output for the Cluster analysis of depth preferences for sea cucumber species on the Red Sea, *1- A.crassa, 2- A.mauritiana, 3- P. graeffei, 4- B.cousteaui, 5- B.tenussima, 6-B.marmorata, 7- H.spinifera, 8- H.edulis, 9- H.atra, 10- H.paradilis, 11- H.leucospilota, 12- H.scabra, 13- H.fuscogilva, 14- H.nobilis, 15- H.fuscocinerea, 16- H.albiventer, 17- H.flavomaculata, 18- H.sp, 19- S. herrmanni, 20- S. horrens, and 21- Synapta maculate.* Inserted table shows mean density of different species collected from different sites regarding to depth profile.

Clear differences have been found in the abundance of the main commercial species when fished and non-fished areas are compared (Figure 6). However, it should be noted that it is almost impossible to find non-fished sites along the coast of Egypt. In this instance, therefore, non-fished refers to sites that are mostly unexploited. These sites benefit from some form of protection, being either too remote, difficult to approach, near to army installations or forming part of the private beach to hotel complexes. Figure 6 highlights the almost complete loss of the most valuable species *H. scabra* and *H. nobilis*,

as well as the removal of most commercial species from most fished areas. Whilst both seagrass and coral sites have been targeted by fishermen, their impact on seagrass areas appears to have been more severe.

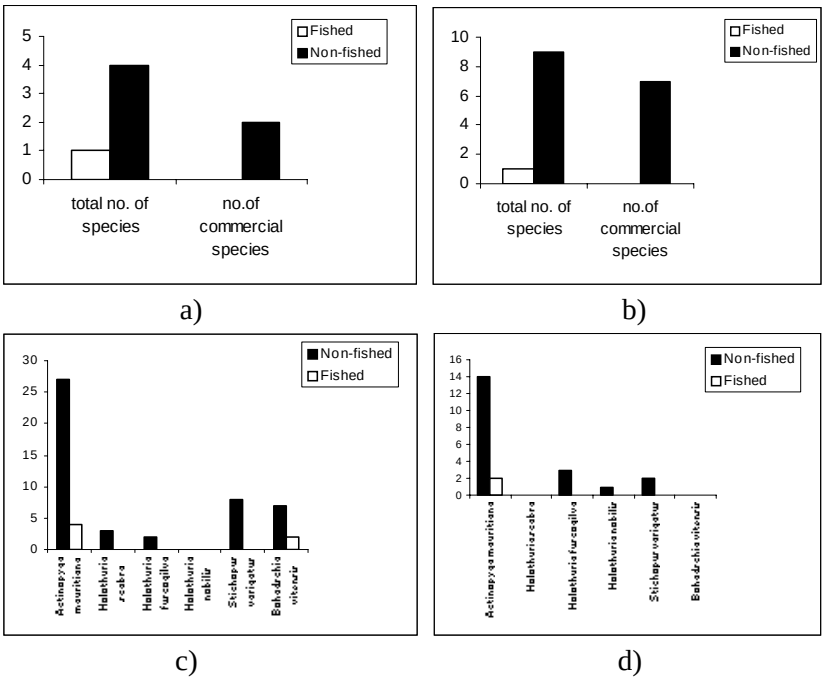


Figure 6. The impact of fishing on the total number of species of sea cucumber, number of commercial species and their composition on seagrass (a & c) and coral (b & d) habitats. Data is expressed as mean number per transect

Based on the numbers of animals counted on each of the transects, the mean density of the main commercial species per hectare has been calculated for sand and seagrass areas (Table 3). For each of the species the density is higher in the southern

Table 3. The density of animals per hectare of the main commercial species of sea cucumber in Egypt, compared with the highest and lowest estimated densities found in the literature from 1 – Preston, 1993, 2- Shelley, 1981; 3- Kinch, 2002.

Species	Gulf of Aqaba Sector	Hurgada Sector	Highest density from literature	Lowest density from literature
H. scabra	0.0	158.0	> 600 (1) 2, 900 (2)	0.00 (3)
H. fuscogilva	4.0	8.0	> 18 (1)	0.42 (3)
H. nobilis	2.2	6.4	> 13 (1)	0.18 (3)
S. hermanni	28.0	46.0	450 (1)	0.09 (3)
S. horrens	4.0	19.6	-	-
P. graeffi	0.0	26.0	-	0.37 (3)
A. mauritiana	70.0	204.0	300 (1)	0.12 (3)
H. atra	950.0	1002.4	> 500 (1)	9.8 (3)

sector than in the northern sector. Also apparent is the low density of most valuable species (Class 1) compared with the less valuable. Generally, the highest density species is *A. mauritiana*, one of the lower valued species. It should be noted that the

value calculated for *H.scabra* is the result of a high density of animals found at one specific site.

All evidence collected to date indicates that the fishery has been severely over-exploited, although not to the level seen in other parts of the world.

3.1.6 Peer reviewed Outputs

At this time, two peer reviewed publications have been produced:

Lawrence, A.J., Ahmed, M., Hanafy, M), Gabr, H., Ibrahim, A and Gab-Alla, A. (2004) Status of the Sea Cucumber Fishery in the Red Sea – The Egyptian Experience. In: Lovatelli, A. (comp./ed.); Conand, C.; Purcell, S.; Uthicke, S.; Hamel, J.-F.; Mercier, A. (eds.). Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper. No. 463. Rome, FAO. 2004. 457p

M. I. Ahmed (2006) Taxonomic and Fishery stock status of sea cucumber in the Egyptian Red Sea, MPhil Thesis, University of Hull, pp

Data from the study has also been presented in:

Mulit-Authored (2004) Conservation of and trade in sea cucumbers in the families Holothuridae and Stichopodidae Technical Report to the CITES Animals Committee. CITES Doc AC20 Inf 14

A second peer reviewed paper will also shortly be submitted:

M. Ahmed and A. Lawrence (in prep) Diversity, distribution, habitat and depth preferences of sea cucumber (Family: Holothuriidae) along the Red Sea coast of Egypt and Gulf of Aqaba.

3.2.1 Bioactivity Study

3.2.2 The Bioactivity Team

Mr Rafat Afifi, Professor Khalifa (Dept of Pharmacy, Suez Canal University), Dr Tim Paget, Dr Andrew Lawrence (Dept Biological Sciences, Hull University) Dr XYZ (School of Chemistry, Hull University).

3.2.3 Training

Mr Rafat Afifi was selected as a member of the Marine Science Department, Suez Canal University who had successfully completed his MSc and was ready to undertake a PhD.

As part of his training at Hull, Mr Afifi had to undertake specific Post-Graduate Training Modules (PGTM) to a minimum of 20 credits per year. The specific modules he completed were: Health and Safety (5 credits, assessed by submission of risk assessment for his work together with appropriate COSHH forms) Numerical Methods and Statistical Analysis (15 credits, assessed by submission of hand calculated and SPSS generated work sheet, including review of the appropriate test to use and why) English for Academic Purposes (10 credits, assessed by submission of an essay and oral presentation) First Year Report (10 credit, assessed by an internal viva by two academic members of the Department) and Scientific Poster (10 credit, assessed by the successful publication of a peer reviewed scientific paper), preparation and delivery of a Departmental Research Seminar (10 credit, assessed by a report submitted by the academic responsible for the departmental seminar programme.

Mr Rafat Afifi also received training in bioactive compound separation

techniques, high performance liquid chromatography (HPLC), thin layer chromatography (TLC) liquid chromatography/ mass spectroscopy (LC-MS), bioassay techniques for bacteria, fungi, protozoan parasite, cancer cell lines, NMR.

3.2.4 Methods

Eleven species representing four holothurian genera, which included the most numerous and those of major commercial importance, were selected to look for bioactivity (*Actinopyga mauritiana, Actinopyga crassa, Bohadschia vitienses, Bohadschia tenuissima, Bohadschia=Pearsonthuria graeffei, Bohadschia cousteaui, Holothuria atra, Holothuria leucospilota, Holothuria nobilis, Holothuria fuscogilva* and *Stichopus varigatus*. The samples were frozen immediately after collection and maintained at - 20 ºC prior to the extraction. Two specimens of each species were kept, one for extraction and the other for identification. For three species *Holothuria atra, Actinopyga mauritiana Bohadschia tenuissima* samples were taken from sites both in the North and the south of the sampling area.

3.2.4.1. Extraction from Sea cucumbers

The frozen samples of sea cucumbers (a single specimen of each species) were left to defrost and dissected using scissors. Internal organs were removed and the body wall was washed with water, broken down into small pieces and overnight extracted at room temperature 4 times with sufficient amount of Methylene chloride/ Methanol, (1: 1) until no further colour was extracted to ensure complete extraction (Wright 1998). The combined extracts were filtered through Whatman no. 1 filter paper and evaporated at 40 ºC and the residue was washed into small vials and labelled. Extracts were stored at - 20^0C for further analysis.

Bioactivity of extracts was assessed against bacteria , (*Pseudomonas aeroginosa* and *Escherichia coli Staphylococcus aureus*) fungi, *Leishmania major* promastigotes and

3.2.4.2 ***Organisms and culture conditions.***

Standard and wild type isolates of two Gram negative (*Pseudomonas aeroginosa* and *Escherichia coli*) were used in this study; these were obtained from either Hull Royal Infirmary or NCTC. The standard Oxford Gram positive (*Staphylococcus aureus*) was also used. They were stored on nutrient agar plates and were propagated in nutrient broth (OXOID, CM1) medium. Batches of medium (10 ml) were inoculated with material from nutrient agar (OXOID, CM3) plates containing freshly grown *Staphylococcus aureus, Pseudomonas aeroginosa* and *Escherichia coli* cells and incubated overnight at 37°C. Fourteen extracts of the body walls of eleven species of holothurians were assayed against the isolates of *Staphylococcus aureus*, *Pseudomonas aeroginosa* and *Escherichia coli*.

3.2.4.3 ***Testing for antifungal activity***

Candida albicans (580(1), 581(2) and MEO47228) and *Trichosporon beigelii* clinical isolates obtained from Hull Royal Infirmary were used throughout this study. They were stored on Sabouraud dextrose agar (OXOID, CM0041) plates and were propagated in yeast extract-peptone-glucose medium (1% [w/v] yeast extract, 1% [w/v] peptone, 2% [w/v] glucose. Batches of medium (20 ml) were inoculated with material from yeast extract-peptone-dextrose agar plates containing freshly grown *C. albicans* and *Trichosporon beigelii* cells and incubated overnight in an orbital shaker at 30°C.

Crude extracts of the eleven species of sea cucumbers were tested against the

isolates of *C. albicans* and *Trichosporon beigelii*. The protocol employed here followed that of the antibacterial assay. Four replicates were used for each concentration. Extracts were regarded as active when amount of growth was less than 10 % of the control assayed after 24 hrs. Absorbance values were converted into growth percentage and the medium lethal concentration (LC_{50}) was obtained graphically from the dose response curves.

3.2.4.4 *Testing for anticancer activity*

Culture of human cell lines

Human cell lines Lovo, breast and CACO-2 were used in this study and were obtained from Dr J.Greeman, Biomedical Research Laboratory, Hull University. All cell lines were stored in liquid nitrogen until required. Cultures of cell lines were maintained in 1x DMEM medium (In Vitrogen). Medium was supplemented with 2mM L-glutamine, 100 U ml^{-1} penicillin-streptomycin mixtures and 10 % (v/v) heat inactivated fetal calf serum (Invitrogen). Culture of the cells was in 150 ml flasks with approximately 20 ml of media. Near confluent, media was removed and cells were prepared for passage by the addition of 1 x trypsin / EDTA (Invitrogen) and incubated at 37 ^{o}C for 5 min. Once cells had detached from the flask, medium was added to inactivate the trypsin. The cells were then recovered by centrifugation (1000 x g, 3 min). The cell pellet was then used in the experimental study or split in a 1:3 ratio and passaged as described. The cells were incubated to maintain growth at 37 ^{o}C in a humidified atmosphere of 95 % air / 5 % CO_2.

Viability (MTT) Assay

The MTT Assay was used here as a rapid and accurate method for assessing cell viability rather than using the traditional counting method. This assay is a sensitive method for the measurement of cell proliferation based upon the reduction of the tetrazolium salt 3,[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT). Changes in cell proliferative activity caused by growth inhibitors may be quantified using the MTT (Cole, 1986). MTT is reduced to an insoluble formazan dye by mitochondrial enzymes associated with metabolic activity. The reduction of MTT is primarily due to glycolytic activity within the cell and is dependent upon metabolic activity. This procedure is for cells in a 96 well plate. Cells were counted using a haemocytometer and resuspended at a density of 1 x 10^4 cell/ well. The protocol was summarized as follows. A solution of 5mg/ml MTT was made and dissolved in PBS, filtered and sterilised**.** Before the end of the incubation (after 20 hours) 50 µl of MTT solution was added to each well containing cells. The plates were incubated at 37ºC for 4 hours. Media was removed with needle and syringe. 200 µl of DMSO were added to each well and pipetted up and down to dissolve the crystals. Plates were put into the 37ºC incubator for 5 minutes to dissolve air bubbles. Plates were then transferred to plate reader and absorbance measured at 570nm.

3.2.4.5 *Antibacterial activity testing*

Fourteen extracts of the body walls from eleven species of holothurians were assayed against a standard and wild type isolate of *Staphylococcus aureus, Pseudomonas aeroginosa* and *Escherichia coli*.

Assays were performed in 96-well microtitre plates as described previously (Haug *et al*., 2002). Briefly, Extracts were first diluted in dimethyl sulphoxide (DMSO) to give 25, 50, 100, 250 and 500 µg/ml. Diluted extracts (10 µl) were incubated with 10 µl of a suspension of an actively growing culture of bacteria diluted to 180 µl of fresh media. For each assay, media only and cells in media with appropriate levels of carrier

solvent were included as controls and all. Four replicates were used for each assay point. Bacterial growth was assayed after 24 hrs by measurement of optical density at 630 nm using a mirotitre (MRX) plate reader. Extracts were regarded as inactive when the optical density was >90 % of the control assayed after 24 hrs.

3.2.4.6 ***Anti-Leishmania assay***

To determine anti-leishmania activity, MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) reduction was used to assess cell viability, the method used was as follows *Leishamania major* 10^5 were added to 190 µl of fresh media in each well of a 96 well Microtitre plate. The plates were then centrifuged at 1000 g for 10 min and resuspended in 190 µl of media. 10 µl of extract or diluted extract in DMSO was added to the appropriate wells.

Plates were then incubated at 24 ^{0}C for 24 hr. For the viability assay 50 µl of 5 mg /ml MTT in PBS was added to each well and the plates incubated for 4 hr in the dark at 24 ^{0}C. After incubation, plates were centrifuged at 750 g for 10 min, the supernatant was removed and cell pellet resuspended in 200 µl DMSO then 25 µl of Sorrenson buffer was added. The plate was mixed and the absorbance of the wells was measured at 570 nm.

The results from the preliminary studies have shown that some of the extracts from the tested holothurians possess some anti-infective activity (Antifungal, antiprotozoal and anticancer activities). The species (*Actinopyga mauritiana*, *Bohadschia casteaui*, *B. graeffei* and *B. vitensies*) showed high biological activity in the primary screening and were bulk collected, extracted and biological activities examined once again.

The crude extracts of the target species (*Actinopyga mauritiana (north and south)*, *Bohadschia casteaui*, *B. graeffei* and *B. vitensies*) were fractionated into non-polar (Hexane), medium (Butanol) and high polar (Water) fractions. All fractions were tested again for biological activity and this was found to be localized in the butanol fraction.

A series of solvent systems including a mixture of different proportions of polar and non-polar solvents was used to determine the polarity of the active components and also to decide the best solvent system suitable to separate the active butanol fraction into different spots or bands on thin layer chromatography (TLC).

Activity was checked using a bioautographic ovelay bioassay method to determine the active compounds on the thin layer chromatograph (TLC).

The active butanol faction was then purified and separated using a Sep-PaK C_{18} silica column to separate the crude active fraction into several fractions of different polarities of methanol in water (10%, 20%, 40%, 60%, 80% and 100% methanol). The activity was checked again using the overlay bioassay technique and was found to be located in the 80% and 100% methanol.

3.2.4.7 HPLC and further Isolation

High Performance Liquid Chromatography (HPLC) analysis was performed using a C_{-18} column and UV detector with a mobile phase of acetonitrile (CH_3CN) and water in a gradient system changing from zero to 100 % of aetonitrile in 70 minutes. The samples were injected through a fixed loop (10 µl), and were monitored for 70 minutes, at a flow of 1 ml/minute. The solvents were filtered and degassed before each analysis. All samples were dissolved in mobile phase and filtered through 0.45 µl filter.

Different fractions have been collected from HPLC and these fractions were tested for activity to determine the semi-purified active fraction. The semi-purified active fraction was taken to the mass spectrometry lab at the chemistry Department using Liquid Chromatograph Mass Spectrometry (LC/MS) to get a preliminary idea of the active components. LC/MS analysis was tried in different modes (negative and positive ion mode) and mass spectra obtained showed that these spectra were well presented in the negative ion modes which correspond with other workers.

The application of multiple-stage tandem mass spectrometry for structure elucidation of active peaks allows us to deduct glycosylated compounds (saponins) based on the primary sequence and branching of the sugar units and mass fragmentation.

3.2.5 Results

The following represent the significant findings from this aspect of the study.

Table 4. The antibacterial, antifungal, anti-leishmanial and anticancer activities determined for the crude extracts of11 species of sea cucumber collected from the Egyptian Red Sea.

Species	antibacterial	antifungal	antileishmanial	anticancer
Actinopyga mauritiana(n)	-	++	++	++
Bohadschia tenuissima (n)	-	+	+	++
Holothuria atra(n)	-	_	+	++
Actinopyga mauritiana(s)	-	+	+	++
Bohadschia tenuissima (S)	-	+	-	+
Holothoria atra(s)	-	_	++	++
Holothuria fuscogilva	-	_	+	-
Stichopus varigatus	-	_	+	+
Actinopyga crasa	-	+	+	++
Bohadschia graeffi	-	++	++	++
Holothuria leocospilota	-	+	+	++
Bohadschia casteaui	-	++	+	++
Bohadschia vitenses	-	+	+	++
Holothuria nobilis	-	+	_	+

Table (5) shows the range of activities seen with the 11 extracts for holothurians. The species showing the greatest activities for each of the bioassay organism is also shown.

	Candida	Leishmania	Tumor cell-lines
Range of LC_{50} activities	400-75 µg/ml	400-91 µg/ml	100-0.50 µg/ml
Most active species	*-Actinopyga mauritiana(n)* *-Bohadschia graeffi* *-Bohadschia casteaui*	*-Actinopyga mauritiana(n)* *-Bohadschia graeffi* *-Holothoria atra(s)*	*-Actinopyga mauritiana(n)* *-Bohadschia graeffi* *-Bohadschia casteaui*

In summary, the majority of extracts from the Red Sea holothurians tested possess some anti-infective activity. Interestingly the pattern of activity seen showed significant variation (Sites and species). None of the extracts gave antibacterial activity. Good antifungal activity was detected as was anti-leishmainal activity. High antitumour activities were also detected in several species. It is thought that saponins in sea cucumbers are produced to protect them against predators, fungi and other protests. It is important that the active components are characterised and the optimum environmental conditions for the production of useful compounds are elucidated**.**

These results have important implications in relation to biodiversity conservation and fishery management, with regard to the taxonomic differentiation of species. They also raise the potential value of sea cucumbers beyond a simple fishery. This has positive implications with regard to raising public awareness of importance of sea cucumbers (stop over fishing) and raises importance with authorities.

3.2.6 Peer Review Outputs

Rafat M. A. Khattab (2006) Isolation and bioactivities of Natural Products from Red Sea Holothurians (Sea Cucumbers). PhD Thesis, University of Hull.

In addition, several scientific papers will shortly be submitted to peer reviewed journals:

A. Lawrence, A., Khattab, R., Ahmed, M., Khalifa, M and Paget, T (in prep) The Application Direct Value, Indirect Value and Future Option Value in the Promotion of Conservation and Sustainable Use of Sea Cucumber in the Egyptian Red Sea.

Khattab, R., Paget, T and Lawrence, A (in prep) Chemotaxonomy of Holothuria

3.3.1 Mariculture Study

3.3.2 The Team

Dr Howaida Gabr, Dr Ashraf Ibrahim, Dr Mahmoud Hanafy, Mr Mohammed Ahmed, Mr Wael Hefny, Dr Andrew Lawrence with support from the staff at Haraz Hatchery

3.3.3 Training

After some delays and personnel changes in Egypt, Mr Wael Hefny was selected to complete an MSc at Hull University related to this aspect of the study. Mr Hefny, an

EEAA Ranger based in Hurghada, was selected in recognition of the "in kind" support given by the EEAA to the project, his previous academic background (BSc Marine Biology) and his selection within the EEAA for a possible future management role.

As part of his training at Hull, Mr Hefny had to undertake specific Post-Graduate Training Modules (PGTM) to a minimum of 20 credits for his year of study. The specific modules he completed were: Health and Safety (5 credits, assessed by submission of risk assessment for his field work) Numerical Methods and Statistical Analysis (15 credits, assessed by submission of hand calculated and SPSS generated work sheet, including review of the appropriate test to use and why) and English for Academic Purposes (10 credits, assessed by submission of an essay and oral presentation).

Mr Wael Hefny received training in field survey, sediment analysis, sediment bacteria analysis, methods for water and sediment chemical analysis. This formed part of his MSc thesis, examined by an Internal and External Examiner.

3.3.4. Aquaculture Methodology

Primarily Drs Howaida Gabr and Ashraf Ibrahim performed the aquaculture study, with support from the technical staff at the Haraz Hatchery. Mr Mohamed Ahmed, supervised by Drs Gabr, Ibrahim and Lawrence, primarily performed the work on the reproductive cycle of *Actinopyga mauritiana*.

Actinopyga mauritiana broodstock for the sexual and asexual reproduction trials were generally collected from first of Jun to first of August from different locations at Red Sea shores. At that time, the seawater temperature varies from 22 to 25 °C. A few individuals were sacrificed and dissected in order to adequately determine the maturation stage of the gonads. Specimens weighing around 240 g and measuring around 16 cm in length were generally preferred. The collection of broodstock was not easy. Over fishing had led to scarce broodstock in the field.

The animals were transported to Ismailia using 1 m^3 fish transporter aerated with oxygen. Transport time was 5 hours. The animals were placed in private hatchery in 2.5 m^3 indoor holding tank containing 10 cm. of coarse sand. The water in the broodstock tank was changed daily and the sand every week. Mixture of different kind of dried algae (*Cystoseira, caulerpa, and Padina*) and fish powder were placed in the tank to enrich the substrate and stimulate the growth of bacteria and fungi to provide a food source for the animals. Before starting any experiment all animals were allowed to adapt to the new habitat for 3 weeks as recommended in the literature. All parameters such as temperature, salinity were monitored and maintained as close as possible to normal to minimize the stress on the animals.

All the experimental work was carried out at private hatchery located on Suez Canal coast called Haraz hatchery. This was the only place suitable for running the breeding experiments. The facilities included indoor hatchery tanks with water supply, filteration, aeration; nursery tanks with water supply and aeration; 6000 m^2 small ponds with water suppply, hatchery equipment (microscope, balances, counting cells, refractometer, DO and pH meters, glassware, blender and grinder, plankton netting, filters, pumps, heaters etc); an algae culture unit (sterile and mass culture); standby generation capacity

3.3.4.1Reproductive Biology

To study the reproductive biology of *Actinopyga mauritiana*, total length was measured immediately on site to the nearest 0.5cm to minimize the expected

changes in animal's size in transit. In the laboratory, total weight to the nearest 0.1g was measured for each specimen and then all the animals were dissected. Gonads were removed, sexed, weighed to the nearest 0.01g and preserved in 7% formalin. The body wall weight was obtained. For each animal, the Gonad Index (GI) was determined.

The gonads' macro- and microscopic features were used to assess maturity stages. four maturity stages was determined: Stage I, premature or recovery; Stage II, maturing; Stage III, mature; and Stage IV, post-spawning. Each gonad was examined and assigned to one of these stages. These four maturity stages were verified by histological examination. Diameters of 30 oocytes from each of the different stages of vitellogenesis (pre-, mid-, and late vitellogenic stages) were also measured.

3.3.4.2. Asexual Reproduction Trials

To determine the potential of *A. Mauritiana* to reproduce asexually, 180 individuals were separated into two size groups, each group was held in separate tank to assess the effect of size on the ability of the individuals to reproduce asexually. The first group contains 100 individuals ranging in length from 5 to15cm and weighing from 50 to 200gm. The second group contained 80 individuals ranging in size from 15 to 28cm and weighing from 220 to 570 gm.

In order to force asexual division, each sea cucumber was fitted with a rubber band across the mid-body. After the individuals had completely divided, the anterior ends were moved to separate tanks from the posterior ends. The tanks were monitored daily and the mortality rate was recorded. The experiment was maintained for three months and terminated when the internal organs except the gonads of all divided halves had regenerated. Scarifying some animals and scanning with ultrasound were used to monitor the regeneration of the internal organs. The percentage of survival was calculated for each size group as following:

Survival percentage S% = [(A+P)/2T]*100

Where (A+P) corresponded to the anterior and posterior ends respectively and (T) to the total number of specimens that had undergone fission.

3.3.4.3. Sexual Reproduction Trials

A number of trials and a number of dissections were performed for spawning attempts. All the trials were carried out during June 2004. The temperature at that time ranged from 22–25°C and the salinity was 42‰.

Spawning induction through thermal shock

Twenty animals were used in these trials (average length 17 cm and average weight 270 g).The animals were placed into a tank with sea water and then they were induced to spawn using thermal shock. The thermal shock were achieved by raising the water temperature 5 degrees above ambient for 1 h in aerated 300-l container. Temperature were raised by use of aquarium heaters or addition of heated seawater. These trials were repeated two times per week.

Spawning induction using blended gonads and thermal shock

In a separate set of trials an alterntive stimulus to spawn was attempted. In these trials blended gametes from gonads of scarified mature males were added to the tanks in an effort to stimulate spawning.

3.3.4.4. Growth trial

Twenty animals *of Actinopyga mauritiana* were used to test the effectiveness of captivity on their growth and maturation. The animals were in gametogenesis out of spawning season and there was no significant difference in the size of these animals (average length 14 cm & average weight 185g). The animals were separated equally into two groups, one group placed in a holding tank (2.5 m^3) while the second group were moved to an earthen pond 10 m x 15m and water depth 1.60 m . The tank animals were fed quantities of dried algae (*Cystoseira, caulerpa, and Padina*). The algae were collected from Suez Canal coast and placed in the tank to enrich the substrate and provide a food source for sea cucumbers. The pond animals were used as a control. The pond contained no added food and their food was limited to naturally occurring detrital matter contained in the sand. The weights of individuals were recorded periodically to determine change in size over time. It was difficult to weight sea cucumber accurately because of the large amount of water retained in the coelom. To reduce this problem we removed the animals prior to weighing, and prodded them until maximum contraction and expulsion of water occurred. The salinity of the water was the same in both the tank and the pond (42 ‰) and the temperature was little lower in the tank (25°C) than in the pond (29 °C).

3.3.5. Results

The following represent the significant findings from this aspect of the study.

3.3.5.1. Reproductive Cycle

A total of 244 *Actinopyga mauritiana* were collected from El-Gemsha Bay. Of the individuals sectioned (102 sexed and 142 unsexed), 53 individuals (52%) were females, and 49 individual (48%) were males. The unsexed individuals either carried unidentified gonads or had no visible gonads. The females and the males weere more or less equal in proportion, giving a sex ratio of 1:1.

Maturity scale

Four stages of sexual maturity for *Actinopyga mauritiana* were defined according to the gross morphology and microscopic development in the gonads. The maturation process was similar morphologically and histologically. Table 6 summarizes the mean dimensions of the gonadal tubules and oocytes diameter at various maturity stages.

Monthly variation in maturity stages

To determine the spawning season, the occurrence of mature females and males throughout the period of study was examined. Figs. 6 and 7 illustrate the monthly percentage composition of the maturity stages of female and male *Actinopyga mauritiana* respectively. Most mature females and males (Stage III) were observed between March and August. Meanwhile, the peak incidence of

mature males (Stage III) coincided with the peak incidence of spawning females (March to May).

Table 6. The mean size of male (M) and female (F) gonadal tubules and oocytes diameters for *A. mauritiana* at different stages of development.

Stage	**1. Gonad tubules**						**Oocyte diameter (µm)**
	Length (cm)		**Weight (g)**		**Diameter (mm)**		
	M	F	M	F	M	F	
Premature or recovery	3.00 ±0.50	2.50±0.40	0.15±0.20	2.51±0.70	0.23±0.01	0.13±0.26	19.60 ±0.37
Maturing	3.50 ± 0.20	4.50± 0.30	5.20±0.45	15.80±0.89	0.42±0.06	0.50±0.04	32.90 ± 0.03
Mature	9.80 ±0.40	12.00± 0.50	15.60±0.98	45.00±0.65	0.53±0.03	0.87±0.02	107.20 ± 0.43
Post-spawning	6.50 ± 0.44	7.30±0.30	8.50±0.84	18.35±1.65	0.45±0.06	0.60± 0.03	84.00 ± 0.91

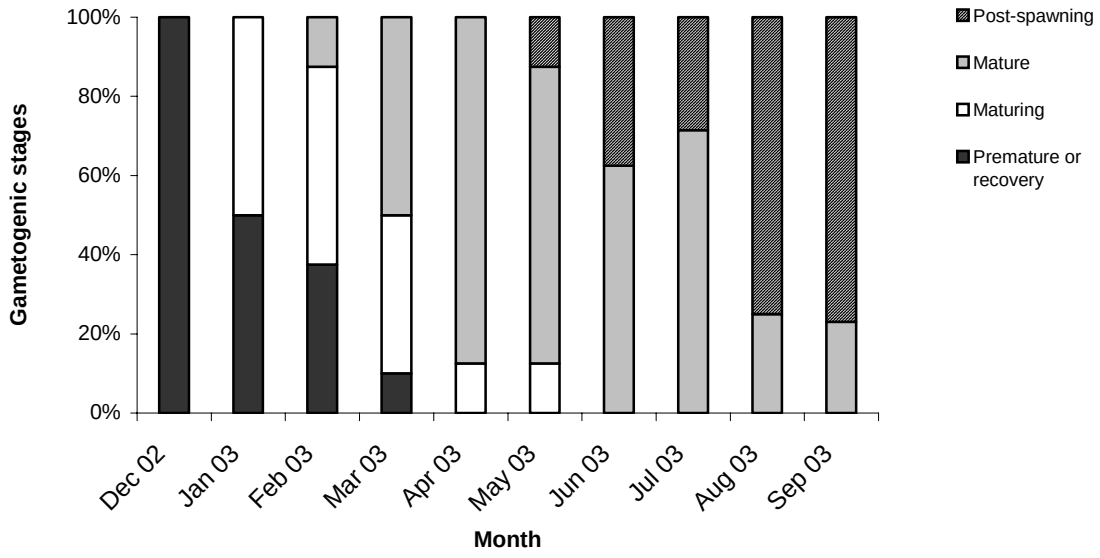


Figure 6. Monthly variation of gametogenic stages in female *A. mauritiana.*

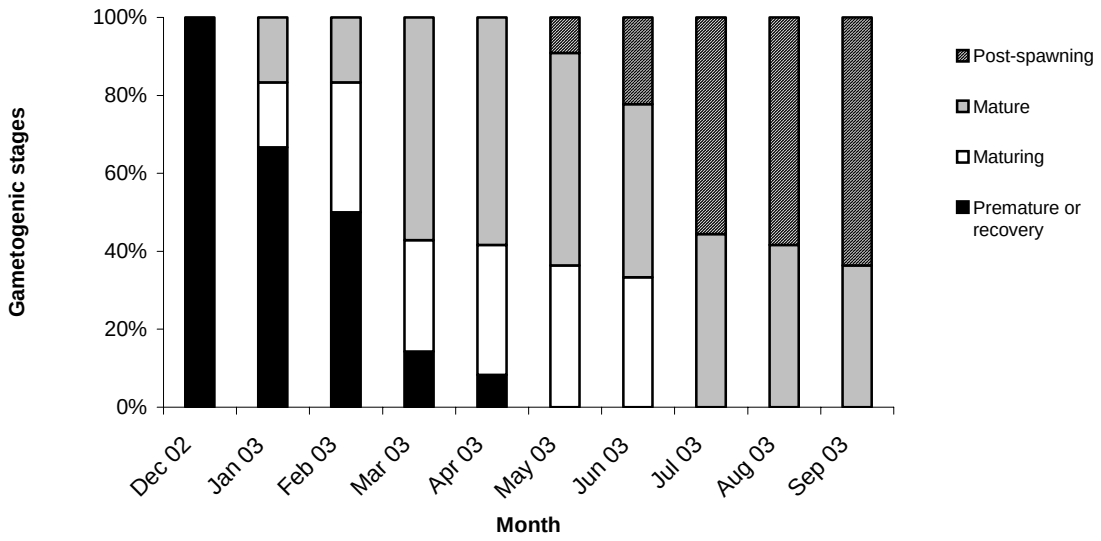


Figure 7. Monthly variation of gametogenic stages in male *A. mauritiana*

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Monthly changes in gonad index

The pattern of maturation indicated by examination of the gametogenic stages was supported by the gonad index (GI). The seasonal variation in the GI was pronounced for both the sexes (Fig. 8). The female and male gonad index increased in March and peaked in April. This coincided with the peak percentage of mature specimens. The GI declined in May and June, then a gradually increased to an intermediate level in July and August. For both the sexes, the GI dropped in September. The drop of GI in May and June was due to the presence of specimens with gonad weights that varied significantly from the mean.

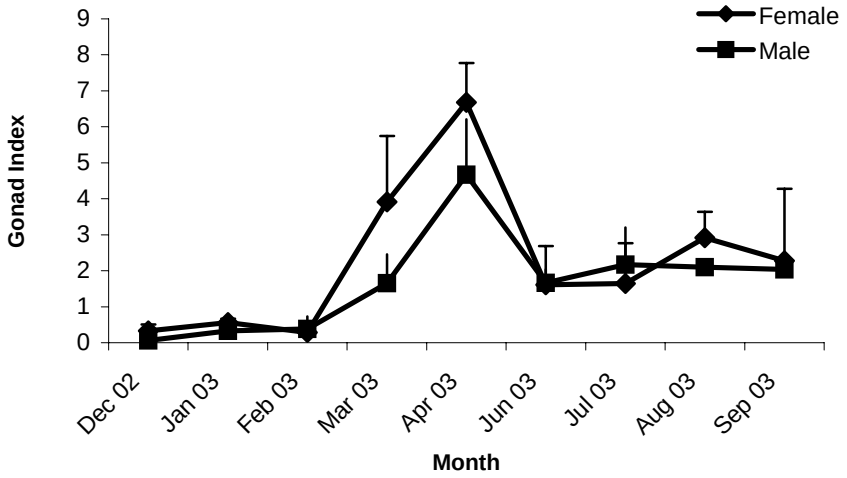

Figure 8. Monthly variation in means gonad index (GI) for female and male *A. mauritiana*.

Asexual Reproduction

It is not known whether *Actinopyga mauritiana* is able to reproduce asexually by transverse fission in addition to its sexual mode of reproduction. However this experiment indicates that this species has some capacity to reproduce asexually.

The survival rate of the small size group (average length 12cm and average weight 130gm) was 75% with 65% survival of the anterior parts and 85% survival of the posterior parts (Figure 9). While, the survival rate of the large size group (average length 19cm and average weight 330gm) was 58% with 50%survival of the anterior parts and 66% survival of the posterior parts.

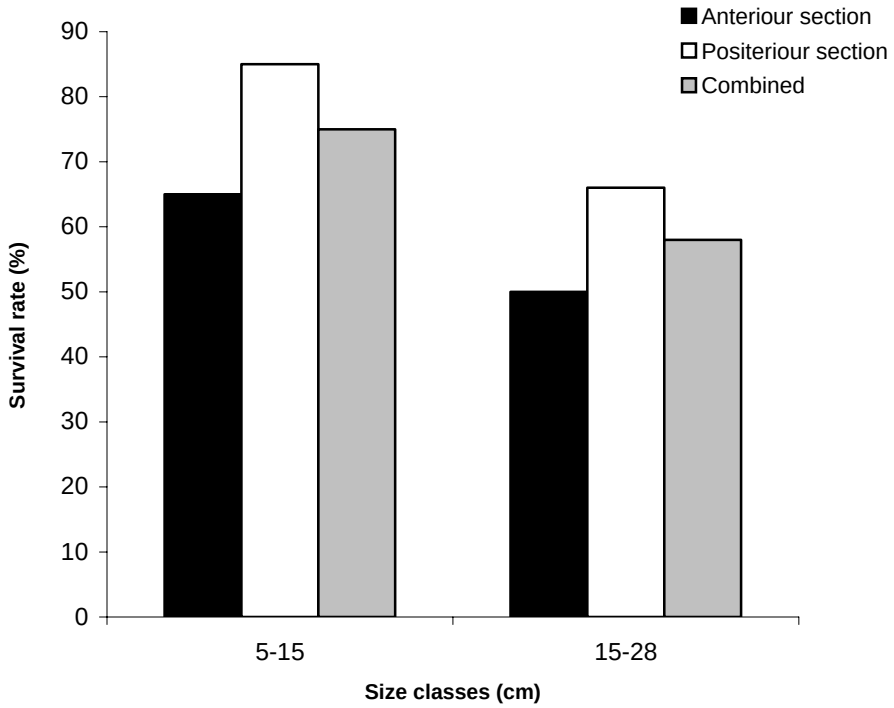

Figure 9 Survival rate of asexual reproduction experiment for two size groups of *A. mauritiana.*

Regeneration rate

Dissection of the sea cucumber on a monthly interval indicated that large individuals of *A. mauritiana* (average length 19cm and average weight 330g) were able to regenerate the posterior and the anterior parts into a whole individual, in around 100 days. In contrast, small animals (average length 12cm and average weight 130g) were able to regenerate both anterior and posterior parts into whole individuals in around 60 to 80 days. The shortest regeneration time was for the posterior parts of the smallest size class.

Sexual Reproduction Trials

Thermal shock failed to induce the animals to spawn at any time during the season. A number of animals showed signs of reproductive activity. They exhibited the typical Cobra position, standing up and waving their posterior part. The animals kept in this position for about 40 minutes but were not seen to spawn. Some animals inverted their bodies, and exposed the buccal podia.

Spawning induction using only blended gonads

None of the animals showed any response to the addition of sperm to sea water

Comment

The conditioning time may delayed the spawning season and the experimental animals may were unable to utilize the nutrient resources available. Minimize the weight loss and adjust the temperature regimes during the conditioning period is likely to be important in obtaining successful spawning. Perhaps only ripe animals should be collected from the natural environment during the spawning season and induce spawning should be conducted once arrive to the hatchery. This is may help to avoid problems associated with starvation and delay the maturation of gonads or reabsorption of gonads over extended periods in captivity.

Growth Trials

After two months, the animal growth in the tank differed significantly from that in the pond. The mean weight of the animals in the tank was static or had declined despite the provision and consumption of added food. There was a clear increase in the size of animals maintained in the pond. The initial weight increased by about 10 percent by the end of second month of captivity. Four animals from each group were dissected to check their reproductive maturation. Of the tank individuals sectioned, 3 were females and 1 was male while of pond individuals sectioned 2 were females and 2 were males. All the sacrificed animals carried gonads. The gonads of tank' animals were in the same maturity stage. The tubules were white and unbranched fine and thread-like and were classified as at an early stage based on their morphology. The gonads of pond animals were longer, thicker and branched and classified as at the growing stage. However, in comparison, wild animals collected at the same time contained ripe gonads.

Consequently, it was determined that captivity affected the growth and maturation of the animals. The animals in the tank did not gain weight due to the captive environment, and/or inadequate nutrition. They did not make progress in gametogenesis because they were unable to compensate entirely for the diversion of resources to reproduction. The difference in physico-chemical parameters between the Red Sea area and Suez Canal may be responsible for delaying the maturation of the pond animals. In view of these observations and the numerous attempts to stimulate spawning, it is felt that ripe animals, caught from the wild, should be induced to spawn immediately on the day of collection. The conditioning period and conditions at the Haraz Hatchery appear not ideal for *A. Mauritiana* and may be the major barrier to induce spawning.

3.3.6. Published Outputs

H.R. Gabr , A. I. Ahmed, M. H. Hanafy, A. J. Lawrence, M. I. Ahmed and S. G. El-Etreby (2004) Mariculture Of Sea Cucumber In The Red Sea - The Egyptian Experience. In: Lovatelli, A. (comp./ed.); Conand, C.; Purcell, S.; Uthicke, S.; Hamel, J.-F.; Mercier, A. (eds.). Advances in sea cucumber aquaculture and management. FAO Fisheries Technical Paper. No. 463. Rome, FAO. 2004. 457p

3.4.1. Ecological Role of *Actinopyga mauritiana*

Due to the difficulties encountered during the mariculture study, the work was broadened out to to examine the impact of sea cucumber removal on its habitat

3.4.2. The Team

Mr Wael Hefny (MSc student), EEAA Ranger Team, Dr Mahmoud Hanafy, Dr Andrew Lawrence

3.4.3 Training

See section 3.3.3.

3.4.4. Methodology

Nine (3x3m) cages were constructed and set up in El Gemsha Bay. The cages were positioned on a sandy area in 5m of water and covered in fish net with a 0.5m mesh. The cages were stocked with high (60 individuals), low (9 individuals) and control (0 individuals) numbers of *Actinopyga mauritiana*. These were maintained for a period of 4 weeks before sampling began.

At this point three sediment cores were collected from each cage, on a 2-weekly basis and for a period of 14 weeks. These cores were used to analyse sediment chemistry and physical parameters. Phosphorus was determine following the method or Bray Kurtiz (1995), ammonia was determined following the method of Book and Labring (1973), nitrite was determined using the cadmium reduction method (Nydhal, 1976) and nitrite following the method of Barnes and Folkards (1951). Total N was determined following the standard method (APHA, 1985) and total organic carbon (TOC) using a muffle furnace to determine ash and ash free dry weight of sediment.

Bacteria count was determined following culture on agar plates and chlorophyll a, spectrophotometrically following acetone extraction (APHA, 1985). Redox potential was determined using electrodes and particle size analysis following sieving through stardard sized sieves. Further cores were collected from each cage and fixed in rose bengal 5% formalin. Meiofauna were decanted and counted using a stereomicroscope.

3.4.5 Results

The following represent the significant findings from this aspect of the study.

3.4.5.1. Total Organic Content

The presence of sea cucumber *A. mauritiana* had a high significant effect on the total organic carbon in sediment. The high-populated cages had the lowest values of total organic carbon in the sediment. The overall mean total organic carbon in the high and low populated cages after 14 weeks was 3.22 mg C g^{-1} and 4.62 mg C g^{-1}, respectively

compared with 5.24 mg C g^{-1} in the non populated cages (Figure 10).

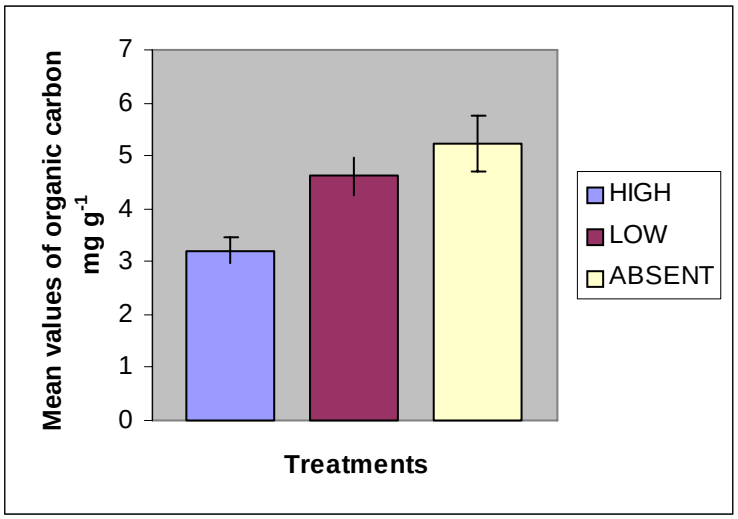

Figure 10 Overall means total organic carbon values in the sediment of three treatments: High A. mauritiana abundance, low A mauritiana abundance and no A. mauritiana (absent).

Two-way ANOVA showed a highly significant effect of *A. mauritiana* on total organic carbon ($p<0.001$). There was also a significant time effect on the total organic carbon levels in the sediment of the treatment cages (Figure 11).

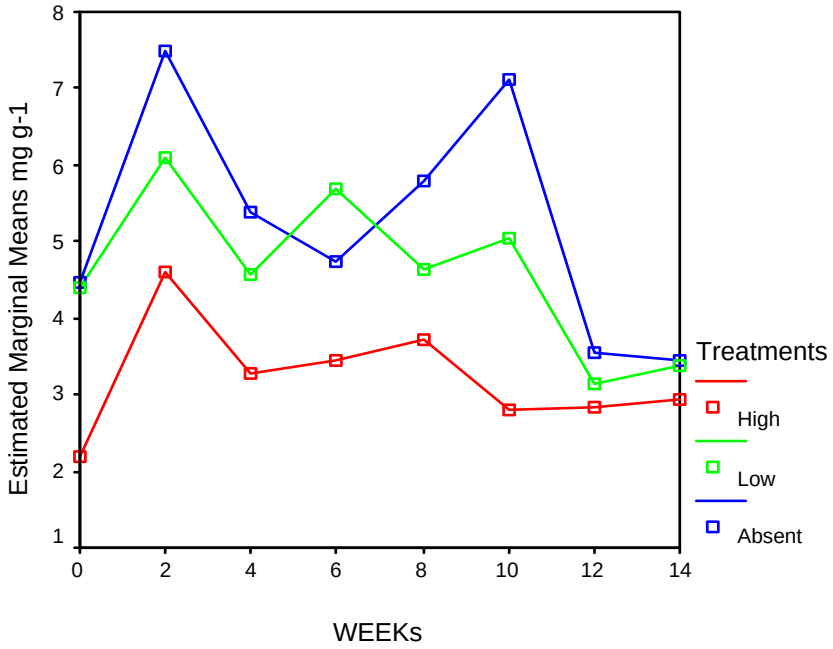

Figure 11. Average total organic carbon measured bi-weekly in the sediment of treatment cages populated with high, low and zero densities of *A. mauritiana* over experimental period of 14 weeks.

3.4.5.2. Bacterial Population

The presence of *A. mauritiana* showed a highly significant effect on the sediment

bacterial population count. The high-populated cages had the lowest count of bacteria in the sediment. The overall mean bacterial population in the high and low populated cages after 14 weeks was 9.2 and 13.9 Cfu g^{-1} respectively compared with 20.4 Cfu g^{-1} in the non-populated cages (Figure 12).

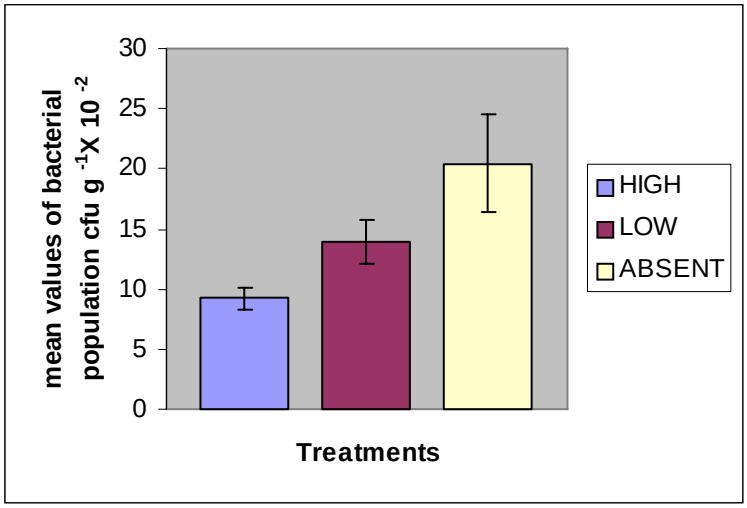

Figure 12. Overall mean bacterial population in the sediment of three treatments: High *A. mauritiana* abundance, low *A mauritiana* abundance and no *A. mauritiana* (absent).

Two way ANOVA showed a highly significant effect of *A. mauritiana* on bacterial population count ($p<0.001$). There was also a significant time effect on the bacterial population count in the treatment cages ($p<0.001$) and interaction between treatment and time.

3.4.5.3. Nitrate

The presence of sea cucumbers *A. mauritiana* had a significant effect on nitrate values in the sediment. The high-populated cages had the lowest values of nitrate concentration in the sediment. The overall mean nitrate levels in the high and low populated cages over the period 14 weeks of the experiment were 0.7 mg g^{-1} and 1.00 mg g^{-1} respectively compared with 1.2 mg g^{-1} in the non-populated cages (Figure 13).

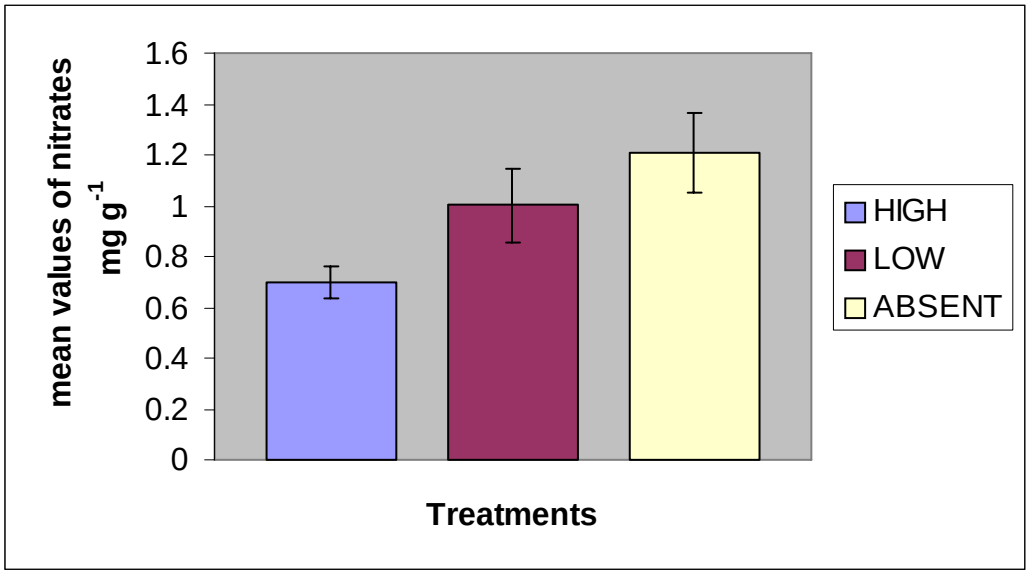

Figure 13. Overall mean nitrate in the sediment of three treatments: High *A. mauritiana*

abundance, low *A mauritiana* abundance and no *A. mauritiana* (absent).

Two way ANOVA showed a significant effect of *A. mauritiana* on nitrate concentration in the sediment. However, there was no significant time or interaction effect on the nitrate levels in the treatment cages.

3.4.5.5. Total Phosphorus

The presence of sea cucumbers *A. mauritiana* had a significant effect on the total phosphorus values in the sediment. The high-populated cages had the highest values of sediment total phosphorus concentration. The overall means of total phosphorus in the high and low populated cages over the 14 weeks period of the experiment was 1.22 mg g^{-1} and 0.84 mg g^{-1} respectively compared with 0.57 mg g^{-1} in the non-populated cages (Figure 14).

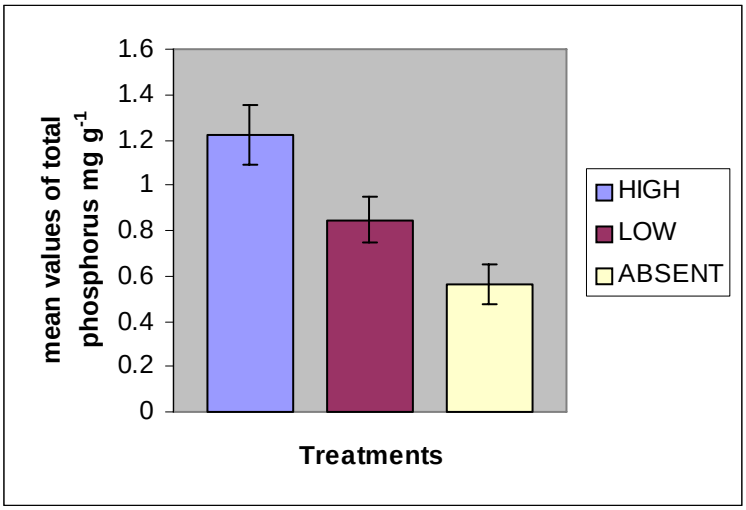

Figure 14. Overall means total phosphorus in the sediment of three treatments: High *A. mauritiana* abundance, low *A mauritiana* abundance and no *A. mauritiana* (absent).

Two way ANOVA showed a significant effect of *A. mauritiana* on phosphorus concentration in the sediment ($p<0.001$). There was also a significant time effect ($p<0.001$) but no interaction effect.

3.4.5.6. Chlorophyll a

The presence of sea cucumbers *A. mauritiana* had a high significant effect on chlorophyll a values in the sediment. The high-populated cages had the lowest values of chlorophyll a concentration in the sediment. The overall mean chlorophyll a levels in the high and low populated cages over the 14 weeks period of experiment was 1.18 µg g^{-1} and 1.49 µg g^{-1} respectively compared with 2.07 µg g^{-1} in the non populated cages (Figure 15). Two way ANOVA showed high significant effect of *A. mauritiana* on chlorophyll a concentration in the sediment ($p<0.001$).

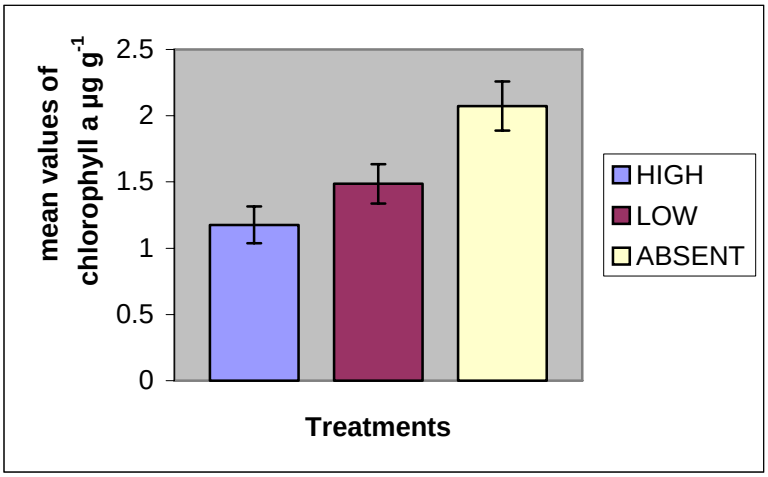

Figure 15. Overall means chlorophyll a in the sediment of three treatments: High *A. mauritiana* abundance, low *A mauritiana* abundance and no *A. mauritiana* (absent).

3.4.5.7. Sediment Particle Size Analysis

Table7. Sediment particle size analysis from treatment cages populated with different densities of *A. mauritiana* (i.e. High =60, Low=9 and Absent= zero individuals /cage).

Treatment	MDΦ	Sorting	Skewness	Kurtosis
High density	+1.4 Medium grained	+1.2 Poorly sorted	+0.016 near symmetrical	+0.55 very platykurtic
Low density	+1.53 Medium grained	+1.24 Poorly sorted	+0.143 Fine skewed	+0.92 Mesokurtic
Absent	+1.66 Medium grained	+1.31 Poorly sorted	+0.2 Fine skewed	+1.01 Mesokurtic

Grain size analysis of El Gemsha Bay sediment showed a change in median Phi between the three treatments (high, low and absent). The presence of *A. mauritiana* in the treatments populated at a high density showed the lowest Phi value grain size (MDΦ +1.4). The low populated and non-populated treatments of *A. mauritiana* showed higher Phi values (MDΦ +1.53 and MDΦ + 1.66) (Table 7). Grain size analysis was symmetrical skewed and platykurtic in the cage populated with high densities of *A. mauritiana* compared to fine skewed and mesokurtic in cages populated with low density and non populated A. *mauritiana.* The presence of *A. mauritiana* at a high density showed a high consumption of clay and fine sediment.

3.4.5.8. Sediment Meiofauna Analysis

Table 8: Estimated means of each meiofaunal group in the different treatment cages of *A. mauritiana* (ie. High =60 , Low=9 and Absent= zero individuals /cage).

2. Group \ Treatments	HIGH		LOW		3. ABSENT	
	Mean no/159cm^3	%	Mean no/159cm^3	%	Mean no/159cm^3	%
Nematoda	81	68.4%	143.1	56.4 %	93	37 %
Copepoda	23.4	20 %	83	33 %	75.2	30 %
Ostracoda	0	0 %	4	1.6 %	0	0 %
Polychaeta	10	8.4 %	16.1	6.3 %	78.4	31 %
Oligochaeta	2	0.02 %	6.1	2.4 %	3	1.2 %
Tardigrada	2	0.02 %	1.4	1 %	3.33	1.32 %
Total	118.4 (0.75 ind / cm^3)		253.7 (1.62 ind / cm$^{3)}$		252.9 (1.61 ind/ cm^3)	

3.4.6 Peer Reviewed Outputs

Wael A. Hefny (2006) A Study of the Impact of Holothuria (*Actinopyga mauritiana*) Overfishing on the Red Sea Marine Environment, MSc Thesis, University of Hull

4. Project Impacts

7.1 Meeting the Project Purpose

The original purpose of the project was to develop the a sustainable sea cucumber fishery along the Red Sea coast of Egypt. To this end, the project has been unsuccessful. It became apparent very early on that the level of fishing that had taken place up to the start of the project had significantly depleted resources. Consequently, it was not possible to work toward a strategy of developing a sustainable fishery but rather to examine the level of impact and status of the remaining stock. The problem of over-exploitation was exacerbated during the project by the continued, illegal, fishing of sea cucumber. At the request of the EEAA, and with its support in the form of resources and man-power, the stock assessment was significantly expanded to assess the level of over-fishing together with its impact.

Despite this, data from the project lead to the initial establishment and then re-instatement of a ban on the fishery to December 2003 within the Red Sea Governorate. Further assessment of the status of the fishery has lead to officials from the EEAA requesting an extension of the ban to allow stock recovery. This request was supported and endorsed by The Red Sea Governorate in a letter to the General Authority for Fisheries Resources and has lead to an extension to the ban to the end of 2005. However, as a result of the project, and the reporting of its outcomes at a meeting held in Sharm El-

Sheikh in 2005, the ban continues to the present time and has been extended to include the whole of the Egyptian coastline. This further ban has the support of all relevant agencies within Egypt. These agencies wait to hear the outcome of a second, follow on, Darwin Initiative project which has established a Monitoring Programme to assess stock recovery.

7.2 Assisting Egypt to Meet its Obligations under the CBD

The project has provided detailed information on the diversity and status of a little known group or marine organisms in Egyptian coastal waters. This has been made available to staff within the EEAA who are in direct and regular contact with Dr Moustafa Fouda (the Egyptian CBD). Furthermore, members from the project team met with Dr Fouda in 2005 to specifically consider the results of the current project. Thus the results of the project are available for incorporation into Egypt's Biodiversity Status Report and Action Plan. This meeting lead to Dr Fouda's stated aim of further expanding the aims of the study regionally with neighbouring countries to try to further protect the resource.

7.3 Training and Capacity Building

The project has undoubtedly improved Egypt's capacity to further biodiversity work. Training has lead to the establishment of capacity within the country to further develop work of this nature. At the current time:

Mr Mohammed Ahmed is studying toward a PhD partly funded by a Darwin Initiative follow up project. This specific study further builds on Mr Ahmed's taxonomic skills and will expand these into the field of molecular biology. During this time Mr Ahmed is also further training Rangers within EEAA and students at Suez Canal University in holothurian taxonomy.

Dr Rafat Khattab is now working in Suez Canal University partly as a lecturer in the Department of Marine Science but also as a Darwin Fellow. He is establishing a biotechnology laboratory within the Marine Science Department and furthering the characterisation of bioactive substances in holothuria. He currently has two undergraduate students working with him who are specifically gaining training in natural product isolation and characterisation techniques.

Mr Wael Hefny is now working in Hurghada as a Senior Ranger within the EEAA. As part of the follow on project, Mr Hefny is overseeing the establishment of a monitoring programme for sea cucumber which will be undertaken by EEAA Rangers.

7.4 Collaboration between UK and Local Partners

Collaboration between the partners has been a strength of the project throughout. The support of the Egyptian Environmental Affairs Agency has been particularly beneficial in relation to provision of facilities, manpower and equipment for stock assessment surveys. The EEAA also supported the visit of Mr Reiner Pitt to Egypt.

The University of Hull also offered to fund a further year of study for the students engaged in the stock assessment and bioactive substance studies. In the case of Mr Khattab, this was accepted by Suez Canal University but in the case of Mr Ahmed it was declined due to his not having a Masters degree. The Egyptian system requires that research students gain a masters qualification before beginning a PhD.

The Red Sea Governorate has given its full support to the project, with the Governor maintaining a ban on the fishery throughout the project. Furthermore, following a meeting held in Sharm El-Sheikh, the Suez Governorate, Gulf of Aquaba Governorate

and Department for Agriculture and Fisheries Resources, highlighted their interest in the project and desire to become involved in the work.

7.5 Social Impact from the Project

The project has had positive and negative impacts on individuals and the local community. It has clearly helped a number of individuals in their personal careers. In addition to the research students, involvement in the project, together with a letter of support from Dr Lawrence, at least in part helped Dr Gabr gain her promotion from Assistant to Associate Professor at Suez Canal University. It has also been helpful to Dr Paget in gaining a Readership in the Department of Pharmacy at Kent University.

The project has also increased the profile of Dr Lawrence and Hull University in Egypt. This has resulted in two additional research students from EEAA working with him. The first (Mr Selim, a Senior Ranger in the EEAA Hurghada) is working towards a PhD supported by a 2 year Ford Foundation Fellowship. The study is examining the impact of development and sedimentation on the coral reefs in the Hurghada region. The second, Mr M. Shabana, is the Head of EEAA Environmental Monitoring Unit in Hurghada. He is performing a study on bacterial disease in the reef community in the Hurghada region.

The ban on the fishery has arguably had mixed impacts. It will certainly have resulted in a loss of revenue to many within the local fishing communities. However, this "perceived" negative impact will have occurred despite the project, through the collapse of the fishery. Furthermore, the ban should have also resulted in a reduction in the number of diving accidents associated with the fishery.

5. Project Outputs

8.1 Outputs in relation to the Agreed Schedule

Most outputs have been achieved against those proposed in the schedule. Several additional outputs were achieved. For example, the schedule included the submission of 3 MSc theses. Two of these were subsequently upgraded to a PhD and MPhil (code 1 and 2). Furthermore, the funding for tuition fees for the third year of PhD were paid by Hull

University. Additional dissemination included a local TV news story on the project in the UK (code 18D) and two national/ international radio interviews in the UK (BBC World Today and Africa Today). Presentations were made at 2 additional international workshops/ conferences at CITES (2004) Kuala Lumpur and Natural Products Conference, Vancouver 2003. In addition, many press releases were sent out to local and national media and these received a high level up uptake.

Some outputs were not or have not yet been fully achieved. First, due to the problems encountered with the mariculture work, no training of local fishermen in mariculture techniques has taken place (codes 6A and 6B). Also, priority has been given to the completion and submission of research student theses. Consequently, the number of peer reviewed submissions is lower than originally proposed (Codes 11A and 11B). However, with the successful defence of each of the theses, priority is now being given to this and a number of papers are in preparation.

On the whole, the project partners feel that the project has been successful and that it has provided good value for money.

8.2 Dissemination of Project Outputs

Outputs from the project have been disseminated within Egypt via the network established through the participants in the Scientific Committee. Establishment and maintenance of the fishery ban has been publicised widely in Egypt both within the national and local media and has consequently reached a wider target audience of fishermen and traders within the local communities.

The project has developed links with the GAFR, and NGOs including the Hurgada Environmental Protection and Conservation Association (HEPCA) and the Red Sea Diving and Watersport Association (RSDWA), The Regional Organisation for the Conservation of the Environment of the Red Sea and Gulf of Aden (PERSGA) all of which has raised awareness of the project within Egypt.

Outputs have also been disseminated internationally within the wider community of scientists, fishery organisations and traders at events held in China and Kuala Lumpur (UNFAO and CITES). The dissemination will continue, particularly within Egypt beyond the time of the original project. Indeed, part of the proposal of the follow up project is to extend dissemination to neighbouring countries in the Red Sea and East Africa region. This cost is being covered partly by the Darwin Initiative, through the follow up project, but also through local agencies which have the funding to support this.

1. Project Expenditure

Table 9.1 Project Expenditure against Proposed budget

Item	*Budget*	*Expenditure*	*Balance*

It should be noted that fieldwork and fuel expenses include a per diem to some technical support staff

9.2 Agreed Changes in Budget and variations in expenditure

Some changes were made in the budget and reported to the Darwin Initiative. For example, some equipment (underwater video, GIS software) were not purchased to save money towards an extended fieldwork programme.

2. Project Operation and Partnerships

10.1 The Project Partners

Three project partners were initially signed up to the project: Suez Canal University, Department of Marine Sciences; The Egyptian Environmental Affairs Agency and the Red Sea Governorate. Each of these were active in outputs of the project in a number of ways. Suez Canal University identified students to work on the project, they supported the fieldwork with manpower and extra facilities, they were instrumental in identifying and working with the Haraz Hatchery on most aspects of the mariculture study.

The Egyptian Environmental Affairs Agency were also very active and supportive in the study. The EEAA has the role within Egypt for monitoring and management of the environment. It's Nature Protectorates Section has responsibility for the management of its natural environmental, development of National Parks and management of these.

It was this Section that the project had closest links with. Through this, the project also had links with Dr M. Fouda (Director for Conservation of the EEAA) and the Egyptian Focal Point for the CBD).

The EEAA provided manpower, equipment and resourses to support the expanded stock assessment work. It also provided one member of staff, Mr W. Hefny, to support the mariculture work and to study the impact of sea cucumber removal on habitat quality.

The EEAA also managed to liaise with other agencies and NGOs in Egypt to promote the project including the General Authority for Fisheries Resources, GAFR, the Hurgada Environmental Protection and Conservation Association (HEPCA) and the Red Sea Diving and Water Sports Association, The Regional Organisation for the Conservation of the Environment of the Red Sea and Gulf of Aden (PERSGA). Through the recommendations of the EEAA and its consultant (Dr M. Hanafy) the Red Sea Governorate were kept informed of the outcomes of the project and continued to make policy decisions regarding the fishery. Most notably it initiated and maintained a ban on the fishery during the lifetime of the project.

Partners were involved in project planning and implementation through regular meetings of the Scientific Committee, for which representatives of each partner were present. Plans were modified significantly at the beginning of the project for reasons outlined earlier. The main effect of this was to divert a larger portion of the budget into fieldwork.

10.2 Collaboration with Similar Projects within Egypt

Collaboration existed between this Darwin Initiative project and a USAID Monitoring and Conservation Project. Indeed, the results of the monitoring programme were mentioned in the USAID Conservation Final Report. There was no direct consultation with Egypt's Biodiversity Strategy Office. However, there were two meetings with Dr Fouda (CBD Focal Point) during the project which specifically reported the status of the project and discussed possible ways forward beyond the time of Darwin Initiative funding.

10.3 International Participants in the Project

Internationally, a number of links have been developed and are resulting in on-going collaborations. For example, a link has been established with Dr Yves Samyn (CBD National Focal Point, The Royal Institute of Natural Science of Belgium). Dr Samyn offered advice and confirmation of some species identifications.

In addition, a link has also been established with Dr Sven Ulthike, from The Australian Institute of Marine Sciences (AIMS) regarding distribution and taxonomy of *H. fuscogilva*. The species collected in the Red Sea appear slightly different to those described in the Indo Pacific. We have sent photographs and samples which are being used in a molecular genetic analysis of the species across their global range being performed by Sven Ulthicke's group. An acknowledgement was received for this in a recent publication by Sven Ulthicke.

10.4 Post Project Local Partner Activity

The partners are still in contact and through the support of a Darwin Initiative follow up project grant the work is currently continuing to develop a monitoring programme to determine stock recovery and to further expand the work of the bioactive

substances study. More community participation would be beneficial. The EEAA have liaised with dive boat operators as a possible mechanism of monitoring stocks and this is being considered.

3. Monitoring and Evaluation, Lesson learning

11.1 Monitoring, Evaluation and Indicators of Achievement

Monitoring and management of the project was primarily via Merlin (an internet system which allows all of the named participants in the project to correspond and communicate on a regular basis). The project participants, and Management Committee, were able to share and disseminate information, provide training and monitor progress against predetermined milestones and the logistic framework via the system. In addition there were regular meetings between the Project Coordinators in Egypt, the UK and China.

Information collected in the study is highlighted in Section 3. Indicators of achievement and project milestones included progress reports submitted by project students/ groups for each of the three topic areas of the project, Submission of PhD, MPhil and MSc theses are all indicators of achievement as are the submission of the Management Plan and use of the Field Guide.

An indicator of the fact that the outputs contributed to the project purpose is highlighted by the establishment and re-instigation of a fishery ban during in 2003 and 2005 whilst any evidence of stock recovery is determined.

11.2 What were the Main Problems

The main problem for the project was in its financial management. This was a result of the mechanism required by the Darwin Initiative clashing with that required by the University, clashing with that required by the Egyptian Partners. Indeed, the partners required cash advances before any work could be undertaken. In addition, there were continual time delays in the reporting of expenses by the main Egyptian Partner which delayed in particular the submission of the final report.

Some of the problems were overcome by the Darwin Initiatives agreement to allow cash advances based on statements of proposed expenditure. No real solution was found to getting statements submitted within the required timeframes required by the Darwin Initiative.

11.3 Internal and External Evaluation of the Project

There has been internal and external evaluation of the project throughout its time frame. For example, aspects of the scientific elements of the project were monitored by the Scientific Committee at regular intervals. Both the PhD student and MPhil student had to submit first year reports to the Department of Biological Sciences. These were examined by viva by two internal members of the department not involved in the project work.

In addition, all three elements of the work: the stock assessment study, the bioactive substances study and the impact of sea cucumber removal study have all been submitted as PhD, MPhil and MSc theses and examined by internal and external examiners and passed (MSc subject to minor corrections).

The financial aspects of the project have been audited on two separate occasions by external auditors.

11.4 Key Lessons learned

The key lessons learned from the project have been that good regular

communications are fundamental to the success of a project. Good systems need to be in place for this between countries and individuals in a way which keeps everyone informed of the progress of the work. The Merlin system used in this project could have been more effective in this if all partners had regularly used it.

One key problem for this project was the loss of the named Egyptian Partner within weeks of the grant being awarded. Whilst new partnerships were forged and links made and whilst the resultant partnership was very effective and friendships made, it possibly never quite had the level of personal commitment and support that would have been anticipated if the original, named partner had remained in post.

A second lesson learned was that most elements of work, particularly overseas and in remote areas and developing countries take longer than anticipated. Work programmes and timetables should probably reflect this but might be less likely to be successful at the proposal stage if they did so.

4. Actions taken in response to annual report reviews (if applicable)

Reviews of the project have mostly been positive and encouraging and were discussed with project partners during meetings of the Scientific Committee. The committee would like to thank the reviewers for their encouraging and constructive comments throughout the time of the project. Where issues were raised, we have tried to respond to these. Thus:

- All major items of equipment were insured by Suez Canal University in Egypt
- The group were aware of the work Reiner Pitt. He visited Egypt and offered advice on aspects of the mariculture work.
- Aspects of GIS work were being performed by EEAA staff at the GIS Unit, Hurgada. In addition, Mr M. Ahmed, the student most directly involved in the stock assessment work received 3 months training in remote sensing and GIS.
- There was a delay in the completion of the Management Plan, partly due to initial slippage. However, the Management and Monitoring Plan was submitted to the Scientific Committee for consideration.
- Mr Wael Hefny (EEAA Ranger) had originally been registered by Hull University to complete a research MSc. The scope of this project was broadened to incorporate aspects of the mariculture and the impact of sea cucumber removal on habitat quality. This was submitted as an MSc thesis.
- The issue of variation of bioactivity in species along their range is a key one. This is now being addressed in the follow up project using molecular genetic tools. This, as the reviewer highlights, has implications for any re-stocking of sea cucumber and also any future management plans for the resources.
- The problem of reporting on the budget was noted by the reviewer. This is a problem that was highlighted earlier in relation to the mismatch between the Darwin Initiative, University and partner reporting methods. The problem is, hopefully overcome, in the expenditure reported in Table 9.1

5. Darwin Identity

Every effort was made to publicise the Darwin Initiative throughout the project. The Darwin Logo was used on the truck purchased for fieldwork and laboratory doors. It was also used on publicity materials (fliers, reports, field guide, etc). In addition, the support of the Darwin Initiative was written into every press release and highlighted in every radio and TV interview.

The Darwin Initiative is familiar to all of the project partners including government agencies within Egypt. In addition, we known that the award of the project was made known to the then Environment Minister who had discussed this with the UK Ambassador to Egypt (reported back via the British Council Office in Cairo). It was also familiar to operational managers of similar projects such as the Coordinator of the USAID Conservation Project. The Darwin Initiative has also been highlighted as the funding agency in several of the sea cucumber reports in local and national news papers in Egypt.

Whilst collaborating with similar projects and working with individuals involved in wider biodiversity and conservation issues, the project has mostly been considered as a distinct project with a clear identity.

6. Leverage

Additional funds to the project were provided by the University of Hull to cover the additional scholarship fee for the PhD student. In addition, the greatly expanded field work was substantially supported by the EEAA “in kind”. The project was invited to submit a proposal to the UNFAO to further the work of the mariculture study. However, given the problems encountered in the study and the need to review possible ways forward, this was deferred until a feasible way forward could be agreed.

The UK partner has been instrumental in highlighting potential sources of further funding to support aspects of the project work. Some of these are currently being progressed. However, it is apparent that it is likely that these will have to be lead by the UK partner.

7. Sustainability and Legacy

At the current time the capacity building, through training of research students and EEAA Rangers will endure and be built upon. Furthermore, resources provided by the project will continue to be used by some of the Egyptian partners, notably those physical resources left with the Department of Marine Sciences, Suez Canal University.

Mr M Ahmed will continue work on holothurian taxonomy and, through the successful follow on project, will gain skills in molecular biology. This, together with the outlined monitoring programme, will ensure that the sea cucumber fishery remains an issue for the Egyptian authorities in the coming years. Furthermore, it is hoped that through the use of EEAA Rangers in the future monitoring programme, that assessment of the stock status of sea cucumber will become imbedded in the work of EEAA monitoring teams.

The work of the natural products group will certainly continue through the employment of Dr R. Khattab as a lecturer in Suez Canal University. This is clearly an aspect of the work that the Egyptian partners are keen to progress. It is anticipated that Dr Khattab will begin training undergraduates in some of the extraction and assay techniques, developed during the project, in the coming months and years.

Partners will clearly remain in touch during the follow up project. However, changing staff will also mean that some links end with the completion of the follow up project. Further partnerships will also continue to develop and it anticipated that other aspects of marine biodiversity conservation may become future focuses of attention between some of the partners.

It is also clear that both Dr Khattab and Mr Ahmed will stay in contact beyond the period of the follow up project and future funding. Very good partnerships have been developed between these staff and the UK partner.

Furthermore, some of the key project conclusions are currently being applied through the funding provided by the Darwin Initiative follow on project. Notably a monitoring programme is being developed to assess stock recovery and the current ban is continuing at the moment. It is hoped that additional funding from other sources might continue and expand upon the legacy left by this Darwin Initiative project. A key element of the current follow on project is that additional funding is applied for from various donor agencies. In the first instance these are being sought to try to properly fund an invertebrate mariculture system in Hurghada (through UNFAO) and secondly further funding will be sought to further the work of the marine natural products group.

8. Value for money

Overall, whilst not every output of the project was achieved, notably the development of a mariculture facility, the majority of outputs were. Furthermore, where outputs were not achieved, alternative work and studies were undertaken which have provided valuable information to the EEAA and other agencies when arguing at a national level to continue the fishery ban. Notable here was the work looking at the impact of sea cucumber removal on habitat physical and chemical parameters. Consequently, overall, the projects has mostly met its goals and purpose, producing the outputs and indicators of achievement that were originally proposed.

Furthermore, the project has clearly begun to leave a lasting legacy in Egypt through the employment of trained personnel and their roles in educational and governmental institutions. These people are now going on to train others in aspects of the original work. The project has had a relatively large profile both in Egypt and the UK further increasing the awareness of sea cucumber, the Darwin Initiative and wider marine biodiversity conservation and management issues. It has gained interest at an international level both within the sea cucumber science and management community and wider legislative agencies such as CITES. It has attracted some additional funding from the partners which expanded the scope of some of the work and allowed some of the research students to gain qualifications higher than those anticipated.

Consequently, on reflection, we feel that the project has been extremely cost effective and represents very good value for money.

9. Appendix I: Project Contribution to Articles under the Convention on Biological Diversity (CBD)

Please complete the table below to show the extent of project contribution to the different measures for biodiversity conservation defined in the CBD Articles. This will enable us to tie Darwin projects more directly into CBD areas and to see if the underlying objective of the Darwin Initiative has been met. We have focused on CBD Articles that are most relevant to biodiversity conservation initiatives by small projects in developing countries. However, certain Articles have been omitted where they apply across the board. Where there is overlap between measures described by two different Articles, allocate the % to the most appropriate one.

Project Contribution to Articles under the Convention on Biological Diversity		
Article No./Title	**Project %**	**Article Description**
6. General Measures for Conservation & Sustainable Use		Develop national strategies that integrate conservation and sustainable use.
7. Identification and Monitoring	40%	Identify and monitor components of biological diversity, particularly those requiring urgent conservation; identify processes and activities that have adverse effects; maintain and organise relevant data.
8. In-situ Conservation	10%	Establish systems of protected areas with guidelines for selection and management; regulate biological resources, promote protection of habitats; manage areas adjacent to protected areas; restore degraded ecosystems and recovery of threatened species; control risks associated with organisms modified by biotechnology; control spread of alien species; ensure compatibility between sustainable use of resources and their conservation; protect traditional lifestyles and knowledge on biological resources.
9. Ex-situ Conservation	10%	Adopt ex-situ measures to conserve and research components of biological diversity, preferably in country of origin; facilitate recovery of threatened species; regulate and manage collection of biological resources.
10. Sustainable Use of Components of Biological Diversity		Integrate conservation and sustainable use in national decisions; protect sustainable customary uses; support local populations to implement remedial actions; encourage co-operation between governments and the private sector.
11. Incentive Measures		Establish economically and socially sound incentives to conserve and promote sustainable use of biological diversity.

12. Research and Training	30%	Establish programmes for scientific and technical education in identification, conservation and sustainable use of biodiversity components; promote research contributing to the conservation and sustainable use of biological diversity, particularly in developing countries (in accordance with SBSTTA recommendations).
13. Public Education and Awareness	5%	Promote understanding of the importance of measures to conserve biological diversity and propagate these measures through the media; cooperate with other states and organisations in developing awareness programmes.
14. Impact Assessment and Minimizing Adverse Impacts		Introduce EIAs of appropriate projects and allow public participation; take into account environmental consequences of policies; exchange information on impacts beyond State boundaries and work to reduce hazards; promote emergency responses to hazards; examine mechanisms for re-dress of international damage.
15. Access to Genetic Resources		Whilst governments control access to their genetic resources they should also facilitate access of environmentally sound uses on mutually agreed terms; scientific research based on a country's genetic resources should ensure sharing in a fair and equitable way of results and benefits.
16. Access to and Transfer of Technology		Countries shall ensure access to technologies relevant to conservation and sustainable use of biodiversity under fair and most favourable terms to the source countries (subject to patents and intellectual property rights) and ensure the private sector facilitates such assess and joint development of technologies.
17. Exchange of Information	5%	Countries shall facilitate information exchange and repatriation including technical scientific and socio-economic research, information on training and surveying programmes and local knowledge
19. Bio-safety Protocol		Countries shall take legislative, administrative or policy measures to provide for the effective participation in biotechnological research activities and to ensure all practicable measures to promote and advance priority access on a fair and equitable basis, especially where they provide the genetic resources for such research.
Total %	**100%**	**Check % = total 100**

10. Appendix II Outputs

Please quantify and briefly describe all project outputs using the coding and format of the Darwin Initiative Standard Output Measures.

Code	Total to date (reduce box)	Detail (←expand box)
Training Outputs		
1a	Number of people to submit PhD thesis	1 (Additional Output)
1b	Number of PhD qualifications obtained	1 (Additional Output)
2	Number of Masters qualifications obtained	2 (MPhil is Additional Output)
3	Number of other qualifications obtained	2 (1 subject to minor corrections)
4a	Number of undergraduate students receiving training	0
4b	Number of training weeks provided to undergraduate students	0
4c	Number of postgraduate students receiving training (not 1-3 above)	0
4d	Number of training weeks for postgraduate students	0
5	Number of people receiving other forms of **long-term** (>1yr) training not leading to formal qualification(i.e not categories 1-4 above)	0
6a	Number of people receiving other forms of **short-term** education/training (i.e not categories 1-5 above)	10 Rangers
6b	Number of training weeks not leading to formal qualification	2 weeks training in ID
7	Number of types of training materials produced for use by host country(s)	1 Field Guide to Red Sea Holothuria
Research Outputs		
8	Number of weeks spent by UK project staff on project work in host country(s)	15 weeks spent in Egypt
9	Number of species/habitat management plans (or action plans) produced for Governments, public authorities or other implementing agencies in the host country (s)	1 Management and Monitoring Plan produced for implementing agencies
10	Number of formal documents produced to assist work related to species identification, classification and recording.	1 Field Guide produced to assist in species identification
11a	Number of papers published or accepted for publication in peer reviewed journals	2 papers published in peer reviewed publication
11b	Number of papers published or accepted for publication elsewhere	Information provided towards one technical document
12a	Number of computer-based databases established (containing species/generic information) and handed over to host country	1 database established for the host country
12b	Number of computer-based databases enhanced (containing species/genetic information) and handed over to host country	
13a	Number of species reference collections established and handed over to host country(s)	1 species reference collection established in Egypt
13b	Number of species reference collections enhanced and handed over to host country(s)	

Dissemination Outputs		
14a	Number of conferences/seminars/workshops organised to present/disseminate findings from Darwin project work	1 workshop organised in Hughada in 2005
14b	Number of conferences/seminars/ workshops **attended** at which findings from Darwin project work will be presented/ disseminated.	6 conferences attended and findings presented as papers or posters
15a	Number of national press releases or publicity articles in host country(s)	6 nationally including Egypt Today
15b	Number of local press releases or publicity articles in host country(s)	5 articles locally in Hurghada
15c	Number of national press releases or publicity articles in UK	3 published – Times Higher and Daily Express and Times
15d	Number of local press releases or publicity articles in UK	2 Hull Daily Mail and Yorkshire Post
16a	Number of issues of newsletters produced in the host country(s)	1 HEPCA
16b	Estimated circulation of each newsletter in the host country(s)	4-5 countries
16c	Estimated circulation of each newsletter in the UK	
17a	Number of dissemination networks established	1 Network established
17b	Number of dissemination networks enhanced or extended	
18a	Number of national TV programmes/features in host country(s)	
18b	Number of national TV programme/features in the UK	
18c	Number of local TV programme/features in host country	
18d	Number of local TV programme features in the UK	1 programme (Additional Output)
19a	Number of national radio interviews/features in host country(s)	
19b	Number of national radio interviews/features in the UK	2 (BBC World Today & World Service Africa Today) (Additional Outputs)
19c	Number of local radio interviews/features in host country (s)	
19d	Number of local radio interviews/features in the UK	2 on BBC Radio Humberside
Physical Outputs		
20	Estimated value (£s) of physical assets handed over to host country(s)	22, 500
21	Number of permanent educational/training/research facilities or organisation established	
22	Number of permanent field plots established	
23	Value of additional resources raised for project	£170, 308 (£82, 612 from Egyptian partners, £87, 696 from Hull partner)
23		£9, 750 (additional years studentship for PhD

11. Appendix III: Publications

Provide full details of all publications and material that can be publicly accessed, e.g. title, name of publisher, contact details, cost. Details will be recorded on the Darwin Monitoring Website Publications Database that is currently being compiled.

Mark (*) all publications and other material that you have included with this report

Type * (e.g. journals, manual, CDs)	**Detail** (title, author, year)	**Publishers** (name, city)	**Available from** (e.g. contact address, website)	**Cost £**
A. Lovatelli, C.Conand, S. Purcell, S. Uthicke, J-F Hamel & A. Mercier (Eds) 2004 Advances in Sea Cucumber Aquculture and Management	Lawrence, A.J., Ahmed, M., Hanafy, M), Gabr, H., Ibrahim, A and Gab-Alla, A. (2004) Status of the Sea Cucumber Fishery in the Red Sea – The Egyptian Experience	UN FAO, Rome		
A. Lovatelli, C.Conand, S. Purcell, S. Uthicke, J-F Hamel & A. Mercier (Eds) 2004 Advances in Sea Cucumber Aquculture and Management	H.R. Gabr , A. I. Ahmed, M. H. Hanafy, A. J. Lawrence M. I. Ahmed and S. G. El-Etreby (2004) Mariculture Of Sea Cucumber In The Red Sea - The Egyptian Experience.	UN FAO, Rome		
SPC Beche-de-Mer Bulletin	Titles and authors as above Beche-de-Mer Issue 19, Jan 2004	SPC	www.spc.int/coastfish/news/BDM/19/ASCAM. pdf	Free
CITES Technical Report to the Animals Committee	Lawrence, A.J., Ahmed, M., Hanafy, M, Gabr, H., Ibrahim, A and Gab-Alla, A. (2004) Status of the Sea Cucumber	CITES	CITES Doc AC20 Inf 14	
PhD Thesis	Rafat M. A. Khattab (2006) Isolation and bioactivities of Natural Products from Red Sea Holothurians (Sea Cucumbers).	University of Hull	The Library, University of Hull, Cottingham Road, Hull, HU6 7RX	Free

MPhil Thesis	M. I. Ahmed (2006) Taxonomic and Fishery stock status of sea cucumber in the Egyptian Red Sea,	University of Hull	The Library, University of Hull, Cottingham Road, Hull, HU6 7RX	Free
MSc Thesis	Wael A. Hefny (2006) A Study of the Impact of Holothuria (*Actinopyga mauritiana*) Overfishing on the Red Sea Marine Environment	University of Hull	The Library, University of Hull, Cottingham Road, Hull, HU6 7RX	Free

12.
13. Appendix IV: Darwin Contacts

To assist us with future evaluation work and feedback on your report, please provide contact details below.

Project Title	Darwin Initiative for the Sustainable use of Sea Cucumber in Egypt
Ref. No.	*162/10/027*
UK Leader Details	
Name	Dr Andrew Lawrence
Role within Darwin Project	Project Leader, Supervisor of each research student, overall management of the project,
Address	Department of Biological Sciences, University of Hull, Hull, HU6 7RX
Phone	
Fax	
Email	
Other UK Contact (if relevant)	
Name	
Role within Darwin Project	
Address	
Phone	
Fax	
Email	
Partner 1	
Name	Dr Howaida Gabr and Dr Ashraf Ibrahim
Organisation	Department of Marine Sciences, Suez Canal University
Role within Darwin Project	To oversee project within Egypt, Manage the financial budget within Egypt, research towards mariculture of sea cucumber at Haraz Hatchery
Address	Department of Marine Science, Suez Canal University, Ismailia, Egypt
Fax	
Email	
Partner 2 (if relevant)	

Name	Dr Mahmoud Hanafy
Organisation	Egyptian Envirionmental Affairs Agency (EEAA)
Role within Darwin Project	Member of Scientific Committee, support fieldwork towards stock assessment with manpower and resources, advisor to Red Sea Governor instrumental in establishment of ban on the fishery
Address	EEAA Hurghada Office, Hurghada, Egypt
Fax	
Email	

Appendix V Project Logical framework.

Project summary	Measurable indicators	Means of verification	Important assumptions
Goal To help Egypt, a country rich in biodiversity but poor in resources, meet its obligations under the Biodiversity Convention.	• After 12 months provide checklist of holothuria. • From 1-24 months, one species reference collection • After 24 months provide recommendations for sustainable fishery and biomedical properties • After 36 months, 10 EEAA rangers and 30 locals trained in stock assessment/ mariculture	• Information included in NBUs NBS • Fieldguide to Holothuria of the Red Sea • Computer database and GIS system • Final Report of project Scientific Committee • 3 MSc theses • Publications in scientific literature • Minutes and reports of all progress meetings	• EEAA to continue monitoring beyond Darwin funding • Mariculture not only to prove viable but local communities to develop and operate their own systems based on training • Additional funding/ sponsor found to support work on bioactive substances
Purpose To develop the first example of a sustainable sea cucumber fishery along the Red Sea coast of Egypt	• After 24 months, fishery management plan • After 24 months, primary analysis of biomedical benefits completed • After 36 months, pilot mariculture system in operation/ feasibility study completed • After 36 months, trained rangers and fishermen	• Sea cucumber management plan • 3 MSc theses related to the fishery resource, mariculture and biomedical compounds • Publications in the scientific literature • Final report of project scientific committee • Press releases/ newsletter articles	• Recommendations are accepted and incorporated into policy • Information generated ie: species, economic value, rational use accepted and incorporated into NBU's NBS • Mariculture to offer a viable alternative to fishing for local communities

Outputs • Produce a fishery management plan for sea cucumbers • Produce a pilot mariculture system • Identify secondary compounds of potential biomedical value • Train EEAA rangers and local fishermen in stock assessment and mariculture respectively	• After 12 months species list and reference collection established • After 24 months stock assessment, database and GIS system established • After 24 months bioactive compounds and their activity identified • After 36 months, pilot mariculture system operating	• 3 MSc theses • Field guide to Holothuria of Red Sea • Papers published in scientific literature • Final report of Scientific Committee • Minutes and reports of all progress meetings • Press releases/ newsletter articles	• Recommendations of the management plan accepted/ incorporated into policy • EEAA to continue monitoring beyond Darwin funding • Mariculture to prove economical and therefore expanded by the trained fishermen
Activities • Stock assessment for Holothuria along Red Sea • Development of mariculture system for sea cucumbers • Isolation of bioactive compounds and their specific activity • Training of Egyptian scientists, EEAA rangers and local fishermen	• £160, 700 requested from Darwin Initiative • £170, 308 donated by partner institutions • species list and collection established • After 24 months database and GIS system established • After 24 months bioactive compounds and activity identified • After 36 months, pilot mariculture system operating	• Cost statement for grant will be available from Hull University Research Office • Minutes and reports of all progress meetings • Press releases/ newsletter articles • Final report of Scientific Committee • Papers published in scientific literature	• Secondary compounds with potentially useful bioactivity are found • In vitro fertilisation and culture of plankton stages proves successful in mariculture